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Investigating Trophic Position Estimates of Shark Plasma and Muscle Tissue Using Compound Specific and Bulk Stable Isotope Analysis

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Introduction

- Investigating trophic interactions between sharks and prey items can offer necessary information about ecosystem health and population controls.[1]
- This study investigated trophic position (TP) estimates based on $\delta^{15}N$ values from bulk stable isotope analysis and compound specific isotope analysis of amino acids (CSIA-AA).
- Glutamic acid has been found to be significantly ^{15}N-depleted in shark tissue[2], as well as in pinnipeds[3], cetaceans[4], and penguins[5].
- This ^{15}N-depletion is significantly higher in plasma tissue than muscle tissue[2].
- ^{15}N-depletion in glutamic acid has contributed to low TP estimates[2,3,4,5].
- Sharks store urea, a waste product, in their tissues as an osmolyte[6,7].
- In sharks, an intermediate of glutamic acid, glutamine, is used as primary nitrogen donator[6].
- Glutamine synthesis deaminates glutamic acid twice, contributing to ^{15}N-depletion[3,6].
- This study considered an alternative trophic amino acid (AA), threonine, to be used in place of glutamic acid in TP estimates for urea-producing species.
- This study also sought to understand the variability in TP estimates between muscle and plasma tissues.

Methods

- Sharks were captured in Biscayne Bay with circle-hook drumlines, sampled for full full blood and white muscle, then released (Figure 1).
- Amino acid sample preparation was carried out in the Close Lab at RSMAES following standardized method[8].
- Samples were analyzed for CSIA-AA using gas chromatography-isotope ratio mass spectrometry (GC-IRMS) instrument with 1σ analytical uncertainty.
- Tissue samples were homogenized and freeze-dried.
- Samples were analyzed for bulk isotope analysis using GC-IRMS instrument with 1σ analytical uncertainty.
- TP estimates were compared using ANOVA single-factor tests (α = 0.05) and Tukey HSD pair-wise comparisons (α = 0.05).
- TP estimates were compared between tissue types using student's t-tests (α = 0.05).

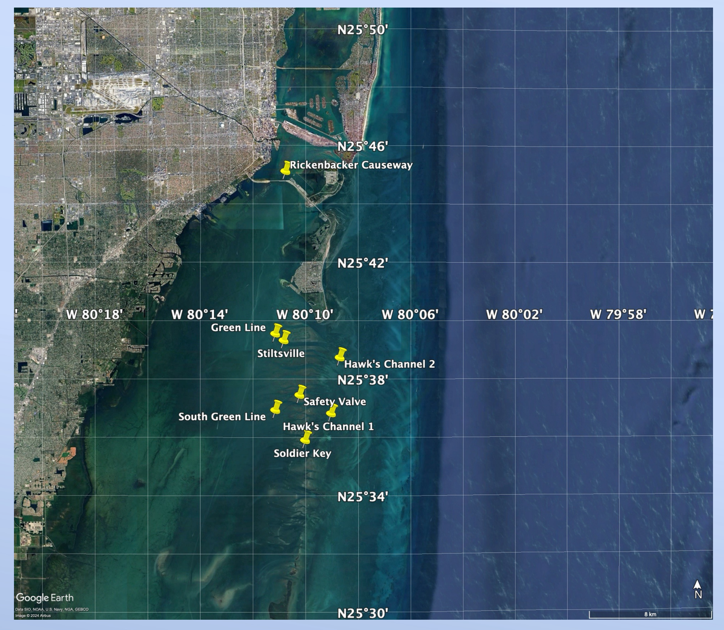

Fig. 1: Diagram of Sampling Locations
GPS coordinates of locations where samples of the following species were collected:
Carcharhinus limbatus (blacktip sharks); n = 6
Carcharhinus leucas (bull sharks); n = 9
Negaprion brevirostris (lemon sharks); n = 7
Rhizoprionodon terraenovae (Atlantic sharpnose sharks); n = 1
Carcharhinus acronotus (blacknose sharks); n = 2
Galeocerdo cuvier (tiger sharks); n = 2
Ginglymostoma cirratum (nurse sharks); n = 6

Equations

TP can be estimated from bulk isotope analysis using the equation[#]:

$$TP_{Bulk} = [(\delta^{15}N_{consumer} - \delta^{15}N_{producer})/3.4] + 1 \quad (Equation\ 1)$$

TP is estimated from CSIA-AA using the general equation[#]:

$$TP_{CSIA-AA} = [(\delta^{15}N_{trophic} - \delta^{15}N_{source} - \beta)/TDF_{AA}] + 1 \quad (Equation\ 2)$$

Table 1: Components and sources of data for TP estimates. Tissue values were determined experimentally for multiple samples, while β and TDF values were chosen from a literature search and remained constant.

TP Estimate Name	Tropic AA or Tissue	Source AA or Tissue	Experimental Source	β (‰)	Literature Source	TDF (‰)	Literature Source
TP (Glu-Phe)	Glutamic acid	Phenylalanine	Horstmann (2020)	3.4	Chikaraishi et al. (2009)	7.6	Chikaraishi et al. (2009)
TP (TAA-SAA)	Glutamic acid, leucine, and alanine	Phenylalanine, lysine, and serine	Horstmann (2020)	2.2	Hannides et al. (2020)	6.3	Hannides et al. (2020)
TP (Thr-Phe)	Threonine	Phenylalanine	Horstmann (2020)	1.7	Ramirez et al. (2021)	-9.3	Bradley et al. (2015)
TP (Thr-Lys)	Threonine	Lysine	Horstmann (2020)	3.3	Ramirez et al. (2021)	-9.9	Bradley et al. (2015)
TP (Bulk)	Consumer whole tissue	Producer tissue estimate (2.9)	Horstmann (2020), Jeng (2024)	N/A	N/A	3.4	Bowes & Thorp (2015)

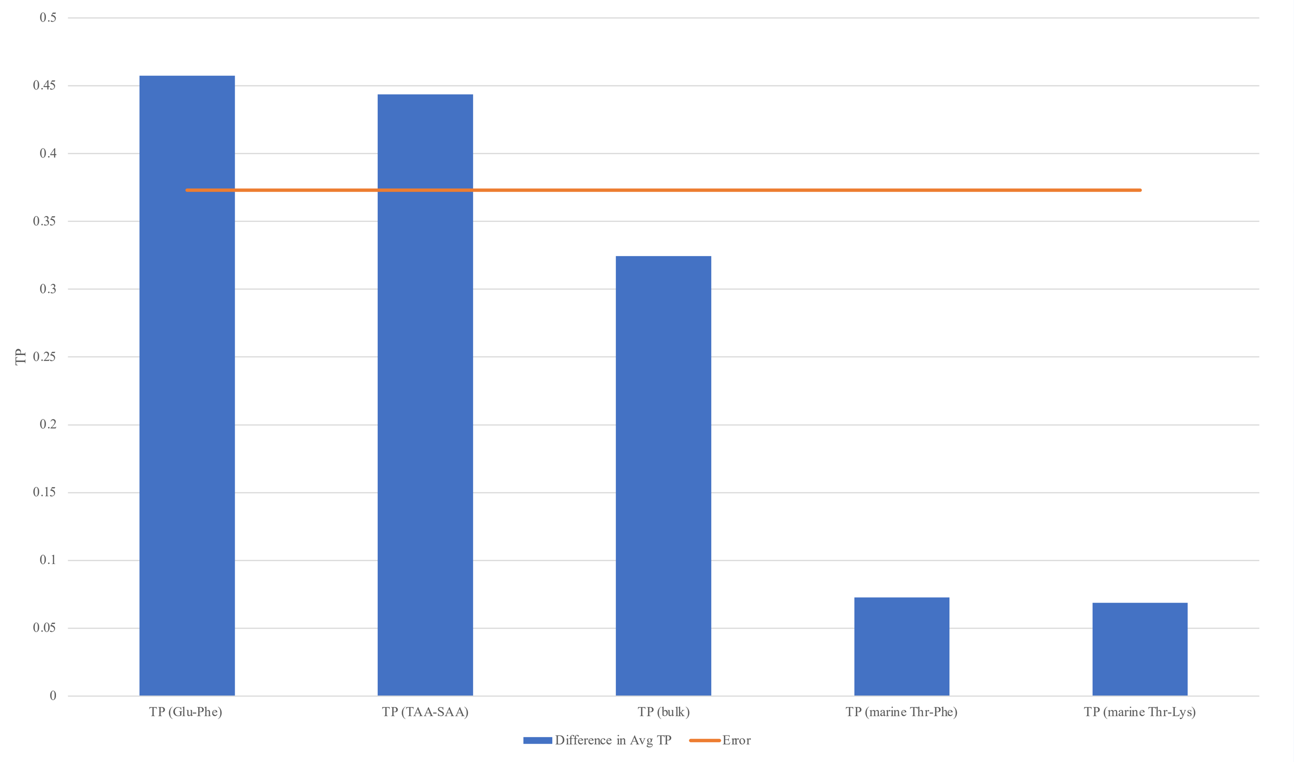

Fig 2. Components and sources of data for TP estimates. Tissue values were determined experimentally for multiple samples, while β and TDF values were chosen from a literature search and remained constant.

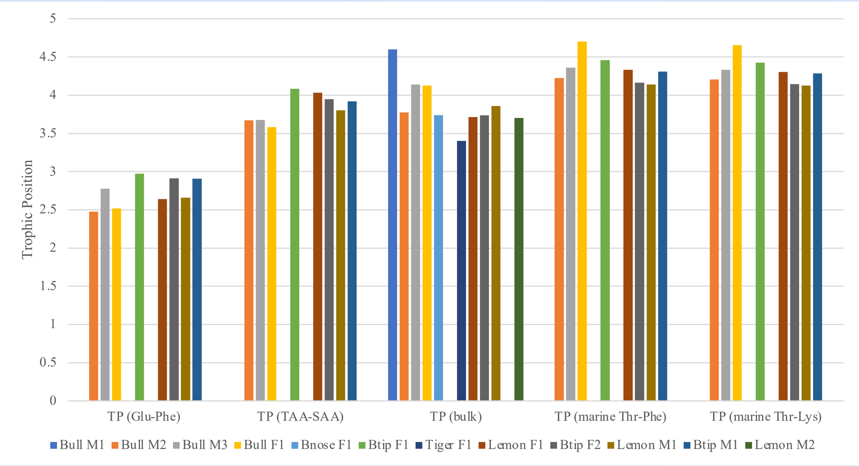

Fig. 3. TP calculations using amino acid nitrogen isotopic values ($\delta^{15}N$; Equation 4, Equation 3, threonine and phenylalanine, threonine and lysine) and bulk isotope analysis ($\delta^{15}N$; Equation 1) for muscle tissue of all paired samples.

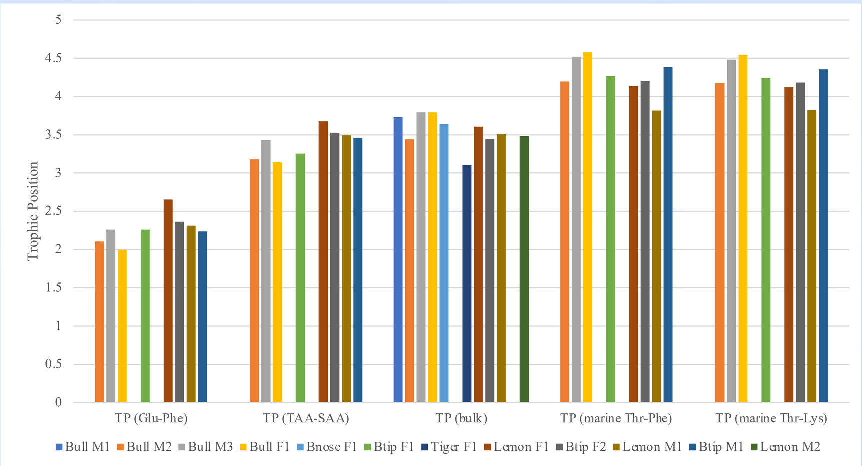

Fig. 4. TP calculations using amino acid nitrogen isotopic values ($\delta^{15}N$; Equation 4, Equation 3, threonine and phenylalanine, threonine and lysine) and bulk isotope analysis ($\delta^{15}N$; Equation 1) for plasma tissue of all paired samples.

	TP (TAA-SAA)	TP (Glu)	TP (marine Thr-Phe)	TP (marine Thr-Lys)
TP (Glu)	P < 0.01			
TP (marine Thr-Phe)	P < 0.01	P < 0.01		
TP (marine Thr-Lys)	P < 0.01	P < 0.01	P > 0.05	
TP (bulk)	P > 0.05	P < 0.01	P < 0.01	P < 0.01

Table 2: A pair-wise comparison of TP calculations using amino acid nitrogen isotopic values ($\delta^{15}N$; Equation 4, Equation 3, threonine and phenylalanine, threonine and lysine) and bulk isotope analysis ($\delta^{15}N$; Equation 1) within a tissue (α = 0.05).

Discussion

- Bulk isotope analysis found the strongest correlation between $\delta^{15}N$ and $\delta^{13}C$ in bull sharks; however, no other significant correlation was noted.
- TP estimates from bulk isotope analysis did not significantly differ from TP calculated from CSIA-AA using an average of TAAs and SAAs (Table 2).
- TP estimates calculated using threonine were significantly higher than all other TP estimates (Figure 3, 4, Table 2).
- TP estimates calculated using threonine made muscle and plasma tissues significantly more similar (Figure 2).

Conclusions

- Sharks are expected to be located at TP 4 or higher, based on observation, SCA, and controlled feeding studies[9,10,11,12,13,14].
- Anomalously low glutamic acid $\delta^{15}N$ values are likely responsible for low TP estimates in bulk and CSIA-AA isotope analysis.
- Higher TP estimates based on threonine may be viable and preferable compared to other TP estimates for urea-producing species.
- TP estimates based on threonine may allow for simultaneous processing of plasma and muscle tissue, rather than requiring a separate estimation for TP in each tissue.
- Multiple tissue analysis may allow for the reconstruction of multiple timescales of feeding data due to differences in $\delta^{15}N$ incorporation rates[10,11].
- Further work will focus on comparing threonine-based TP estimates to controlled feeding study data and SCA estimates.

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