Cold Stunned Sea Turtle Diet Analysis in Cape Cod Bay from 2015-2020

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Abstract

As water temperatures drop in November, Kemp's Ridley, Loggerhead, and Green sea turtles cold-stun in Cape Cod Bay. The foraging ecology of these sea turtles remains an understudied area of research. In this study, we aim to assess the diet of these turtles using a multi-tissue stable isotope analysis of cold-stunned kemp's ridley, loggerhead, and green sea turtles stranded from 2015 to 2020. Stable isotope ratios of carbon and nitrogen were measured in blood, front and rear flipper, liver, muscle, skin, and scute tissue samples. We predict an elevated level of Nitrogen isotope ratios in kemp's ridley and loggerhead turtles compared to green turtles due to the carnivorous loggerheads and kemp ridleys' carnivorous diet and the greens herbivorous diet. We anticipate empty stomachs due to starvation while stranded, and a variety of foraging strategies, migration patterns, and trophic positions between these species. Data collected from this study will add to the knowledge of these turtles' prey species and aid managers in the preservation of these species as a mitigation strategy for these turtles' extinction.

Introduction

The fluctuation of environmental factors, such as temperature and atmospheric pressure associated with climate change, is caused by the buildup of pollutants, including excess carbon dioxide and fossil fuels (Ottersen, G. 2001). The increasing levels of these substances traps solar radiation in the atmosphere due to the greenhouse effect (Ottersen, G. 2001). In a healthy atmosphere, solar radiation enters the atmosphere and is released back as needed (Ottersen, G. 2001). With the increasing greenhouse gases in our atmosphere, solar rays become trapped contributing to fluctuating global temperatures, thus causing oceans to stay warmer later in the autumn. These shifts impact the migratory patterns of sea turtle species in the Western North Atlantic Ocean region (Ottersen, G. 2001).

Cold stunning is a condition comparable to a hypothermic reaction that occurs when sea turtles, specifically, are exposed to prolonged cold water temperatures (V Burke et al. 1991). Cold stunning can result in lethargic or comatose sea turtles often becoming trapped on the shoreline, putting them at risk of complications such as hypoglycemia, pneumonia, starvation, and bradycardia (V Burke et al. 1991). While cold stunning is a significant problem, this phenomenon does not affect a large population (Griffin, L. P. 2019). Cold stunned turtles are often able to recover if rehabilitation measures are taken immediately, thus stressing the importance of rehabilitation and volunteer efforts (Allman P.E. 2000).

The Kemp's Ridley sea turtles, *Lepidochelys kempii*, migrate from the gulf of Mexico to the New England coastal waters during their migration season spanning from late June through early December (Lazell, J. 1980)(Griffin, L. P. 2019). Due to our oceans remaining warmer through early fall, immediately followed by a rapid decline in temperature, the Kemp's Ridley's migration season has been drastically altered (Lazell, J. 1980)(Griffin, L. P. 2019). When sea surface temperatures reach 10°C, cold stunning events have the potential to begin (Griffin, L. P .2019)(Witherington, B. E. 1989)(Schwartz, F. J. 1974). Additionally, when sea surface temperatures reach 5°C conditions are often fatal (Griffin, L. P .2019)(Witherington, B. E. 1989)(Schwartz, F. J. 1974). Kemp's ridley sea turtles typically begin to cold stun at the higher latitudes where ocean temperatures are generally lower and volatile cold snaps occur (Lazell, J. 1980)(Griffin, L. P. 2019). Kemp's ridley sea turtles are the most cold-stun-prone species in the Northwest Atlantic due to their small size (Rainey WE 1981). Kemp's ridley turtles have a strong jaw to help them crush and grind crabs, clams, mussels, and shrimp (Burke, V. J. et al. 1993). Additionally, these turtles' diet includes multiple fish species, sea urchins, squid and jellyfish (Burke, V. J. et al. 1993).

Kemp's ridley turtles have an oceanic–neritic developmental pattern recruiting to benthic–neritic habitats at 20+ cm CL (Snover et al., 2007). Post-hatchling Kemp's ridleys that were stranded in the Gulf of Mexico had Gastropoda (sea snails), Malacostraca (crabs), and algae (e.g., Sargassum sp.) in their gut (Shaver, 1991). Witzell and Schmid (2005) observed that immature Kemp's ridleys that recruited to benthic–neritic waters of southwestern Florida fed primarily on Ascidiacea (e.g., tunicates, Molgula occidentalis). Adult Kemp's ridleys are found primarily in continental shelf waters of the Gulf of Mexico and feed on Malacostraca (i.e., crabs) (Frick and Mason, 1998; Morreale et al., 2007). This diet analysis and movement data suggest that Kemp's ridleys feed on epipelagic organisms during their oceanic period and once recruiting to benthic–neritic habitats, as juveniles, their diet is dominated by bottom-dwelling fauna throughout maturation and adulthood.

Green sea turtles, Chelonia mydas, have a serrated lower jaw which allows them to better digest their primarily herbivorous diet (Rainey WE 1981). Green sea turtles have an oceanic-neritic developmental pattern (Bolten 2003) that has a concomitant shift in diet from omnivory to herbivory (Jones 2013). Their diet fundamentally consists of invertebrates, algae and seagrasses (Seminoff, J. 2006). Due to their larger size, they are less prone to cold-stunning events when compared with other sea turtle species (Seminoff, J. 2006). However, some Green sea turtles are cold-stunning at higher latitudes due to delayed or failed migration south by late autumn (Seminoff, J. 2006). Green sea turtles experience a diet shift concomitant to changes in size and habitat. Ontogenetic shifts can be reflected in stable isotope studies(e.g., Reich et al. 2007; Arthur et al. 2008; Howell et al. 2016). Most marine species who have been found to have ontogenetic diet shifts follow a pattern of feeding in productive or neritic habitats (Barnes and Hughes 1988; Polis et al. 1996). As many marine species age, they switch to a less productive or pelagic habitat (Barnes and Hughes 1988; Polis et al. 1996). Green turtles, however, have previously been found to shift from feeding in diet from a less productive habitat to a more productive habitat. Suggested benefits to having an ontogenetic diet shift include: minimizing intraspecific competition for food among different age classes and the distribution of the impacts of foraging across a diversity of foods (Werner and Gilliam 1984), differences among individual preferences, foraging behaviors, relative abundance, as well as ecological trade-offs. These factors are predicted to influence the food choices of individuals (Estes et al. 2003; Svanback and Persson 2004; Araujo et al. 2009, 2011). There is no evidence that Green sea turtles consume all potential foods in proportion to their relative availability, and there is in fact high variation among individuals or locations. Where seagrass is scarce, juvenile turtles exploit macroalgae and other foods (Cardona et al. 2009; Carman et al. 2012; Russell and Balazs 2015; Santos et al. 2015; Howell et al.2016).

Loggerhead sea turtles, *Caretta caretta*, are found in coastal tropical/subtropical waters, often traveling to temperate waters searching for food (Bass, A. L. 2004). Loggerhead sea turtles have a strong jaw to aid them in digesting their carnivorous diet (Rainey WE 1981). These sea turtles generally eat meat and forage for shellfish living in the deep ocean (Tomas, J. 2006). Loggerheads also eat invertebrate species, including horseshoe crabs, clams, mussels, and shrimp (Tomas, J. 2006).

Loggerhead turtles follow an oceanic–neritic developmental pattern departing nesting beaches and entraining in prevailing oceanic currents as float-and-wait foragers (Bolten, 2003, Witherington, 2002) until recruiting to neritic foraging grounds. Although recent evidence suggests a prolonged oceanic stage or perhaps plasticity in shuttling between habitats persisting even through the commencement of breeding (Parker et al., 2005). During the oceanic stage loggerhead post-hatchlings and juveniles are primarily carnivorous (Bjorndal, 1997). Loggerhead post-hatchlings of the southwest Pacific feed on similar items (unidentified Cnidaria, Gastropoda, Malacostraca, and seagrasses) (Boyle and Limpus, 2008). The diet of oceanic stage loggerheads is purely pelagic/epipelagic unlike the mixed diet of neritic stage loggerheads (Bolten, 2003). Recruitment to the neritic, however, may not come with a concomitant change in diet. There is a transitional stage where new recruits continue to feed on epipelagic organisms while gradually including benthic organisms in their diet (Bolten, 2003; Casale et al., 2008). The length that loggerheads recruit to neritic areas also varies geographically (Bjorndal et al., 2000; Seminoff et al., 2004; Boyle and Limpus, 2008; Casale et al., 2008; Wallace et al., 2009).

Kemp’s Ridley, Loggerheads, and Greens tend to cold stun at higher latitudes where temperatures are lower (Lazell, J. 1980)(Griffin et al 2019). Kemp’s Ridleys are the most prone species to cold-stunning due to their small size (Rainey WE 1981). Sea turtles are a keystone species; thus, the ecosystem would drastically change and suffer if they are removed (Butler, J. et al 2012). These species are responsible for nutrient cycling and grazing of seagrass beds (Butler, J. et al 2012). Seagrass typically has a slow germination rate, however, seagrass seeds excreted from a sea turtle digestive tract can have near-complete germination in less than 30 days (Tol, S. J. et al 2021). As seagrass becomes more stressed due to the impacts of global climate change, understanding the interactions between mega-herbivores and seagrasses may be key to successful management and conservation of both (Tol, S. J. et al 2021).

Stable isotope analysis is a technique that utilizes the identification of isotope signatures found within a tissues sample to determine diet, habitat, trophic position, and migration patterns (Bean, S.B. 2019)(Zanden, H. B. V. 2010)(Dodge, K. L. 2011). Diet can be deduced using heavier isotopes, such as carbon and nitrogen found in tissues, where stable isotope ratios are elevated relative to their diet (Dodge, K. L. 2011) (Bean, S.B. 2019). Stable isotope values reflect the turtle's metabolic activity over different time scales (Dodge, K. L. 2011). This information can be collected by analyzing multiple tissue samples with varying turnover rates (Bean, S.B. 2019). Tissues with faster turnover rates, such as liver tissue and blood plasma, typically reflect their recent diet. In comparison, tissues with slower turnover rates, such as muscle tissue and whole blood, reflect the turtle’s metabolic activity over more extended time periods (Dodge, K. L. 2011). It is more challenging to do a stable isotope analysis on cold-stunned Kemp’s ridleys’ diet due to the empty stomach from starvation once they are stranded on the shorelines (Bean, S.B. 2019)(Zanden, H. B. 2010)(Dodge, K. L.2011)(Reich, K. J. 2008). Most sampling opportunities come from stranded and cold-stunned turtles (Bean, S.B. 2019). Therefore, not all samples are representative of a healthy turtle (Bean, S.B. 2019). Due to lethal sampling methods required to obtain the tissue samples for the stable isotope analysis, data can only be collected from deceased turtles, as these Kemp's Ridley are critically endangered, and Green and Loggerhead sea turtles are threatened (Bean, S.B. 2019). Some sea turtles are highly migratory species, consequently making it difficult to investigate diet and movement based on bulk (i.e., whole tissue) stable isotope values (Bean, S.B. 2019). Bulk isotope values can also be influenced by starvation, which can cause an increase of 15N in tissues due to preferential excretion of 14N during the catabolic breakdown of body tissues (Hobson et al. 1993) (Gannes et al. 1997) (Bean, S.B. 2019). Such effects can further complicate the interpretation of stable isotope data derived from cold-stunned individuals (Hobson et al. 1993) (Gannes et al. 1997) (Bean, S.B. 2019). However, a bulk stable isotope analysis can still provide crucial generalized information on the ecology of the cold-stunned community given the current lack of knowledge (Hobson et al. 1993) (Gannes et al. 1997) (Bean, S.B. 2019).

Kemp's ridley, Green, and Loggerhead sea turtles are all currently on the endangered species list. Cape Cod is at the northern end of their current range, but coastal waters in MA are likely to become increasingly important to these species as their ranges continue to shift further north with climate change. Over the past two decades, abundant new research techniques and aspects such as stable isotope analysis, biologging, and physiological monitoring have emerged and provided new insights into sea turtle's nutrient acquisitions (Jones, T. 2013). Because of techniques such as stable isotope analysis being relatively new, there is still a significant amount of research required to strengthen the understanding of these species' foraging strategies and life cycle to help mitigate their population decline (Jones, T. 2013).

Goal of the Research

This study aimed to identify prey species of Loggerhead, Green, and Kemp's Ridley sea turtles and the diets of these prey species in the Western North Atlantic Ocean. Using a stable isotope analysis of samples from Loggerhead, Green, and Kemp's Ridley sea turtle species and their prey that died from 2015 to 2020, we predicted to find a significantly increased value of nitrogen in Kemp's Ridley and Loggerhead sea turtles that have been found cold-stunned on the Northeastern Atlantic coast (Jones, T. 2013)(Bean, S.B. 2019). Furthermore, we anticipated that the cold-stunned Kemp's Riddles would have high rates of starvation, more isotopic signatures indicating the consumption of fish, clams, mussels, and shrimp, and higher values of nitrogen. Loggerheads would have a carnivorous diet consisting of more clams and muscles with a higher isotopic nitrogen value. Green sea turtles were hypothesized to have increased amounts of algae and lower values of isotopic nitrogen due to their herbivorous diet.

Methods

Stable Isotope Analysis

The samples included in the Stable Isotope Analysis were collected from Necropsy sessions of Kemp's Ridley, Green, and Loggerhead sea turtles that had died while stranded on beaches in the Massachusetts Bay area as a result of cold stunning, along with lady and spider crabs, ctenophores, moon snails, and eelgrass in the same region (Bean, S.B. 2019). The samples were frozen for storage until they were prepared in a laboratory setting for the Stable Isotope Analysis. Samples were dried at 50- 60 degrees celsius for 72 hours and homogenized.

Sample identification information recorded from each sample was transferred into a spreadsheet. Once the sample preparation was complete, samples were sent to the Stable Isotope Lab at UC Davis. An Isotope Ratio Mass Spectrometer was used to identify the nitrogen 15 and carbon 13 isotope markers and record their values within each sample. Data was plotted using standard deviations from naturally occurring values of these isotope markers and compared to species standards and community baselines (McMahon et al. 2014).

Data Analysis

The data was compiled into excel where it was organized clearly. Variables were plotted such as stranding date, weight, gender, and species versus their respective carbon 13 and nitrogen 15 ratios. This data was then transferred to Data Classroom to make graphs and further analyses. T-Tests were conducted to find statistical significance.

Results

An elevated level of nitrogen isotope ratios (δ15NAir (‰)) (Figure 1) in Kemp's ridley and Loggerhead turtles compared to Green turtles and slightly higher levels of isotopic carbon (δ13CVPDB (‰)) (Figure 2) ratios in Green Sea turtles was observed, although the sample size for this species is fairly small due to their lower tendency to cold stun. A t-test conducted a found a statistically significant relationship between the Kemp's ridleys and Green sea turtle δ15NAir (‰) ($p=0.0016$) and between Loggerheads and Green sea turtle δ15NAir (‰) ($p=0.0089$). Additionally, this t-test showed a statistically significant relationship between Loggerheads and

Green sea turtles δ13CVPDB (‰) (p=0.0325). This higher value of δ13CVPDB (‰) indicates a larger proportion of eelgrass (*Zostera marina*) and other invertebrate species prior to stranding.

Cold-stunned Kemp's ridley sea turtles had the lowest average total Carbon to total Nitrogen ratio, which is indicative of the amount of fat in the sample. Loggerheads, being the largest of these three species had the highest average Carbon to Nitrogen ratio.

Discussion

Nitrogen isotope values typically increase with increasing trophic position, reflecting diet (Figure 1), while carbon isotope values tend to be generally consistent with a marine phytoplankton baseline (Figure 2) (Dodge, K. L. 2011) (Bean, S.B. 2019). Lower isotopic nitrogen in green sea turtles was observed (Figure 1), as well as slightly higher levels of isotopic carbon (Figure 2), reflecting their herbivorous-based diet as they primarily feed on species with a lower trophic position such as eelgrass (*Zostera marina*) and other invertebrate species prior to stranding. In Kemp's Ridley and Loggerhead turtles, we observed elevated nitrogen isotope levels (Figure 1), reflecting their carnivorous based diet, reflecting the large proportion of prey species with a higher trophic level. ratios in Green Sea turtles were observed, although the sample size for this species is fairly small due to their lower tendency to cold stun.

The carbon 13 to nitrogen 15 ratio tends to be a good proxy for the fat contents in a sample (Figure 3). Samples with a higher carbon 13 to nitrogen 15 ratios have higher fat contents. These samples with a higher fat content are theoretically healthier. Because most of the turtles included in this study were unhealthy due to their cold stunning conditions, the ratios were relativity low. Despite these low ratios, Kemp's ridley sea turtles had the lowest average total

Carbon to total Nitrogen ratio compared to the other three species, most likely due to their small size and starvation endured while stranded.

The cold-stunned population doesn't have a homogenous migratory and trophic history, and this study observed a variety of foraging strategies, migration patterns, and trophic positions between these species. These techniques could provide further insight into individual turtle trophic history and aid in the development of potential mitigation strategies and improve our knowledge of the northern species in danger of cold-stunning. A comprehensive view of food webs is fundamental for predicting likely responses of marine ecosystems to potential environmental changes. This study contributes to our overall understanding of these endangered species to help save them from extinction.

Due to the lethal sampling methods and ethical implications of studying endangered species of sea turtles, this study is only representative of dead sea turtles. The soft tissues used in this analysis, liver, and muscle, are commonly used in fish isotope studies but may be less useful for sea turtle research given the challenges of obtaining these samples in a non-lethal and minimally invasive manner (Bean, S. B. 2019).

Conclusion

The goal of this research was to determine the prey species of Loggerhead, Green, and Kemp's Ridley sea turtles, as well as their diets, in the Western North Atlantic Ocean. We predicted that Kemp's Ridley and Loggerhead sea turtles found cold-stunned on the Northeastern Atlantic coast would have a significantly increased nitrogen value based on stable isotope analysis of samples from Loggerhead, Green, and Kemp's Ridley sea turtle species and their prey that died between 2015 and 2020 (Jones, T. 2013)(Bean, S.B. 2019). Furthermore, we expected the cold-stunned Kemp's Riddles to exhibit greater rates of starvation, more isotopic signatures indicating a carnivorous diet, and higher nitrogen levels. Loggerheads would consume clams and muscles with a higher isotopic nitrogen value as part of their carnivorous diet. Because of their herbivorous diet, green sea turtles were thought to have more algae and lower isotopic nitrogen values.

Future Research

To continue this study, we plan to conduct a Gut Content Analysis. This technique will be used to quantify sea turtle diet composition based on the frequency of occurrence of stomach contents in our dataset of stomach samples from cold-stunned sea turtles (Revelles 2007). This data will provide a valuable complement to the stable isotope data, supplying direct evidence of diet preferences to help inform the isotope data and our general understanding of sea turtle diet in the Western North Atlantic Ocean (Revelles 2007).

Additionally, we plan to conduct a sulfur isotope analysis of scute samples. Scutes record information over the turtles' lifespan and so can be used to get both recent and earlier life history information. This additional test will provide a good estimate for whether turtles are feeding more in estuaries or coastal waters in Massachusetts.

Appendix

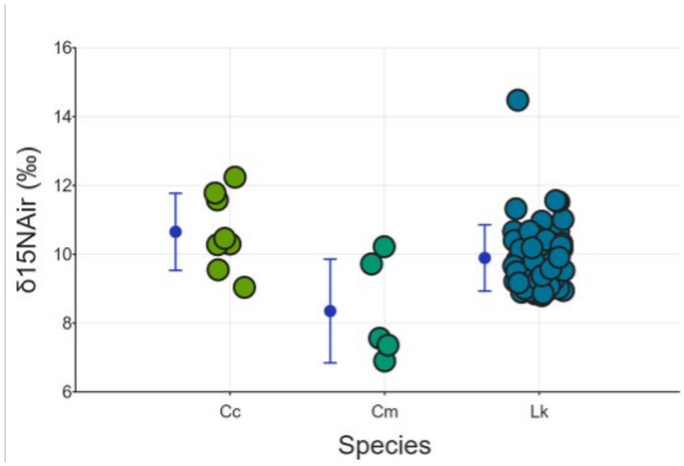

Figure 1. Isotopic nitrogen ratio levels within a sample (δ15NAir (‰)) plotted across species. δ15NAir (‰) tends to increase with increasing trophic levels.

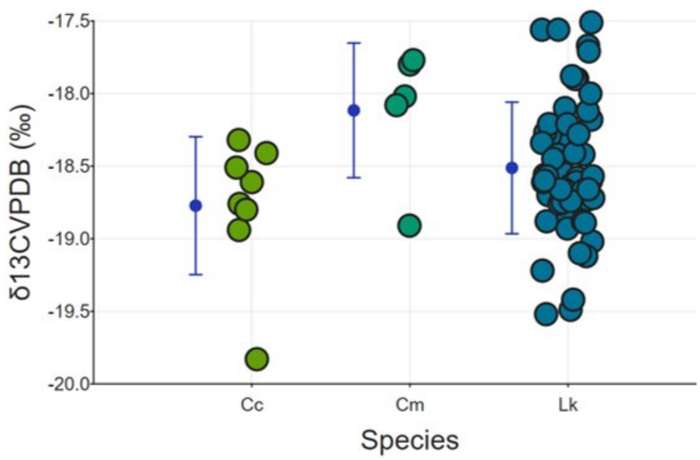

Figure 2. Isotopic carbon ratio levels within a sample **(**δ13CVPDB (‰)) plotted across species. δ13CVPDB (‰) tends to be generally consistent with a marine phytoplankton baseline.

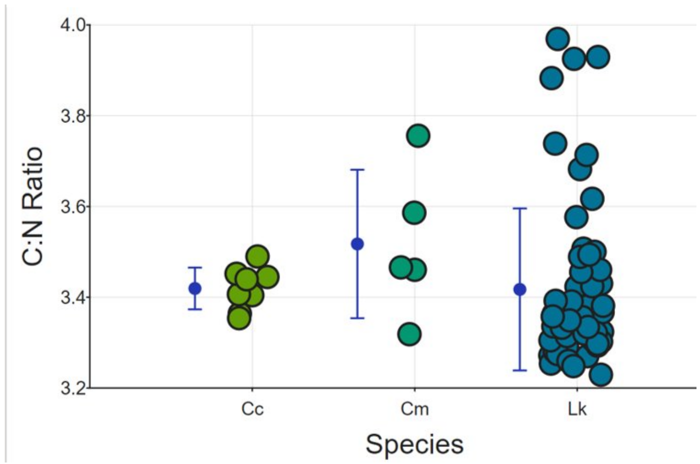

Figure 3. Total Carbon to total Nitrogen ratio in samples plotted versus species. This statistic is generally a good proxy for the amount of fat within a sample.

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