Toward a chemical tracer for fish feces in the biological pump: Amino acid-specific nitrogen isotope analysis of mesopelagic fish hind-gut contents

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Abstract and Introduction

Fish feces have been highlighted recently as potentially important contributors to the ocean's biological carbon pump, but their importance is poorly constrained. Fishes release both dissolved and particulate organic matter as waste, and considering the biomass and abundance of fishes in open ocean environments, there is interest in determining how much the particulate waste (feces) may contribute to sinking particles. Recently, a unique chemical signature of zooplankton feces was identified using compound-specific nitrogen isotope analysis of amino acids (nitrogen CSIA-AA; [1]), and this has been used to trace the contribution of zooplankton feces in sinking particles [2]. We present CSIA-AA results from the hindguts of three species of fish that were captured between 200-1750 meters depth from the southern California Current, representing zooplanktivorous and piscivorous trophic guilds, both vertical migrators and non-migrators. We assess the usefulness of CSIA-AA as a chemical tracer for fish feces by comparing to previous results from micronekton and zooplankton tissues, zooplankton feces, phytoplankton, and microbially reworked particles. We plan to further consider the role of diet and depth of feeding in shaping the chemical signature of expelled waste, as well as how mesopelagic fish ecology influences the potential contribution of fish feces to sinking particles and carbon cycling throughout the ocean's water column.

Research Questions

- What are the differences in amino acid isotopic signature between fish tissue and fish hind-gut contents?
  - Is there a statistically significant difference between these two?
  - How do fish hind-gut contents compare to zooplankton biomass, zooplankton feces, phytoplankton, and microbial degraded matter?
- How do fish hind-gut contents contribute to the ocean's biological pump? Why is this important?

Methods/Materials

Mesopelagic fish hind-gut contents were collected in 2021 in the southern California Current from 4 species between 700-200 m depth. Hind-gut contents were freeze-dried and homogenized. The CSIA-AA protocol is standard in the Close Lab (University of Miami) [2], [3].

The sample preparation steps:

- Hydrolysis (6N HCl, 20 hrs, 110 C)
- Separating AA hydrosylate from solids (0.01N HCl)
- Purifying AA's utilizing cation exchange columns (2N NH_4OH)
- Reprotonation (0.2N HCl, 5 min, 110 C)
- Derivatization (standards join in preparation):
  - ➢ Esterification (4:1 Isopropanol/Acetyl chloride, 1 hour)
  - ➢ Trifluoroacetylation (3:1 DCM/TFAA, 15 min)

An internal standard (norleucine & aminoadipic acid) and an external standard (14 protein-forming amino acids) were analyzed alongside samples on a Thermo Trace 1310 gas chromatograph coupled to a Thermo MAT 253 isotope ratio-monitoring mass spectrometer via an Isolink II combustion/reduction interface and a Conflo IV low-flow open split interface.

An ANOVA test used to determine statistically significant differences in data sets.

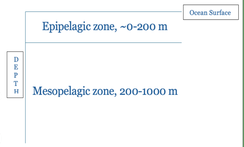

Figure 1. Generalized depth profile of open ocean water column displaying epipelagic and mesopelagic ("deep") zones.

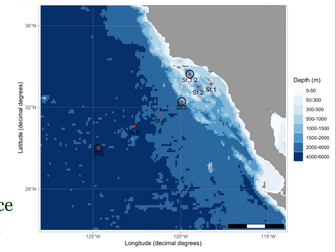

Figure 2. Sample collection sites in the southern California Current.

Results

Species and Family ID	Diet	Vertical migrator?	Depth (m)	MAX Length (cm)
Melamphaes acanthomus *Melamphaidae* (data forthcoming)	Zooplanktivore - crustacean	No	700-400	8.4
Lampanyctus tenuiformis *Myctophidae*	Zooplanktivore – crustacean (TP ~2.8*)	Yes	700-400 and 400-200	8.5
Stomias atriventer *Stomiidae*	Piscivore (TP ~3.3*)	Yes	700-400 and 400-200	8.9

Table 1. The mesopelagic fish species, general diet component, whether they vertically migrate, the depth they were caught, and length. Species sizes and images of sampled species (below) from [4],[5],[6],[7],[8]. *TP=estimated trophic position [10].

Melamphaes acanthomus

Lampanyctus tenuiformis

Stomias atriventer

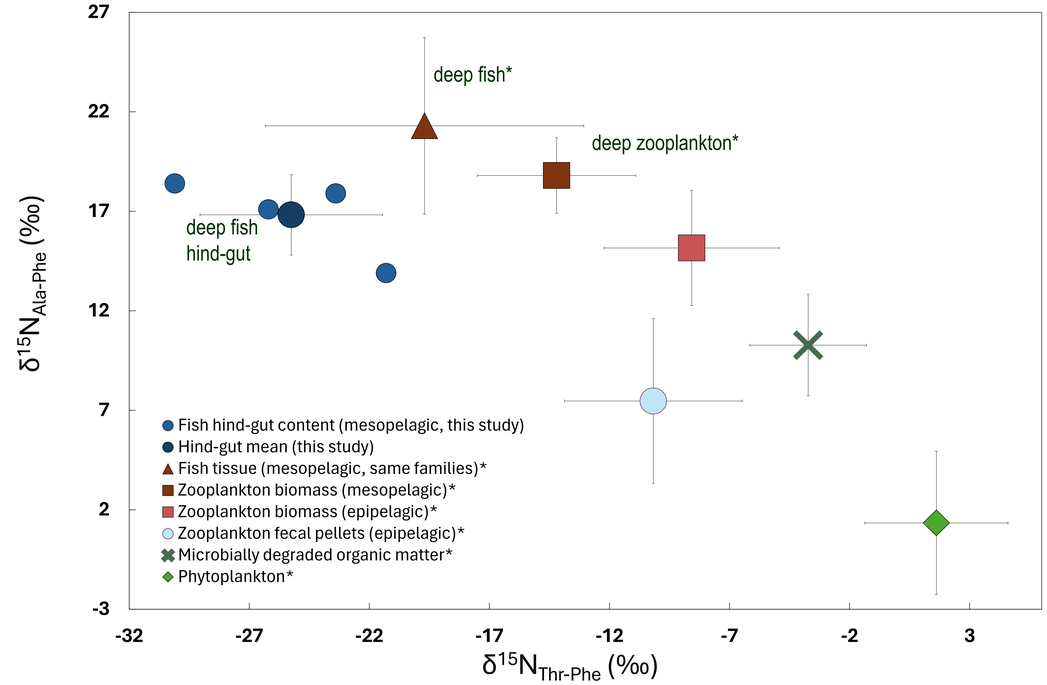

Figure 3. Natural-abundance nitrogen isotope ratios ($\delta^{15}N$ values) of alanine (Ala) and threonine (Thr), each normalized to that of phenylalanine (Phe), obtained from purified amino acid fractions extracted from mesopelagic (deep) fish hind-gut contents (this study) and from fish, zooplankton, and phytoplankton biomass, zooplankton feces, and degraded organic matter from the literature (indicated by *), after Doherty et al. (2021, [1]) and references therein. Literature data are plotted as means and 1σ standard deviation across available data sets in that category. Additional data sources include [9, mesopelagic zooplankton] and [10, mesopelagic fish].

Statistical Analysis

A one-way ANOVA Test was calculated for both $\delta^{15}N$ Threonine - Phenylalanine and $\delta^{15}N$ Alanine – Phenylalanine using F-distribution $\alpha = 0.05$. In both cases, the F-stat was greater than the F-critical, resulting in rejecting our null hypothesis. There is a statistically significant difference between the seven groups in both parameters. Both p values are $< 1.0 \times 10^{-5}$.

Discussion

- Natural-abundance nitrogen isotope ratios ($\delta^{15}N$ values) of alanine and threonine are normalized to those of phenylalanine to account for geographic baseline variations and to quantify changes during trophic transfers (Fig. 3, [1]).
  - ➢ $\delta^{15}N$ values of phenylalanine remain relatively constant during trophic transfer but vary across environments due to N source at base of food web [11].
  - ➢ $\delta^{15}N$ values of alanine increase at each trophic step due to cleavage of amine during first step of metabolism [11].
  - ➢ $\delta^{15}N$ values of threonine decrease at each trophic step due to threonine ammonium lyase's increase in activity [12].
- Doherty et al. (2021) found a distinction between zooplankton, their feces, and their potential food sources using the relationship between $\delta^{15}N_{Ala\text{-}Phe}$ and $\delta^{15}N_{Thr\text{-}Phe}$; preliminarily, we find a parallel distinction between mesopelagic micronekton and the hind-gut contents of similar species, both distinct from potential food sources (Fig. 3).
  - ➢ The consistency of this relationship in zooplankton & micronekton hints at fundamental aspects of nitrogen isotope fractionation during animal digestion/waste production.
- Saba et al. (2021) suggested that fish feces could contribute significantly to the ocean's carbon pump (sinking organic particles). However, evidence to quantify this importance is lacking.
  - ➢ Our AA-CSIA results suggest that fish feces can be distinguished chemically as a component of sinking particles.

Future Work

- Experiments with digestive isotope fractionation of epipelagic fish (aquaculture setting)?
- Analysis of more individuals (diet types, depths, vertical migration versus nonvertical migrators)
  - ➢ Variations in isotopic signature of hind-gut contents?
  - ➢ Possibility of detecting signature in sinking particles?
    - ➢ At what depth do fish release feces?

References

[1] Doherty et al. (2021) *Limnol Oceanogr* 66(7), 2827-2841
[2] Wojtal et al. (2023) *Limnol Oceanogr* 68(9), 1965-1981
[3] Hannides et al. (2013) *Limnol Oceanogr* 58(6) , 1931-1946
[4]Andrew D. Williston, Museum of Comparative Zoology, Harvard University, https://mcz.harvard.edu/database
[5] *MBARI's Deep-Sea Guide*. dsg.mbari.org.
[6] https://fishbase.mnhn.fr/summary/7415
[7] Marine Vertebrate Collection Database
[8] https://sioapps.ucsd.edu/collections/mv/
[9] Shea et al. (2023) *Limnol Oceanogr* 68(8), 1949-1963
[10] Choy et al. (2012) *PLOS ONE* 7(11), e50133
[11] Ohkouchi et al. (2017) Organic Geochemistry 113, 150-174
[12] Fuller and Petzke. (2017) *Rapid Communications in Mass Spectroscopy* 31(8), 705-718
[13] Saba et al. (2021) *Limnol Oceanogr* 66(5), 1639-1664

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