Finding Your Bacteria Sources: Microbial Source Tracking

CWI Workshop: Microbial Source Tracking
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Problem

Fecal pollution entering the watershed
Impacts on Community

Health Impacts
❖ Nausea, vomiting, diarrhea, fever

Economic Impacts
❖ Beach Closures ($ Millions)
❖ Shellfish Harvest Closures
❖ Capital Improvement Costs
“Effective management and mitigation requires knowledge of the source(s) of fecal contamination.”

–California MST Manual
Microbial Source Tracking - Tiered Approach

1. Build a hypothesis based on existing evidence
2. Collecting fecal indicator bacteria data
3. Verify hypothesis with Source Tracking test methods
4. Interpretation and conclusions
Forming a Good Hypothesis

❖ **Examples**
"A sanitary sewer is leaking and sewage is entering the watershed"
"Chicken farming or aging septic are the most likely contributors to elevated bacteria"
'Wildlife sources, not SSI, are the major contributors to fecal contamination"

❖ **Avoid definitive hypothesis that will be difficult to prove**
"All of the FIB comes from Farm X. "
"No human fecal pollution exists in our watershed."
Hypothesis

Gather Existing Evidence

❖ Insight from local partners

❖ Location, age, and condition of Sanitary Sewer and Septic Systems
  • Land Use
  • GIS Data

❖ FIB History

❖ Sanitary Surveys and Field Reconnaissance
Fecal Indicator Bacteria

Finding bacteria "hot spots"

WHERE?

- High then low bacteria levels across geographic area?
- Geographic Features (public bathrooms, land spreading, homeless)?
- Connections between watersheds and flow patterns

WHEN?

- Dry vs Wet – Indicates stormwater influence
- Seasonal- Farm patterns, Use patterns (fairs, horse racing season)
- Beaches - Tides
Microbial Source Tracking Tests

- Fecal Indicator Bacteria – Simple, culture-based, general bacteria levels
- Microbial Source Tracking – Specialized, qPCR-based, source specific results
- US EPA Developed/Patented Methods
  - Human
  - Cattle
  - Chicken
- US EPA Upcoming MST Standardized Methods (Human)
- California MST manual recommended qPCR tests for:
  - Human, Dog, Ruminant, Cow, Pig, Horse, and Gull.
Microbial Source Tracking Tests Available

NEW - Beaver
Sampling & Test Plan

Locations?

❖ Start with FIB hotspots
❖ Absent hotspots, use obvious physical sources (closest to potential sources)

When to collect?

❖ Wet weather events (stormwater and septic influence)
❖ Dry sampling events (sanitary collection systems)
❖ Seasonal Changes
Sampling & Test Plan

How many samples?
- Trade off between cost and certainty
- Proving a host is not a major contributor requires more sampling
- Frequency at a location allows for a stronger interpretation

Which tests?
- Priority (Typically human or agriculture)
- Likelihood of occurrence
- Selection of 2 tests for a host increases certainty

Other Considerations…
- Are tests properly validated for your potential sources?
- Are there major sources with tests not available?
- Which hypothesis or locations should take priority?
Submitting Samples

Microbial Source Tracking hold time (typically 24-48 hours)

Shipping Water Overnight

❖ 500 ml sterile bottles, coolers, ice packs and packing material provided
❖ Advantages: Simpler and requires less logistics

Sending Filtered Water

❖ Local lab filters and freezes samples
❖ Procedures and support provided by SM
❖ Advantages: Reduced shipping costs and ability to select samples for microbial source tracking
Cost

$175 per test - 4 or more tests per sample
$225 per test - 3 tests per sample
$275 per test - 2 tests per sample
$375 per test - 1 test per sample
Measure quantity - additional $40 per test

Projects $10,000 to >$50,000 scaling 15%-35% price reduction.
MST Results

Qualitative Results
- "Present or Absent"
- 5-10 working days
- Less expensive than quantification

Quantitative Results
- Concentration of host-associated biomarker
- Results reported in "Genetic Copies/100ml"
- 5-10 working days
- High or low magnitude can be integrated into interpretation
Likelihood & Result Interpretation

Existing Lines of Evidence (FIB, Sanitary Survey, GIS Data)
Number of samples increases certainty
Magnitude of marker concentration influences certainty
Number of tests per host increases certainty
Proving "innocence" more difficult than proving "guilt"
Case Study

Problem
State of Delaware was observing high levels of Enterococcus at an estuarine inland recreational beach.

Existing Evidence
Dog: Visual observation of dogs at the beach
Gull: Often large concentrations of gull.
Human: Geographic proximity to septic systems.

Hypothesis
Gull, Dog, or Human are likely sources of high bacteria.

Sampling Plan
Bacteria levels tested on each sample
3 sample locations (composited)
3 sampling events per week for ~3 months
49 samples total

Test Plan
Quantification: If present
2 tests for Human
1 test for Dog
1 test for Gull
Case Study

Submission
Samples filtered locally. Shipped on dry ice.

Results

Human
92% of all samples were negative (N=49)
   Quantification - 8% had low concentrations of human fecal indicator (≤ 112 genome equivalents/100ml)

Dog
96% of all samples were negative (N=49)
   Quantification - 4% had low concentrations of dog fecal indicator

Gull
100% of samples positive (N=48)
   Quantification - abundant concentration of gull fecal indicator (≤ 25,365 gene copies/100ml)

Interpretation
Based on the existing lines of evidence and the genetic qPCR results gulls were likely the major contributed to the elevated levels of Enterococcus (Delaware's water quality indicator) at this beach.
About Source Molecular

- Founded in 2002 to fill the void between research and implementation

- Active in Research & Development of new MST methods
  Only commercial laboratory to perform qPCR MST as part of the California Source Identification (SIPP) Method Evaluation Study

- Strict focus on Microbial Source Tracking

- Detection of 13 fecal pollution sources

- US EPA-patented methods (Human, Cattle, Chicken)

- Pending ISO 17025 laboratory quality accreditation
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