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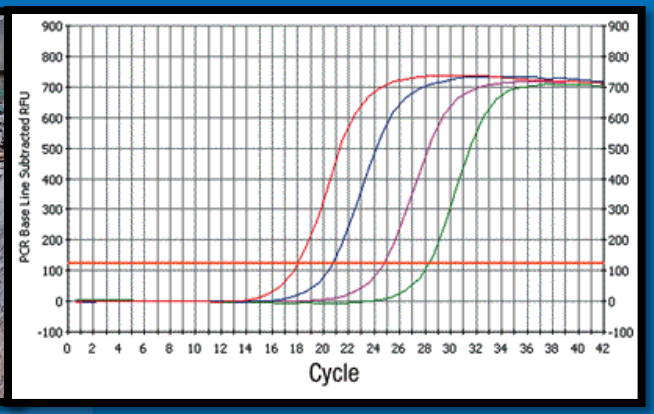
Microbial Source Tracking with qPCR:
Applications and Technology Transfer

Orin C. Shanks



Office of Research and Development
National Risk Management Research Laboratory, Water Supply and Water Resources Division

Presentation Overview

1. Microbial Source Tracking Background
2. MST qPCR in Action
3. EPA MST qPCR Technology Transfer Activities

Fecal Pollution is a Nationwide Challenge

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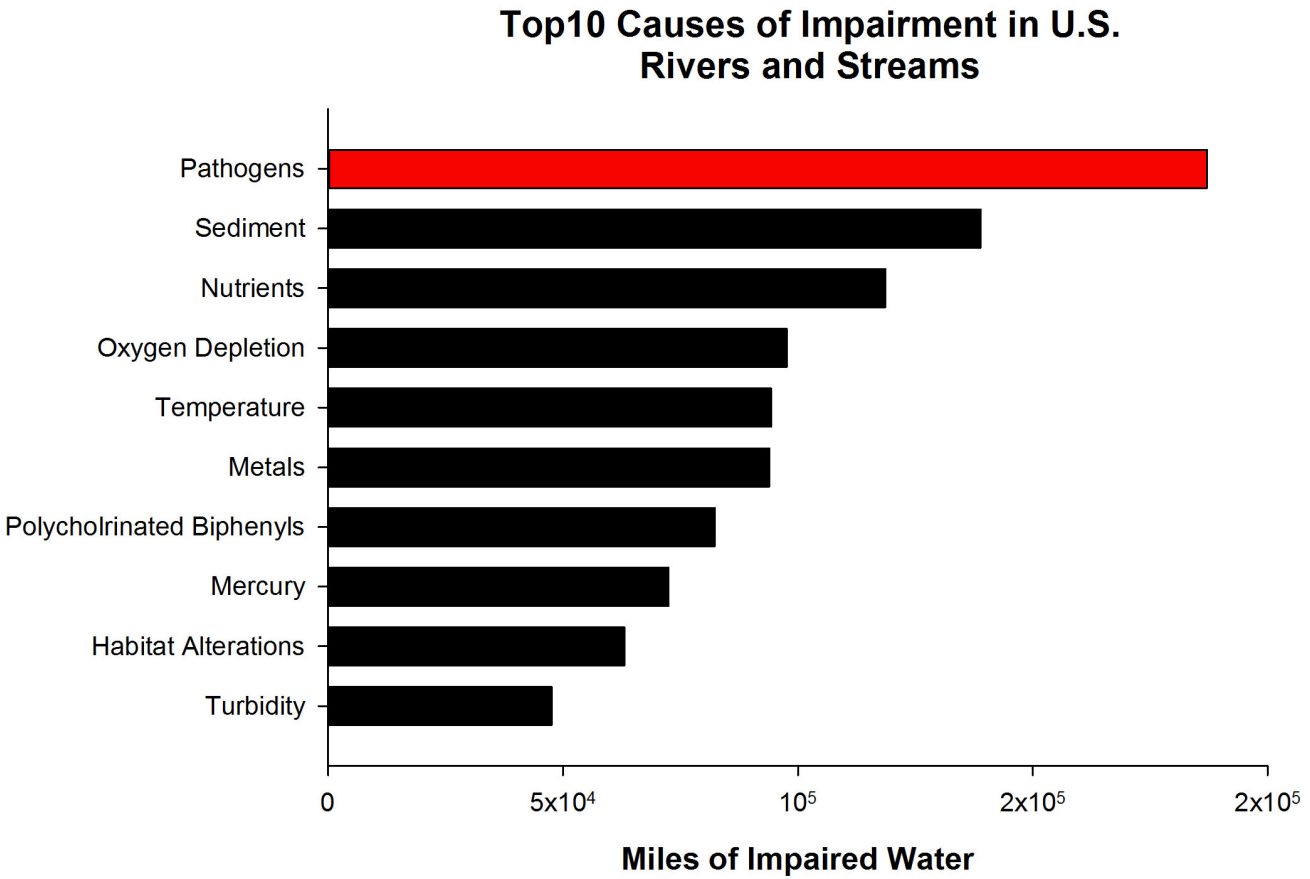
- Fecal microbes are most common biological contaminant in storm and surface waters
- Public health, economic, and ecological impacts



https://ofmpub.epa.gov/waters10/attains_nation_cy.control#causes

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EPA Responsibilities

Protect and Restore Waters for Recreational Use

- Clean Water Act 1972

Risk Assessment of Beach Contaminants

- BEACH Act (2000)
- Development of new or revised ambient water quality criteria (AWQC)

Management of Point and Non-Point Pollution Sources

- Total Maximum Daily Load (TMDL) programs
- National Pollutant Discharge Elimination System (NPDES) programs
- National Estuary Program (NEP)
- Combined Sewer Overflow (CSO) consent decrees

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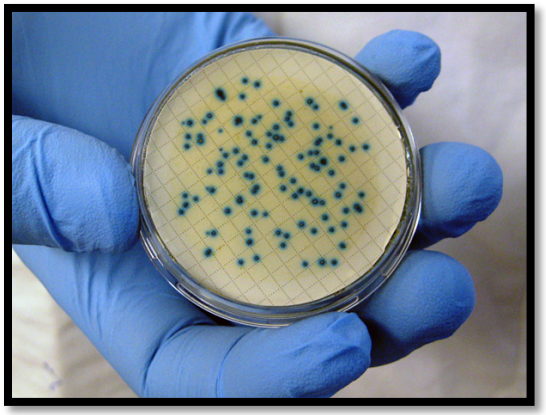
Current Fecal Pollution Management Tools

- Based on general fecal indicators
- Measure of total fecal pollution
- Presence in water is a warning signal of public health risk
- Do not discriminate between sources



Source of Fecal Pollution is Important

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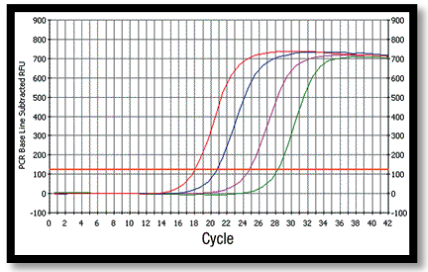
- Public health risk can vary by source
- Mitigation strategies can vary by source
- Source information improves management and public safety

A Microbial Source Tracking Solution

SOLUTION … Method designed to collect, isolate, identify, and measure a **host-associated identifier** from an environmental sample



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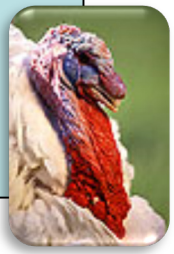
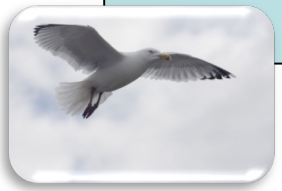
The Science Behind a Host-Associated Identifier

- **Gut Condition Differences**
  - Diet
  - Digestive physiology
  - Temperature
- **Resource Competition**
  - Space
  - Nutrients

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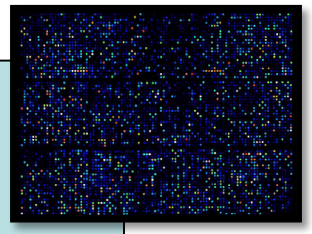
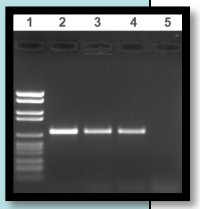
Many Microbial Source Tracking Technologies Available

- Microarray
- Next generation sequencing
- End-point PCR
- Quantitative real-time PCR
- Digital PCR
- Terminal restriction fragment length polymorphism
- Antibiotic resistance analysis
- Chemical detection
- Canine scent detection





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Technology Selection by Expert Consensus

- Source Identification Protocol Project
  - 5 organizations formed technical lead team
  - Public challenge via blinded study
  - 27 expert laboratories
  - 41 methods
- Majority of experts (>90%) favor a **qPCR-based technology**
- Identification of top methods for pollution sources

Boehm *et al.* (2013) Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research* 47: 6812-6828.

Layton *et al.* (2013) Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. *Water Research* 47: 6897-6908.

Stewart *et al.* (2013) Recommendations following a multi-laboratory comparison of MST methods. *Water Research* 47: 6829-6838.

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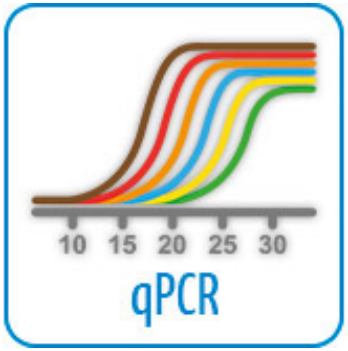
Benefits of MST with qPCR



- Mainstream scientific technology
- "Gold standard" for many applications
- No cultivation requirement
- Sensitive and specific in complex systems
- Highly reproducible when standardized
- Established quality control guidelines (Bustin et al. 2010)
- Specialized reagents for environmental testing

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Many Water Quality Management qPCR MST Applications

- Urban stormwater management
- Impaired site prioritization for remediation
- Evaluation of a best management practices
- Total Maximum Daily Load planning
- Hazardous event response
- Waterborne disease outbreak response

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Importance of Field Studies

- **One MST qPCR procedure will not work for all applications**
  - Sampling strategies
  - Ancillary data requirements
  - Data analysis procedures
- **Real-world examples are crucial**
  - Application tailored methodology
  - Peer-reviewed
- **Implementation Strategy**
  - Develop core procedure
  - Conduct field studies
  - Provide tailored methods to public

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MST in Action:
Identification of Septic Pollution with MST qPCR

Question: Does human fecal pollution originate from leaky sewer lines or failing septic systems in my watershed?

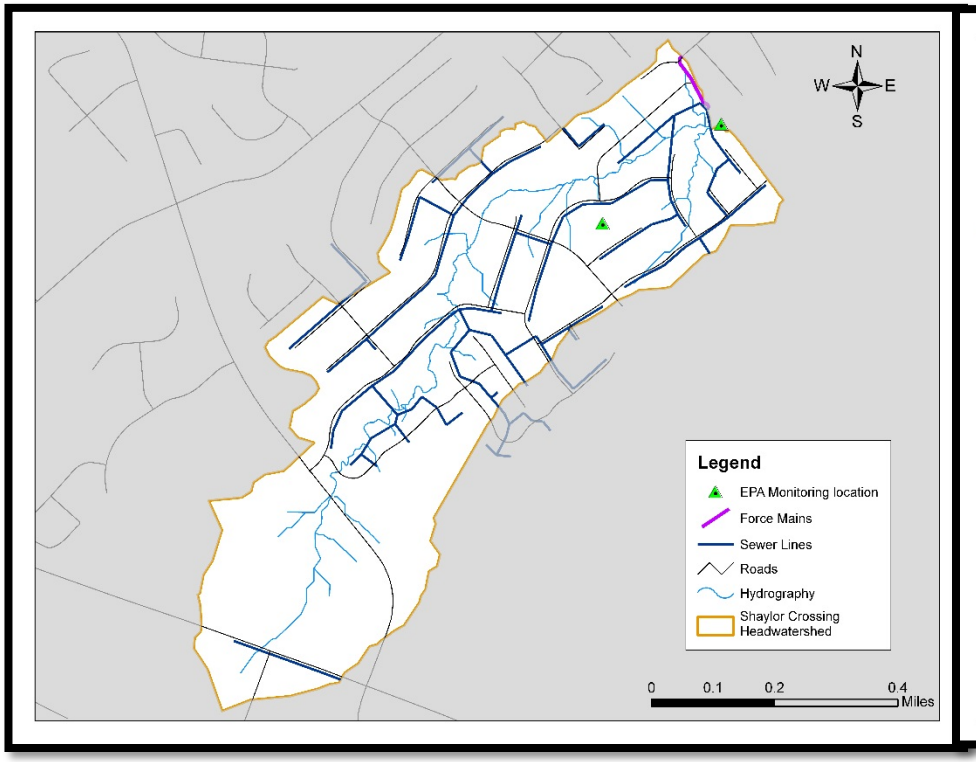
East Fork Little Miami Watershed

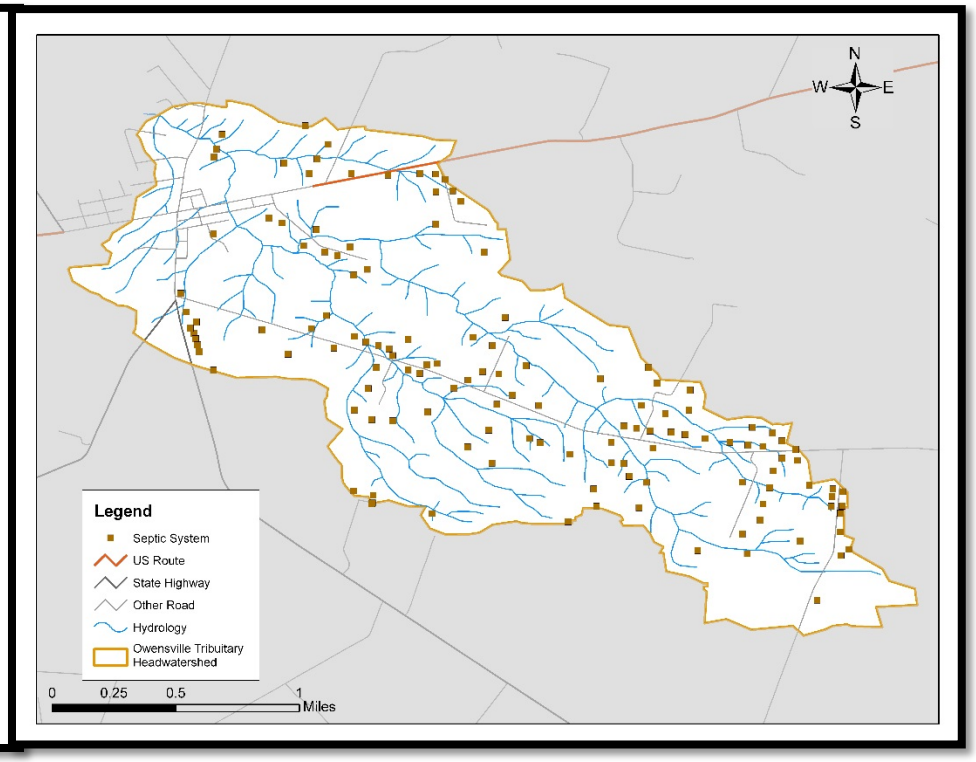
- 1,295 km^2 Southeastern Ohio watershed
- Range of septic/sewer use intensity
- 9 catchment areas
- Small stream sampling
- 24-month sampling period
- 3 human-associated qPCR methods
- Unsafe levels of fecal pollution > 40% of time

  (*E. coli* and enterococci MPN cell counts)

Peed *et al.* (2011). Combining land use information and small stream sampling with PCR-based methods for better characterization of diffuse sources of human fecal pollution, *Environmental Science & Technology* **45:5652-5659**.

Quantifying Catchment Land Use with GIS



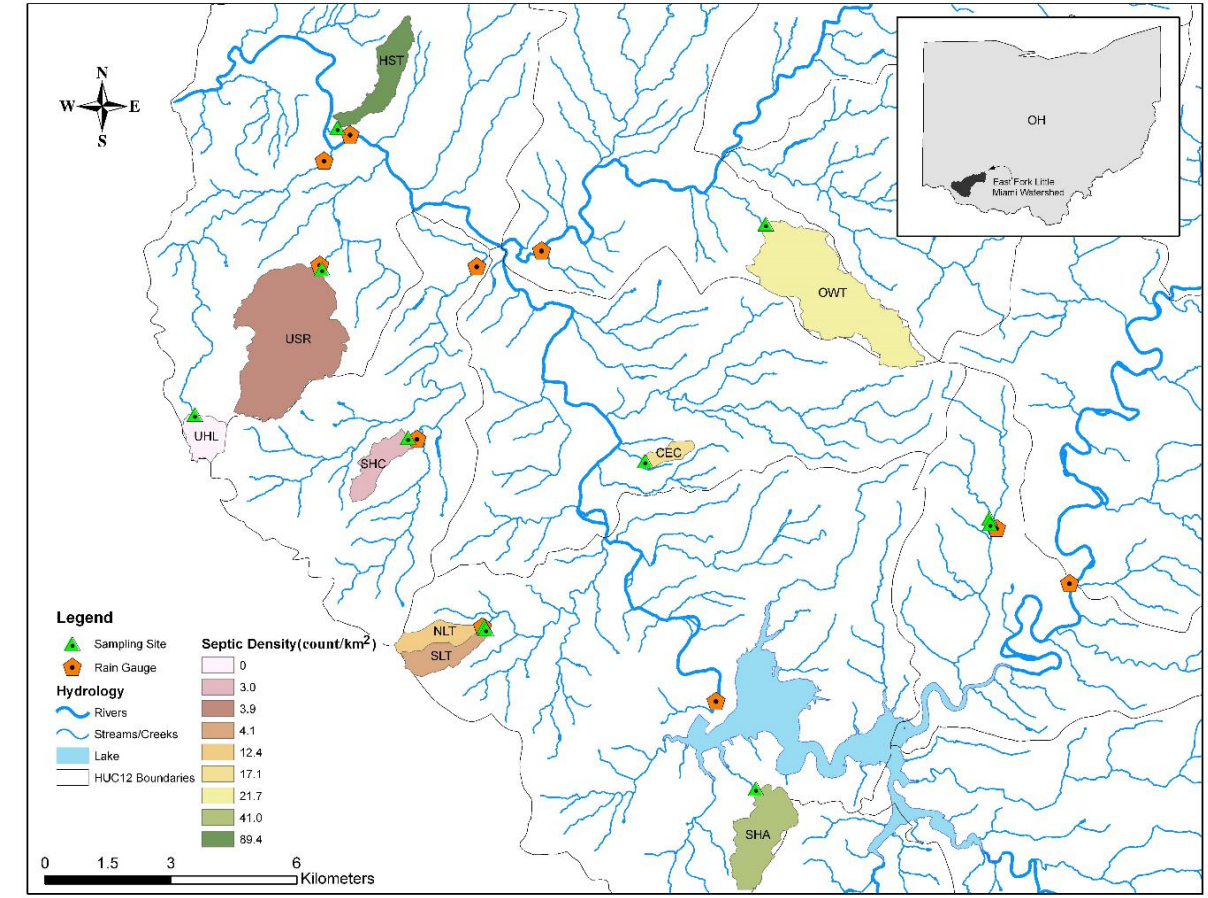


- Estimate sewer and septic densities
- Normalized by catchment area

Peed *et al.* (2011). Combining land use information and small stream sampling with PCR-based methods for better characterization of diffuse sources of human fecal pollution, *Environmental Science & Technology* **45:5652-5659**.

Experimental Design to Address Question

- Catchments represent gradient of sewer and septic use
- Negative correlation between septic and sewer densities (R^2 = -0.69)
- Does human pollution trend with sewage, septic, or neither?



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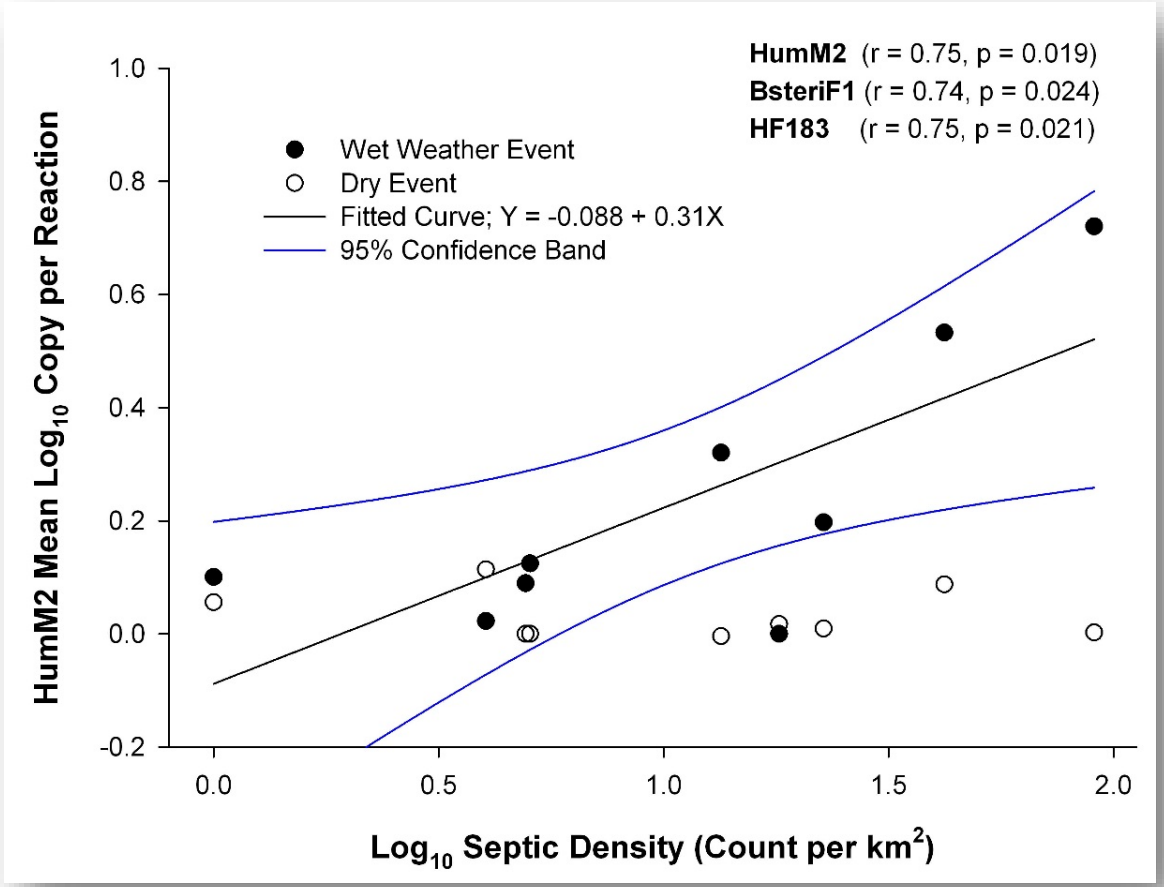
Identifying Human Fecal Pollution Trends



- Human fecal pollution increases with septic density (wet weather events only)
- Trend supported by all 3 human-associated qPCR methods
- Potential Actionable Outcome: septic site inspections

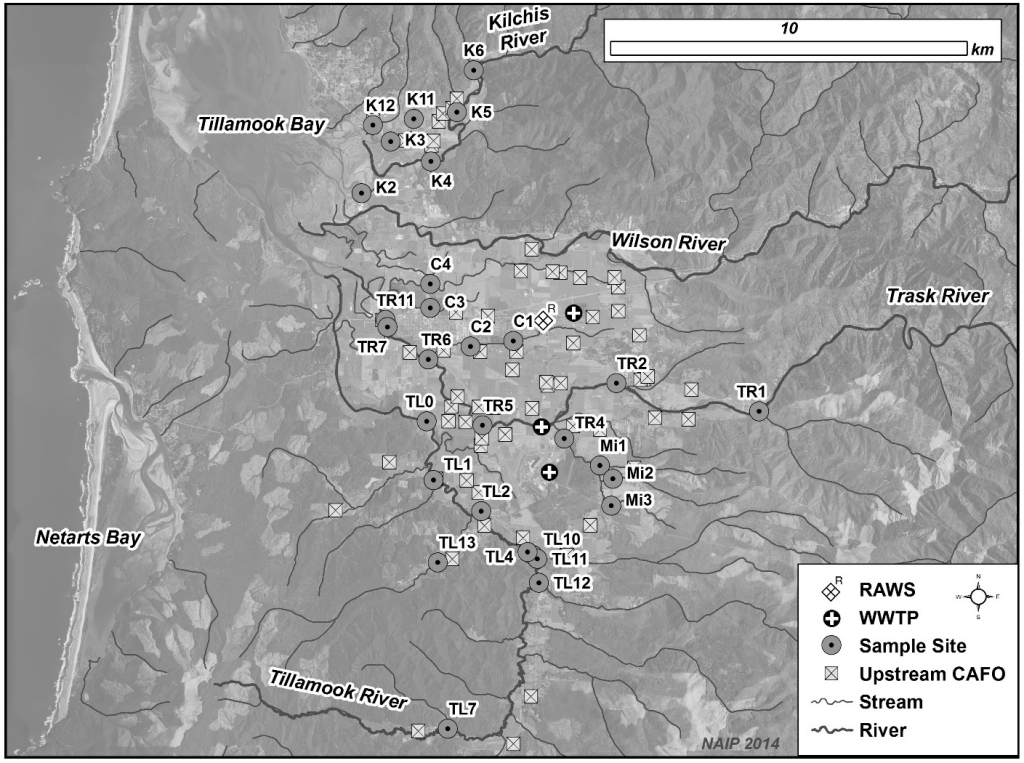
Peed *et al.* (2011). Combining land use information and small stream sampling with PCR-based methods for better characterization of diffuse sources of human fecal pollution, *Environmental Science & Technology* **45:5652-5659**.

MST in Action:
Agriculture and Wildlife Impacts with MST qPCR

Question: Does wildlife and agricultural practices contribute to chronic fecal pollution in my watershed?

Tillamook Basin

- 1,500 km^2 northern Oregon coast
- Active dairy industry
- 29 catchment areas
- 12-month sampling period
- 8 host-associated qPCR methods
- Chronic fecal pollution



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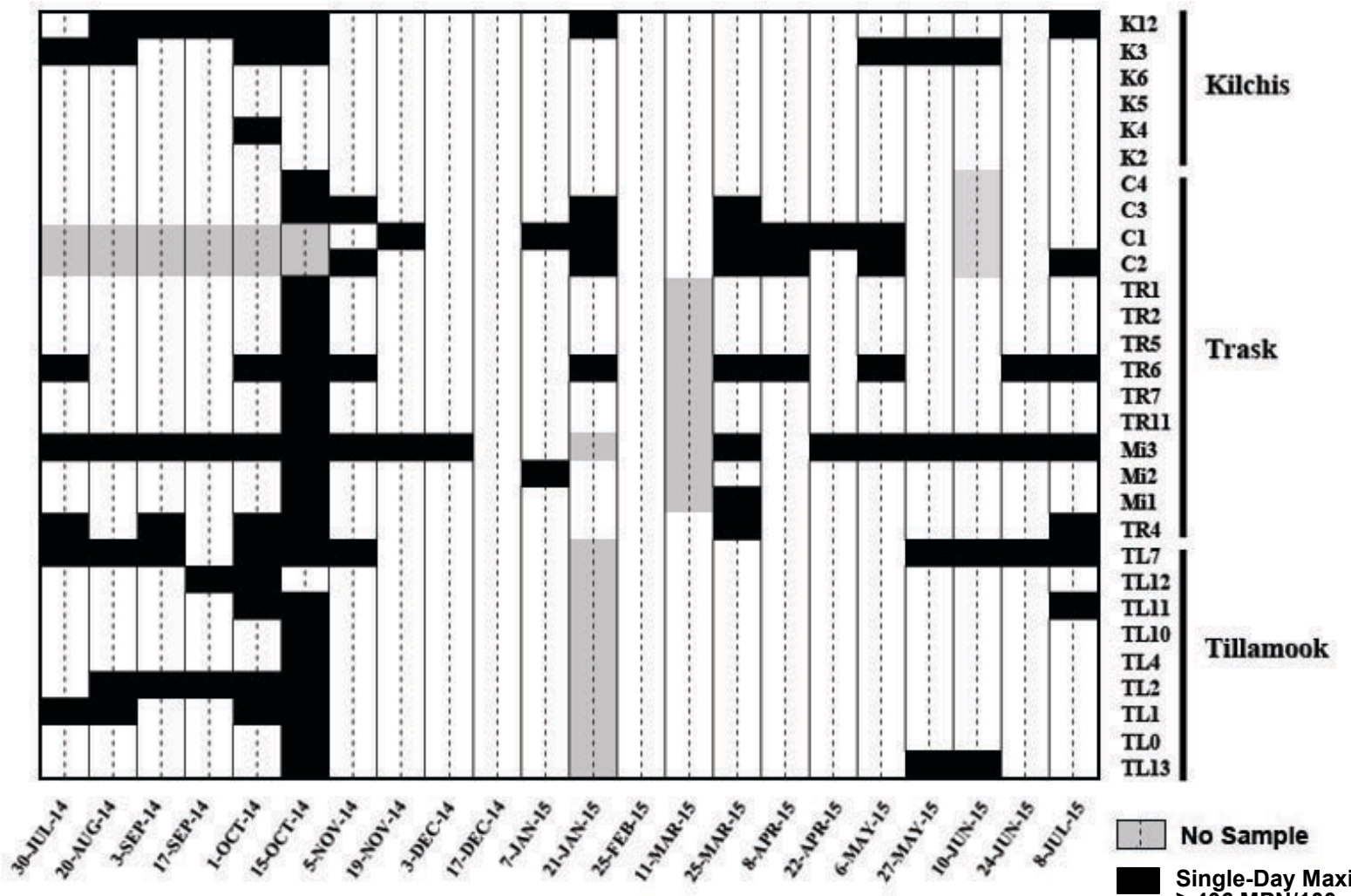
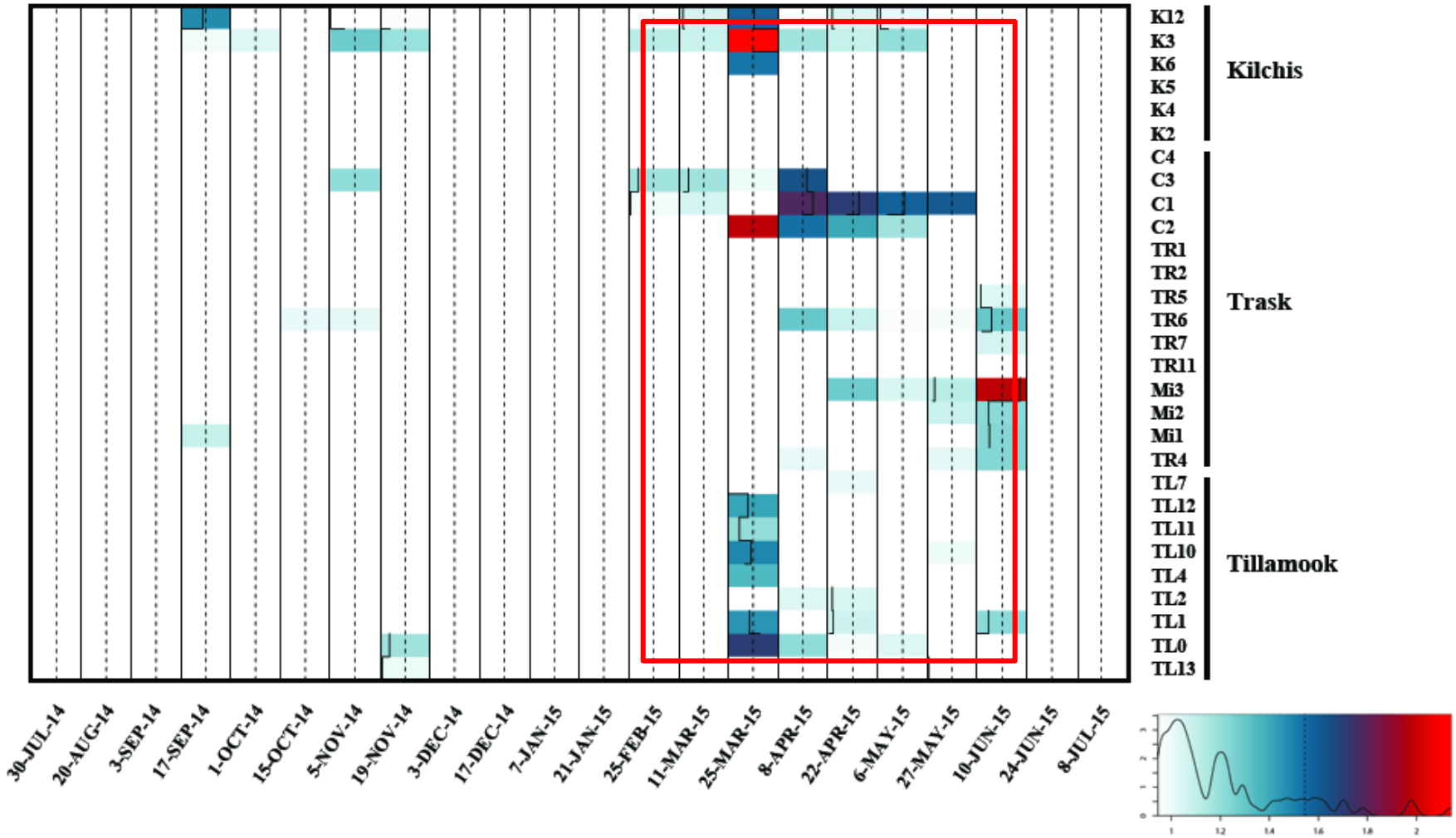
Water Quality Management with *E. coli*



Avian Pollution Spatial and Temporal Trends



Potential bird migration water quality impact

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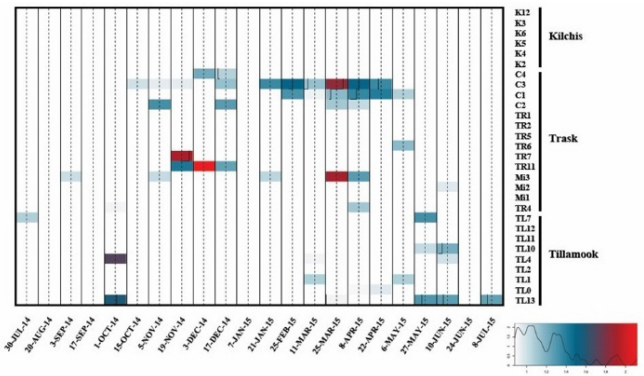
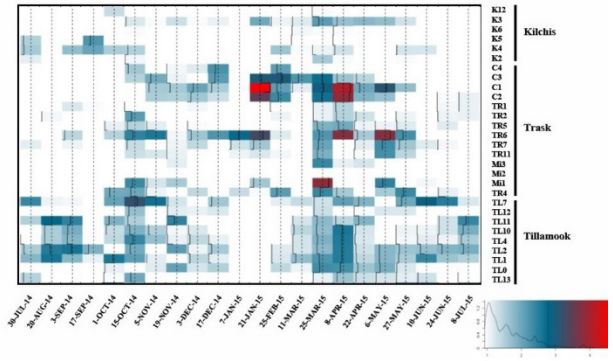
Spatial and Temporal Trends in Other Fecal Sources

- Spatial trends
  - *Land use*
  - *Waste management practices*
- Temporal trends
  - *Agricultural practices*
  - *Wildlife activities*
- Varies by source

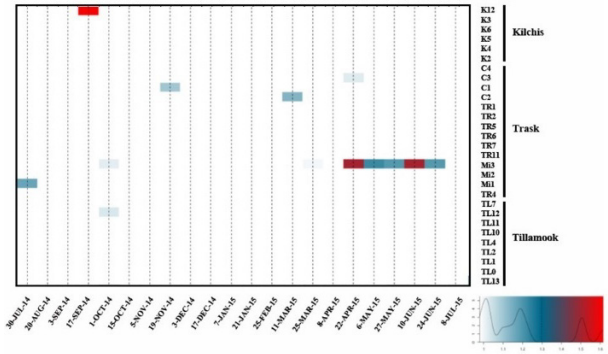
Human Source (HF183/BacR287)



Ruminant Source (Rum2Bac)



Dog Source (DG3)



Sampling Site Fecal Pollution Profile

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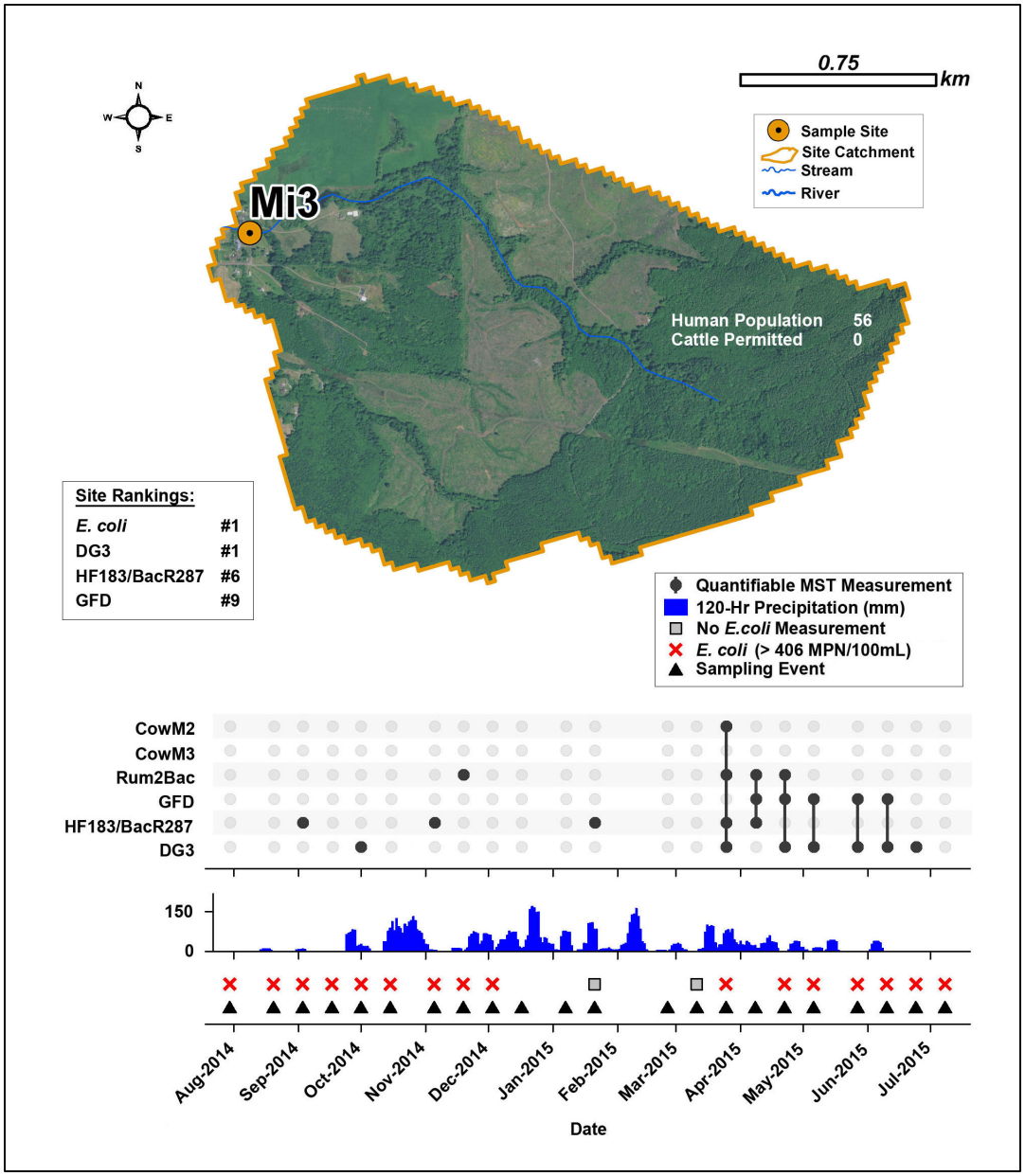
- *E. coli* exceedance (80%)
- Seasonal dog pollution, target local breeding facility
- Possible bird migration impact
- Possible rain event human impact
- Ruminant in spring, likely beef cattle AFO
- Potential actionable outcomes:
  - Site inspection in survey in Spring
  - Target AFO, septic system, and dog facility



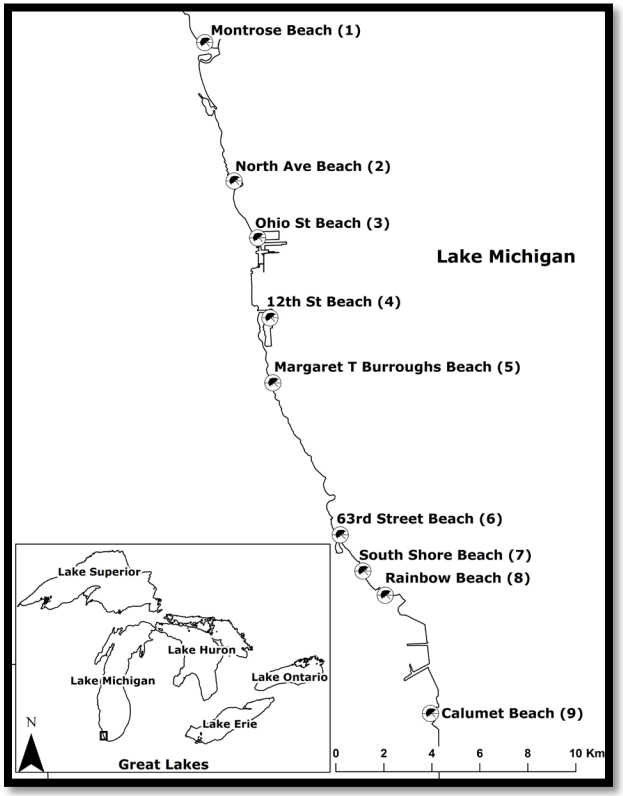
Recreational Beach Management with MST qPCR

- Recreational activity annual public health and economic impacts
  - About 90 million illnesses[1]
  - Approx. $2.9 billion medical expense[1]
- Managed with general fecal indicators (*E. coli* or enterococci)
  - Identifies problem
  - No source information
- Control strategies can vary by source
- MST qPCR applications
  - Linking pollution source to general indicator
  - Site prioritization by pollution source

[1] DeFlorio-Barker et al. (2018) Environmental Health 17:3

MST in Action:
Recreational Beach Management with MST qPCR

Question: Are there any links between my MST qPCR and general indicator measurements?

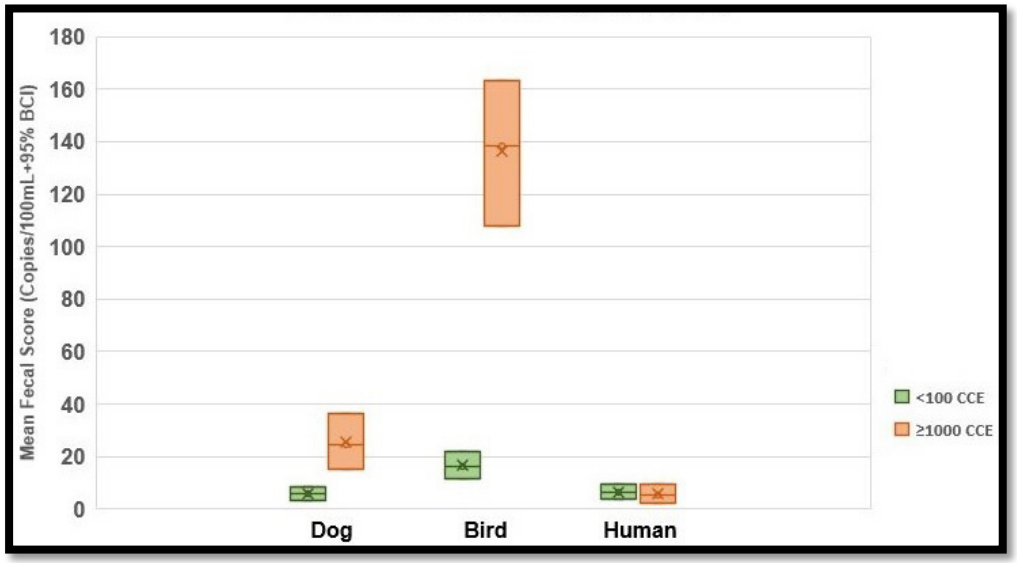


- University of Illinois at Chicago School of Public Health study
  - Sam Dorevitch (Principal Investigator)
  - Abhilasha Shrestha (PhD Candidate)
- 9 beaches sampled 5 days/week over beach season
- *E. coli* and enterococci general indicator testing
- MST qPCR testing for human, bird, and dog sources

Linking General Indicator and MST Findings

Enterococci qPCR Weighted-Averages

Group #1: ≥ 1,000 CCE (US EPA recommended BAV)
Group #2: < 100 CCE



[1] Shrestha et al. manuscript in preparation

- Group samples based on local recreation criteria
- Calculate weighted-average for each group
- Compare differences between groups:
  - Bird 8.4x higher
  - Dog 4.2x higher
  - Human similar
- Potential actionable outcomes:
  - Minimize bird activity
  - Restrict dog access

MST in Action:
Recreational Beach Management with MST qPCR

Question: How do I prioritize sites based on human fecal pollution levels?

- Partners:
  - City of Racine Health Department
  - Northeast Ohio Regional Sewer District
  - Scientific Methods, Inc.
- 6 sampling sites
- Potential pollution sources (human, bird and dog)
- Sampled 5 days/week over beach season
- 16 water quality and beach area parameters

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Urban Stormwater Management with MST qPCR

- > 80% of U.S. population live in communities with MS4 discharges
- 7,550 regulated communities
- MS4 permittees required to develop, implement, and mitigate stormwater management programs
- MS4 discharges can contain fecal waste
- Control strategies can vary by source

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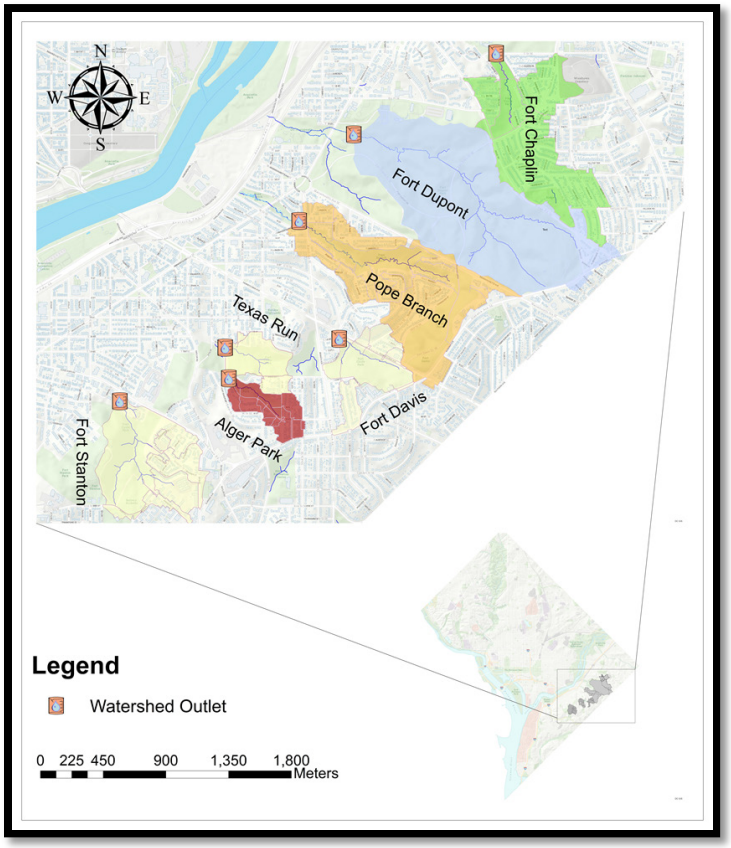
Growing Interest in MST qPCR and Urban Stormwater Management

- Charles River and Boston Harbor
  (Boston Water and Sewer Commission)
- City of Santa Barbara
  (State Clean Beach Initiative)
- Hampton Roads Sanitation District
  (Virginia Beach, VA)
- Oklahoma Stormwater Quality Program
  (City of Tulsa Streets and Stormwater Dept)
- Colorado *E. coli* Toolbox: A Practical Guide for Colorado MS4s (Urban Drainage & Flood Control District City and County of Denver)

MST in Action:
Urban Stormwater Management with MST qPCR

Question: What are the sources of fecal pollution in my MS4 outfalls?



- Partners:
  - Department of Energy & Environment
  - ORISE
  - EPA Region 3 Laboratory
- 7 first order catchments
- 32 MS4 outfalls
- Routine and event sampling
- Potential pollution sources (human, ruminant, bird and dog)

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MST qPCR: Implementation Status

- Many examples in scientific literature
- No nationally standardized methods or application guidance yet
- Some qPCR MST methods closer to "prime time" than others
  - Human > Ruminant, cattle > swine > dog > avian
- Recommend confirming performance with local reference samples
- Ideal to consult expert for assay selection, experiment design, and result interpretation
- Need for improved data visualization and communication tools

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EPA qPCR MST Technology Transfer Activities

- National validation of two human-associated qPCR methods
- Towards standardized EPA Methods
- Development of implementation tools
- EPA outreach activities

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EPA Multiple Laboratory Validation - Overview

- Formal study conducted by EPA
  - Office of Water
  - Office of Research & Development
- Two qPCR Methods
- 14 Laboratory Participants
  - Fresh and marine water matrices
- Supplied with:
  - Standard protocols
  - Reference DNA materials
  - Sewage spike material
  - Blinded filter set (n = 18)
  - All reagents and consumables

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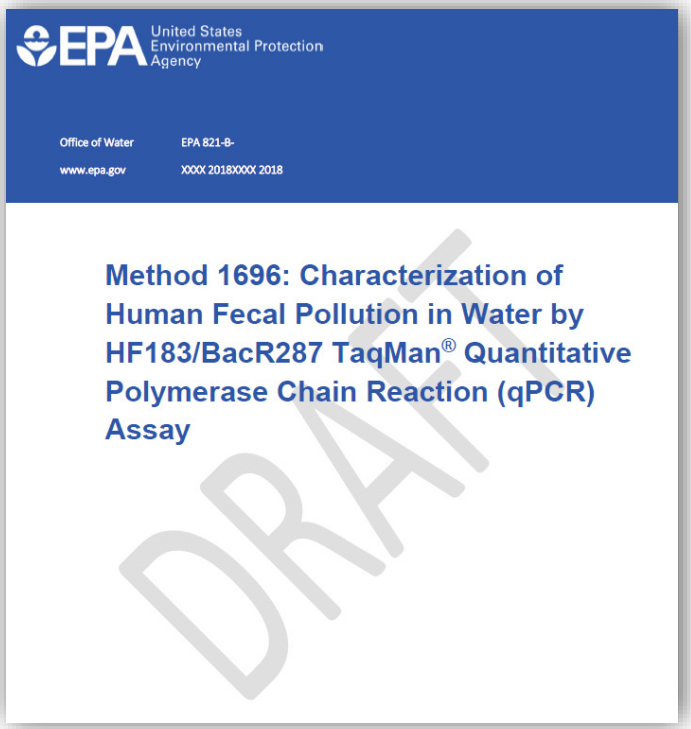
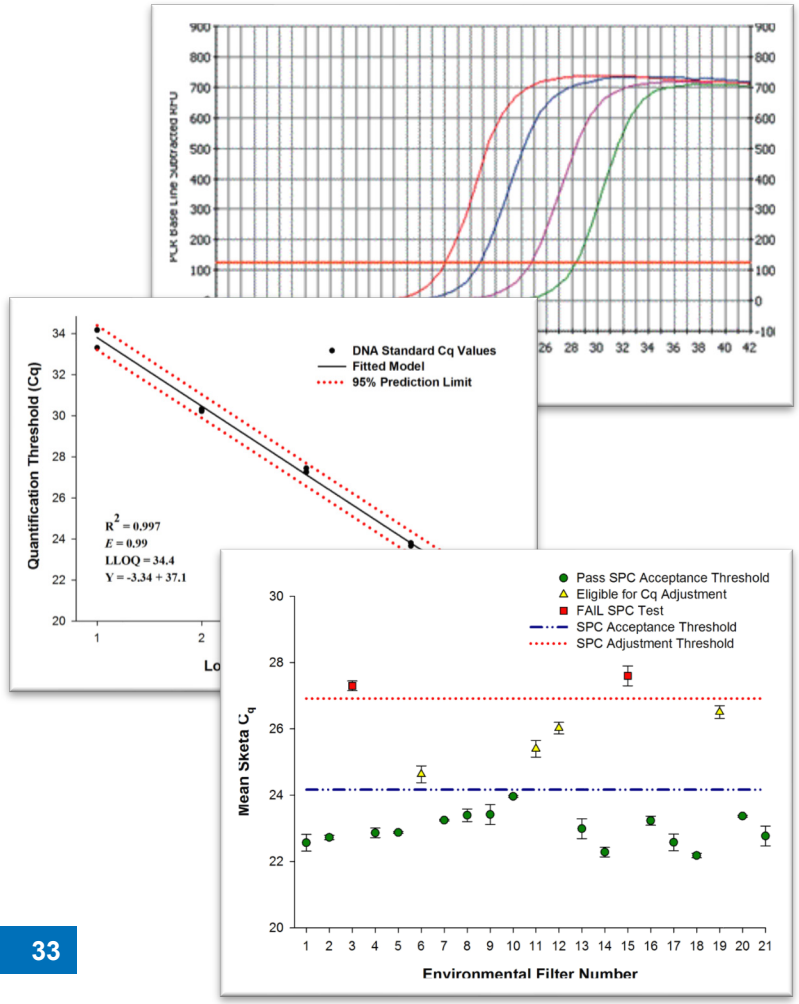
Draft EPA Methods 1696 and 1697: Content Overview

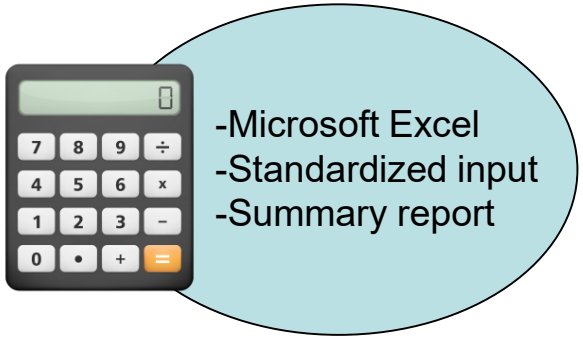


- Safety
- Laboratory organization
- Equipment, reagents, and supplies
- Sample collection, handling and storage
- Standardized laboratory procedures
- Quality controls
- Data analysis and calculations

qPCR Automated Data Analysis Tool



- Simplify complex calculations
- Ensure standardized analysis
- Implement data acceptance metrics
- Concentration estimates with error



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Self-Administered Method Proficiency Test

- Successfully complete:
  - Prior to environmental sample testing
  - After new reference material preparations
- Six metrics based on:
  - National laboratory validation
  - Reagent manufacturer recommendations
  - qPCR experts
- Training and management tool

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Reference DNA Material Development

- National implementation requires a high quality reference DNA material
- Centralized and standardized source
- Not feasible for EPA to manufacture and distribute
- Interagency Agreement with National Institute of Standards and Technology

National Institute of Standards and Technology

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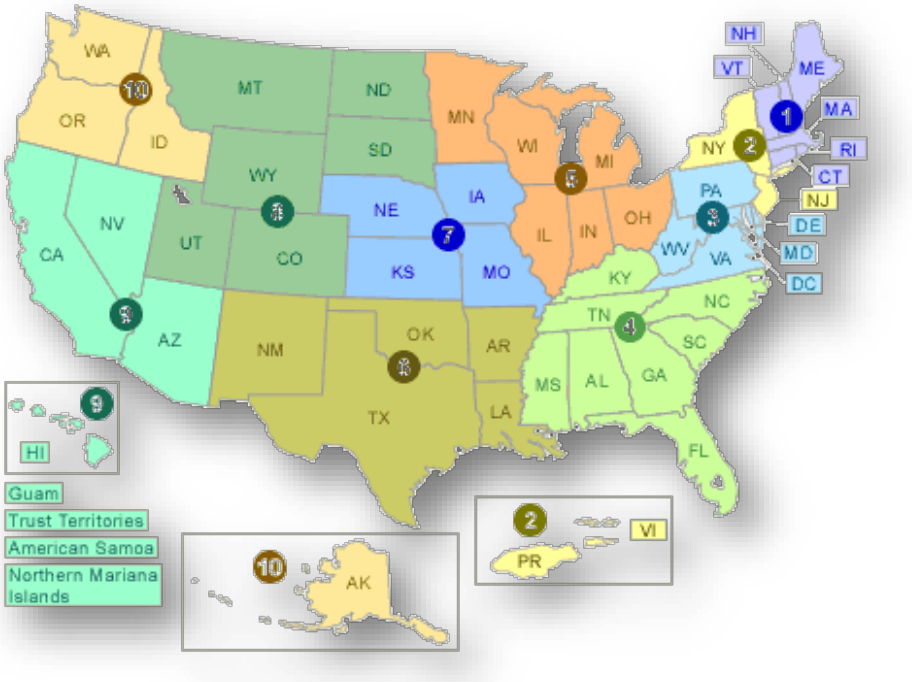
EPA Outreach Activities



- Building a support network
- Communication
- Training opportunities
- Cooperative partnerships
  - States, tribes, and other local labs
  - Association of Pubic Health Laboratories MOU
  - Federal agencies

Acknowledgements

Septic Study:

- Lindsay Peed (EPA)
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- Mark Meckes (EPA)
- Thomas Mooney (EPA)

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- Cathy Kelty (EPA)
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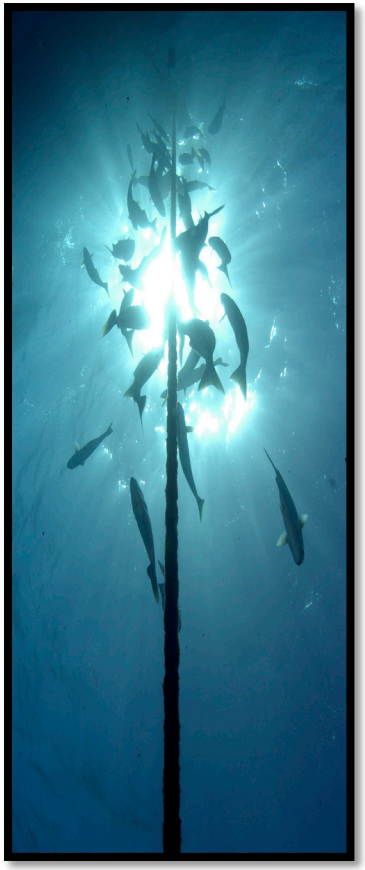
- Pat Clinton (EPA)
- Jay Reichman (EPA)
- York Johnson (ODEQ)
- Wym Matthews (ODA)
- Stephanie Bailey (EPA Region 10)

MS4 Study:

- Amir Sharifi (DOEE)
- Mano Sivaganesan (EPA)
- Cathy Kelty (EPA)



Chicago Beach Study:

- Abhilasha Shrestha (UIC)
- Sam Dorevitch (UIC)
- Mano Sivaganesan (EPA)
- Cathy Kelty (EPA)

Great Lakes MST Study:

- Xiang LI (EPA)
- Mano Sivaganesan (EPA)
- Cathy Kelty (EPA)
- Mike Cyterski (EPA)
- Kevin Oshima (EPA)

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- Richard Haugland (EPA)
- Sally Gutierrez (EPA)
- Scott Jackson (NIST)
- Validation Study Volunteer Labs

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