Insights into Tuna Trophic Level and Physiology from Compound-Specific Nitrogen Isotope Analysis of Proteinaceous Amino Acids

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Brief Outline

- Introduction and inspiration
- Analytical methods
- Sample analysis: proof-of-concept example
- An intriguing example
- Conclusions and implications
Nitrogen Isotope Fractionation

More of the lighter isotope \(^{14}\text{N}\) is excreted than the heavier isotope \(^{15}\text{N}\) during metabolism.

\[ \delta^{15}\text{N}_{\text{predator}} = 3.0 + \delta^{15}\text{N}_{\text{prey}} (\text{‰}) \]
Inspiration

Method Overview

- **Hydrolysis (Cowie and Hedges 1991, L&O 37:703)**
  - Boil in 6N HCl, ~1 h (150°C)
  - Clean up
    - Filtration
    - Ion exchange

- **Esterification (Hofmann et al. 2003, Isotopes Environ. Health Studies, 3:233)**
  - Heat at 110°C, 1 h acetyl chloride:isopropanol (1:4)

- **Acylation**
  - Heat at 100°C, 15 min methylene chloride:trifluoroacetic anhydride (3:1)
5 mg protein
1 μL/1000 μL
10:1 split ratio
Stable Isotope Analysis

- **Analysis time**
  - 35-40 minutes; ~12 amino acids

- **Sensitivity**
  - 5 mg protein (0.5 mg)
  - 0.5 µL/100 µL (0.5 µL/10 µL)

- **Accuracy and Precision**
  - Accuracy (standards) ~ ±0.5‰ (0.2–0.8‰)
  - Precision (standards) ~ ±0.2‰ (0.1–0.4‰)
  - Precision (samples) ~ ±0.3–1.0‰
$\delta^{15}N$ of Bulk White Muscle Tissue

Eastern Tropical Pacific Sashimi

$y = 0.2x + 11.6$, $r^2 = 0.96$
$\delta^{15}N$ of Amino Acids?

Eastern Tropical Pacific Sashimi

**Non-Essential AA**

**Shift in TL**

**Essential AA**

**Small $\delta^{15}N$ shift in base of food chain**

Latitude: -10 to 30

$\delta^{15}N$, ‰: 9 to 17
$\delta^{15}N$ of Amino Acids?

Eastern Tropical Pacific Sashimi

- Non-Essential AA
- Essential AA

No shift in TL

Same TL

Shift at the Baseline
\( \delta^{15}N \) of Amino Acids in WMT

Eastern Tropical Pacific Sashimi

Trophic Level = 4.1

- Aspartic acid
- Glutamic acid
- Alanine
- Phenylalanine
- Bulk
- Glycine

Latitude
Ontogenic Shift in Yellowfin Tuna from Oahu

Shift in base of food chain?
TL shift?
Starvation?

Graham et al. 2005 submitted

(Graham et al. 2005 submitted)
Conclusions and Implications

- $\delta^{15}$N of amino acids in sashimi
  - Essential - track $\delta^{15}$N of base of the food chain
  - Nonessential - track trophic level and physiology
  - Characterize $\delta^{15}$N of base of food web and trophic level in same tuna sample

- Implications
  - Test if tuna trophic level changed with time
  - Allows migration reconstruction for comparison with archival tags
  - Monitor tuna health - recognize starvation and natural mortality?
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