A Multifaceted Approach to Microbial Source Tracking Within Secondary Environments

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Boulder Creek Watershed
Boulder Creek
Boulder, CO

Recreation Class 1A
Primary Contact

Aquatic Life Class 1
Cold Water
Stretch of Boulder Creek

“Impaired”

Area of Study:
On 303(d) list for *E. coli*;
TMDL required.
Storm Drain Outfalls
Outfall Environments

Dynamic & Diverse:

- Motor oil
- Lawn care products
- Sediment
- Pet waste
- Plastics/garbage
Boulder Creek Monitoring Goals

1. Identify concern temporal & spatial.
   ✓ Target Sampling

2. Examine persistence & background levels of \( E. \text{coli} \).

3. Implement multiple analyses to accurately identify wastewater contamination.
   ✓ Toolbox Approach

“Currently one method cannot answer all of the questions”
USEPA 2005.
Environmental Trends: 
*E. coli* vs. Temperature

\[ y = 0.0037x + 0.5862 \]

\[ R^2 = 0.4002 \]
Environmental Contribution?
Persistence Research
E. coli Concentrations: Sediment vs. Water

\[ y = 0.8547x + 1.5473 \]

\[ R^2 = 0.8619 \]
Boulder Creek Benchtop Sediment Microcosms

E. coli Concentrations (CFU/100mL)

Date

Variable
BC CAN
BC CU
OUT POM
OUT SKI

115 days post sampling
Source Tracking Guide

Bacterial
- Idexx - *E. coli*
- *Bacteroids*

Chemical
- Optical Brighteners
- ELISA
- GCMS
Why *Bacteroides*?

✓ 1/3 of fecal flora

✓ **Anaerobe** - Limited survival in environment

✓ **Host-specific** variation in animal host (library independent)

✓ Only found in feces, rumen, and body cavities

✓ Found to **correlate more often** than *E. coli* to **pathogens** (Savichtcheva 2007).
Bacteroides
Research & Method Development

✓ Don Stoeckel
✓ Bernhard & Dick
✓ Layton
✓ Field
✓ Seurinck
✓ Ahmed
✓ McQuaig
Boulder Creek – Bacteroides qPCR

- 15 significant detects.
- 10 positive *E. coli* correlated with 10 positive *Bacteroides*.
- 7 positive *E. coli* NOT associated with *Bacteroides*.
- Only positive *Bacteroides w/o E. coli* at WWTP.
Relative Fluorescence

✓ Need to quantify levels.

✓ Standards most common FWA (Tenopal CBS-X)

✓ USGS, Boulder, CO
Larry Barber
Relative Fluorescence: Hydrologic Connection

[Graph showing relative fluorescence over time with peaks and valleys, labeled for West to East Boulder Creek.]
ELISA
(Enzyme-Linked Immunosorbent)

*Competitive reaction* → *Fluorescence*

- Triclosan
- Estradiol
ELISA Results

**Triclosan**
- Antimicrobial
- Resistant strains

Levels found within:
- **Outfalls:** 0-343 ng/L
- **In-stream:** 34.46 ng/L

**Estradiol**
- Naturally occurring hormone.
- Estrogenic in nature

Levels found within:
- **Outfalls:** 0-38.8 ng/L
- **In-stream:** ave 3.3 ng/L
Gas Chromatography -
Mass Spectrometry

✓ Solid Phase Extraction
✓ Surrogate Standards
✓ Full scan for standards
✓ SIM scan for samples
✓ Peer reviewed
GC-MS: Caffeine

Levels found within:

Outfalls:
183-19,000 ng/L

In-stream:
42 ng/L
GC-MS Detects

4-Methylphenol
✓ Disinfectant
✓ Solvent

Levels found within:
Outfalls: 0-256 ng/L
In-stream: 19 ng/L

Bisphenol-A
✓ Plasticizer
✓ PVC piping
✓ Toilet paper
✓ Endocrine disruptor

Levels found within:
Outfalls: 9-204 ng/L
In-stream: 38 ng/L
## Comparison #1: Out-Pom

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>26.2 CFU/100mL</td>
</tr>
<tr>
<td>TOC</td>
<td>89 mg/L</td>
</tr>
<tr>
<td>OB</td>
<td>98 ug/L</td>
</tr>
<tr>
<td>Caffeine</td>
<td>12,275 ng/L</td>
</tr>
<tr>
<td>Triclosan</td>
<td>95 ng/L</td>
</tr>
<tr>
<td>Estradiol</td>
<td>5.4 ng/L</td>
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</tbody>
</table>
## Comparison #2: Out-Ski

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>7,701 CFU/100mL</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>0.1 copies/mL</td>
</tr>
<tr>
<td>TOC</td>
<td>1.8 mg/L</td>
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<tr>
<td>OB</td>
<td>45 ug/L</td>
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<tr>
<td>Caffeine</td>
<td>0 ng/L</td>
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<tr>
<td>Triclosan</td>
<td>10 ng/L</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.9 ng/L</td>
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<tr>
<td>Substance</td>
<td>Value</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>9,804 CFU/100mL</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>304 copies/mL</td>
</tr>
<tr>
<td>OB</td>
<td>45 ug/L</td>
</tr>
<tr>
<td>Caffeine</td>
<td>297 ng/L</td>
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<tr>
<td>Triclosan</td>
<td>31 ng/L</td>
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<tr>
<td>Estradiol</td>
<td>3.5 ng/L</td>
</tr>
</tbody>
</table>
What it all means....

✔ Relationships between indicators could not be established.
   ➢ Does not weaken alternative indicators

✔ Raises further **doubt in the utility of E. coli** as an indication of wastewater contamination.

✔ Due to **environmental persistence**, *E. coli* is not completely accurate in identifying recent contamination.

✔ **Multiple constituents** must be used in order to accurately detect a broad range of human-derived contamination.
Questions...?