Utility of biofilm fatty acid signatures as a tool to characterize watershed environmental stress

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Population predicted to shift from 17.7 to 20 million people by 2030
Associated demands for food, energy, clean water.

Chesapeake TMDL program (a pollution diet)
Watershed specific (EPA mandates to the state level)

“generate less pollution yet plan for the same levels given a 3-5 million population increase”
Can we complement nutrient monitoring?

(1) How do we characterize disturbed ecosystems?

(2) What benchmarks:
   Do we use to characterize the extent of disturbance?
   Do we use to evaluate the success of restoration?
Biomass accrual (C, N, P)

Biogeochemical Removal as gas?

Nutrient Flux (C, N, P)

Biomass accrual (C, N, P)

Microbial biomass (C, N, P)

Algal biomass (C, N, P)

Environmental Supply (N and P)

Approaches to ecological assessment
Biomass accrual (C, N, P)

Biogeochemical Removal as gas?

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Environmental Supply (N and P)

Approaches to ecological assessment

Supply side-monitor point sources and total maximum delivered loads
Biomass accrual (C, N, P) 

Biogeochemical Removal as gas? 

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Microbial biomass (C, N, P) 

Algal biomass (C, N, P) 

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Environmental Supply (N and P)

Approaches to ecological assessment

Indicator species and indices of biotic integrity

Supply side-monitor point sources and total maximum delivered loads
Biogeochemical Removal as gas?

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Environmental Supply (N and P)

Approaches to ecological assessment

Population assessment

Indicator species and indices of biotic integrity

Supply side-monitor point sources and total maximum delivered loads
What about linkages among compartments?

Supply side - monitor point sources and total maximum delivered loads

Approaches to ecological assessment

Population assessment

Biogeochemical Removal as gas?

Microbial biomass (C, N, P)

Algal biomass (C, N, P)

Environmental Supply (N and P)
Fatty acids are a group of lipids with a distinct chemical structure. Often used to assess the quality of carbon.

Typically are bookended by a methyl and carbonyl group.

SAFA: Saturated fatty acid
MUFA: Mono-unsaturated fatty acids
PUFA: Poly-unsaturated fatty acids
HUFA: Highly unsaturated FA
w3 and w6: placement of the first double bond

ECOLOGICAL NUTRITION: Study of macromolecules (lipids, carbohydrates, proteins); nutrients (carbon, nitrogen, phosphorus); and vitamins within a foodweb or ecosystem.
Fatty acids serve many physiological and ecological functions:

- Fundamental building blocks (macromolecule and growth)
- Promote physiological processes (visual acuity)
- Govern physiological processes (membrane fluidity)
Ecologically important FA’s

HUFA, PUFA and long chain fatty acids are generated via elongation and desaturation.

Only certain taxa at the base of the food web can do it de novo.

Ecologically important FA’s are an important commodity within foodwebs.

How do watershed stressors influence fatty acid composition?
Can we use biofilms to characterize nitrogen, phosphorus, and sediment stressors?

Approach:

• Flow through 110 L mesocosms connected to solenoids.

• Manipulated sediment, nitrogen, phosphorus

• Colonized each stream with the same slurry collected from nearby Straight Run.

• Incubated the streams for 30 days
Experimental design
*Analogous to a 3 way ANOVA*

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**Individual treatments**
- nps, Nps, nPs, NPs, npS, NpS, nPS, NPS
  - $n = 4$

**Specific treatments**
- n, N
- p, P
- s, S
  - $n = 16$
**Response measures (conventional)**

- Biomass accrual
- Algal community structure
- Total and dissolved nutrients

**Functional responses**

**Fatty acids**

- Unsaturated fatty acids
- Monounsaturated (MUFA)
- Saturated (SAFA)
- Stearic acid (18:0)
- Polyunsaturated (PUFA)
- Omega-3
  - α-linolenic (18:3 ω3)
- Omega-6
  - (18:2 ω6) Linoleic

**Enzyme activity**

- P-ase
- N-ase
- C-ase

**Stable isotopes**

- Ambient labeling study
  - (N uptake)
  - 14N: 14.00307 (99.63%)
  - 15N: 15.0001 (0.37%)
  - 12C: 12.00000 (98.89%)
  - 13C: 13.00335 (1.11%)

**Metabolism**

- GPP
- Respiration
Fatty acids

Stress = 0.15
Fatty acids

Stress = 0.15
SIMPER Analysis
(Similarity of Percentages)

Characterize which fatty acids explain differences in treatment assignment.

Low versus high nitrogen
Treatments organized according to low versus high nitrogen.

- **c18:3n3**
  - $F = 6.6$, $P = 0.01$

- **c18:2n6c**
  - $F = 5.1$, $P = 0.03$

- **c16:1n7**
  - $F = 12.1$, $P = 0.002$
Treatments organized according to low versus high phosphorus
Treatments organized according to low versus high phosphorus.
Treatments organized according to low versus high sediment
Can we detect a threshold?

ANCOVA approach

Mussels
Eels
Control (no mussels or eels)
Mussels + eels

24 different nitrogen loading regimes

0.005-6 mg L NO$_3^{-1}$

Blocks of eight loadings per treatment per round
Can we detect a threshold of ecological function?

Biogeochemical Removal as gas?

Nutrient Flux (C, N, P)

Biomass accrual (C, N, P)

Microbial biomass (C, N, P)

Algal biomass (C, N, P)

Consumer excretion (N and P)

Environmental Supply (N and P)

Measured:

1) Nutrient uptake (isotope labeling)
   Gas emission (N₂O, N₂, CH₄, CO₂)

2) Macroinvertebrate community structure

3) Microbial and algal community (phenotype and fatty acid composition)

4) Mussel/Eel physiology and excretion

5) Dissolved nutrient flux (inflow vs. outflow)
Can we detect a threshold of ecological function?

(1) What factors best explain nutrient retention/loss along a gradient of nutrient loading?

(2) Do consumers have the ability to alter this threshold?

Gas emission ($N_2O$, $N_2$, CH4, CO2)

2) Macroinvertebrate community structure

3) Microbial and algal community (phenotype and fatty acid composition)

4) Mussel/Eel physiology and excretion

5) Dissolved nutrient flux (inflow vs. outflow)
Key fatty acids that may be indicative of nitrogen stressors
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ANOVA (N,S,P) study

Threshold = 2.8 mg N L$^{-1}$
Key fatty acids that may be indicative of nitrogen stressors

ANOVA (N,S,P) study

Threshold study

Threshold = 2.5 mg N L\(^{-1}\)
Key fatty acids that may be indicative of nitrogen stressors

ANOVA (N,S,P) study

Threshold study

Threshold = 2.4 mg N L\(^{-1}\)
Scaling up to the real world!

Collaboration with DEP 2014 and 2015
Biofilm Fatty acids
Gradient of watershed land-use and nutrient loading
Integrating nutritional indices into other USGS capabilities

Linking nutrition to ecological function and SPARROW
Nutritional studies in other systems with different stressors
Simulated brine spill

Ecosystem approach to understanding unconventional gas exploration

- High [ ]
  - 24 hours
  - n = 6

- Low [ ]
  - 24 hours
  - n = 6

- Low [ ]
  - 7 days
  - n = 6

- Control
  - n = 6

Time
Collaboration with Susquehanna River Basin Commission (SRBC)
Fatty acid profiles of biofilms with and without Didymo
Still a lot of science needs to be done!

Methodological caveats

- How to sample and where to sample?
- Contamination?
- Lab-specific variability in chemistry and interpretation?
Thank you!
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Andrew Hughes
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Matt Shank