Tracking Juvenile Kemp's Ridley Sea Turtles With Stable Isotopes

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Abstract

Every fall, juvenile sea turtles become trapped in Cape Cod Bay and strand as the cooling waters lowers their metabolism into a hypothermic state called cold-stunning. Kemp's ridleys are the most endangered sea turtle species and account for the majority of cold-stunned sea turtles in Massachusetts. Stable isotope analysis has been used to track migratory animals including other sea turtles species except for Kemp's ridleys. For marine life, the ratios of $\delta^{13}C$ and $\delta^{15}N$ correlate to latitude and distance from shore, respectively. Since tissues do not incorporate stable isotopes at the same rate, an individual's migration route can be identified by analyzing the same isotopes in different tissues. Skin and whole blood samples from fifteen deceased Kemp's ridleys that were recovered in November and December 2017 were analyzed and compared to existing isoscapes of the northwestern Atlantic Ocean to identify their summer feeding grounds. The skin sample ratios showed that the Kemp's ridleys were likely feeding off the coast of the mid-Atlantic states in June through July. The whole blood samples put the sea turtles in Georges Bank, southeast of Cape Cod, in August through September, but it is possible that the turtles were feeding further north in the Gulf of Maine. The creation of a Gulf of Maine isoscape would help determine just how far north juvenile Kemp's ridley sea turtles migrate, identifying important feeding habitat for a vulnerable life stage.
Dedication

For the volunteers of the New England Aquarium and Wellfleet Bay Wildlife
Sanctuary who work tirelessly to save sea turtles.
Acknowledgments

My gratitude for their guidance, encouragement, and emails to keep me on track during this process goes to my research director, Dr. Noreen Tuross, and advisor, Dr. James Morris. I would also like to thank Dr. Marshall Otter, the manager of the MBL Stable Isotope Laboratory, for the tour of his lab and sharing the view of Woods Hole from it.

I would not have been able to complete this thesis without the staff at the New England Aquarium seeing the value in this study, particularly the Director of Rescue and Rehab, Constance Merigo, and the Director of Animal Health, Dr. Charles Innis. I would also like to thank the following individuals in the Rescue/Rehab department for their assistance: Adam Kennedy and Linda Lory, Senior Biologists; Katie Pugliares-Bonner, Necropsy Coordinator; and Rebecca Visnick, Necropsy Volunteer.

Lastly I would like to thank the following people for helping fuel my sea turtle obsession over the last decade: Dr. Terry Norton of the Georgia Sea Turtle Center; Robert Prescott and Dennis Murley of the Wellfleet Bay Wildlife Sanctuary; and Kerry McNally of the New England Aquarium.

Of course I would not have even gotten to this point without my family and all of the support they provided. I would especially like to thank my parents for nurturing a love of science and the natural world throughout my life.
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Chapter I

Introduction

Post-hatchling sea turtle migration in the Atlantic Ocean is controlled by oceanic currents until the Gulf Stream brings them to New England. Once the turtles have reached the northwest Atlantic, "they would have grown in size and strength to be active swimmers instead of just drifting" (Marquez-M, 1994, p. 14). Four species of the seven species of sea turtle migrate as far north as Massachusetts to feed: leatherback (*Dermochelys coriacea*), green (*Chelonia mydas*), loggerhead (*Caretta caretta*), and Kemp's ridley (*Lepidochelys kempii*). Green, loggerhead, and Kemp's ridley sea turtles can be trapped in the cooling waters of Cape Cod Bay during the migration and become cold-stunned, washing ashore (Innis et al., 2009; Marquez-M, 1994; Still, Griffin, & Prescott, 2005). From 1979-2002, Kemp's ridleys accounted for approximately 78% (984 out of 1280) of stranded sea turtles on Cape Cod beaches (Still et al., 2005).

Definition of Terms

"Anthropogenic": caused or influenced by humans.

"Bycatch": organisms caught in fishing lines or nets that are not the targeted species.

"Carapace": the dorsal portion of a turtle shell.

"Cold-stunned": hypothermic state.
"Delta 13 Carbon (δ¹³C)": stable isotope ratio of carbon (¹³C:¹²C).

"Delta 15 Nitrogen (δ¹⁵N)": stable isotope ratio of nitrogen (¹⁵N:¹⁴N).

"Isoscape": a spatial prediction of isotope ratios in a landscape.

"Life history": the events in an organism's lifetime

"Lost years": juvenile sea turtles. The term usually references the time between post-hatchlings and sub-adults.

"Natal beach": hatching location that will be returned to as an adult to reproduce.

"Neritic": description for the ocean above the continental shelf and the species within it.

"Pelagic": description for the open ocean and the species within it.

"Plastron": the ventral portion of a turtle shell.

"Scutes": modified scales on a turtle shell.

"Stable isotopes": atoms that do not decay.

"Trophic level": an organism's position on the food chain.

The Most Endangered Sea Turtle Species

Kemp's ridley sea turtles are protected under both the Endangered Species Act (ESA) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix I (National Marine Fisheries Service [NMFS], U.S. Fish and Wildlife Service, & SEMARNAT, 2010). There were fewer than 550 adult females in 1990, resulting in Kemp's ridleys being listed as critically endangered (Spotila, 2004). Sea turtles return to their natal beaches to nest, and the majority of Kemp's ridleys nest on
Mexican and Texan beaches on the Gulf of Mexico (Marquez-M et al., 2005; NMFS et al., 2010; Spotila, 2004). Because of cooperative conservation efforts between Mexico and the United States, Kemp's ridleys may have the smallest population size of sea turtles but as of 2009 there were an estimated 8,000 nesting females (Marquez-M et al., 2005; NMFS et al., 2010).

The juvenile years are often referred to as the "lost years" because sea turtles are difficult to observe, swimming from their natal beaches to the open ocean where they feed and grow (Carr, 1987; Reich, Bjorndal, & Bolten, 2007). If the "lost years" habitat of Kemp's ridleys can be identified, regulations could be created to help increase survivorship of a life stage that is more affected by anthropogenic threats than natural predators (NMFS et al., 2010). When Kemp's ridley sea turtles hatch, their estimated survival rate is only 34% (NMFS et al., 2010), but once they reach the size that strand on Cape Cod every year (mean straight carapace length [SCL] of 26.9 cm), their survival rate increases to 76% (NMFS et al., 2010; Still et al., 2005). If the migration routes and feeding sites of juvenile Kemp's ridleys can be identified, it could be used to draft new regulations for fisheries or boating policies. Protecting their habitat could help increase survivorship of the juvenile age class. Once a Kemp's ridley grows to sub-adult size, it has a 91% chance of surviving until reproductive maturity at approximately 12 years old (NMFS et al., 2010; Witherington & Witherington, 2015).

The coastal waters of the eastern United States provide important feeding habitat for juvenile Kemp's ridley sea turtles. Kemp's ridleys transition from pelagic foragers to benthic hunters at around 2 years old (NMFS et al., 2010). Juvenile Kemp's ridleys
captured in New York waters and recaptured within 90 days grew an average of 1.2 cm SCL with the highest growth at 2.4 cm SCL (Morreale & Standora, 2005). Morreale and Standora (2005) estimated that Kemp's ridleys could grow 4.1 cm SCL during the 4 month period in which they are feeding in coastal New York waters. A comparative study by Schmid (1995) calculated growth rates ranging from 4.26 to 7.84 cm SCL per year when the Kemp's ridleys were recaptured after more than 180 days near Cape Canaveral, Florida. The abundance of prey in the northwest Atlantic is important for juvenile Kemp's ridleys during a time in which their diets change and the turtles need to gain experience hunting crabs (Morreale & Standora, 2005).

Kemp's ridleys strand annually on beaches along Cape Cod Bay, Massachusetts in November and December (NMFS et al., 2010; Still et al., 2005), but it is unknown if Cape Cod Bay is a key feeding ground for juvenile Kemp's ridleys in the northwest Atlantic Ocean or if a subset of that age class gets incidentally trapped by the natural barrier of Cape Cod as they try to migrate south to warmer water. Cold-stunned Kemp's ridleys in the northwest Atlantic are not exclusive to Cape Cod as they are also known to strand on Long Island, NY (Morreale, Meylan, Sadove, & Standora, 1992). A total of 97 Kemp's ridleys stranded on beaches along Long Island Sound between 1985-1987, and the turtles are known to be feeding in those waters during the summer (Morreale et al., 1992; Morreale & Standora, 2005). The topography of Long Island Sound allows turtles entering those waters to have a better chance of being blown out to the open ocean by winds as compared to animals within Cape Cod Bay (Still et al., 2005). Cape Cod is not the furthest north a Kemp's ridley has been found. In November 2015, a cold-stunned
Kemp's ridley was even found alive on the coast of Nova Scotia (CBC News, 2015).

Determining the summer feeding habitat of the Kemp's ridley sea turtles could aide conservation efforts for the most endangered sea turtle species at a vulnerable time in their life history (Marquez-M et al., 2005; NMFS et al., 2010). Juvenile Kemp's ridleys are believed to spend their time in water no deeper than 50 meters along the continental shelf of the eastern United States (NMFS et al., 2010). Between 2001-2008, fishery observers documented two Kemp's ridley sea turtles in Georges Bank (southeast of Cape Cod), one in August and the other in September (Murray, 2011). Sea turtles that were satellite tagged and released from Massachusetts did not enter Cape Cod Bay the following summer (Appendix: Figures 13, 14, 15). The hypotheses explored in this study are: 1) Juvenile Kemp's ridleys are feeding in the Atlantic Ocean around Cape Cod, even north of it, and 2) the individuals that strand in the fall in Cape Cod Bay because of their attempts to return south when the water temperature started to drop and not because they utilized it as feeding habitat. Using Kemp's ridleys that had stranded on Cape Cod as a representative sample of the juvenile age class, I analyzed the stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ to determine whether or not the turtles fed within Cape Cod Bay during the summer.

Stable Isotope Analysis

Stable isotope analysis has become an alternate method of tracking migratory animals instead of equipping individuals with a satellite tag or establishing a mark-recapture program (Rubenstein & Hobson, 2004). Several investigators have suggested
the potential for stable isotope analysis as a tool for tracking sea turtle species during their juvenile years (Lopez-Castro, Bjorndal, Kamenov, Zenil-Ferguson, & Bolten, 2013; Reich et al., 2007; Reich, Bjorndal, & Martinez del Rio, 2008) and identifying habitat for sea turtle conservation efforts (Wallace, Avens, Braun-McNeill, & McClellan, 2009).

Stable isotope analysis has been used to track the following species of sea turtles: loggerhead (Allen et al., 2013; Ceriani, Roth, Evans, Weishampel, & Ehrhart, 2012; Hatase et al., 2002; McClellan, Braun-McNeill, Avens, Wallace, & Read, 2010; Pajuelo, Bjorndal, Reich, Arendt, & Bolten, 2012; Wallace et al., 2009; Zbinden et al., 2011), leatherback (Caut, Guirlet, Angulo, Das, & Girondot, 2008; Seminoff et al., 2012; Wallace, Seminoff, Kilham, Spotila, & Dutton, 2006), and green (Reich et al., 2007; Vander Zanden, Bjorndal, Mustin, Ponciano, & Bolten, 2012) but not Kemp's ridley. This
study would attempt to use stable isotope analysis not to only track a species that has not been previously studied but also an age class that is difficult to track (Marquez-M, 1994).

Stable isotopes occur naturally in the environment and do not decay (Hobson, 2007). An organism, such as a sea turtle, will accumulate stable isotopes from its environment, and the ratios of those isotopes will vary by location (Pajuelo et al., 2012).

Figure 2. $\Delta^{13}$N of particulate organic matter (POM) (top) along the east coast of the United States by season (McKinney, Oczkowski, Prezioso, & Hyde, 2010).
One way for an organism to acquire stable isotopes is by feeding, and since the isotope does not decay, it will maintain the same $^{13}$C ratio throughout the food chain in the same area (McMahon, Hamady, & Thorrold, 2013). The signature of $\delta^{13}$C can be correlated with latitude (Figure 1) (Best & Schell, 1996; Hobson, 2007; Hofmann et al., 2010; Rubenstein & Hobson, 2004), although there is a good deal of equifinality at midlatitude. The ratio of $^{15}$N, however, varies significantly depending on the trophic level as well as distance from land. Primary producers use nitrogen as a nutrient source, which causes an increase in the $\delta^{15}$N ratio (or higher values) as trophic level increases (Hobson, Barnett-Johnson, & Cerling, 2010; France, 2015). Land runoff can also add nitrogen to the ocean, causing the ratio decrease as distance from shore increases (Hobson et al., 2010). The stable isotope for nitrogen can be used to determine neritic (more enriched, lower ratio) or pelagic (less enriched, higher ratio) feeding (Figure 2) (Hobson, 2007; McKinney, Oczkowski, Prezioso, & Hyde, 2010; Rubenstein & Hobson, 2004).

Stable isotope ratios will vary in the ocean by latitude as well as between neritic versus pelagic habitat (Rubenstein & Hobson, 2004). Best and Schell (1996) were able to use baleen plates from southern right whales in order to determine migration patterns. The newest sections of baleen on an individual were near the gum line, and the sample represented a more distant point in time down to the tip of the baleen (Best & Schell, 1996). This allowed multiple time points to be recovered from the same individual whale, representing nearly the entirety of its life history (Best & Schell, 1996).

Instead of using one tissue that grows continuously, such as baleen, to get isotopic ratios from different points of time, different tissues can also be used. Stable isotopes are
accumulated into different tissues in an organism's body based on growth and cell turnover rates (Lopez-Castro et al., 2013; Reich et al., 2008). Changes between tissue turnover time within the same individual animal may have different isotopic values, providing a tracking method for that animal by using the different tissues to represent different points of time. This technique has been used to successfully track loggerhead, green, and leatherback sea turtles (Allen et al., 2013; McClellan et al., 2010; Reich et al., 2007; Rubenstein & Hobson, 2004; Seminoff et al., 2012; Zbinden et al., 2011). For the Kemp's ridley sea turtles, I will not attempt to track their entire life but the more recent feeding sites prior to stranding.
According to a study by Reich et al. (2008), analyzing different tissues will result in different time points for both $\delta^{13}$C and $\delta^{15}$N. Figure 3 shows the effect of a diet change on stable isotope ratios in juvenile loggerhead sea turtles. Based on the results published by Reich et al. (2008), whole blood should provide a more recent location than skin. Using two tissues with different metabolic rates (whole blood and skin), the analysis of two stable isotopes ($\delta^{13}$C and $\delta^{15}$N) should be able to provide locations on where individual turtles were feeding at two different points of their recent life history.

These isotopic ranges will document where the Kemp's ridley sea turtles were feeding in the summer and early fall. The isotopic signatures should not represent the time right before their strand dates as cold-stunned turtles typically have reduced fat and empty digestive systems (Innis et al., 2009). Valente et al. (2008) found that it took juvenile loggerhead sea turtles approximately 3 weeks to pass 85% of simultaneously ingested markers in water temperatures of about 16.3°C (61°F) (Valente et al., 2008). Since Kemp's ridleys strand when Cape Cod Bay reaches 10.4°C (51°F) and lower (Still et al., 2005), the whole blood isotopic values should represent close to the last feeding location.

Research Implications

Stable isotope ratios (C and N) from a hypothetical Kemp's ridley with a whole blood sample that yields high $\delta^{13}$C and high $\delta^{15}$N and skin that yields low $\delta^{13}$C and low $\delta^{15}$N could have spent early summer in the Gulf Stream off the mid-Atlantic coast before swimming to the coast of the United States and traveling north. My aim was to analyze
stable isotopes from a minimum of fifteen Kemp's ridley sea turtles of varying sizes to see if the turtles were following a single migration route or if they were taking different trajectories before washing ashore on Cape Cod Bay beaches in the fall. If the juvenile Kemp's ridleys share a summer feeding habitat, the information could be used to support regulations that would help protect sea turtles in that area.

It is known that the sea turtles are in the northwestern Atlantic Ocean since they strand on beaches facing Cape Cod Bay and Long Island Sound every fall (Still et al., 2005; Morreale et al., 1992), but unlike in Long Island Sound, the habitat they using prior to washing ashore on Cape Cod is unknown. The stable isotope signatures, when matched with the approximate corresponding locations in the North Atlantic Ocean, can be applied to a map in order to determine the range of juvenile Kemp's ridley sea turtles. The map could then be used to create or expand fishery and boating regulations. Kemp's ridley sea turtles are affected directly by these industries when they are caught as bycatch; dredging reduces their prey species; they are killed or injured by boat propellers; chemical waste causes sick turtles as they bioaccumulate toxins; or they are stressed by low frequency noise pollution (NMFS et al., 2010). Protecting juvenile Kemp's ridley sea turtles from human impacts will help increase survivorship as anthropogenic factors are the only threat left from the juvenile age class through adulthood other than sharks (NMFS et al., 2010).
Chapter II

Materials and Methods

As samples were taken from dead sea turtles, this study required neither a U.S. Fish and Wildlife Service ESA section 10(a)(1)(A) recovery permit (D. Carter, personal communication, February 23, 2017) nor an approved animal research protocol with Harvard (S. Niemi, personal communication, January 26, 2017).

Turtle Selection

Sea turtles that strand in Massachusetts are found and collected by the MassAudubon's Wellfleet Bay Wildlife Sanctuary (WBWS) to be brought to the New England Aquarium (NEAQ) for emergency care and rehabilitation (Innis et al., 2009; Still et al., 2005). When they get to NEAQ, the turtles are photographed (Figure 4); given short, supervised swims; and have blood drawn so the veterinarians can determine which turtles are in poorer health and what treatments to prescribe. Turtles were confirmed dead on arrival when no cardiac activity was viewed on an ultrasound by an NEAQ veterinarian. Turtles that arrived alive at NEAQ and were given medication but died afterwards were not included in this study due to the potential for confounding effects on the stable isotope signatures.

The turtles stranded during the same 2017 cold-stunning event on Cape Cod to omit the possibility of annual variation in ocean temperatures affecting the migration
Figure 4. NEAQ intake photos for NEST-17-116-Lk (ST2017-256) carapace (top) and plastron (bottom) views.
route or isotope values within the sample cohort. The first sampled turtle stranded on
November 16\textsuperscript{th} and the last on December 13\textsuperscript{th} (Appendix: Table 2). Each turtle had been
assigned two accession numbers: 1) An "ST" number (formatted ST-year-number i.e.
ST2017-256) by the state stranding coordinator at WBWS and 2) a "NEST" number
(formatted NEST-year-number-species code i.e. NEST-17-116-Lk) on admittance to
NEAQ. Turtles only receive a NEST number if they are transported to NEAQ. The
stranding information, including measurements, are provided on the "Sea Turtle
Stranding and Salvage Network - Stranding Report" (Appendix: Figure 16) that is filled
out for every stranded sea turtle in Massachusetts. Turtles are measured with calipers
(accurate to 0.1 cm) and weighed (to 0.1 kg) at WBWS prior to transport. Figure 17 in the
appendix shows how SCL is measured on a sea turtles. I hoped for a representation of
different sized cold-stunned Kemp's ridleys as the SCL range is 18.4-37.2 cm (Still et al.,
2005). Turtles at the smaller end of the spectrum may have utilized different feeding
habitat than the larger turtles.

Sample Collection and Processing

Skin and whole blood samples were taken from fifteen deceased Kemp's ridley
sea turtles in the necropsy room of NEAQ's Rescue/Rehab department. Turtles were
sampled at NEAQ and not at WBWS as WBWS only sends live turtles to NEAQ. Any
turtle that arrived deceased would likely have died during transport, and sample
collection required recently deceased turtles. Whole blood was taken from the jugular
vein in a 3.0 mL syringe with a 22-gauge needle for a minimum total volume of 0.5 mL
(Pajuelo et al., 2012). Skin was taken from the dorsal aspect of the neck (Figure 5) with a fresh scalpel blade, at least 100 mm\(^2\) in area, avoiding skin defects such as scar tissue. All samples were stored in cryovials labeled with the turtle's NEST number and frozen until they could be processed.

To prepare the samples for stable isotope analysis, skin samples had fat or blood vessels removed before being cut finely with a new scalpel blade. The samples were freeze dried in the cryovial with the cap slightly off until no moisture remained before being ground into a powder with a mortar and pestle (Pajuelo et al., 2012; Reich et al., 2008). A minimum dry weight of 0.7 mg was measured in a tin capsule that was then loaded into a 96-well tray for submission to a contract stable isotope laboratory. Fifteen

Figure 5. NEAQ photo of NEST-17-236-Lk prior to necropsy. Note skin missing on the dorsal surface of the neck due to my sample site.
wells with skin samples were mailed to the UC Davis Stable Isotope Facility in Davis, CA. The whole blood samples took longer to dry, so those samples were later hand-delivered in fifteen wells along with two wells of skin samples to the Marine Biological Laboratory Stable Isotope Laboratory in Woods Hole, MA.

Both batches of samples were analyzed for $^{13}\text{C}$ and $^{15}\text{N}$ in a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) that was connected to either a Europa ANCA-GSL (Davis) or ANCA-SL (Woods Hole) analyzer (University of California Davis Department of Plant Sciences, 2017; University of Chicago Marine Biological Laboratory, 2015).

Analysis

The results of $\delta^{15}\text{N}$ were analyzed to determine pelagic versus neritic feeding sites, and the $\delta^{13}\text{C}$ results gave approximate latitude. This was done by comparing results to previous stable isotope studies performed in the northern Atlantic Ocean. Since the sampled turtles stranded between mid-November and mid-December, the isotopic signatures from skin should reflect that of feeding locations approximately five months prior (June-July) and the whole blood that of approximately three months prior (August-September). Graphs original to this thesis were created using RStudio (RStudio, 2018) and maps were created in Google Earth (Google, 2013).
Chapter III

Results

The Kemp's ridley sea turtles sampled for this study all stranded between November 16 and December 13, 2017 and arrived at NEAQ either the same day they were recovered off the beach or the following morning. The smallest turtle had an SCL of 20.9 cm and a mass of 1.3 kg while the largest was 36.1 cm with a mass of 5.9 kg (Appendix: Table 2).

The ranges for the ratios of $\delta^{13}$C between skin (-17.5 to -15.0‰, variance 0.39) and whole blood (-20.3 to -18.1‰, variance 0.45) did not overlap. The whole blood samples yielded lower results than the skin samples (Table 1). The $\delta^{15}$N ratios had a wider range for whole blood (8.18 to 14.41‰, variance 3.64) than they did for skin (8.7 to 12.2‰, variance 1.01). In Figure 6, the isotopes were graphed against each other with $\delta^{13}$C on the x-axis and $\delta^{15}$N on the y-axis to illustrate the separation of $\delta^{13}$C values

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<th>Skin (N = 15)</th>
<th>Whole blood (N = 15)</th>
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<td>Range (max., min.) (‰)</td>
<td>Variance</td>
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<tr>
<td>$\delta^{13}$C</td>
<td>2.5 (-15.0, -17.5)</td>
<td>0.39</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>3.5 (12.2, 8.7)</td>
<td>1.01</td>
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between the tissue types as well as the difference of spread between the tissues' $\delta^{15}$N ratios. The best fit line indicated a negative correlation between the two isotopes. When running a statistical analysis on Figure 6 in RStudio (2018), it gave an adjusted R-squared value close to 0 (0.131) and a p-value less than 5% (0.02797). This indicates a strong statistical value in the isotope ratios when all 30 samples are grouped together. Figure 7 displays the ratios by tissue type. When only looking at the isotopic relationship of the 15 samples from each tissue type, skin had a negative correlation while whole blood had a positive one. The smaller sample sizes did not demonstrate strong statistical significance, however. The adjusted R-squared values were closer to 0 (skin 0.05599, whole blood -0.004863) but the p-values increased significantly (skin 0.1992, whole blood 0.3519).

Looking at the isotopic signature changes in each individual turtle (Appendix:}
Figure 7: Kemp's ridley isotope results for skin and whole blood by tissue type demonstrated trends but were not statistically significant.
Table 3), δ^{13}C decreased from the skin sample to the whole blood while δ^{15}N increased for 11 turtles. The remaining 4 turtles either had δ^{15}N ratios that stayed similar to the skin ratios (032-Lk, 094-Lk, and 208-Lk) or decreased (268-Lk). Those 4 turtles also accounted for the lowest δ^{15}N ratios for whole blood. The only turtle that exhibited the decrease not only had the lowest δ^{15}N whole blood signature (8.18‰) but was also the largest turtle in this study (SCL 36.1 cm, 5.9 kg).
Chapter IV
Discussion

Comparing the isotopic signatures to isoscapes previously created for the Atlantic Ocean demonstrated a migration path for the fifteen Kemp's ridley sea turtles from approximately June through December 2017. All of the sampled turtles were recovered from Cape Cod Bay beaches between 42.03 to 41.76 N latitude and -70.48 to -70.00 W longitude.

Isotopic Signatures of Skin Samples

The isotopic signatures from the skin samples would represent approximately 150 days prior to stranding (Reich et al., 2008), which would encompass mid-June to mid-July for the turtles used in this study. Because the ocean temperature around New England in early summer averages 15.0°C (59°F) or lower (National Oceanic and Atmospheric Administration's [NOAA] National Data Buoy Center [NDBC], 2018a), it was expected that the stable isotopes from the skin samples would indicate waters further south.

Carbon

Since the ratios for $\delta^{13}C$ of POM are correlated to latitude, the range of -15.0 to -17.5‰ is likely to fall between 32.5 (approximately midway between Savannah, GA and
Figure 8: $\delta^{13}$C ratios from adult male loggerhead sea turtle skin samples correlated with latitude in the Northwest Atlantic Ocean (Pajuelo et al., 2012).

Charleston, SC) to 40.0 N latitude (near Delaware's southern border) (Figures 8 and 9) (Ceriani et al., 2014; Pajuelo et al., 2012). While it is possible that the turtles could have been further south in this time frame based on the Ceriani et al. (2014) isoscape (Figure 9), it is unlikely that the turtles were further north. Schmid and Barichivich found that Kemp's ridleys tend to be found in water temperatures above 20.0°C (68°F) outside of cold-stunning events (as cited in NMFS et al., 2010, p. I-13). Due to sea surface temperatures in 2017, it was unlikely that the Kemp's ridley sea turtles would have been further north than New Jersey by mid-July if they were in coastal waters (NOAA NDBC, 2018b). If the turtles were still pelagic and within the warmer waters carried by the Gulf Stream, however, they may have been found further north (NMFS et al., 2010).
Nitrogen

Per Rubenstein and Hobson (2004), organisms feeding closer to shore would have a lower $\delta^{15}N$ ratio that gets higher as they get further away. The $\delta^{15}N$ ratios are not directly tied to longitude since the distance the distance from shore along the same line is not consistent, and a source of nitrogen in the ocean is runoff from land (Ceriani et al., 2012; Rubenstein & Hobson, 2007). The skin sample ratios ranged from 8.7 to 12.2‰, which seemed to indicate that the turtles were in the vicinity of 75.0 W longitude. That line of longitude, when running parallel to the locations indicated by the carbon results, has a varying range of $\delta^{15}N$ ratios due to the shape of the U.S. coastline (Ceriani et al., 2014). Turtles feeding near that longitude off of South Carolina would have lower nitrogen than turtles at the same line from Maryland to New Jersey due to the latter being much closer to the coastline (Figure 9).

Isotopic Signatures of Whole Blood Samples

Whole blood was expected to provide feeding sites from approximately 100 days prior to stranding, which would cover August and September for the turtles that were sampled. Based on my hypothesis, these ratios should not match Cape Cod Bay as I did not believe the turtles to be actively feeding in that body of water during the summer. The resulting whole blood ratios for $\delta^{13}C$ were lower than covered in the isoscapes from Ceriani et al. (2014), and the ratios for $\delta^{15}N$ were higher (Figure 9).
Figure 9: $\delta^{13}$C (top left) and $\delta^{15}$N (bottom left) isoscapes with associated standard errors (right) from juvenile and adult loggerhead sea turtle skin samples (Ceriani et al., 2014).
Carbon

A study by Dodge, Logan, and Lutcavage (2011) that involved live capture of leatherback sea turtles around Massachusetts in July through October 2007-2009 yielded a mean whole blood $\delta^{13}C$ ratio of -18.51‰ (± 0.44) (Appendix: Table 4). If leatherback sea turtles also have an incorporation rate of 100 days for $\delta^{13}C$ in whole blood, the ratio measured by Dodge et al. (2011) off of Massachusetts would account for approximately April through June. Since $\delta^{13}C$ ratios in the ocean do not exhibit an offset with trophic level (Graham, Koch, Newsome, McMahon, & Aurioles, 2010), the values from the sample turtles were compared to non-sea turtle data in order to determine a feeding location. Dodge et al. (2011) also sampled Ctenophore and Cnidarian jellyfish species that the leatherbacks would have possibly been feeding on around Massachusetts. The jellyfish sampled in August-October 2007 and July-September 2008 had $\delta^{13}C$ ratios ranging from -22.67 to -18.37‰ (Dodge et al., 2011).

The $\delta^{13}C$ range of -20.3 to -18.1‰ for the fifteen Kemp's ridleys in this study had signatures more similar to those of the zooplankton collected by Fry and Quiñones (1994). Zooplankton collected in August 1988 from Georges Bank had ratios ranging from approximately -23.0 to -18.5‰, and 2 sampling sites in the Sargasso Sea the following month ranged from -21.5 to -18.5‰ (Figure 10) (Fry & Quiñones, 1994).

Figure 18 in the appendix shows the locations of the sample site coordinates from Fry and Quiñones (1994) along with the turtles' stranding location of Cape Cod Bay indicated by flags entered in Google Earth. As $\delta^{13}C$ is also linked to temperature, comparing the water temperature readings at 1 meter below NOAA Station 44011 in Georges Bank gave
Figure 10. Georges Bank (left, open circles), Gulf of Maine (left, open squares) and both Sargasso Sea sites (right, filled circles and squares). It was not specified which coordinates went with each Sargasso Sea plot (Fry & Quiñones, 1994).
a range of 15.4-24.9°C (60-77°F) for August 1988 compared to 16.7-25.5°C (62-78°F) for August 2017 (NOAA NDBC, 2018a).

Nitrogen

The whole blood $\delta^{15}$N had the largest variance of isotopic values ranging from 8.18 to 14.41‰. The Gulf of Maine zooplankton signatures in Figure 10 (approximately 5.5 - 9.0‰) could account for the lower end of the spectrum, but the jellyfish in Nantucket Sound (8.82 - 12.29‰) were a closer match (Appendix: Table 4) (Dodge et al., 2011). When also considering that ocean upwelling can cause an increase in $\delta^{15}$N signatures, it is possible that the Gulf of Maine and Georges Bank had higher $\delta^{15}$N ratios in 2017 than they did in 1988 due to hurricanes (Graham et al., 2010; Oczkowski, Kreakie, McKinney, & Prezioso, 2016). In August and September 1988 there was one named storm in the Atlantic that travelled through Georges Bank on August 7th, Tropical Storm Alberto (NOAA National Hurricane Center [NHC], 2018). In 2017, Hurricanes Gert (August 16th) and Jose (as a tropical storm on September 20th, downgrading on the 22nd but lingering until the 25th) may have caused an increase in $\delta^{15}$N, particularly Hurricane Gert as Jose was later than the expected time frame of the Kemp's ridley whole blood signatures (NOAA NHC, 2018).

As $\delta^{15}$N also changes with different trophic level feeding by 3-4‰, it is possible that the lower range ratios (8.18-11.22‰) are going to more closely match the regional zooplankton for where these turtles had been feeding while the higher range ratios (11.57-14.41‰) were from the same location but the turtles were eating prey at a higher
trophic level (Ceriani et al., 2012; France, 2015). This could mean that to the turtles were feeding on large zooplankton and one trophic level higher in Georges Bank and the Gulf of Maine or that they were feeding one to two trophic levels higher than the zooplankton in the Sargasso Sea (France, 2015; Fry & Quiñones, 1994).

Correlations Between the Isotopes

Figure 6 demonstrated a statistically significant trend that $\delta^{15}$N decreased as $\delta^{13}$C increased, but the trend did not hold when the graphs were separated out by tissue. The skin samples maintained the same trend in Figure 7 as they did in Figure 6 ($\delta^{15}$N became lower, or more enriched; $\delta^{13}$C became higher, or less enriched). For the whole blood samples, however, the trend line showed that $\delta^{15}$N became less enriched as $\delta^{13}$C also became less enriched. Since an increasingly negative $\delta^{13}$C is linked to an increase in latitude (Ceriani et al., 2014; Pajuelo et al., 2012), the combined sample trend line as well as the one for only the skin samples indicate higher $\delta^{15}$N ratios at higher latitudes, but the trend line for whole blood by itself shows lower $\delta^{15}$N at higher latitudes.

The $\delta^{15}$N ratio can be representative of both distance from shore and feeding at different trophic levels. The low statistical significance for the tissues when isolated from each other in Figure 7 could be because of the small sample size. It could have also been caused by the turtles changing their feeding behavior as they moved further north. Post-hatchling Kemp's ridley sea turtles live and feed in floating beds of algae, such as in the Sargasso Sea, for their first 1 to 4 years of life (NMFS et al., 2010). The turtles will then move to coastal waters where they switch to a diet mainly comprised of crabs and other
invertebrates (Morreale & Standora, 2005; NMFS et al., 2010).

If the skin sample $\delta^{13}C$ was showing the turtles as being somewhere in the mid-Atlantic Ocean 150 days prior to stranding, they may have moved closer to shore as they travelled north. With a skin $\delta^{15}N$ range of 3.5‰, the values were within the range that may or may not indicate a shift of trophic level. At the lower latitudes in the 100 day range, the trend line indicating higher $\delta^{15}N$ means that the turtles are either feeding closer to shore, or at a higher trophic level, or some combination thereof. In comparison, the whole blood $\delta^{13}C$ likely putting the turtles somewhere between the Sargasso Sea and the Gulf of Maine approximately 150 days prior to stranding. The turtles exhibited a wider range of $\delta^{15}N$ (6.23‰) when they were further north, which more strongly demonstrated a trophic level shift than the skin $\delta^{15}N$ range.

Figure 7 showed $\delta^{15}N$ decreasing along with $\delta^{13}C$. An explanation for this result could be explained by the way in which New England curves west after the hook of Cape Cod. Turtles feeding in Georges Bank to the east of Cape Cod at latitude N42°, longitude W68° would be closer to land than Kemp's ridleys feeding at the same longitude but one degree latitude further north. This would put the turtles at a lower $\delta^{15}N$ because of pollution having a reduced effect on $\delta^{15}N$ in the ocean as you moved away from shore.

Conclusions

I had hypothesized that the Kemp's ridley sea turtles are not feeding in Cape Cod Bay in the summer. Based on the stable isotopes ratios, specifically whole blood samples that represent a time point when the turtles would be expected to be in the waters around
Figure 11. Cyan fields are skin estimates and magenta fields are whole blood estimates. The more saturated area is where the carbon and nitrogen ratios from each tissue overlap. The small yellow patch at approximately N42°, W70° is the area encompassing the stranding beaches for the sample turtles on Cape Cod Bay.

Massachusetts, it seems unlikely that they are feeding in Cape Cod Bay. The juvenile Kemp's ridleys would appear to start off in the mid-Atlantic in late spring to early summer and migrate as far north as the Gulf of Maine by late summer to early fall. Figure 11 shows the theoretical regions indicated by the skin stable isotope ratios (cyan) and whole blood (magenta) with the darker areas of each color indicating the overlap of carbon and nitrogen results for each tissue. The red markers are the same coordinates that were given in the zooplankton study from Fry and Quiñones (1994) as shown in Figure 18 in the appendix.
Looking at the fifteen sampled Kemp's ridleys as individuals (Appendix: Table 3), all of their $\delta^{13}$C values decreased from skin to whole blood, which is consistent with a northern migration from early to mid-summer. For $\delta^{15}$N values, eleven turtles had an increase in value that could indicate the turtles moving closer to shore, feeding at a higher trophic level, or both. The other four turtles’ $\delta^{15}$N ratios for whole blood either remained similar the skin ratios (032-Lk, 094-Lk, and 208-Lk) or decreased (268-Lk). The only turtle that exhibited a decrease had the lowest $\delta^{15}$N whole blood signature (8.18‰) and was also the largest turtle in this study (SCL 36.1 cm, 5.9 kg) with the second to last latest stranding date (December 11th) (Appendix: Table 2). If 268-Lk had been feeding closer to shore in the mid-Atlantic and then was further out in Georges Bank later in the summer, that could account for the decrease in $\delta^{15}$N. It is also possible that 268-Lk could have been feeding on spider crabs (*Libinia emarginata*), which Kemp's ridleys have been documented feeding on in New York (Chesapeake Bay Program, 2018; Morreale & Standora, 2005). Spider crabs walk instead of swim, which makes them easier for sea turtles to catch (Morreale & Standora, 2005). They also feed on algae and will attach it to themselves for camouflage, which would put them at a lower trophic level than other crab species (Chesapeake Bay Program, 2018). Spider crabs captured in the estuaries North Carolina had $\delta^{15}$N ranging from 10.17-11.13‰ (Wallace et al., 2009). Those spider crabs would likely have higher $\delta^{15}$N values than spider crabs in Georges Bank due to how they were captured amongst the barrier islands of North Carolina (Wallace et al., 2009).
Sea Turtles in the Gulf of Maine

The northern boundary of the whole blood area in Figure 11 is likely further south than the habitat the Kemp's ridleys are using. The Kemp's ridley that stranded in Nova Scotia, Canada in November 2015 was found in Halls Harbour (CBC News, 2015). Halls Harbour (Figure 12) is in the northeastern corner of the Bay of Fundy. If sea turtles are stranding in Cape Cod Bay because they are obstructed while attempting to swim south to warmer waters, the Bay of Fundy does not have a barricading land mass to the south the way Cape Cod Bay does. Since wind direction is "the most important factor in

Figure 12. Gulf of Maine (Cape Cod to Nova Scotia) and Bay of Fundy (between Nova Scotia and mainland Canada) in reference whole blood area (magenta) and Cape Cod Bay stranding area (yellow).
determining the beach recovery location" (Still et al., 2005, p. 876), it was more likely that turtle was carried into the Bay of Fundy. The Hills Harbour turtle was likely blown into the Bay of Fundy from the Gulf of Maine as the land mass of Nova Scotia would make it difficult for the wind to bring a turtle from the open Atlantic Ocean in the east.

Research Limitations

Both the small sample size and the variation in animal age are limiting factors in this research. This is most clearly shown when considering the statistical significances for the plots shown in Figures 6 and 7.

In regards to animal age, the isoscapes used to compare the Kemp's ridley results were both from loggerhead sea turtles (Ceriani et al., 2014; Pajuelo et al., 2012). Pajuelo et al. (2012) used data from adults that were feeding coastally. Ceriani et al. (2014) used a combination of smaller juveniles that were a similar size to my Kemp's ridleys as well as larger juveniles and adults. When making different isoscapes, Ceriani et al. (2014) made one for all sampled turtles and another made up of only the larger, coastal juveniles and adults. The two isoscapes "fundamentally generated the same isotopic patterns" (Ceriani et al., 2014, p. 8), so there may not be discrepancies in applying isoscapes to different age classes, at least if the Kemp's ridley iscoscapes mirror loggerhead sea turtle data.

The stable isotope turnover rates from Reich et al. (2008) were taken from juvenile loggerhead sea turtle juveniles that were smaller (9.0-13.1 cm SCL) than the Kemp's ridleys I sampled. Those loggerheads were also on a consistent diet in an
artificially controlled habitat (26.5°C or approximately 80°F), so the turnover rates for
the Kemp's ridley tissues might be slower due to the decreased metabolism in colder
waters as well as having to forage actively instead of being fed regularly.

Since Kemp's ridleys are predators, and the diet of juveniles consists largely of
invertebrates (NMFS et al., 2010), the stable isotope values of $\delta^{15}$N could also exhibit a
wider variance because of a non-homogenous diet (Godley, Thompson, Waldron, &
Furness, 1998).

The lipid content of tissue samples can have an effect on $\delta^{13}$C values, yielding
results that are more negative than if the lipids had been chemically extracted or adjusted
for mathematically (Post et al., 2007). Chemical lipid extraction was not performed as it
can fractionate $\delta^{15}$N results (Post el al., 2007). Post et al. (2007) recommends correcting
for lipids in aquatic organisms when the C:N ratio is $\geq 3.5$ although they also noted that,
"Some analyses have found an effect of lipids on $\delta^{13}$C, while others have not" (p. 180).
Post et al. (2007) did not recommend the use of their lipid formula for tissue-specific
studies such as this one.

Future Directions

This study hopefully demonstrates the value in stable isotope analysis on juvenile
sea turtles in New England, but it also adds more questions. The width of the Gulf of
Maine reaches from approximately W70.5° to W65.5° and Cape Cod only juts out into
1/10th of it. Do the turtles feed close to New England beaches, hugging the shore and
getting stuck in Cape Cod Bay? Or do they end up in Cape Cod Bay because of storm
systems that they cannot swim against pushing them into the bay from the northeast? Is
the wide range of $\delta^{15}N$ seen in the whole blood ratios caused by turtles feeding at
different distances from shore or because of a change in trophic level? Where are the
Kemp's ridleys feeding at a point in time closer to stranding? Should New England states
consider legislature that requires trawl fisheries in their waters to use turtle excluder
devices as Georgia does (NMFS et al., 2010)? Or should trawling seasons be shifted to
when the turtles are not in the area as South Carolina does (NMFS et al., 2010)?

Isoscapes, particularly of nitrogen, for the Gulf of Maine would help answer some
of these questions, as would taking samples from different possible prey species in the
area. It would even be possible to get a more recent time point out of live turtles as blood
can be used for three different time points: whole blood, red blood cells, and plasma
(Reich et al., 2008). Red blood cells would give a timepoint between skin and whole
blood (approximately 120 days) and plasma would reflect approximately 60 days before
stranding (Reich et al., 2008). A study analyzing stable isotopes from the different parts
of blood would likely require live turtles. Red blood cells will not separate when blood is
being centrifuged if they have started to breakdown due to either decomposition or cold
exposure causing them to freeze. Since the cold-stunned turtles also usually dehydrated
when they strand, it may also take multiple blood draws to get the amount of plasma
needed (Keller et al., 2012; Rockwell, Innis, Merigo, & Prescott, 2017). This would be
easier to do on live turtles.

The fisheries question would be difficult to address as only two Kemp's ridleys
have been documented caught by or observed near a trawling vessel from 2001-2008
(Murray, 2011). The number of cold-stunned sea turtles on Cape Cod has increased in the last decade, however. In 2014 a new record was reached with over 1,200 turtles recovered in Massachusetts (Appendix: Figure 19). If the cold-stunned sea turtles represent a fraction of the population that is off of New England in the summer and fall, how many are at risk of drowning in fishing gear before dropping water temperatures become a threat?
Figure 13. Satellite tracking map for 1 green sea turtle (Quiddick, black track, 416 days) after September 30, 2005 release. The other track terminates earlier than the summer following release (Seaturtle.org, 2009).
Figure 14. Satellite tracking map for 1 Kemp's ridley (Fossil Butte, navy blue track, 322 days after August 18, 2010 release) and 1 green sea turtle (Goose, red track, 713 days after August 26, 2009) post-release. All other tracks terminate earlier than the summer following their release (Seaturtle.org, 2013).
Figure 15. Satellite tracking map for 1 green sea turtle (Nate the Great, darker blue track) released on August 21, 2013 until 365 days post-release. Another green sea turtle (Kay Scarpetta, green track) did follow the southern coast of MA to swim north into Cape Cod Bay, re-stranding in the 2014 cold-stun season (Seaturtle.org, 2016).
Table 2: All turtles arrived at NEAQ the same day as stranding except for ST17-226 who spent the night at Mass Audubon and was driven up the next morning.

<table>
<thead>
<tr>
<th>ST2017-</th>
<th>Strand Date</th>
<th>Strand Location (all adjacent to Cape Cod Bay, MA)</th>
<th>NEST-17- SCL (cm)</th>
<th>Weight (kg)</th>
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<tr>
<td>128</td>
<td>Nov. 16</td>
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<td>26.6</td>
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<tr>
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<td>032-Lk</td>
<td>21.0</td>
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<tr>
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<td>400</td>
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Figure 16. Sample stranding form for turtle NEST-17-208-Lk (ST2017-376).
Figure 17. Location of SCL measurement on a sea turtle (Wyneken, 2001).
Table 3: Kemp's ridley sea turtles used in this study listed by their ST numbers, NEAQ accession number, 2017 strand date, carapace straight length, and stable isotope ratios of $\delta^{13}$C and $\delta^{15}$N for skin and whole blood (WB) samples.

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<th>NEST-17-</th>
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<th>$\delta^{15}$N (‰)</th>
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<tr>
<td>273-Lk</td>
<td>29.0</td>
<td>3.4</td>
<td>-16.1</td>
<td>-18.9</td>
</tr>
</tbody>
</table>
Table 4. Stable isotope ratios of leatherback sea turtles as well as their potential prey species from the coastal waters of Massachusetts. The asterisk indicates that the sample was a combination of multiple individuals (adapted from Dodge et al., 2011).

<table>
<thead>
<tr>
<th>Sample</th>
<th>δ^{13}C</th>
<th>δ^{15}N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leatherback sea turtles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Whole blood (n = 15)</td>
<td>-18.51 (± 0.44)</td>
<td>10.61 (± 1.03)</td>
</tr>
<tr>
<td>- Skin (n = 27)</td>
<td>-17.84 (± 0.67)</td>
<td>11.13 (± 1.29)</td>
</tr>
<tr>
<td><strong>Potential prey</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- <em>Cyanea capillata</em> (n = 16)</td>
<td>-20.31 (± 0.82)</td>
<td>10.90 (± 1.39)</td>
</tr>
<tr>
<td>- <em>Chrysaora quinquecirrha</em> (n = 9)</td>
<td>-19.30 (± 0.53)</td>
<td>10.83 (± 0.82)</td>
</tr>
<tr>
<td>- <em>Pelagia noctiluca</em> (n = 1)</td>
<td>-20.81</td>
<td>7.59</td>
</tr>
<tr>
<td>- <em>Beroe ovata</em> (n = 2*)</td>
<td>-20.55 (± 2.12)</td>
<td>9.65 (± 1.61)</td>
</tr>
<tr>
<td>- <em>Mnemiopsis leidy</em> (n = 1*)</td>
<td>-18.37</td>
<td>9.48</td>
</tr>
<tr>
<td>- <em>Pleurobrachia pileus</em> (n = 1*)</td>
<td>-20.83</td>
<td>8.82</td>
</tr>
</tbody>
</table>
Figure 18. Georges Bank, Gulf of Maine, and both Sargasso Sea sites with Cape Cod Bay for reference (Fry & Quiñones, 1994).
Figure 19. Cold-stunned sea turtles recovered by WBWS 1979-2016 (WBWS, 2018).


Morreale, S. J. & Standora, E. A. (2005). Western North Atlantic waters: crucial developmental habitat for Kemp's ridley and loggerhead sea turtles. *Chelonia Conservation and Biology*. 4(4), 872-882. This study goes into why it is beneficial for the sea turtles to enter our waters and highlights the importance of preserving those habitats.


