



A Multifaceted Approach to Microbial Source Tracking Within Secondary Environments



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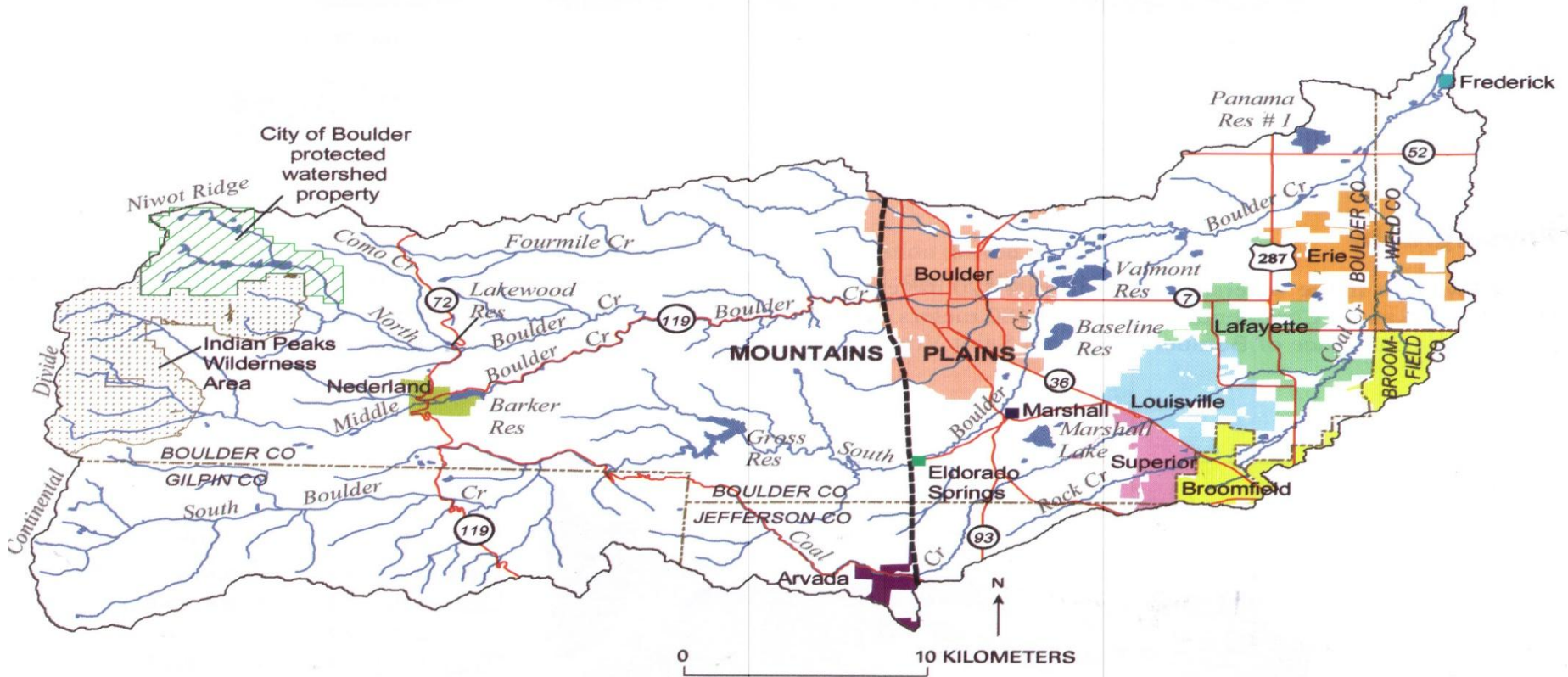


Acknowledgments

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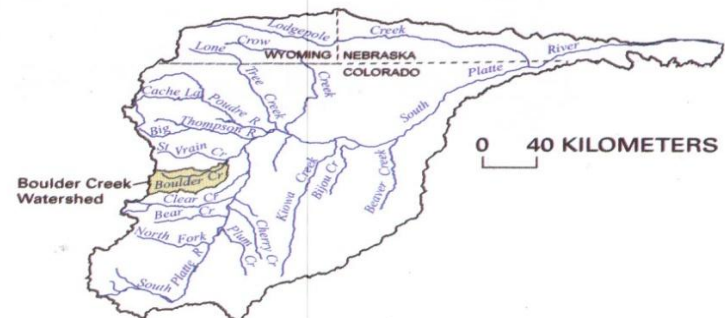
Boulder Creek Watershed



• Boulder Creek Watershed (from Murphy and others, 2003)



Mississippi River Watershed



South Platte River Watershed

Boulder Creek Boulder, CO



Recreation Class 1A
Primary Contact

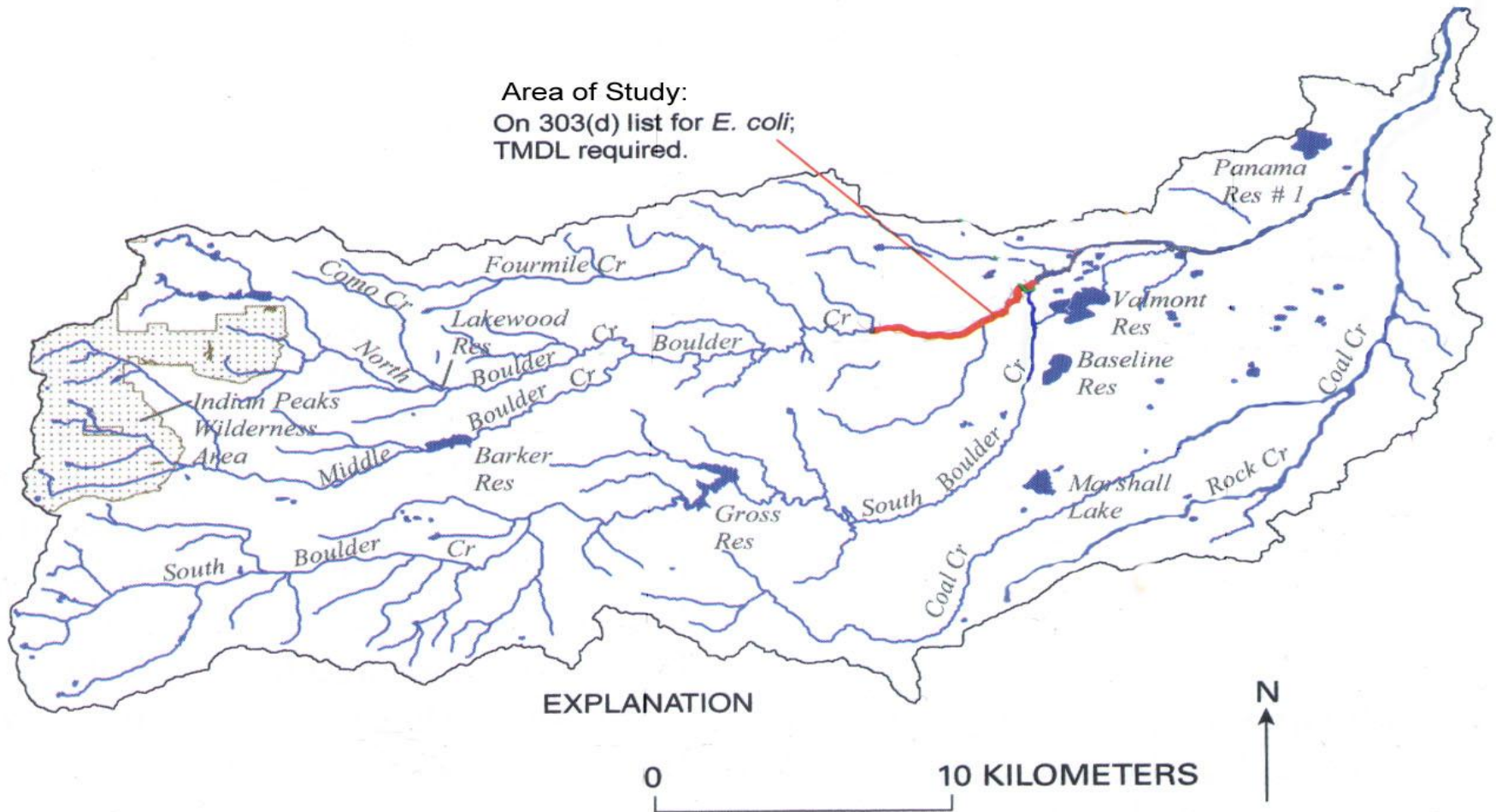
Aquatic Life Class 1
Cold Water



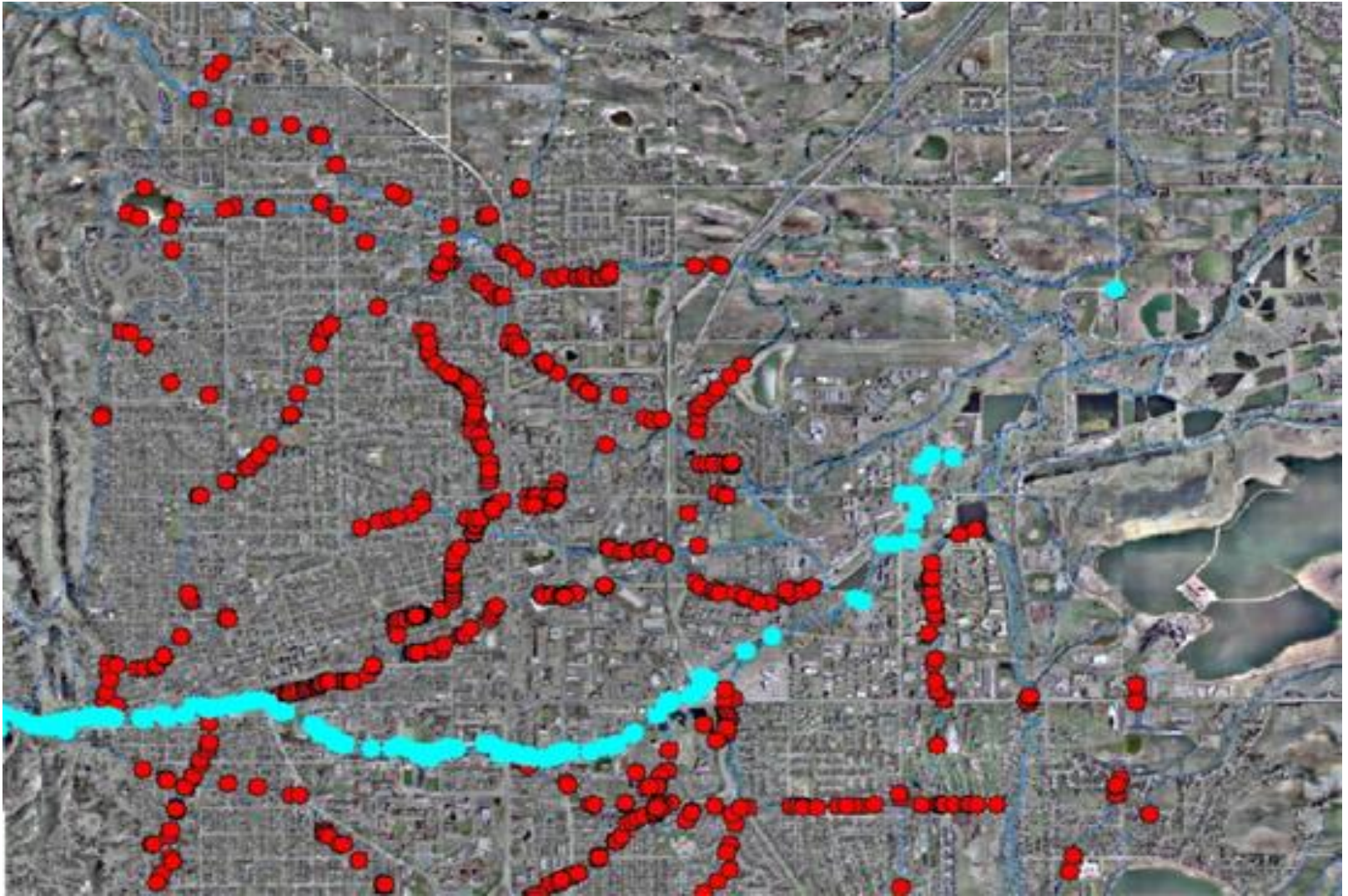
Stretch of Boulder Creek

“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

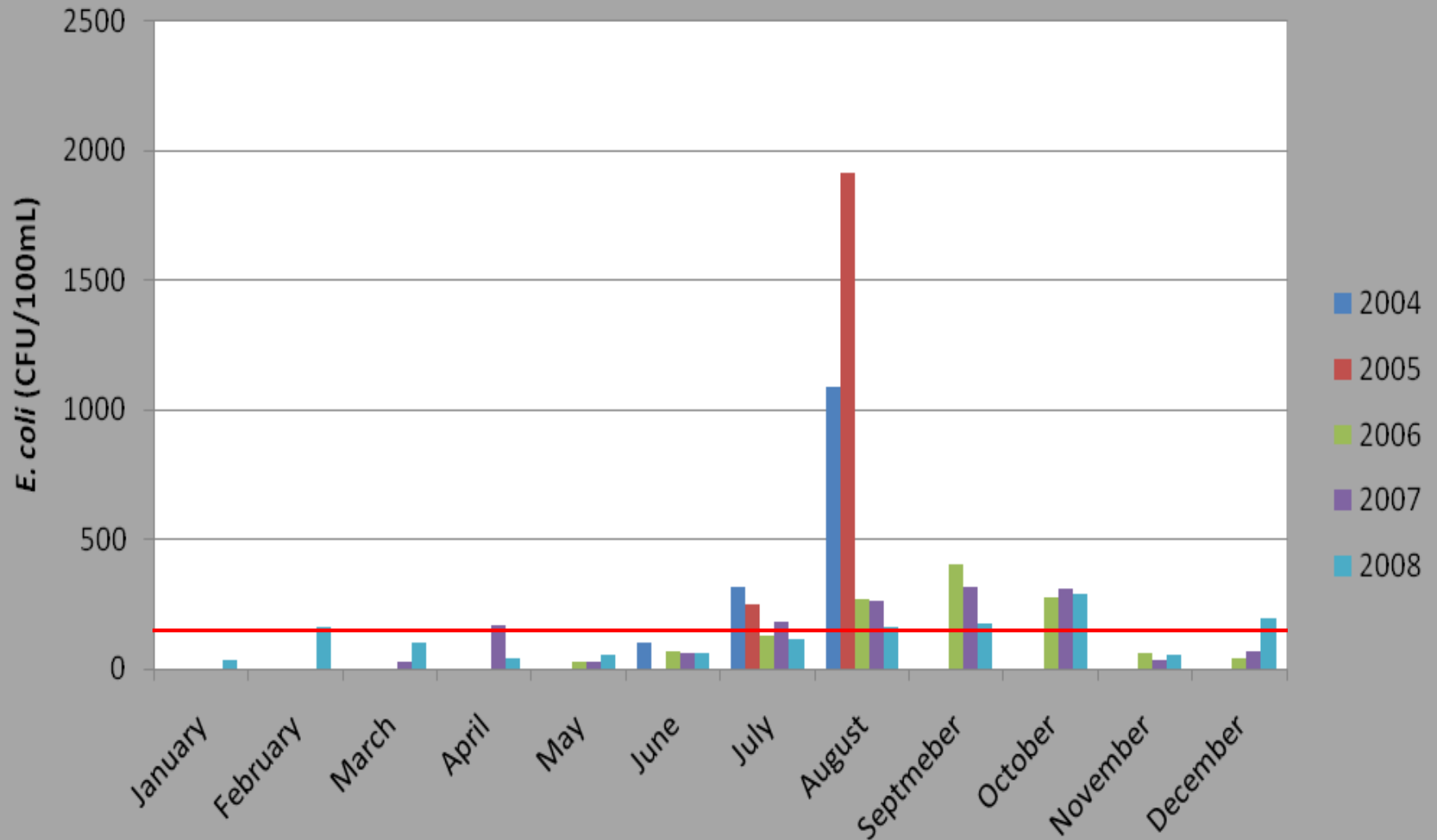


1. Identify concern temporal & spatial.
✓ **Target Sampling**
2. Examine persistence & background levels of *E. coli*.
3. Implement multiple analyses to accurately identify wastewater contamination.
✓ **Toolbox Approach**

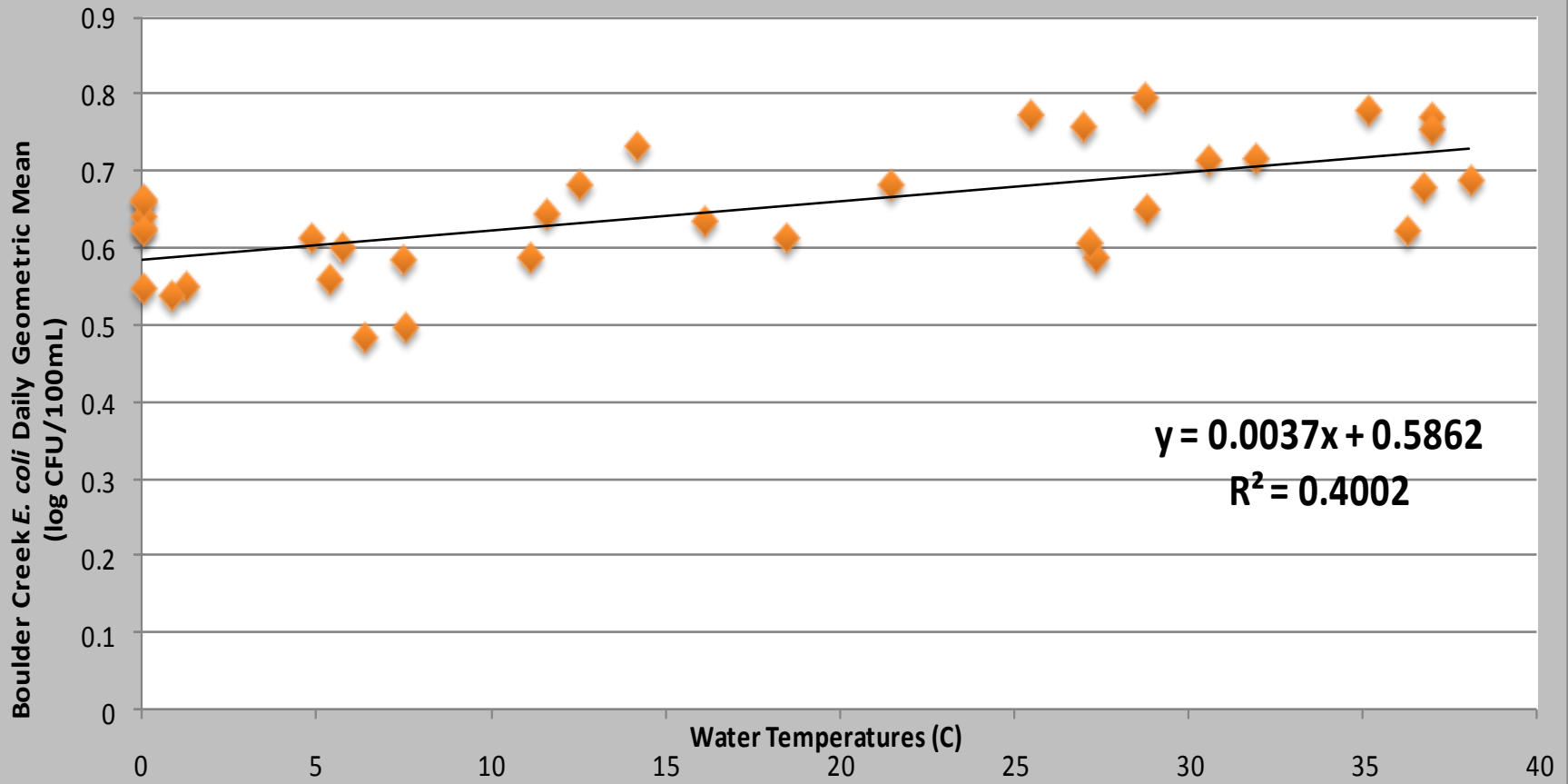
“Currently one method cannot answer all of the questions”

USEPA 2005.

Boulder Creek Temporal Trends



Environmental Trends: *E. coli* vs. Temperature

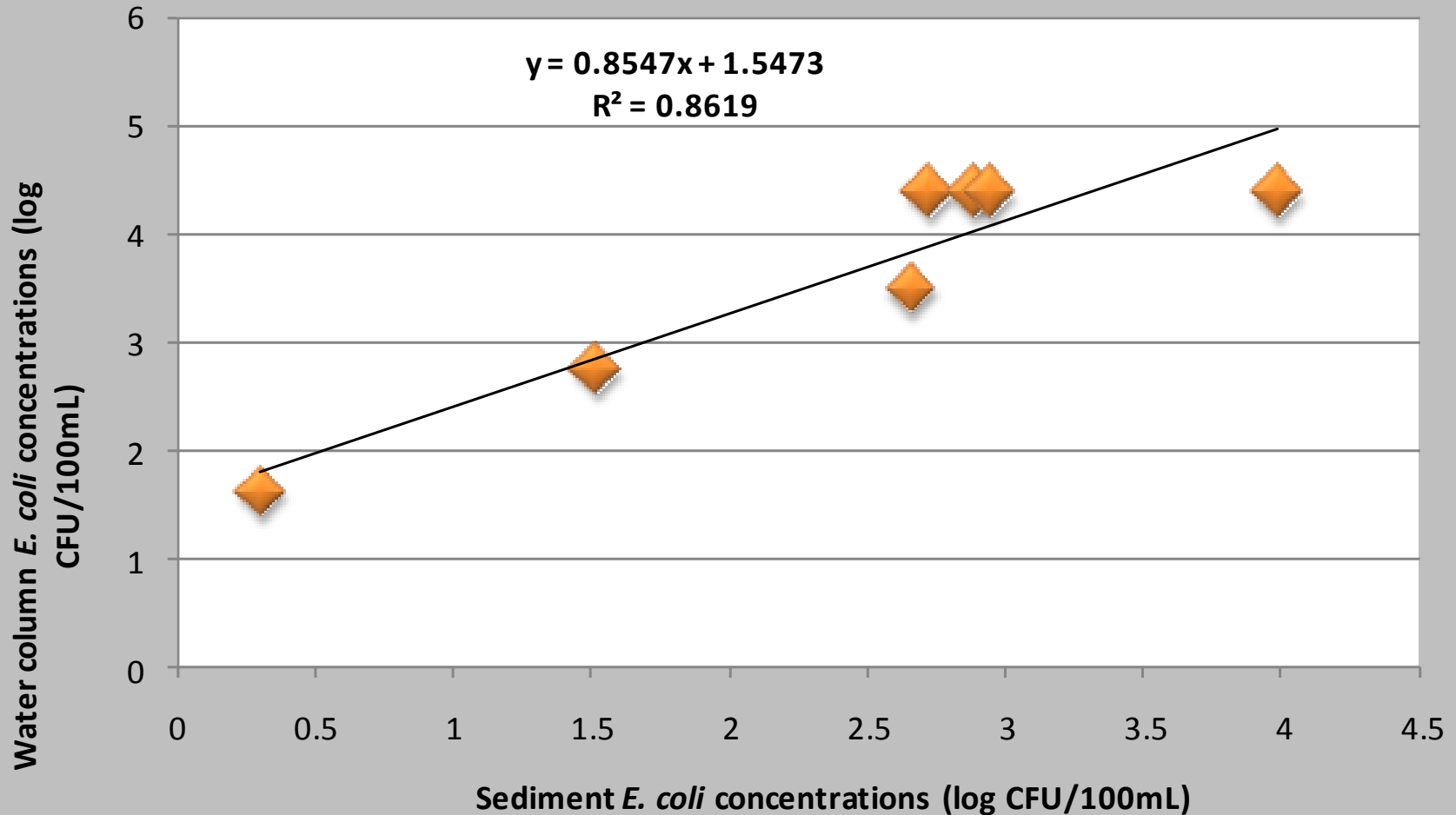


Environmental Contribution?

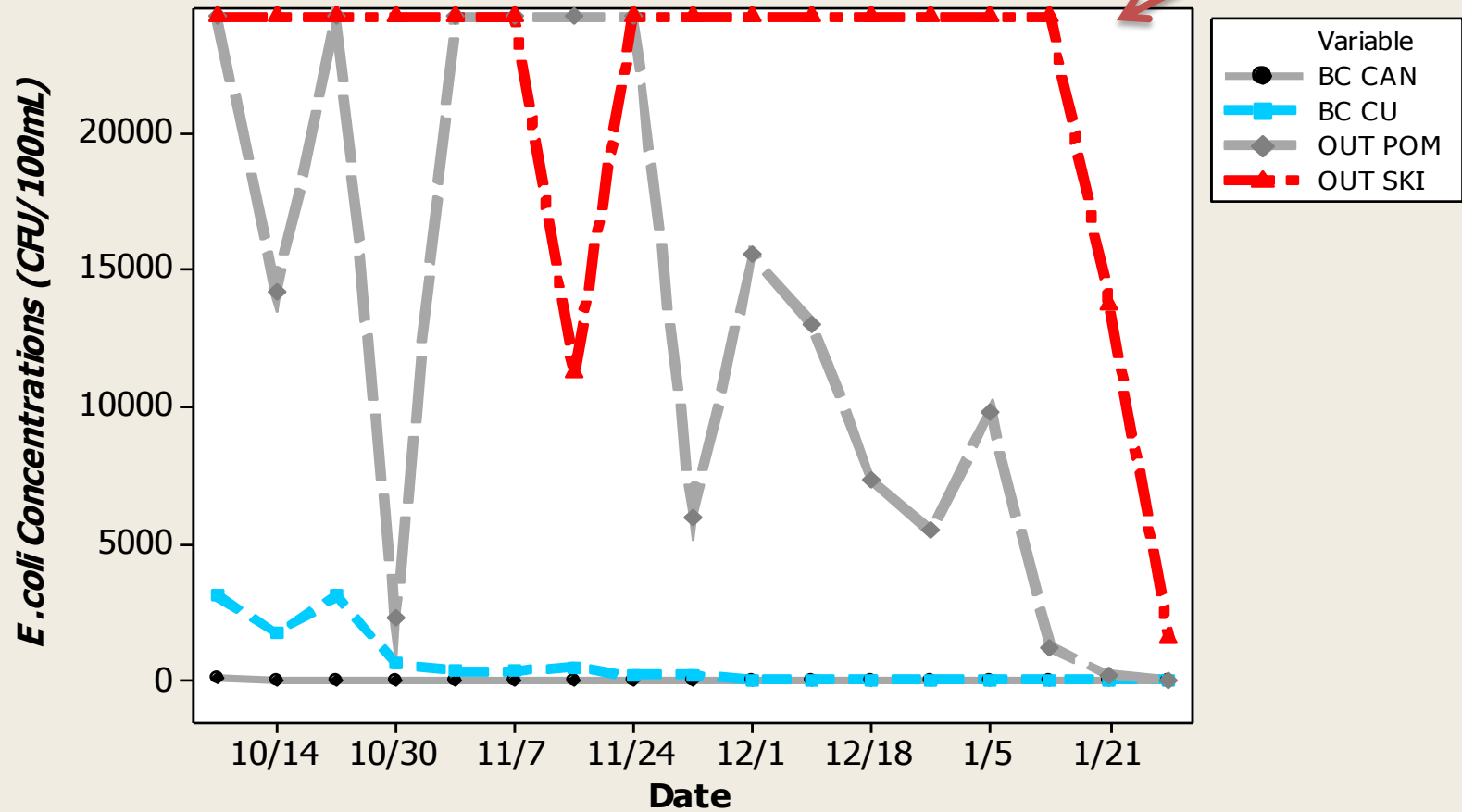
Persistence Research



E. coli Concentrations: Sediment vs. Water



Boulder Creek Benchtop Sediment Microcosms



115 days post sampling

Source Tracking Guide

Bacterial

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

Chemical

- Optical Brighteners
- ELISA
- GCMS



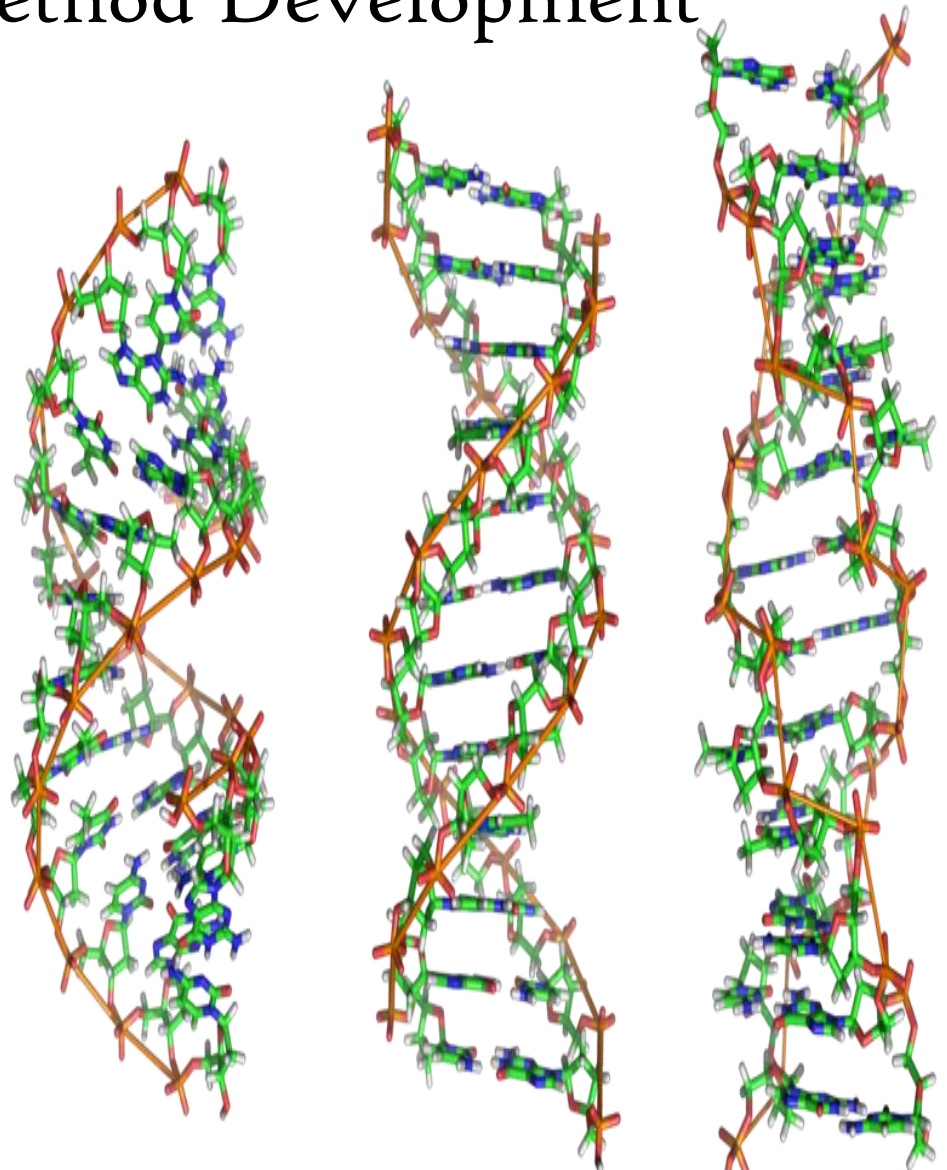
Why *Bacteroides*?

- ✓ **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

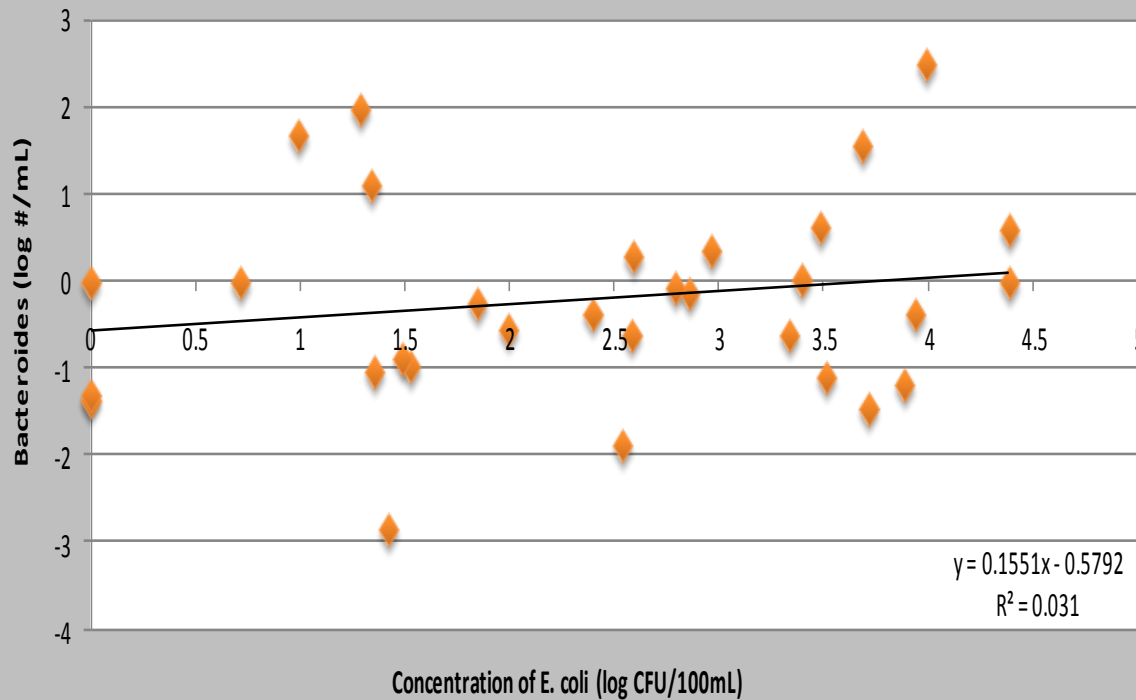
Research & Method Development

- ✓ **Don Stoeckel**
- ✓ Bernhard & Dick
- ✓ Layton
- ✓ Field
- ✓ Seurinck
- ✓ Ahmed
- ✓ McQuaig



Boulder Creek – *Bacteroides* qPCR

Boulder Creek *E. coli* vs *Bacteroides*



- ✓ 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.

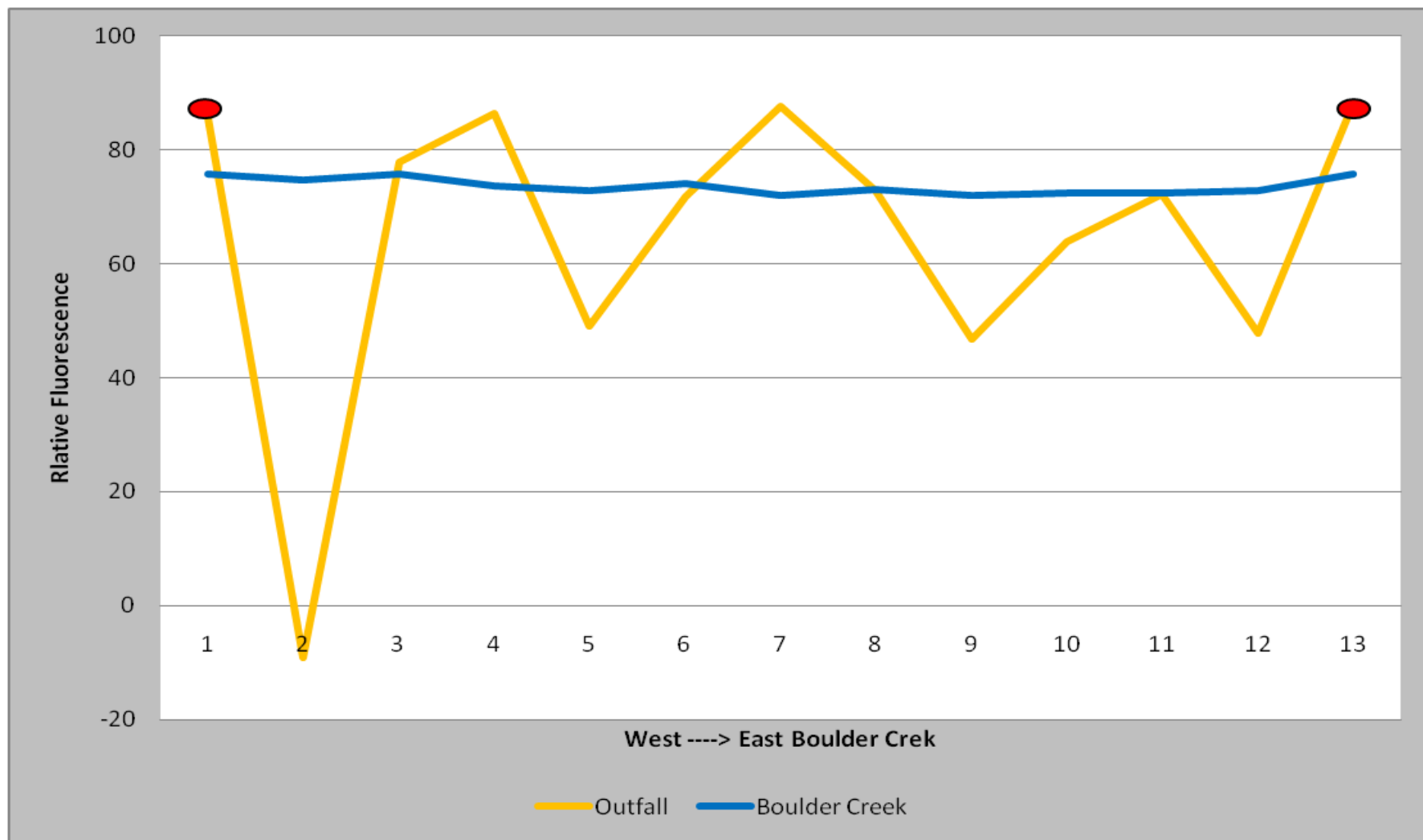
Relative Fluorescence

- ✓ Need to quantify levels.
- ✓ Standards most common FWA (*Tenopal CBS-X*)
- ✓ USGS, Boulder, CO
Larry Barber




Photos: Hartel

Relative Fluorescence: Hydrologic Connection



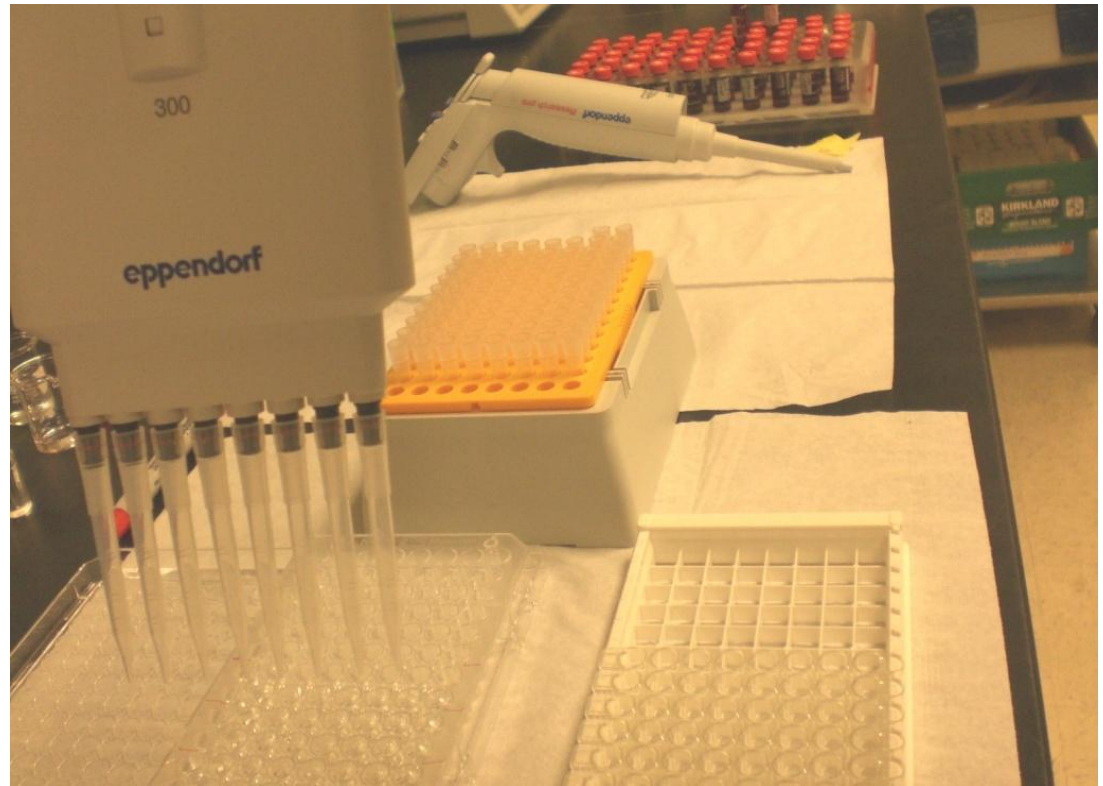
ELISA

(Enzyme-Linked Immunosorbant)

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

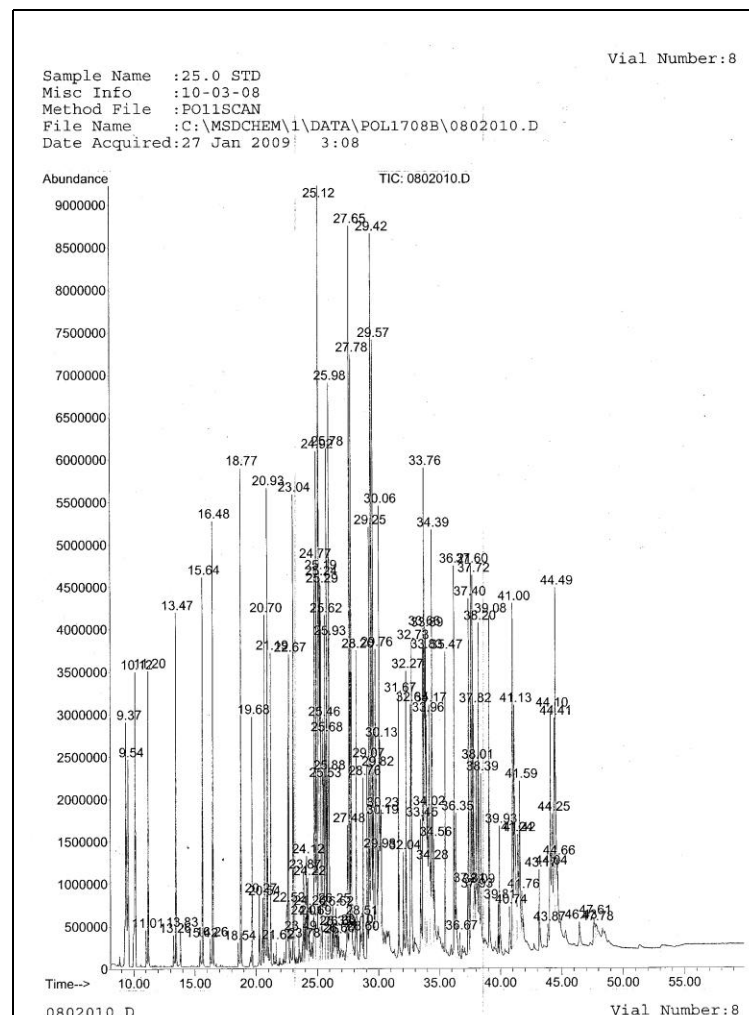
In-stream:

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



Bacterial

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

Chemical

- Optical Brighteners
- ELISA
- GCMS

**ENVIRONMENTAL
TRENDS**



Comparison #1: Out-Pom

E. coli 26.2 CFU/100mL

TOC 89 mg/L

OB 98 ug/L

Caffeine 12,275 ng/L

Triclosan 95 ng/L

Estradiol 5.4 ng/L





Comparison #2: Out-Ski

E. coli 7,701 CFU/100mL

Bacteroides 0.1 copies/mL

TOC 1.8 mg/L


OB 45 ug/L

Caffeine 0 ng/L

Triclosan 10 ng/L



Estradiol 0.9 ng/L





Comparison #3: Out-Fol

<i>E. coli</i>	9,804 CFU/100mL
<i>Bacteroides</i>	304 copies/mL
OB	45 ug/L
Caffeine	297 ng/L
Triclosan	31 ng/L
Estradiol	3.5 ng/L



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...?

