Fatty Acids as Biomarkers for Food Web Structure in the Eastern North Pacific Ocean

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1. ABSTRACT

- Gas chromatography-mass spectrometry (GC-MS) was used to analyze fatty acid composition of suspended particulate organic matter (POM) and zooplankton (ZP; primarily copepods).
- Investigated if essential fatty acids in ZP reflected diet, in particular, distinguishing contributions from a microbial versus traditional food web.
- 2-dimensional GCxGC with time of flight MS used to distinguish polyunsaturated fatty acid (PUFAs) isomers.

2. INTRODUCTION

- Fatty acids (FAs) prevail in nearly all organisms. Essential FAs synthesized chiefly by primary producers (Phy) and some bacteria (Bac) are transferred throughout higher trophic levels.[1] Therefore, FAs can be used to trace food web connections in aquatic environments.
- Certain FAs are found predominantly in Bac, such as the odd numbered FAs 15:0, 17:0 and 21:0.[2]
- Gradients in nutrient concentration across the California Current Ecosystem alters food web structure; low nutrient concentrations elongates the food web favoring the microbial loop.[2] (Fig. 1)

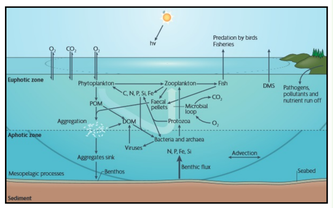


Figure 1[3]: Representation of (a) *Classic food chain: Phy ➔ ZP ➔ Fish. versus* (b) *Microbial Loop: Phy ➔ Bac ➔ Protozoa ➔ ZP ➔ Fish*

- Studying food web interactions in this dynamic environment is difficult. A proxy of food web structure that integrates space and time is a valuable contribution.[4]

3. SAMPLE COLLECTION

Samples Analyzed

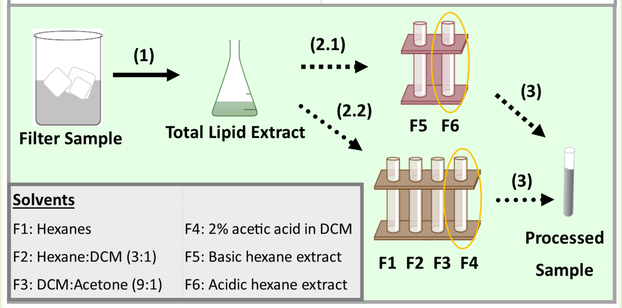
1. Particulate Organic Matter (POM)
   300L of water from a depth of 4m were filtered through 0.7μm Whatman Glass Fiber Filter. Filtrate was composed of mixed phytoplankton, detritus and other unknown particles.
2. Zooplankton (ZP)
   ZP were collected with a 200μm MOCNESS net tow. The collected sample consisted of zooplankton (mostly copepods).

Figure 2: *Samples were collected in the California Current Ecosystem, approximately 9 miles off the coast of San Diego in June 2015.*

4. METHOD

Fatty Acid Extraction Methods

Fractionation Variation 1	*Fractionation Variation 2*
(1) Modified Bligh & Dyer[7] extraction	(1) Modified Bligh & Dyer[7] extraction
(2.1) Saponification then fractionation	(2.2) Column fractionation
(3)Methylation	(3) Methylation



Solvents

F1: Hexanes	F4: 2% acetic acid in DCM
F2: Hexane:DCM (3:1)	F5: Basic hexane extract
F3: DCM:Acetone (9:1)	F6: Acidic hexane extract

5. DATA

Figure 4a: *1D-GC TIC Comparison of POM and ZP*

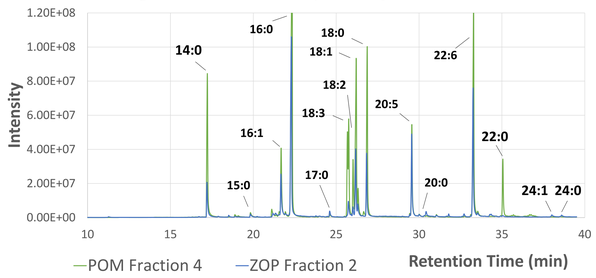
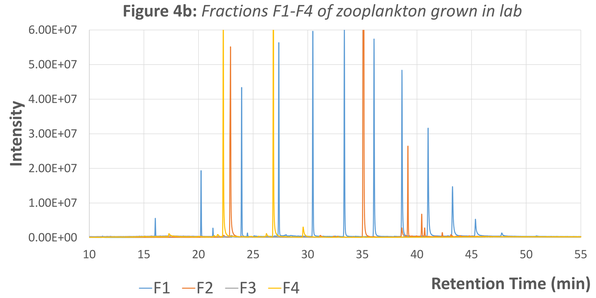


Figure 4b: *Fractions F1-F4 of zooplankton grown in lab*



Polyunsaturated fatty acids (PUFAs) are difficult to separate by normal 1D GC, and so, 2D GC was explored as a better separation tool. As can be seen in Figure 5a and 5b, a number of PUFAs can be detected and quantified in each sample. Figure 6a and 6b show FA mass spectra used to identify FAs in both 1D and 2D GC.

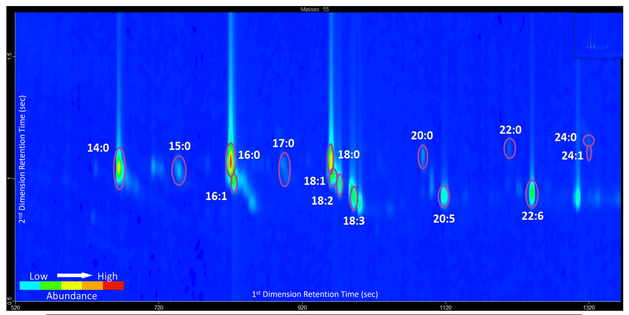


Figure 5a: *2D GC Analysis of Particulate Organic Matter*

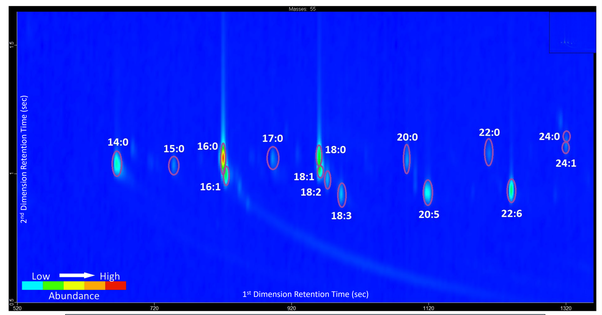


Figure 5b: *2D GC Analysis of Zooplankton*

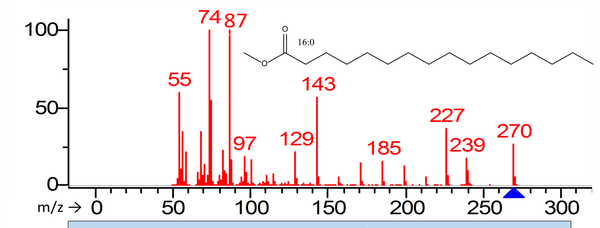


Figure 6a: *Mass Spectrometry of the 16:0 saturated FA*

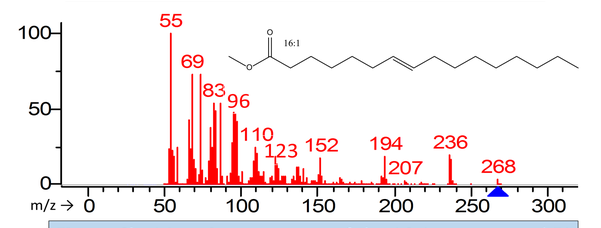


Figure 6b: *Mass Spectrometry of the 16:1 unsaturated FA*

6. RESULTS & DISCUSSION

Total Lipid Extract Composition

Figure 7a: *Bacterial FAs*[7]		
FA	*15:0*	*17:0*
POM	*0.5%*	*0.4%*
ZP	0.8%	1.2%

- FAs were conserved across trophic levels as illustrated by the shared FAs in the ZP and POM samples. (Fig. 4a, 5a, 5b)

Figure 7b: *Saturated FAs*						
FA	*14:0*	*16:0*	*18:0*	*20:0*	*22:0*	*24:0*
POM	8.8%	24.7%	9.1%	0.3%	2.7%	0.3%
ZP	5.5%	27.2%	7.1%	0.4%	0.8%	1.2%

Figure 7c: *Unsaturated FAs*							
FA	*16:1*	*18:1*	*18:2*	*18:3*	*20:5*	*22:6*	*24:1*
POM	6.0%	0.8%	14.9%	10.1%	6.0%	14.9%	<0.5%
ZP	8.4%	0.1%	11%	3.3%	12.2%	19.6%	<0.5%

Literature Review

- The presence of 20:5 and 16:1 serves as an indicator of phytoplankton in the POM sample.[6] The large proportion of 20:5 and 16:1 in ZP is highly likely due to the consumption of diatoms by copepods and other zooplankton in this environment.
- The low relative abundance of short chain PUFAs such as 18:3 and higher relative abundance of long chain PUFAs such as 20:5 indicate biosynthetic processes by secondary consumers.[5]
- The presence of 22:6 provides a reasonable indicator of dinoflagellates (protozoa) in the POM and zooplankton diet.[6] This is tentative evidence of an elongated food web, and is consistent with the relatively oligotrophic (low nutrient) conditions in the region at time of sampling.

7. CONCLUSIONS & FUTURE RESEARCH

- FAs were conserved across the trophic levels examined in this study. Thus FAs serve as a reasonable tracer of food web interactions.
- 2D GCxGC analysis appears to be a promising method for separating and quantifying PUFAs.
- Further research will analyze the hydrogen isotope composition of FAs in ZP and suspended POM collected simultaneously.
- Hydrogen isotopes analysis will help distinguish bacterial from algal sources if FA composition alone cannot.
- FA analysis coupled with isotope analysis may be applied to archived ZP specimens to examine dominant food web interactions in the Northeastern Pacific.

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ACKNOWLEDGEMENTS: Funds were provided from the NSF OCE-REU program and the Scripps Summer Undergraduate Research Fellowship. Special thanks to all members of the Aluwihare lab for their support, especially Jenan Kharbush.