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.
- This study investigated trophic position (TP) estimates based on δ¹⁵N values from bulk stable isotope analysis and compound specific isotope analysis of amino acids (CSIA-AA).
- Glutamic acid has been found to be significantly N-depleted in shark tissue, as well as in pinipercids, cetaceans, and penguins.
- This N-depletion is significantly higher in plasma tissue than muscle tissue.
- N-depletion in glutamic acid has contributed to low TP estimates.
- Sharks store urea, a waste product, in their tissues as an osmolyte.
- In sharks, an immediate glutamic acid, glutaminase, is used as primary nitrogen donor.
- Glutaminase synthesis deaminates glutamic acid twice, contributing to N-depletion.
- 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 analyzed the variability in TP estimates between muscle and plasma tissues.

Methods

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

Equations

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

$$\text{TP}_{\text{bulk}} = \left(\delta^{15}N \text{tissue} - \delta^{15}N \text{prey}\right) / 3 + 1 \quad (\text{Equation 1})$$

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

$$\text{TP}_{\text{CSIA}} = \left(\delta^{15}N \text{amino} - \delta^{15}N \text{source} - \delta^{15}N \text{prey}\right) / \delta^{15}N \text{TDF}_{\text{AA}} + 1 \quad (\text{Equation 2})$$

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

<table>
<thead>
<tr>
<th>Source AA or Tissue</th>
<th>Component Description</th>
<th>TP (NAA)</th>
<th>TP (TDF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine Thr-Phe</td>
<td>Threonine</td>
<td>1.57</td>
<td>2.15</td>
</tr>
<tr>
<td>Marine Thr-Lys</td>
<td>Threonine</td>
<td>1.63</td>
<td>2.22</td>
</tr>
<tr>
<td>Marine Thr-Lys</td>
<td>Phenylalanine</td>
<td>2.09</td>
<td>2.67</td>
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<tr>
<td>Marine Thr-Lys</td>
<td>Lysine</td>
<td>2.34</td>
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<td>2.44</td>
<td>3.02</td>
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</tr>
<tr>
<td>Marine Thr-Lys</td>
<td>Isoleucine</td>
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<td>3.21</td>
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</table>

Discussion

- Bulk isotope analysis found the strongest correlation between δ¹⁵N and δ¹⁵C 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 TAs and SAs (Table 2).
- TP estimates calculated using threonine were significantly higher than all other TP estimates (Figure 3, 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 ± 1 or higher, based on observation, SCA, and controlled feeding studies.
- Anomalously low glutamic acid δ¹⁵N values are likely responsible for low TP estimates in bulk and CSIA-AA isotopic analysis.
- Higher TP estimates based on threonine may be viable and preferable compared to other TP estimates for urea-producing species.
- TP estimates 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 δ¹⁵N incorporation rates.
- Further work will focus on comparing threonine-based TP estimates to controlled feeding studies and SCA estimates.

Acknowledgements

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