Developments in Microbial Source Tracking

Digital PCR, California’s Source ID Manual, and US EPA tests
<table>
<thead>
<tr>
<th>Experience</th>
<th>Research</th>
<th>Methods</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Leading commercial practitioner of</td>
<td>• Commercial partner for California Source Identification Project</td>
<td>• US EPA-Developed</td>
<td>• Pending ISO 17025</td>
</tr>
<tr>
<td>Microbial Source Tracking services</td>
<td>(SIPP)</td>
<td>• Cattle</td>
<td>laboratory accreditation</td>
</tr>
<tr>
<td>• &gt;70 Source Tracking Studies Completed</td>
<td>• Participated in US EPA Method Standardization Study</td>
<td>• Human</td>
<td></td>
</tr>
<tr>
<td>in 2014</td>
<td></td>
<td>• Chicken</td>
<td></td>
</tr>
<tr>
<td>• Founded in 2002</td>
<td>• Collaboration with SCCWRP on Digital PCR study (publication pending)</td>
<td>• Dog</td>
<td></td>
</tr>
</tbody>
</table>
Presentation Overview

1. Method Background/Developments
   - Microbial Source Tracking (MST)
   - Digital PCR
   - California Guide Manual
   - US EPA Tests

2. Applying Microbial Source Tracking
   - Gathering Information
   - Sampling/Test Plan
   - Results and Interpretation

3. Case Studies
   - San Juan Watershed Project
   - San Diego River Project
   - DNREC Beach Project

4. Conclusion and Questions
   - Questions and Discussion
Problem
Fecal pollution entering the watershed

(NC Department of Health and Human Services, 2011)
Method Background

Traditional Fecal Indicator Methods

- Water Sample
- Enumeration
- General Fecal Indicator

Microbial Source Tracking Methods

- Water Sample
- Filtration
- DNA Isolation
- qPCR
- Host Specific Fecal Indicator
- Host Specific Fecal Indicator
- Host Specific Fecal Indicator
Developments

Digital PCR

http://youtu.be/S5SvaPxwXiU

Study Link: http://www.sciencedirect.com/science/article/pii/S0043135414008409
California Microbial Source Identification Manual

- **Link to Document**
- Created by SCCWRP for CA Water Resource Control Board
- Tiered Approach to Identifying Bacteria Sources
- Scientific Consensus! 27 Participating Labs (including Source Molecular)
- Set precedent for Microbial Source Tracking to be used as a mainstream tool in California
Developments

US EPA Developed/Patented Tests

Upcoming US EPA Approved Method

EPA Method Standardization:
Development Plan

1. Method Selection
   - Which pollution source?
   - What detection technology?
   - Which host-associated indicator?

2. Technical Evaluation
   - Peer-reviewed

3. Method Validation
   - Establish Standard Operating Procedure (SOP)
   - Establish quality assurance metrics
   - Conduct multiple lab studies

4. EPA Method
   - Decision made by EPA Office of Water
   - If selected, then eligible for use in future EPA policies and programs
Initial Hypothesis for Cause of Bacteria

- Fecal Bacteria Levels at Each Sampling Site
- Insight From Local Partners
- Age, Location, and Condition Sewage/Septic
- Land Use within Watershed
- Sanitary Surveys
- Field Reconnaissance

Applying Microbial Source Tracking
Sample and Test Plan

- **Sampling Sites**
  - Fecal Bacteria Hotspots
  - Collecting Near Physical Sources

- **Sampling Events**
  - Wet/Dry Weather Sampling
  - Seasonal Changes
  - Statistically Significant Number of Events

- **Tests Per Sample**
  - Focus on Anthropogenic Sources (Human, Dog, Agriculture)
  - Most Likely Wildlife Source (Birds, Deer, etc.)
Applying Microbial Source Tracking

Submitting Samples

Pre-Filtered Samples

Ship Water Samples

Samples Shipped Overnight

Source Molecular
Leader in Microbial Source Tracking
Applying Microbial Source Tracking

Results

Example Results for Site 1
6 Sampling Events

<table>
<thead>
<tr>
<th>SM #</th>
<th>Client #</th>
<th>Analysis Requested</th>
<th>General Marker Quantified</th>
<th>Human Specific Marker Quantified</th>
<th>DNA Analytical Results</th>
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</thead>
<tbody>
<tr>
<td>SM 10267</td>
<td>01032011D</td>
<td>Human Bacteriodes 10</td>
<td>4.97E+03</td>
<td>9.70E+03</td>
<td>Positive</td>
</tr>
<tr>
<td>SM 10268</td>
<td>01032011E</td>
<td>Human Bacteriodes 10</td>
<td>5.44E+03</td>
<td>4.19E+02</td>
<td>Positive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SM #</th>
<th>Client #</th>
<th>Approximate Contribution of Human Fecal Pollution in Water Sample</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM 10267</td>
<td>01032011D</td>
<td>Major Contributor</td>
<td>High levels of human biomarker detected</td>
</tr>
<tr>
<td>SM 10268</td>
<td>01032011E</td>
<td>Major Contributor</td>
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Applying Microbial Source Tracking

Interpreting Results

Scenario 1
- High Fecal Bacteria
- Potential Sources:
  - Human
  - Gull

Non-Human Pollution Source
- Fecal Indicators Present Only During Wet Weather
- Presence of Gull Fecal Indicators
- High Concentrations of Gull Fecal Indicators
- Sanity Surveys show systemic presence of Gulls on the beach

Human Sources
- Absence of Human Fecal Indicators
- Sanity Surveys Showed No Evidence of Leaking Sewers
Interpreting Results

Scenario 2
- High Fecal Bacteria
- Potential Sources:
  - Human
  - Bird
  - Cattle

Non-Human Pollution Source
- Absence of Bird Fecal Indicators
- Absence of Cattle Fecal Indicators

Human Sources
- Presence of Human Fecal Indicator, High Quantity
- Presence of EPA Human Fecal Indicator, High Quantity
- Sanity Surveys show aging infrastructure
- Fecal Indicators present during dry & wet weather
Case Study 1

San Juan Watershed Case Study

http://www.kob.com/article/11687/?v=5035312&v=1
Case Study 2

San Diego River MS4 Project

LINK: Pathogens in Urban Stormwater Systems

Figure 5-4. MS4 Microbial Source Identification Investigation Approach
(Source: Brandon Steets, Geosyntec Consultants)
Problem

• 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
Case Study 3

DNREC Beach Source Tracking Project

Test Plan
• 2 tests for Human
• 1 test for Dog
• 1 test for Gull

Submission
• Samples filtered locally. Shipped on dry ice.

Results
• Human
  • 92% of samples negative (N=49)
  • Quantification – low levels
• Dog
  • 96% of samples negative (N=49)
  • Quantification - low levels
• Gull
  • 100% of samples positive (N=48)
  • Quantification - abundant levels

Interpretation
• Based on the existing lines of evidence and the genetic qPCR results gulls were likely the major contributor to the elevated levels of Enterococcus (Delaware’s water quality indicator) at this beach.
Questions

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