Microbial Source Tracking in Surface Water

Department of Natural Resources
Surface Water Quality Monitoring Program

Matt Zupich, Water Quality Specialist
Island County Board of Health, Coupeville, June 21, 2016
“Sorry. I thought a joke would break the tension.”
Why Monitor Water Quality?

To ensure clean water for the protection of public health and natural resources
What Do We Monitor?

- 11 parameters including:
  - Temperature
  - pH
  - Dissolved Oxygen
- Stream flow
- Fecal coliform bacteria
Fecal Coliform Bacteria

- Common to all warm-blooded animals
- Indicator of pollution
- Standards set to protect human health

## State Standards

<table>
<thead>
<tr>
<th>Designated Use</th>
<th>Parameter</th>
<th>Standard</th>
</tr>
</thead>
</table>
| Core summer Salmonid habitat                | Temperature                | 7 day average of the daily maximum temperatures no greater than 16 °C
                                                                      (60.8°F)                                                               |
|                                             | Dissolved Oxygen (DO)      | 9.5 mg/L minimum*                                                        |
|                                             | pH                         | Between 6.5 and 8.5                                                      |
|                                             | Turbidity                  | Shall not exceed 5 NTU over background when background turbidity is 50 NTU or less |
| Extraordinary Primary Contact Recreation    | Fecal Coliform Bacteria    | Geometric mean < 50, with not more than 10% of samples exceeding 100     |
|                                             | (colonies per 100 mL)      |                                                                          |
| Salmonid spawning, rearing and migration    | Temperature                | 7 day average of the daily maximum temperatures no greater than 17.5 °C
                                                                      (63.5°F)                                                               |
|                                             | Dissolved Oxygen (DO)      | 8.0 mg/L minimum*                                                        |
|                                             | pH                         | Between 6.5 and 8.5                                                      |
|                                             | Turbidity                  | Shall not exceed 5 NTU over background when background turbidity is 50 NTU or less |
| Primary Contact Recreation                  | Fecal Coliform Bacteria    | Geometric mean < 100, with not more than 10% of samples exceeding 200    |
|                                             | (colonies per 100 mL)      |                                                                          |
# Fecal Coliform Sources

<table>
<thead>
<tr>
<th>Domestic animals</th>
<th>Median CFUs per gram (wet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Cattle</td>
<td>180,000</td>
</tr>
<tr>
<td>Sheep</td>
<td>660,000</td>
</tr>
<tr>
<td>Horse</td>
<td>38,000</td>
</tr>
<tr>
<td>Pig</td>
<td>7,100,000</td>
</tr>
<tr>
<td>Poultry</td>
<td>110,000,000</td>
</tr>
<tr>
<td>Cat</td>
<td>2,300,000</td>
</tr>
<tr>
<td>Dog</td>
<td>31,000,000</td>
</tr>
</tbody>
</table>

CFU = Colony Forming Units

An Example

• The average dog produces 340 grams of feces per day (3/4 pound)
• Assume a water quality standard of 100 CFU per 100mL
• One dog can pollute 3.5 Olympic swimming pools each day (2.8 million gallons)
Identify Sources of Fecal Contamination

- Pet feces
- Septic systems
- Livestock feces
- Wildlife feces
- Wastewater treatment plant
- Seagull and goose droppings
- Vessel sewer discharges
- Runoff from impervious surfaces
- Combined sewer overflows

Swimmers
Holmes Harbor Shellfish Protection District

- Elevated fecal coliform bacteria levels

Holmes Harbor Shellfish Protection District (HHSPD) established

- Bacteria Source Identification/Remedy Project
  - Source Identification
    - On-site Septic Evaluation & Compliance
  - Public Education/Outreach
  - Effectiveness Monitoring

Legend:
- HHSP District Boundary
- Shellfish Harvest Closure Boundary
- Roads
- WA State
- Water

1 inch = 1,500 feet
0 750 1,500 Feet
Holmes Harbor Source ID

- Initiated Source Identification
- Methodical, intensified monitoring
- Work in upstream direction
- Attempt to locate specific sources
- Results used to focus efforts and guide corrective actions
Evaluated septic system inspection compliance

Mapped and analyzed with Source ID data

Conducted site visits, dye testing and enforcement actions

Enhanced by 2007 OSS inspection regulation update and HOST program implementation

Very successful compliance effort (98%)
Public Education & Outreach
Effectiveness Monitoring

- Evaluates effectiveness of corrective actions
- Bacteria levels decreased to acceptable levels
- Shellfish harvest in South Holmes Harbor was re-opened last year

BUT...

- Despite continued efforts, bacteria levels are increasing
The Smoking Gun

- Conventional monitoring only *quantifies* fecal bacteria
- Cannot distinguish *who* it is coming from...i.e. human, pet, deer, goose
- DNA analysis suggested to definitively *qualify* the source

Microbial Source Tracking

- MST uses DNA analysis
- Tests for genetic markers in fecal bacteria
- EPA selected Island County for pilot study
  - Analyzed for presence or absence
    - Ruminant,
    - Human, and/or
    - General

Photo source: http://www.tzw.de/timm_images/abteilungen/altlasten/mst-prinzipskizze.jpg
MST Limitations

- Considered “experimental science”
- No standardized methods
- No regulatory approval
- Cost prohibitive
- Time consuming

- False negative and false positive results
- Few known markers
- Does not quantify contamination
- Does not replace conventional testing
- No known correlation between MST and FC

Selected study watersheds based on:

- Fecal coliform exceedances
- Shellfish harvest closures
- Swimming advisories

Holmes Harbor and Cultus Bay Selected

MST Site Selection
Island County Department of Natural Resources

Legend

- 303d Listed Streams
- 303d Listed Marine Water
- Streams
- Recreational Shellfish Beaches
  - Harvest Advisory
  - Marine Biotoxin or Pollution Closure
  - No Health Closures

Watersheds

- 134
- 150
- 154
- 157
- 159

Roads

- Collector
- Highway

4/22/2016

SAC

The following map is intended to be used as a GUIDE. Island County is providing this information as a general geographic representation that should not be used for precise measurements or calculations. Some of the features on this map are not accurately depicted. Any user of this map assumes all responsibility for use and agrees to hold Island County harmless for liability, damages, or loss incurred by use of this information.
Study Objectives

- Evaluate MST as a source identification tool
- To plan effective mitigation strategies, public education and outreach, and compliance enforcement
- To address public and environmental health issues in these watersheds
Procedure Overview

- 8 locations, sampled once a month
- 12 month study - August 2014 through July 2015
- Sent samples to EPA Region 10 Laboratory
- Polymerase Chain Reaction analysis
Quality Control

- Accuracy = closeness to true value
  - Control samples with known positive and negative values
  - Blind samples with presence of horse, cow, and human feces

- Precision = repeatability
  - Field duplicate samples
    - Below detection limit

Island County Public Health and EPA Region 10, OEA Laboratory. 2014. Quality Assurance Project Plan for Island County Public Health Microbial Source Tracking Study.

Bailey, S. Memo to Operations Office faculty, USEPA Region 10 Laboratory, Port Orchard, WA. November 30, 2015.
### 159a
- **Sample Date**: 2/18/15
- **Result**: GB
- **Sample Date**: 3/24/15
- **Result**: GB
- **Sample Date**: 4/15/15
- **Result**: H

### HHC1
- **Sample Date**: 8/19/14
- **Result**: GB
- **Sample Date**: 11/18/14
- **Result**: R
- **Sample Date**: 2/18/15
- **Result**: GB
- **Sample Date**: 3/24/15
- **Result**: GB
- **Sample Date**: 4/15/15
- **Result**: GB
- **Sample Date**: 5/13/15
- **Result**: R
- **Sample Date**: 6/17/15
- **Result**: H
- **Sample Date**: 7/15/15
- **Result**: GB

### HHM
- **Sample Date**: 8/19/14
- **Result**: R
- **Sample Date**: 9/30/14
- **Result**: R
- **Sample Date**: 10/28/14
- **Result**: R
- **Sample Date**: 11/18/14
- **Result**: R
- **Sample Date**: 12/16/14
- **Result**: GB
- **Sample Date**: 1/14/15
- **Result**: R
- **Sample Date**: 2/18/15
- **Result**: R
- **Sample Date**: 3/24/15
- **Result**: GB
- **Sample Date**: 4/15/15
- **Result**: GB
- **Sample Date**: 5/13/15
- **Result**: R
- **Sample Date**: 6/17/15
- **Result**: GB
- **Sample Date**: 7/15/15
- **Result**: GB

### 134ab
- **Sample Date**: 10/28/14
- **Result**: R
- **Sample Date**: 12/16/14
- **Result**: GB
- **Sample Date**: 1/14/15
- **Result**: R
- **Sample Date**: 2/18/15
- **Result**: H
- **Sample Date**: 3/24/15
- **Result**: GB
- **Sample Date**: 4/15/15
- **Result**: GB
- **Sample Date**: 5/13/15
- **Result**: R
- **Sample Date**: 6/17/15
- **Result**: R
- **Sample Date**: 7/15/15
- **Result**: R

### Hhab
- **Sample Date**: 8/19/14
- **Result**: H/R
- **Sample Date**: 9/30/14
- **Result**: R
- **Sample Date**: 10/28/14
- **Result**: R
- **Sample Date**: 11/18/14
- **Result**: R
- **Sample Date**: 12/16/14
- **Result**: GB
- **Sample Date**: 1/14/15
- **Result**: R
- **Sample Date**: 2/18/15
- **Result**: GB
- **Sample Date**: 3/24/15
- **Result**: GB
- **Sample Date**: 4/15/15
- **Result**: GB
- **Sample Date**: 5/13/15
- **Result**: GB
- **Sample Date**: 6/17/15
- **Result**: GB
- **Sample Date**: 7/15/15
- **Result**: GB

### Notes:
- **A**: Absent
- **GB**: General Bacteroides
- **R**: Ruminant
- **H**: Human
- **H/R**: Human/Ruminant
### Cultus Bay MST Results

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/19/14</td>
<td>R</td>
</tr>
<tr>
<td>9/30/14</td>
<td>GB</td>
</tr>
<tr>
<td>10/28/14</td>
<td>GB</td>
</tr>
<tr>
<td>11/18/14</td>
<td>R</td>
</tr>
<tr>
<td>12/16/14</td>
<td>R</td>
</tr>
<tr>
<td>1/14/15</td>
<td>GB</td>
</tr>
<tr>
<td>2/18/15</td>
<td>GB</td>
</tr>
<tr>
<td>3/24/15</td>
<td>A</td>
</tr>
<tr>
<td>4/15/15</td>
<td>GB</td>
</tr>
<tr>
<td>5/13/15</td>
<td>GB</td>
</tr>
<tr>
<td>6/17/15</td>
<td>GB</td>
</tr>
<tr>
<td>7/15/15</td>
<td>H/R</td>
</tr>
</tbody>
</table>

### Notes:
- **A**: Absent
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- **R**: Ruminant
- **H**: Human
- **H/R**: Human/Ruminant
- **R**: Ruminant

*Map created: December 2015*
Discussion

- 92 field samples:
  - 4 + for human biomarker only = 4%
  - 35 + for ruminant biomarker only = 38%
  - 5 + for both human & ruminant biomarkers = 5%
  - 33 + for the general *Bacteroides* biomarker = 36%
  - 15 negative = 16%
  - No human biomarker at site 157a
Conclusions

- MST can be a useful tool for differentiating between human, ruminant, or other sources in areas with known fecal contamination.
- There were some human influences in this study, although the majority of biomarkers present were from ruminants and other sources.
- Fecal coliform samples could be collected simultaneously to correlate concentration with the source.
- This is a cost-prohibitive method, with few labs providing this type of analysis.
- The water quality program will continue using conventional source ID methods, until MST becomes more accessible or a specific situation warrants it, to include:
  - Increase of fecal coliform sample frequency & number in a watershed.
  - Deploying the Fluorometer.
  - Dye-testing of failed septic systems.
Questions?

Stephanie Croan  
s.croan@co.island.wa.us  
360.678.2347

Matt Zupich  
m.zupich@co.island.wa.us  
360.678.7987

Department of Natural Resources  
Surface Water Quality Monitoring Program