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ROSENSTIEL SCHOOL of MARINE & ATMOSPHERIC SCIENCE

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Effectively Determining Trophic Position of Plasma and Muscle Tissue of Sharks in South Florida

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UNIVERSITY OF MIAMI

Introduction

- Understanding complex trophic interactions among sharks and their prey allows scientists to make ecological inferences that are crucial for management and conservation.[1,2]
- In this study, trophic position (TP) was calculated using $\delta^{15}N$ values of specific amino acids (AAs) to understand if the enrichment factors of AAs was different among variable tissue type.
- Two different types of tissue were chosen: tissue with higher levels of metabolic activity and therefore quicker isotopic turnover rates (plasma), and tissue with lower levels of metabolic activity and therefore slower isotopic turnover rates (muscle).[4, 5]
- This study also sought to understand the variability in TP estimates among species, size, and sex.
- TP was calculated using the following equation[3]: $TP_{AA(TAA-SAA)} = [(\delta^{15}N_{TAA} - \delta^{15}N_{SAA} - 2.2) / 6.3] + 1$
- TAAs: "trophic amino acids"; fractionate significantly with movement through the food web (glutamic acid, leucine, and alanine).
- SAAs: "source amino acids"; do not change significantly from the $\delta^{15}N$ at the base of the food web (Phenylalanine, Lysine, Glycine, and Serine).
- Amino acid compound specific isotope analysis (AA-CSIA) is used in this study as a powerful tool to distinguish between trophic and baseline differences in order to understand ecological relationships and population dynamics in South Florida waters.

Methods

- Sharks captured in South Florida in Biscayne Bay via a circle-hook drumline system and then released once samples were obtained.

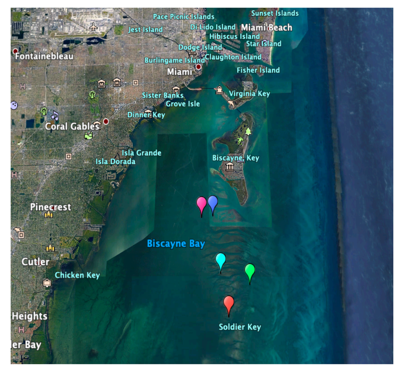


Figure 1: GPS coordinates from various locations where samples from following species were collected:

Charcharhinus leucas (bull shark); n=3
Charcharhinus limbatus (blacktip shark);n=3
Negaprion brevirostris (lemon shark); n=2
Rhizoprionodon terranovae (Atlantic sharpnose shark); n=1
Ginglymostoma cirratum (nurse shark); n=1

- 10 mL of whole blood gathered by caudal venipuncture and centrifuged to isolate plasma.
- Muscle biopsy collected by puncturing body cavity below dorsal fin and using 19 mm melon ball scooper to extract sample.
- Amino acid sample preparation was carried out in the Close Lab at RSMAS following standardized method.[6]
- Samples analyzed using gas chromatography-isotope ratio mass spectrometry (GC-IRMS) instrument via triplicate injections and 1σ analytical uncertainty.

Figure 2 (left): Whole blood being drawn from nurse shark.[7]

Figure 3 (right): Muscle biopsy being extracted from blacktip shark.[7]

Results

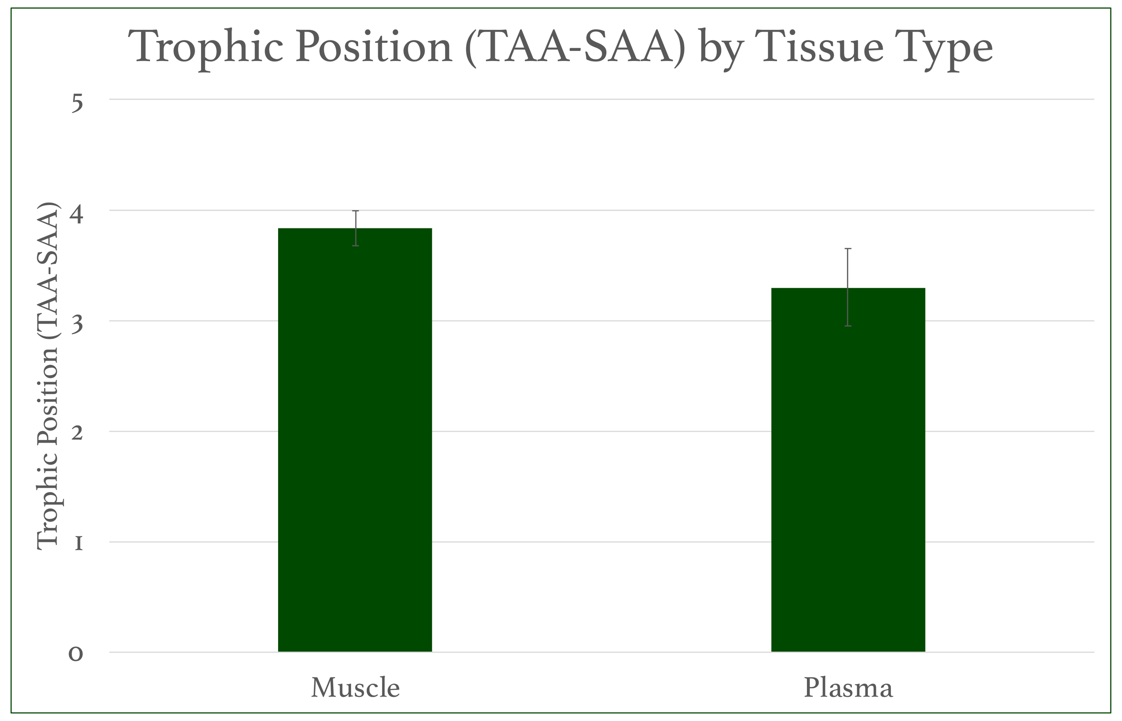


Figure 4: Mean TP calculations using amino acid nitrogen isotopic values ($\delta^{15}N$) for muscle (3.83 ± 0.16; mean ± SD) and plasma (3.3 ± 0.35). Used a t-test to prove statistically significant difference between the tissue types among individuals (p-value < 0.004; alpha level = 0.05).

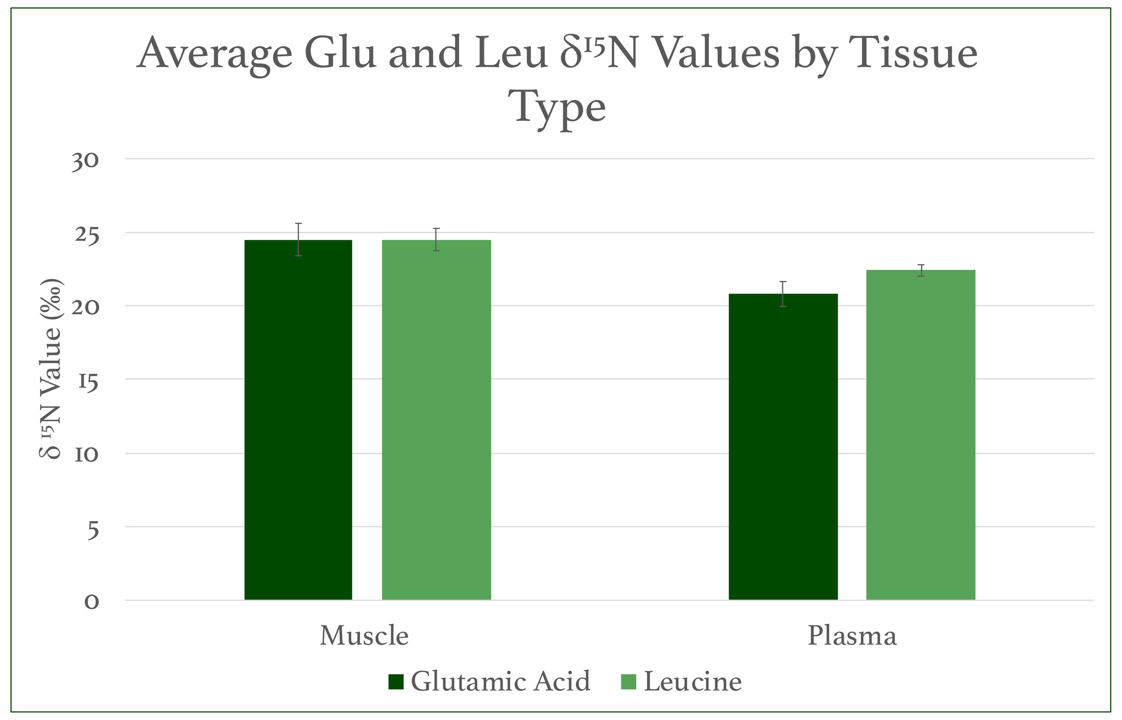


Figure 5: Mean glutamic acid (Glu) and leucine (Leu) $\delta^{15}N$ values of muscle (Glu: 24.5 ± 1.1; Leu: 24.3 ± 0.8; average difference = 0.2 ‰) and plasma (Glu: 20.8 ± 0.8; Leu: 22.4 ± 0.4; average difference = 1.6 ‰). A t-test was used to prove statistically significant difference (p-value < 0.001; alpha level = 0.05).

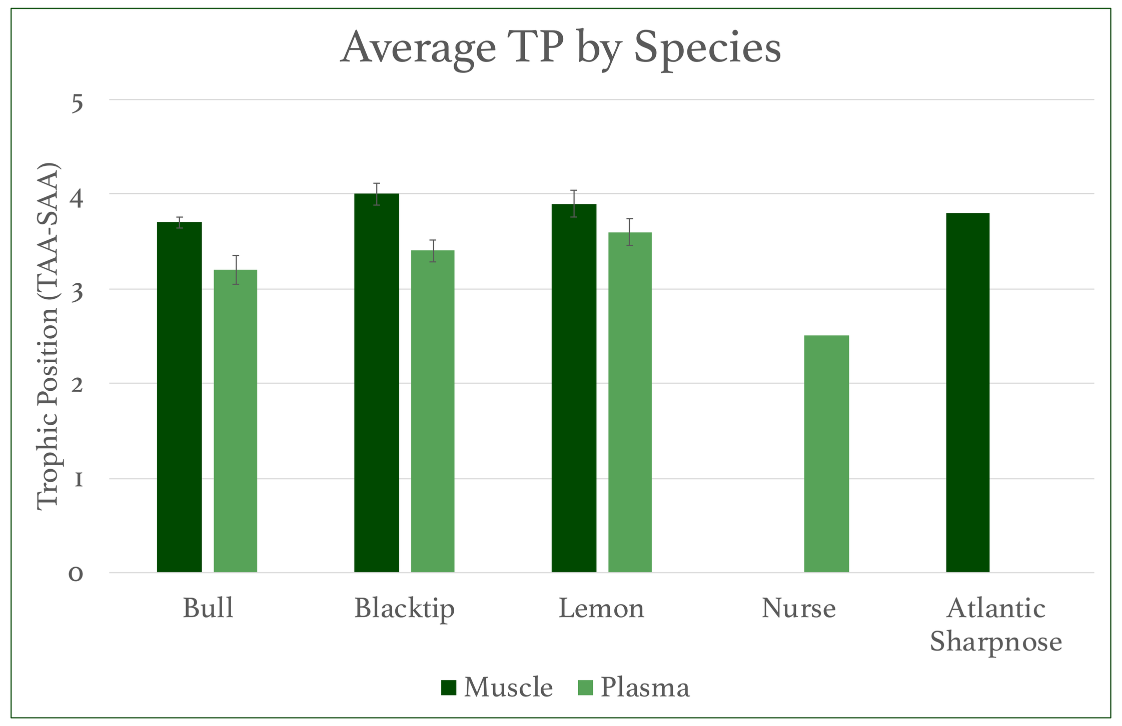


Figure 6: Mean TP estimates using $\delta^{15}N$ values for muscle and plasma. Muscle TP ANOVA: F-statistic (5.11) < F-critical (5.79); 0.1 < p-value < 0.05. Plasma TP ANOVA: F-statistic (4.53) < F-critical (5.79); 0.1 < p-value < 0.05. Average muscle and plasma TP ANOVA: F-statistic (1.26) < F-critical (3.81); p-value < 0.1.

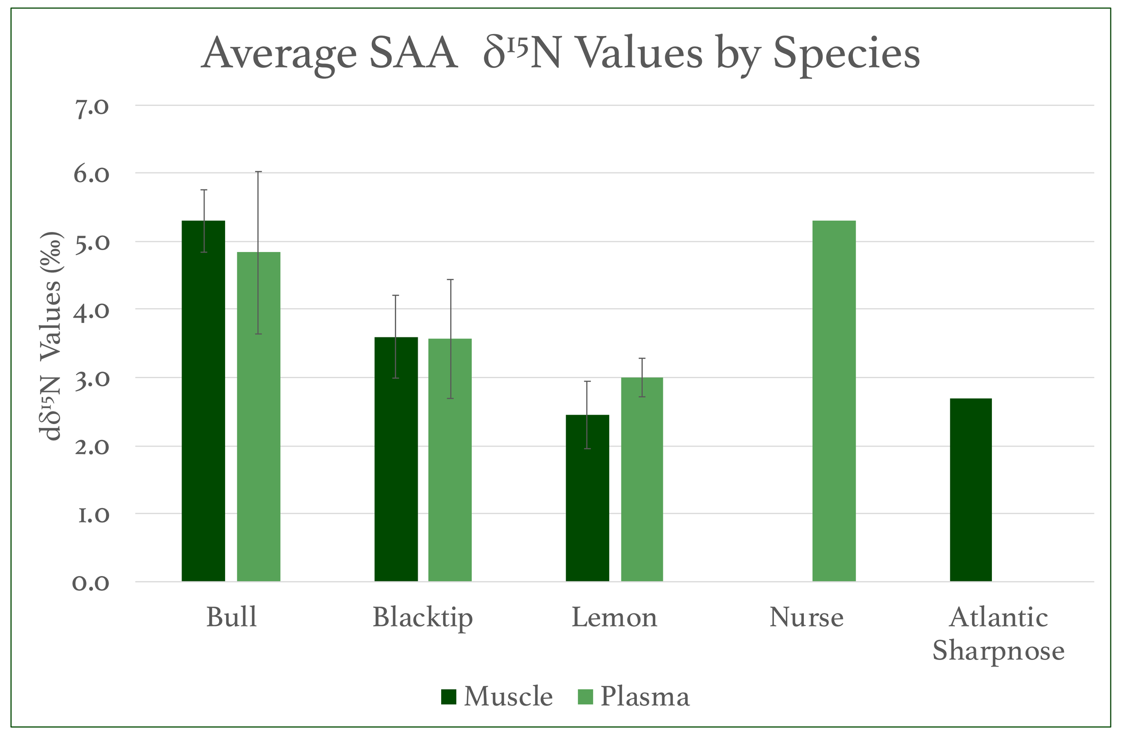


Figure 7: Mean SAA $\delta^{15}N$ values for muscle and plasma. Muscle SAA $\delta^{15}N$ ANOVA: F-statistic (19.001) > F-critical (5.79); p-value > 0.01. Plasma SAA $\delta^{15}N$ ANOVA: F-statistic (2.31) < F-critical (5.79); p-value < 0.1. Average muscle and plasma SAA $\delta^{15}N$ ANOVA: F-statistic (13.44) > F-critical (3.81); p-value > 0.001.

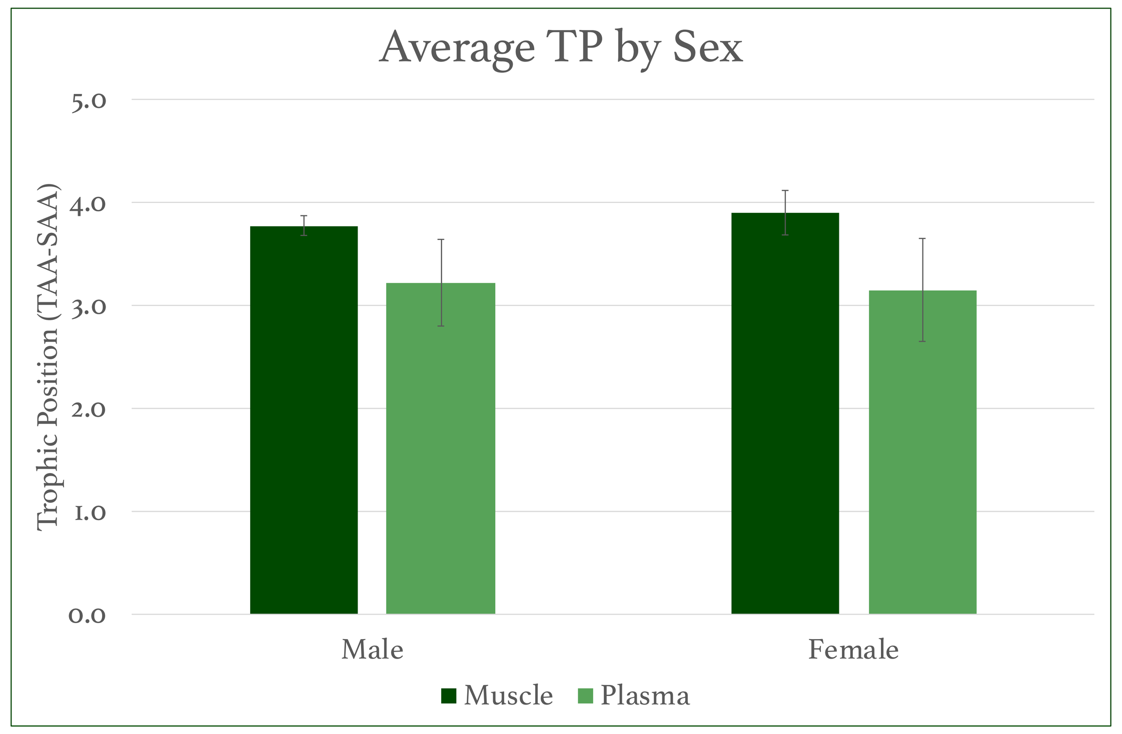


Figure 8: Mean TP calculations using amino acid nitrogen isotopic values ($\delta^{15}N$) for muscle (male: 3.8 ± 0.1; female: 3.9 ± 0.2) and plasma (3.2 ± 0.4; female: 3.2 ± 0.5). A t-test (alpha level = 0.05) was used to analyze; neither muscle (p-value > 0.15) or plasma (p-value > 0.4) tissue showed a statistically significant difference.

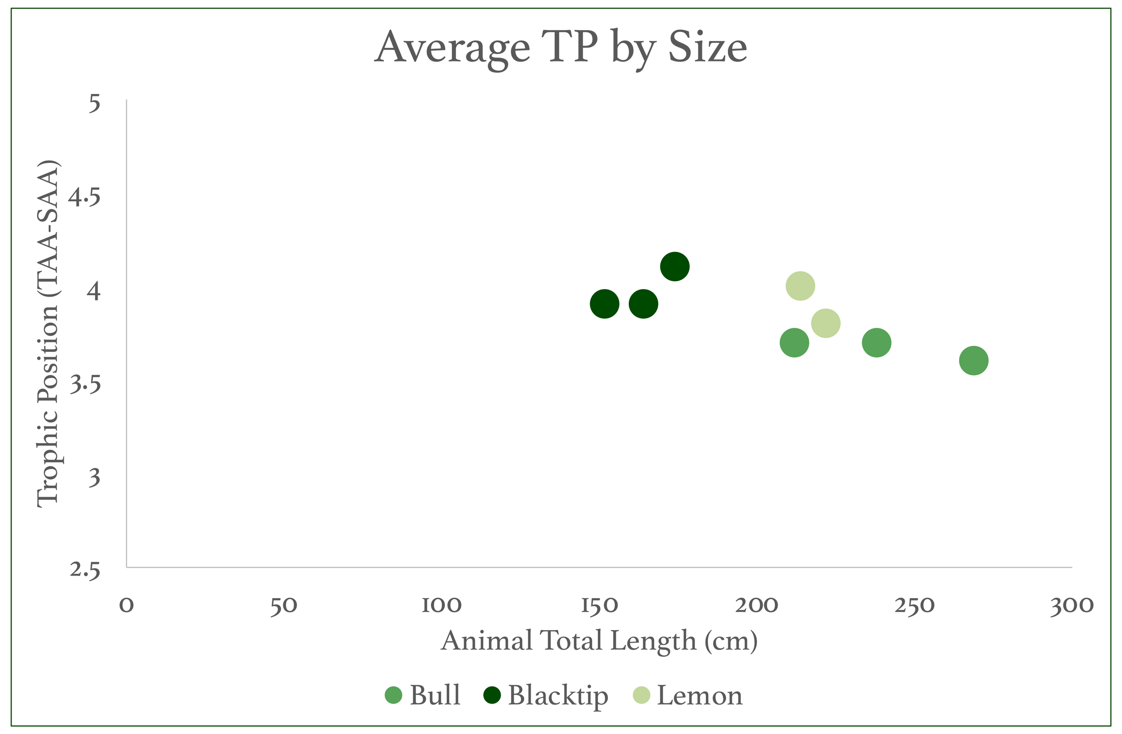


Figure 9: Mean TP calculations using amino acid nitrogen isotopic values ($\delta^{15}N$) by size among species. A Pearson correlation test was used to analyze the relationship and a moderate negative correlation was found ($R = -0.723$; $R^2 = 0.523$; p-value < 0.05).

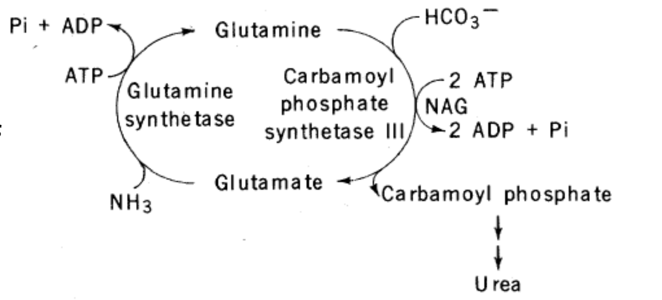
*** Nurse shark and Atlantic sharpnose shark samples were not used in any statistical analysis because only single tissue types were obtained ***

Discussion

Intra-Tissue Comparison

- Statistical analysis of inter-tissue comparison among individuals revealed a significant difference in TP estimates between plasma and muscle tissue (figure 4).
- I hypothesize this is due to variable urea concentrations in muscle and plasma – plasma has higher and more variable concentrations.[8,9]
- Urea is important for the purpose of maintaining osmotic balance among elasmobranchs.[10,11]
- The variable urea concentrations may alter $\delta^{15}N$ values because glutamic acid (Glu) is an important intermediate in the urea synthesis pathway in the form of glutamine.[12]

Figure 10: Urea synthesis pathway in elasmobranchs.[12] In this process, cleaved isotopically light ammonium adds its N onto Glu molecules through transamination.



- We suggest that a fraction of Glu molecules are present in plasma as urea intermediates and therefore reflect lower $\delta^{15}N$ values than other TAAs not used in the urea synthesis pathway, such as Leucine (figure 5).
- We also suggest the role of Glu as an intermediate in the urea pathway is the reason for plasma TP estimates being artificially low, and that a new formulation for plasma TP calculations in elasmobranchs should be created using other TAAs.

Inter-Species Comparison

- No significant difference in the TP calculations among species (figure 6).
- A significant difference was present in the average SAA $\delta^{15}N$ values; likely due to different feeding habits and diet among species (figure 7).
- Bull sharks reflected lowest TP calculations due to their "low-quality diet", meaning their variable diet[13] and reflects AA compositions vastly different from their own AA compositions, making it difficult to incorporate dietary AAs.[14] High SAA $\delta^{15}N$ values reflect the high baseline N values of their diet.
- Blacktip sharks reflected highest TP calculations and mid SAA $\delta^{15}N$ values due to their specialist feeding habits. These sharks are highly efficient feeders and the high $\delta^{15}N$ values for these fish is most likely due to their consistent N-rich diet of teleost fishes.[15]
- Lemon sharks reflected similar TP calculations and SAA $\delta^{15}N$ values to blacktip sharks. This is most likely due to their spatially and compositionally consistent feeding behavior. Lemon sharks also feed on mesopredators such as teleost fishes and are responsible for being specialist feeders.
- Although not included in statistical analysis, the nurse shark reflected the lowest TP (2.5) of all samples. This is likely due to their suction feeding apparatus and diet of crustaceans and benthic invertebrates, which occupy TPs lower than the prey of other species sampled in this study.[16]

Size Comparison

- TP and size reflected a negative correlational relationship through statistical analysis, which was not expected (figure 9).
- More research on TPs of juvenile sharks in comparison with sexually mature sharks should be done due to the difference in isotope turnover rates with age.[17]

References

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Acknowledgments

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