An underwater photograph of a coral reef. In the foreground, a large sea turtle, likely a Kemp's ridley, is swimming towards the left. Its head is in focus, showing its eye and the pattern on its head. In the background, another sea turtle is visible, swimming towards the right. Several striped fish are also present in the water. The overall scene is set against a backdrop of coral and clear blue water.

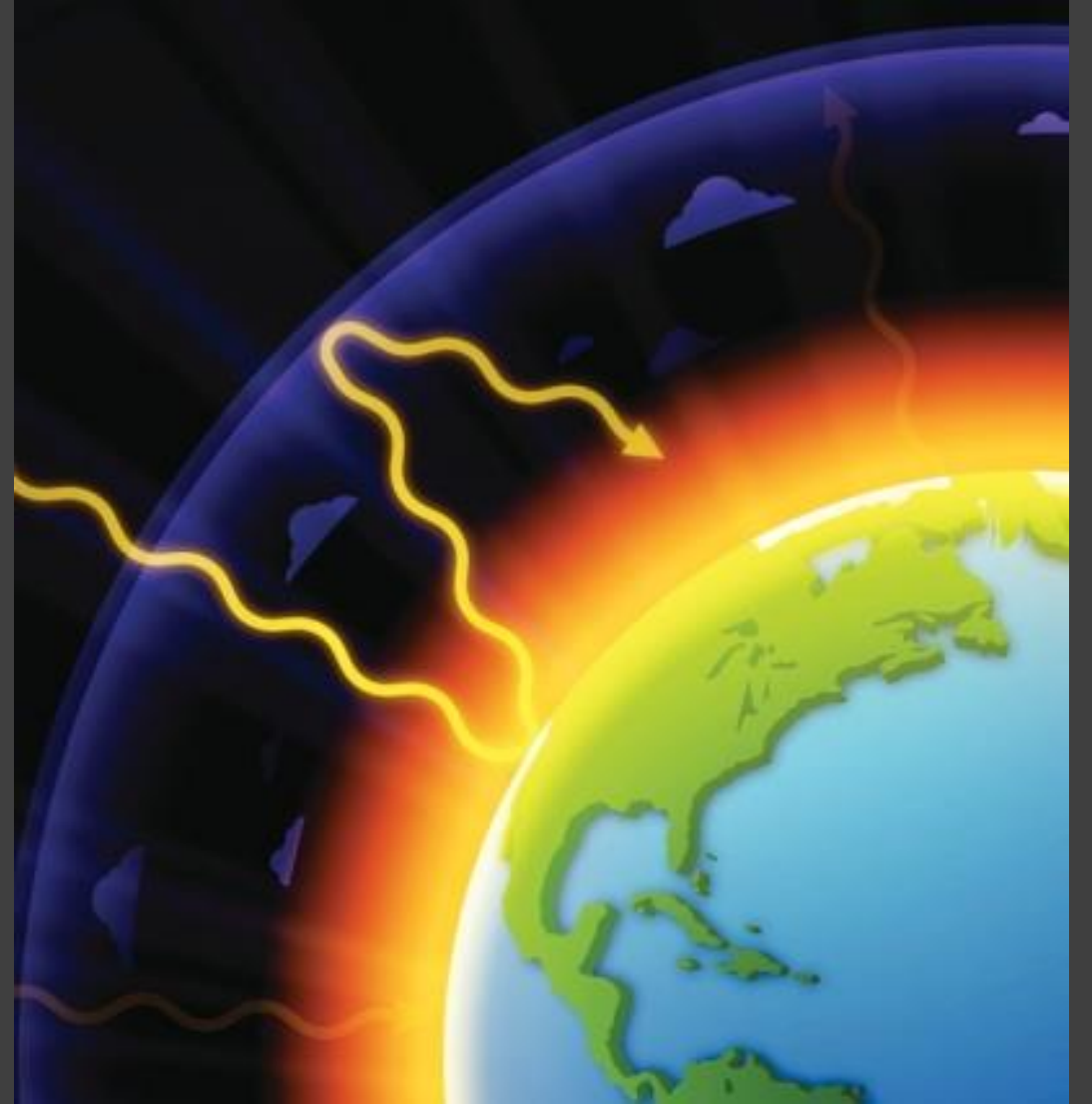
Multi-Tissue Stable Isotope Analysis of Kemp Ridley, Green and Loggerhead Sea Turtles

Lucille McWilliams



Introduction

- Fluctuation of environmental factors caused by buildup of pollutants
 - Fossil Fuels
 - Carbon dioxide
 - Temperature
 - Atmospheric pressure
- Greenhouse effect- traps solar radiation in atmosphere
- Increased greenhouse gases- increases global climate change (Ottersen, G. 2001)



Introduction

Fluctuations
in temperature
and greenhouse effect
impacts

Oceans
temperatures decrease
earlier autumn

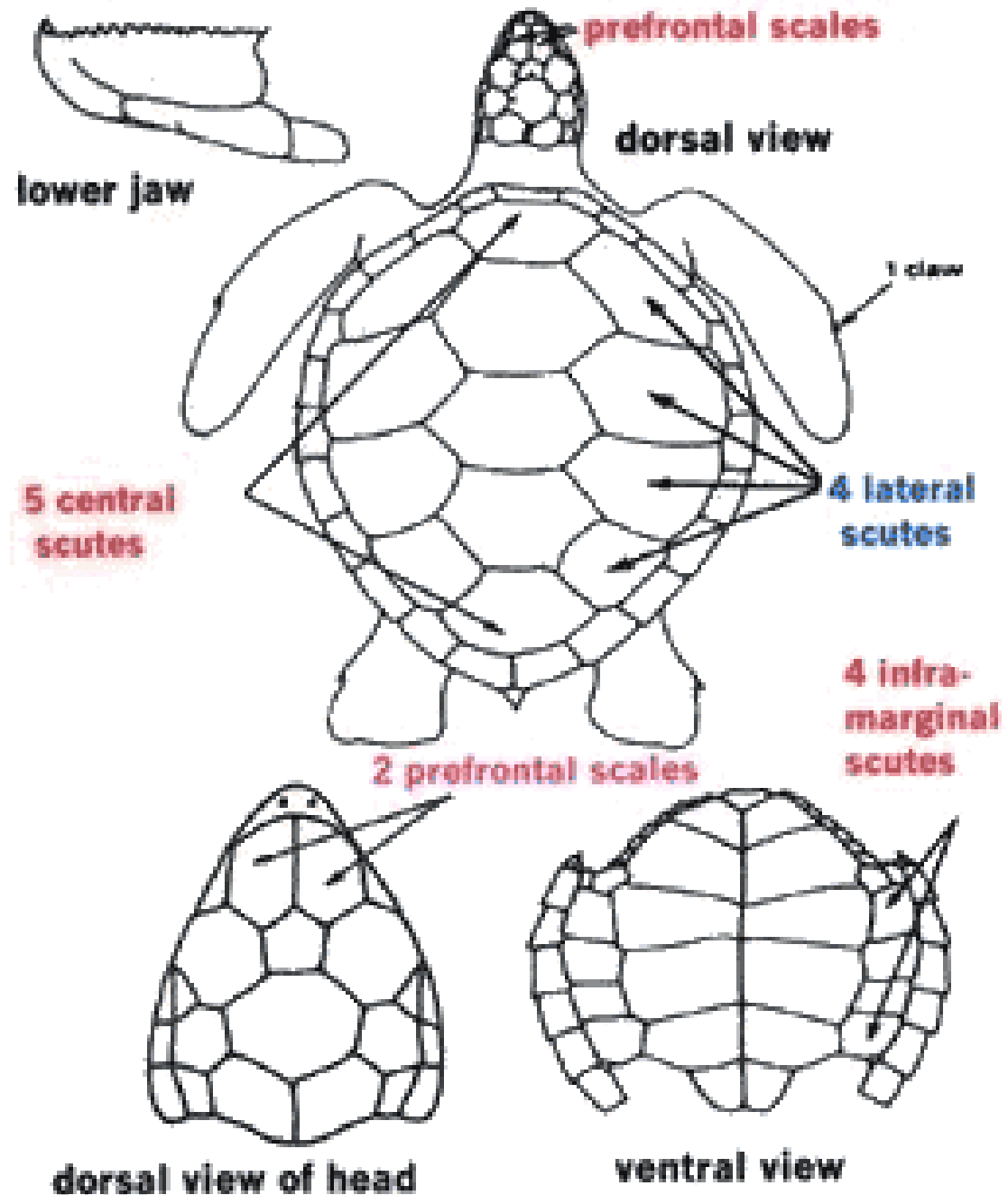
Harsher winters

Migratory patterns



Green Sea Turtles (*Chelonia mydas*)

- Small overall shell
- Brown/Yellowish color
- 4 pairs of lateral scutes
- 5 central scutes
- 2 prefrontal scales
- Serrated lower jaw





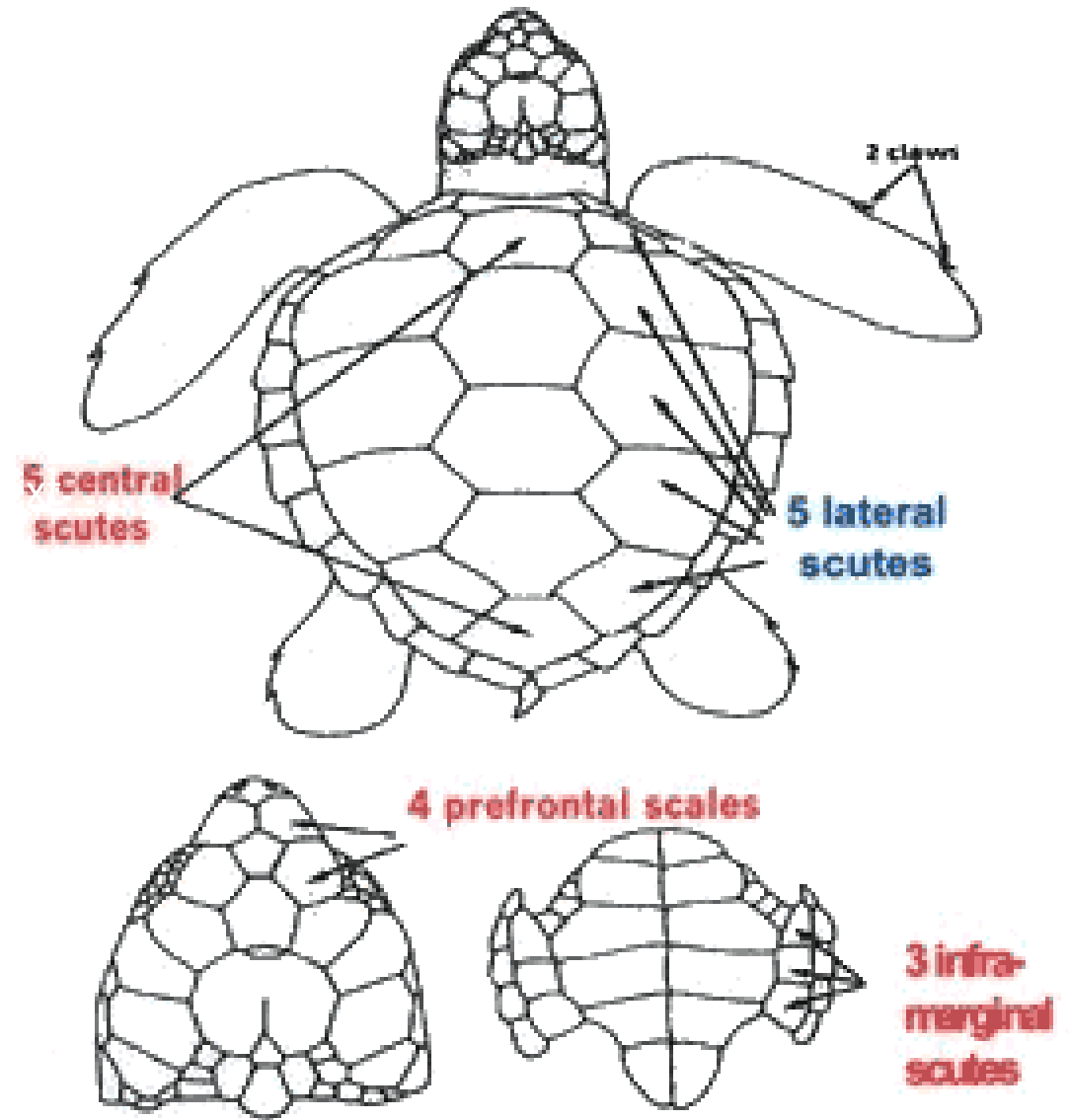
Green Sea Turtles (*Chelonia mydas*)

- Stay in shallow waters until the breeding season
- Females make a long migration back to natal beach to breed
- Travel long distances across oceans to return to preferred breeding site



Loggerhead Sea Turtles (*Caretta caretta*)

- Heart shaped shell
- Reddish/Orange color
- 5 pairs of lateral scutes
- 4 prefrontal scales
- 3 inframarginal scutes





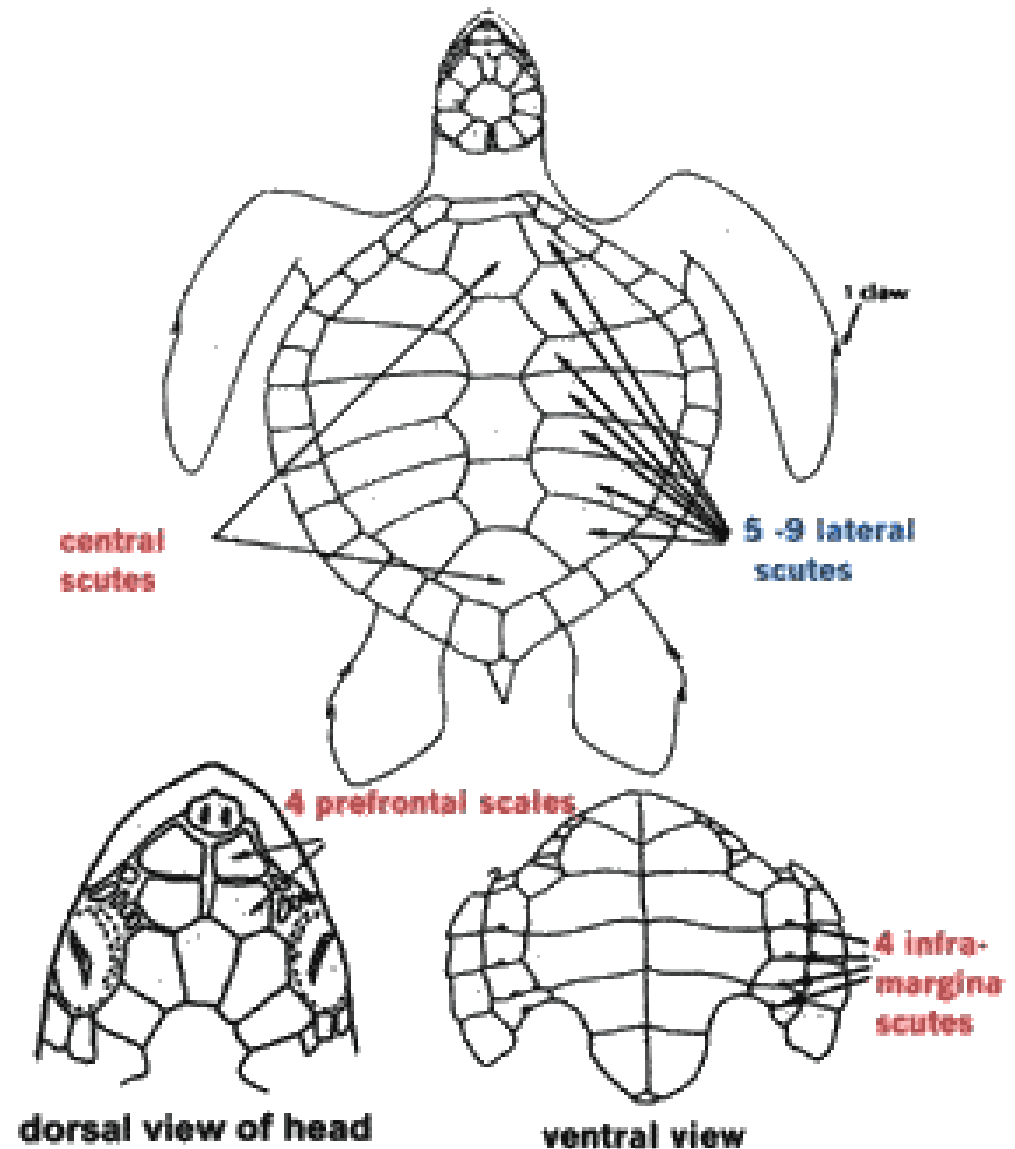
Loggerhead Sea Turtles (*Caretta caretta*)

- Migrate between nesting and foraging grounds
 - Travel thousands of kilometers during their lifetime
- Coasts of Florida to other side of Atlantic and back



Kemp's Ridley Sea turtles (*Lepidochelys kempii*)

- Oval carapace
- Olive-grey color
- 5 pairs of coastal scutes
- 4 inframarginal scutes
- 5-9 pairs of lateral scutes





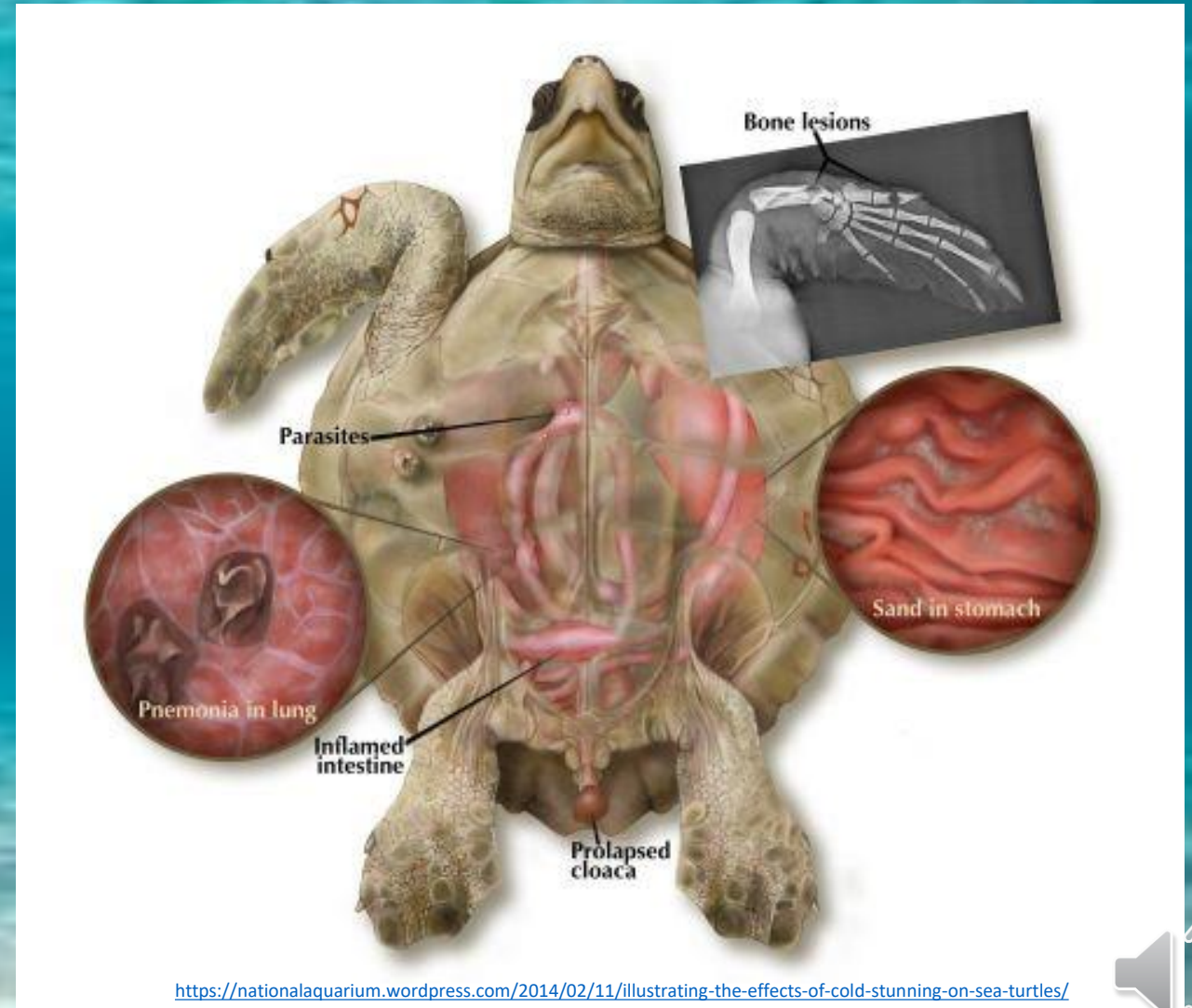
Kemp's Ridley Sea turtles (*Lepidochelys kempii*)

- Males typically stay within Gulf of Mexico
- Some males migrate annually between feeding and breeding grounds
- Others don't migrate at all
 - Mate with females found at feeding grounds or near nesting beaches
- Females migrate to and from nesting beaches off of Gulf of Mexico
- Females migrate to foraging areas from Yucatán Peninsula to southern Florida



Introduction

- Cold stunning
 - Hypothermic reaction when sea turtles exposed to prolonged cold water temperatures
- Cold stunning results
 - Lethargic sea turtles
 - Comatose sea turtles
 - Trapped on the shoreline
 - Risk of complications
 - Hypoglycemia
 - Pneumonia
 - Starvation
 - Bradycardia



<https://nationalaquarium.wordpress.com/2014/02/11/illustrating-the-effects-of-cold-stunning-on-sea-turtles/>

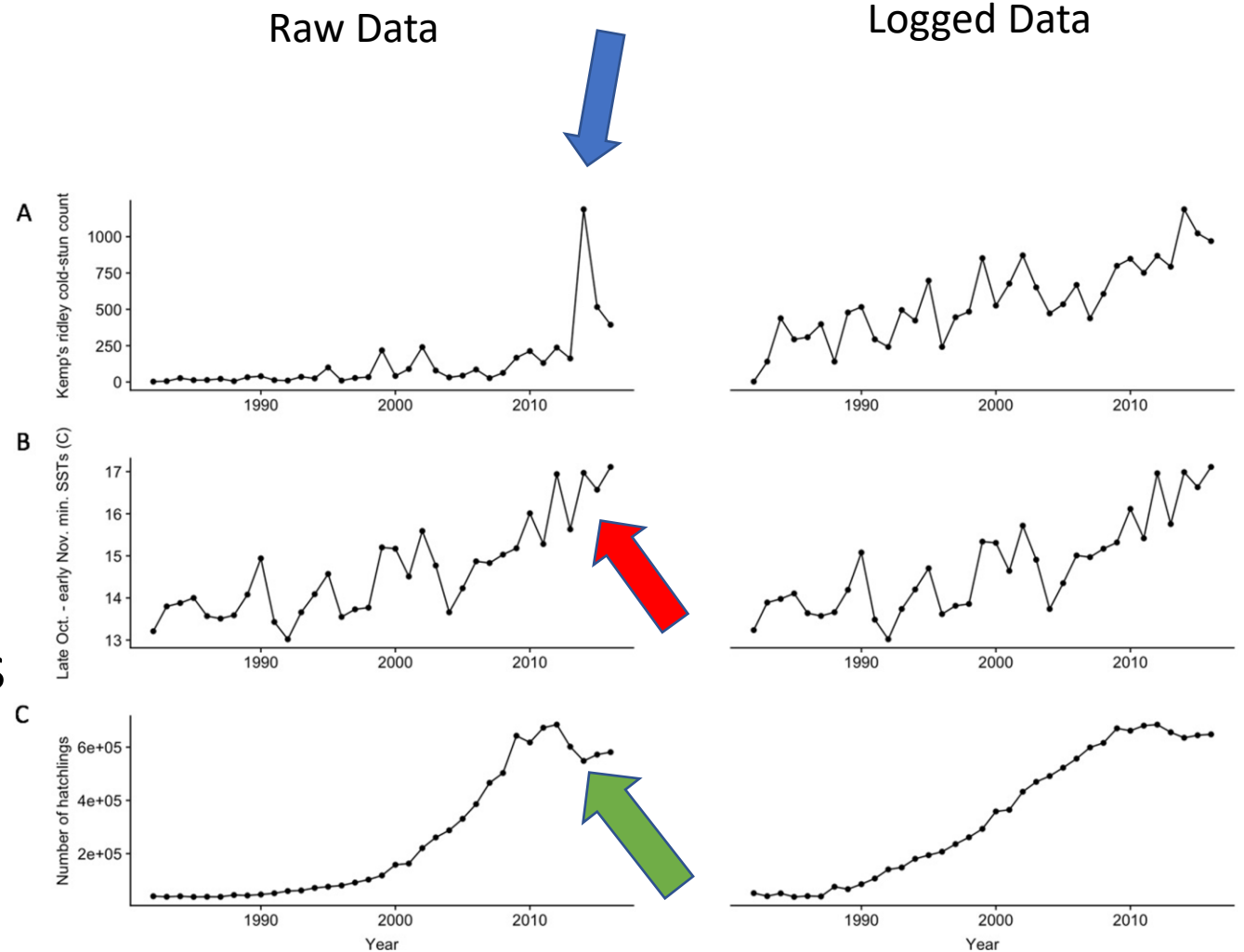
Introduction

- Migration season shortened
- Turtles begin to cold stun at **higher latitudes** where **temperatures are lower**
- Kemp's Ridleys **most prone** species to **cold stunning** due to **small size**



Literature Review

- Kemp's Ridley sea turtles, *Lepidochelys kempii*, migrate from gulf of Mexico to New England coastal waters
 - Migration season from late June through early December
 - Lazell, J. 1980)(Griffin, L. P. 2019
- When sea surface temperature reaches 10°C, cold stunning events can begin
 - Deaths may occur when sea surface temperatures reach 5°C
 - Griffin, L. P .2019
 - Witherington, B. E. 1989
 - Schwartz, F. J. 1974



A. Kemp Ridley Cold Stunned Turtle Count

B. Sea Surface Temperatures

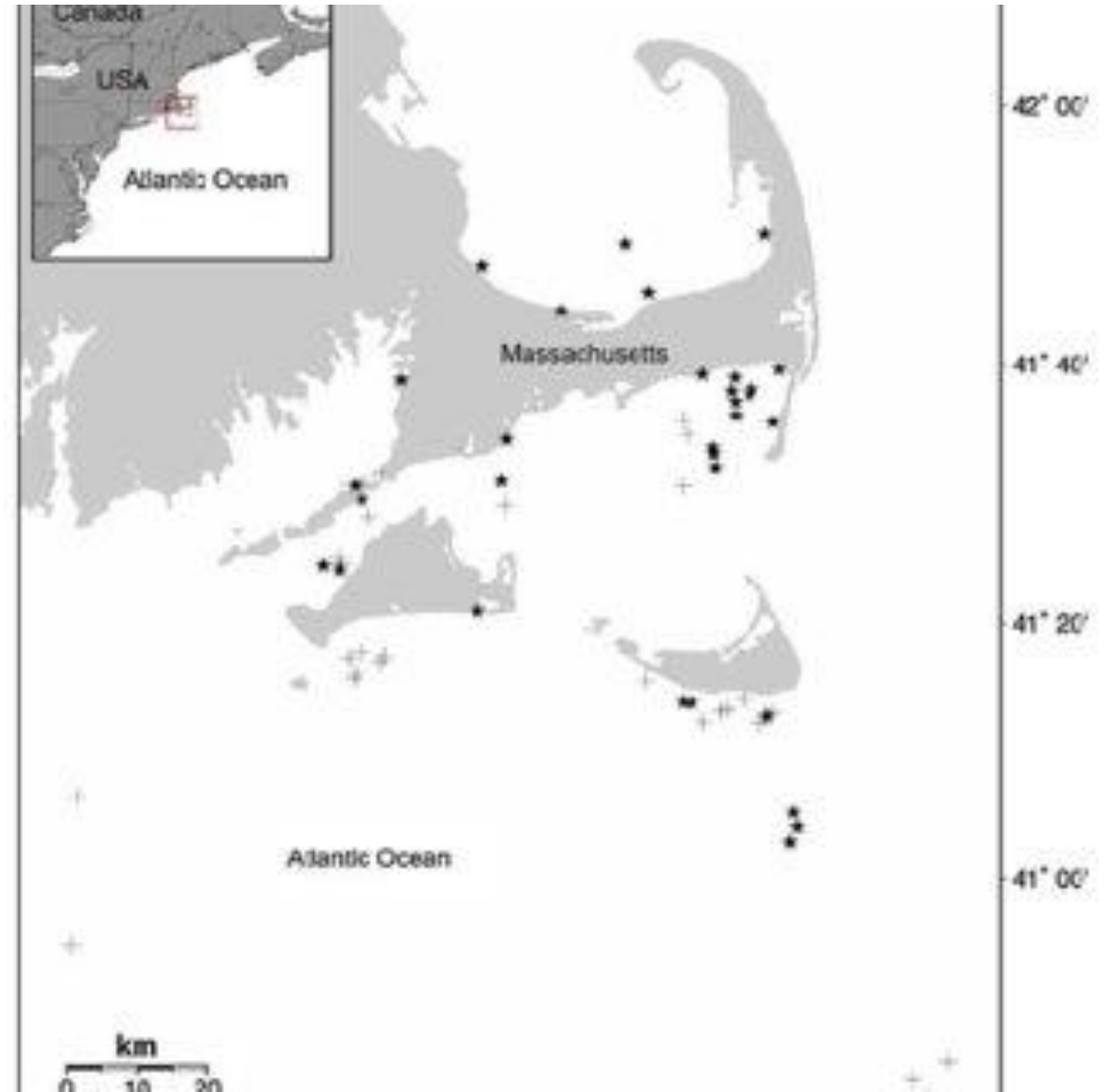
C. Number of Hatchlings of Kemp Ridley Sea Turtles

Griffin, L. P .2019



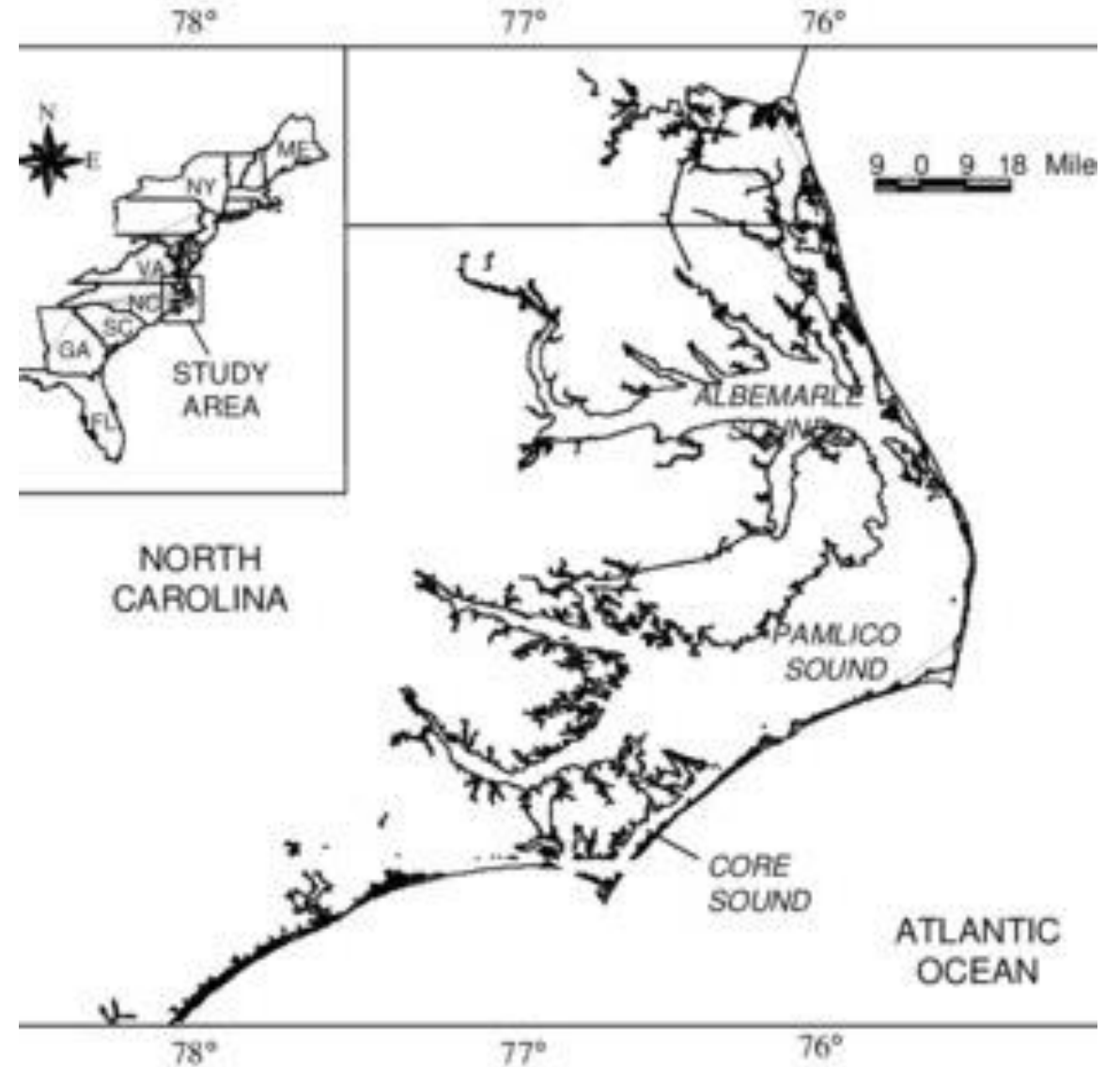
Literature Review

- Multi-tissue stable isotope of leatherback sea turtles in the western North Atlantic to analyze foraging ecology
- Found diet to consist primarily of scyphozoan jellyfish and ctenophores
 - Smaller portion of diet consists of holoplanktonic salps and sea butterflies
 - Logan J. M. 2011



Literature Review

- Loggerheads found in coastal, tropical and subtropical waters often extending to temperate waters in search of food
- Migrate between nesting and foraging grounds
- Migratory locations typically range from Coasts of Florida to other side of Atlantic and back
 - Bass, A. L. 2004





Gap in Research

- 3 types of endangered turtles (Loggerhead, Green and Kemp Ridley's)
- Scientists need to investigate causes of their population decline
- Learn more about their life cycle to develop mitigation strategies





Goal of Research

Identify **prey species** of Loggerhead, Green, and Kemp's Ridley sea turtles in Western North Atlantic Ocean using **Isotope** and **Gut Content Analysis**

Identify **diet** and **foraging** ecology of prey species



Methodology

Stable
Isotope Analysis

Gut
Content Analysis



Methodology -Stable Isotope Analysis

Identification of isotopic markers within a sample

- Turnover rates differentiate between recent vs. past diet
 - Faster turnover rates
 - Liver
 - Plasma
 - Recent diet
 - Slower turnover rates
 - Muscle
 - Whole blood
 - Metabolic activity over longer time periods



Methodology

Stable Isotope Analysis

- Species migrate a lot SIA approach difficult
- Aquatic ecosystems have different carbon and nitrogen level baselines
 - Find these levels in turtles to find feeding areas



Methodology -Stable Isotope Analysis

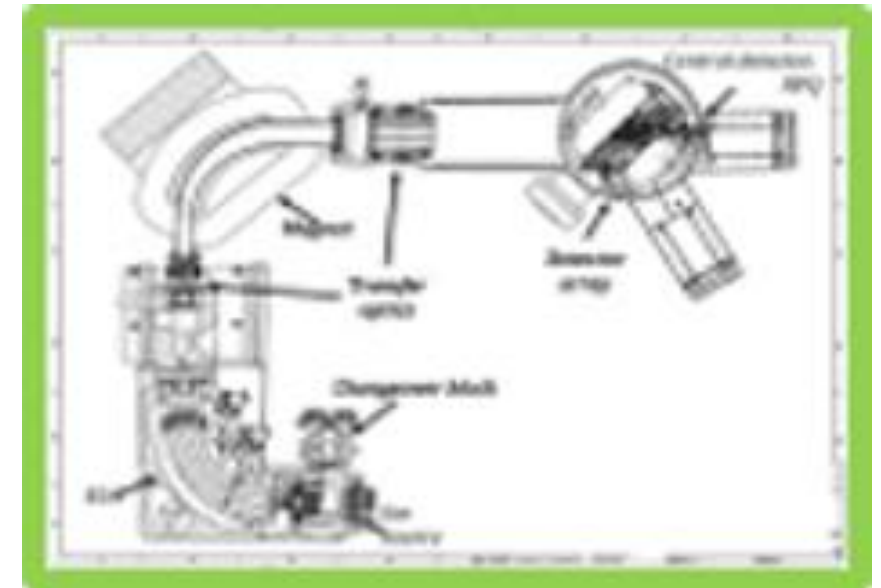
- Tissues collected from turtles
 - Necropsies
- Tissue samples frozen for storage and transportation to lab
- Samples
 - Thawed
 - Rinsed
 - Dried in drying oven
 - Weighed
 - Grinded to uniform composition



Methodology

Stable Isotope Analysis

- Isotopic values collected using an elemental analyzer and an isotope ratio mass spectrometer
- Elemental analyzer
- Isotope ratio mass spectrometer
 - Analyze tissue samples for isotope markers
 - Find stable isotope values





Methodology

Stable Isotope Analysis

- Data plotted using standard deviation from natural occurring values of isotope markers
- Isotope values compared to species standards community baselines
 - Identify
 - Trophic position
 - Migration patterns
 - Diet
 - Habitat
 - Evaluate specific turtle sampled



Methodology -Gut Content Analysis

- Provides insight into:
 - Nutritional requirements
 - Trophic position
 - Material and energy dynamics
 - Food webs
 - Food chains
 - Material and energy transfers between and within ecosystems



Methodology

Gut Content Analysis

- Gut samples collected from turtles
 - Necropsies
- Stored in ethanol
- Placed under Dissecting Microscope
 - Identify prey species within gut sample
- Can be done remotely and pictures sent to lab for further analysis



Previous Results

- Loggerhead-
 - Carnivorous diet
 - Forage for shellfish and invertebrates in deep ocean
 - Horseshoe crabs, clams, mussels
 - (Tomas, J. 2006)
- Green-
 - Herbivorous diet
 - Algae and sea grasses
 - (Seminoff, J. 2006)

- Kemp's Ridley-
 - Carnivorous diet
 - Multiple fish species, sea urchins, squid crabs, clams, mussels, shrimp, and jellyfish
 - (Burke, V. J. et al. 1993)



Previous Results

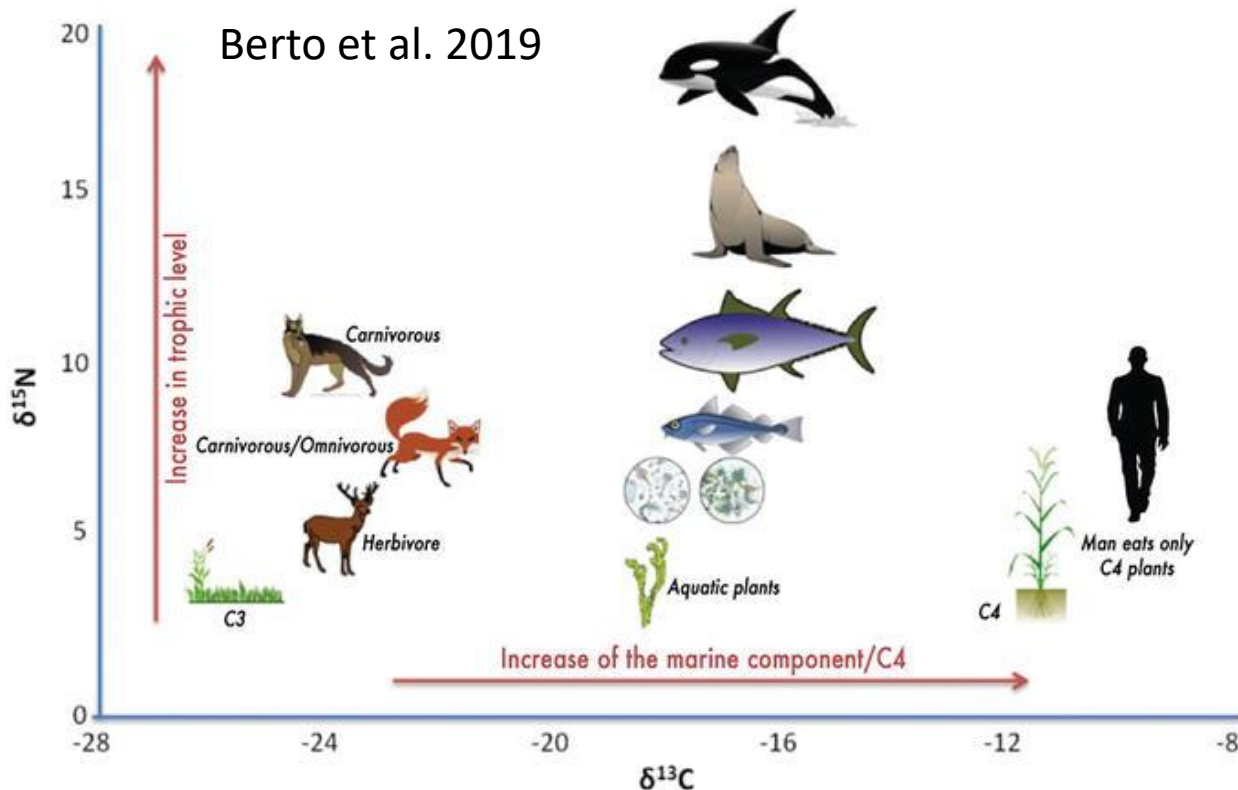
- Horseshoe crabs-
 - Clams, crustaceans, worms, algae
 - (Botton, M.L. 1984)
- Clams/ Mussels-
 - Filter Feeders
 - Plankton, detritus, and bacteria
 - (Mamat, N.Z. 2014)
- Sea urchins-
 - Kelp, decaying matter, algae, dead fish, sponges, mussels, and barnacles
 - (Larson, B.R. 1980)
- Squid-
 - Fish, crabs, and shrimp
 - (Pierce, G. J. et al. 1994)

- Shrimp-
 - Algae and plankton
 - (Fry, B. et al. 1982)
- Jellyfish-
 - Fish eggs and fish larvae; they also eat planktonic eggs and plankton larvae
 - (Underwood, A. H. (2007)



Anticipated Results

- Predicted results will closely mimic findings of previous studies conducted
- Empty stomach contents due to starvation from cold-stunning



- Loggerhead- Carnivorous diet
 - Larger amounts of Clams and Mussels based on availability in northern hemisphere
 - Higher isotopic nitrogen levels than herbivores
- Green- Herbivorous diet
 - Increased amounts of algae
 - Lower isotopic nitrogen levels than carnivores
- Kemp's Ridley- Carnivorous diet
 - Larger amounts of fish, clams, mussels and shrimp
 - Higher isotopic nitrogen levels than herbivores



Discussion

- Adjustment for trophic discrimination and anterior scute carbon and nitrogen isotopic values
 - Represent recent feeding patterns
- Anticipated results reflect significant increase in $\delta^{15}\text{N}$ values for scute edge relative to scute interior samples
 - Suggest local foraging prior to stranding and cold stunning
- Cold-stunned population doesn't have homogenous migratory and/or trophic history
- Techniques could provide further insight into migratory, foraging, trophic and mitigation strategies to improve knowledge of northern species in danger of cold stunning



Figure 1. A. Turtle before the necropsy. The area surrounding the eyes suggests signs of starvation. B. Gas pocket in the blocked digestive tract. C. Detail of contents recovered from the digestive tract after rinsing. D. Contents before rinsing.



Discussion

- Application/Mitigation
 - Contributes to the overall understanding of these endangered species to help save them from extinction
- Limitations
 - Lethal sampling method- only representative of deceased turtles
 - Not always healthy
 - Empty stomach contents due to starvation from cold-stunning



Conclusion

Research Goal

Identify **prey species** of Loggerhead, Green, and Kemp's Ridley sea turtles in Western North Atlantic Ocean using **Isotope** and **Gut Content Analysis** and investigate **diet and foraging ecology**

Methods & Results

Stable Isotope
Analysis of
Tissue
Samples and
Gut Content
Analysis

Higher
Amounts of
Nitrogen in
Carnivorous
Cold
stunned Turtle
species

Empty
stomachs due
to starvation

Variety of
foraging
strategies,
migration
patterns and
trophic
positions



Future Research

- Integration of satellite tracking to model migratory patterns
- Increase ecological data collection on turtles prior to stranding
- Utilize technological advancements to gain better understanding of cold stunning in a variety of global regions



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- Friends
- Science Research Peers



Bibliography

- Bass, A. L., Epperly, S. P., & Braun-Mcneill, J. (2004). Multi-year analysis of stock composition of a loggerhead turtle (*Caretta caretta*) foraging habitat using maximum likelihood and Bayesian methods. *Conservation Genetics*, 5(6), 783–796. doi: 10.1007/s10592-004-1979-1
- Bean, S. B., & Logan, J. M. (2019). Stable isotope analyses of cold-stunned Kemp's ridley (*Lepidochelys kempii*) sea turtles at the northern extent of their coastal range. *Marine Biology*, 166(5). doi: 10.1007/s00227-019-3516-2
- Burke, V. J., Standora, E. A., & Morreale, S. J. (1993). Diet of Juvenile Kemps Ridley and Loggerhead Sea Turtles from Long Island, New York. *Copeia*, 1993(4), 1176. doi: 10.2307/1447107
- Burke, V. J., Standora, E. A., & Morreale, S. J. (1991). Factors Affecting Strandings of Cold-Stunned Juvenile Kemps Ridley and Loggerhead Sea Turtles in Long Island, New York. *Copeia*, 1991(4), 1136. doi: 10.2307/1446115
- Dodge, K. L., Logan, J. M., & Lutcavage, M. E. (2011). Foraging ecology of leatherback sea turtles in the Western North Atlantic determined through multi-tissue stable isotope analyses. *Marine Biology*, 158(12), 2813–2824. doi: 10.1007/s00227-011-1780-x
- Griffin, L. P., Griffin, C. R., Finn, J. T., Prescott, R. L., Faherty, M., Still, B. M., & Danylchuk, A. J. (2019). Warming seas increase cold-stunning events for Kemp's ridley sea turtles in the northwest Atlantic. *Plos One*, 14(1). doi: 10.1371/journal.pone.0211503
- Lazell, J. (1980). New England Waters: Critical Habitat for Marine Turtles. *Copeia*, 1980(2), 290-295. doi:10.2307/1444006
- Luschi, P., Hays, G. C., Seppia, C. D., Marsh, R., & Papi, F. (1998). The navigational feats of green sea turtles migrating from Ascension Island investigated by satellite telemetry. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1412), 2279–2284. doi: 10.1098/rspb.1998.0571
- Ottersen, G., Planque, B., Belgrano, A., Post, E., Reid, P. C., & Stenseth, N. C. (2001). Ecological effects of the North Atlantic Oscillation. *Oecologia*, 128(1), 1–14. doi: 10.1007/s004420100655
- Rainey WE 1981 Guide to sea turtle visceral anatomy NOAA Technical Memorandum NMFS SEFSC 82 82pp
- Rainey, W. E. (1981). Guide to sea turtle visceral anatomy. Panama City, FL: U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Center, Panama City Laboratory.
- Reich, K. J., Bjorndal, K. A., & Bolten, A. B. (2007). The 'lost years' of green turtles: using stable isotopes to study cryptic life stages. *Biology Letters*, 3(6), 712–714. doi: 10.1098/rsbl.2007.0394
- Reich, K. J., Bjorndal, K. A., & Rio, C. M. D. (2008). Effects of growth and tissue type on the kinetics of ¹³C and ¹⁵N incorporation in a rapidly growing ectotherm. *Oecologia*, 155(4), 651–663. doi: 10.1007/s00442-007-0949-y
- Seminoff, J., Jones, T., Eguchi, T., Jones, D., & Dutton, P. (2006). Stable isotope discrimination ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) between soft tissues of the green sea turtle *Chelonia mydas* and its diet. *Marine Ecology Progress Series*, 308, 271–278. doi: 10.3354/meps308271
- Schwartz, F. J. (1974). The Marine Leech *Ozobranthus margoi* (Hirudinea: Piscicolidae), Epizootic on *Chelonia* and *Caretta* Sea Turtles from North Carolina. *The Journal of Parasitology*, 60(5), 889. doi: 10.2307/3278927
- Tomas, J., Aznar, F. J., & Raga, J. A. (2006). Feeding ecology of the loggerhead turtle *Caretta caretta* in the western Mediterranean. *Journal of Zoology*, 255(4), 525–532. doi: 10.1017/s0952836901001613
- Witherington, B. E., & Ehrhart, L. M. (1989). Hypothermic Stunning and Mortality of Marine Turtles in the Indian River Lagoon System, Florida. *Copeia*, 1989(3), 696. doi: 10.2307/1445497
- Wyneken, J. 2001. The Anatomy of Sea Turtles. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470, 1-172 pp.
- Zanden, H. B. V., Bjorndal, K. A., Reich, K. J., & Bolten, A. B. (2010). Individual specialists in a generalist population: results from a long-term stable isotope series. *Biology Letters*, 6(5), 711–714. doi: 10.1098/rsbl.2010.0124

