Microbial Source Tracking

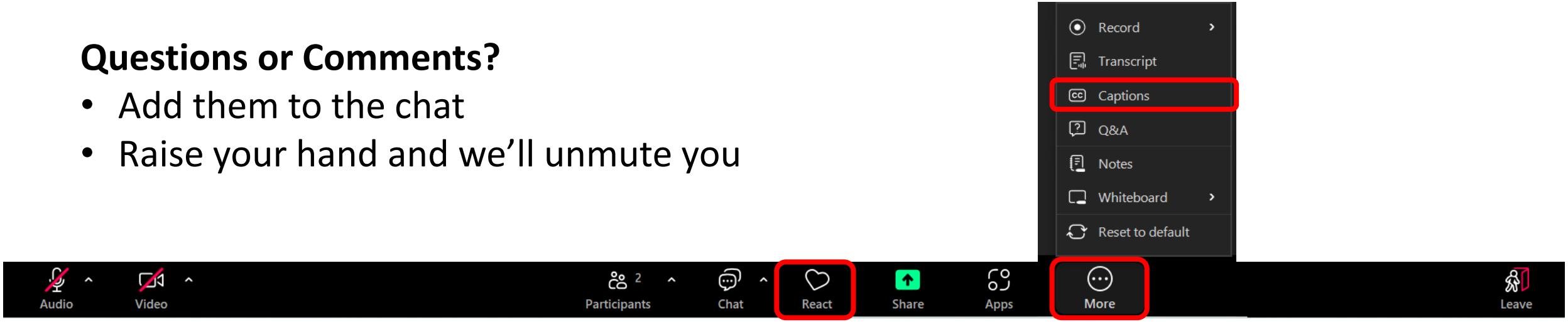
Navigating the Workshop

Welcome! While we wait, please:

- Update your name to include your pronouns and organization
- Introduce yourself in the chat
- Message Mraielle with any access needs

Questions or Comments?

- Add them to the chat
- Raise your hand and we'll unmute you



The slides and recording will be available on Puget Sound Institute's website

Land Acknowledgement

The UW Tacoma community acknowledges that we learn, teach, work and live on the ancestral land of the Coast Salish people. In particular, our campus is situated on traditional lands of the Puyallup Tribe of Indians. We recognize that this is a difficult and painful history, and we understand we must play an active role in remembering, not just what happened to Indigenous communities; post settlement, but also the rich history that existed long before colonization. This land acknowledgement is one small act in an ongoing process of honoring the past while working together with local Tribes to build a more inclusive and thoughtful community.

AGENDA

Time	Topic	Presenter
9:30 AM	Welcome	**Marielle Kanojia** UW Puget Sound Institute
9:35 AM	Microbial Source Tracking for informed decision-making	**Amy Zimmer-Faust** The Nature Conservancy
10:15 AM	Microbial Source Tracking: Origins, Technology and Application	**John Griffith** Southern California Coastal Water Research Project
10:55 AM	The Virridy Lume	**Alex Johnson & Whitney Knopp** Virridy
11:05 AM	Q&A	Amy Zimmer-Faust, John Griffith, Alex Johnson, and Whitney Knopp
11:20 AM	Break	
11:25 AM	Lightning talks	• **Karen DuBose**, Skagit County • **Cameron Chapman**, King County • **Cynthia May**, Whatcom Conservation District • **Natalie Prystajecky**, GEMSTONE
11:45 AM	Panel discussion	All
12:25 PM	Wrap-up	

Microbial Source Tracking for informed decision-making

Presentation to the Puget Sound Microbial Source Tracking Workshop

Amy Zimmer-Faust
September 12, 2025

The Nature Conservancy

Presentation Overview

1. Microbial source tracking overview
2. Methodology and study design
3. Data Analysis
4. Case Study: Cross Border Pollution Study
5. Case Study: Tracking sources of bacteria and nutrients in Tillamook Bay

Fecal Pollution is a Nationwide Problem

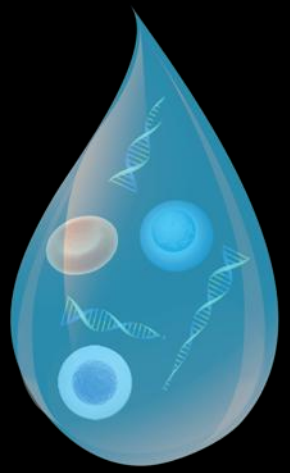
- Fecal microbes are a common biological contaminant in U.S. surface waters
- Public health, economic, and ecological impacts
- Unknown and co-located sources of fecal pollution complicate management and mitigation

Source of Fecal Pollution is Important

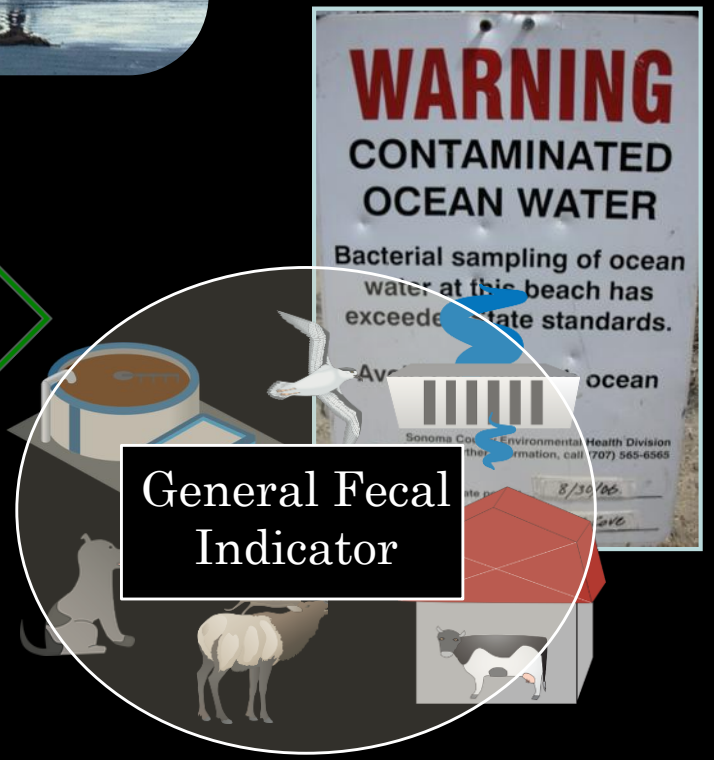
- Public health risk can vary by source with non-human sources less likely to contain pathogens infectious to other humans
- Mitigation strategies can vary by source: knowing source allows for targeted action [i.e., repairing aging infrastructure, adjusting agricultural practices]




Microbial Source Tracking Concept



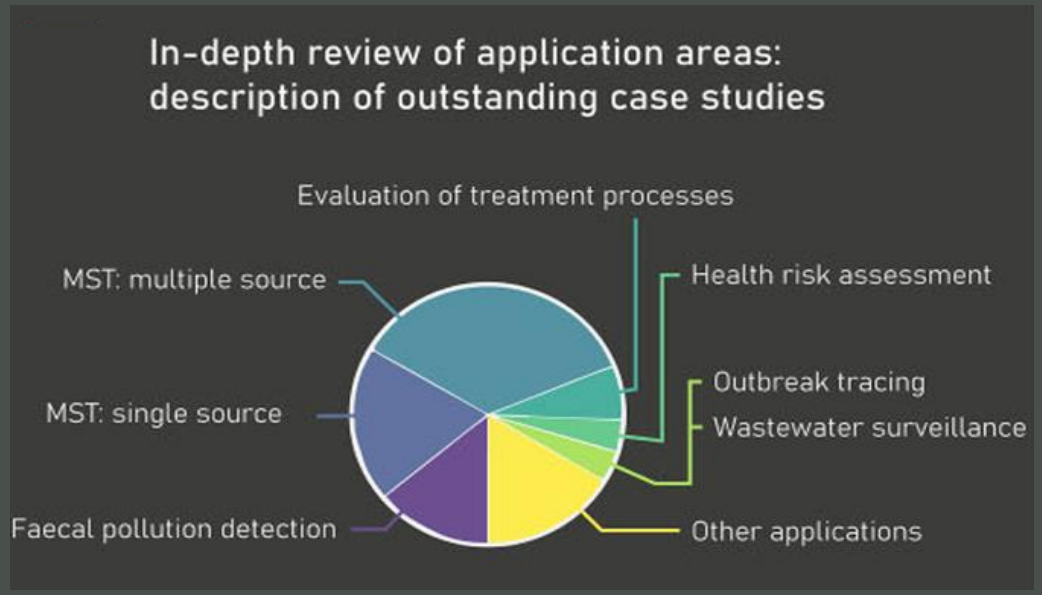
Culturing for FIB
(*E. coli*, *Enterococcus*, Fecal Coliform)
- **24- 48 hours**
- **No information on source**



Microbial Source Tracking Concept

- **Concept: Detection and quantification of RNA/DNA-based markers associated with different fecal sources by PCR-based method**
  - **Source markers:**
    - **Human:** Contamination from failing septic systems, sewage treatment overflows, or broken wastewater infrastructure.
    - **Livestock:** Runoff carrying manure from cattle, poultry, or other farm animals.
    - **Wildlife:** Naturally occurring inputs from birds, deer, and other local fauna.
    - **Pets:** Domestic animal waste not properly disposed of that finds its way into storm drains and waterways.

Microbial Source Tracking Applications

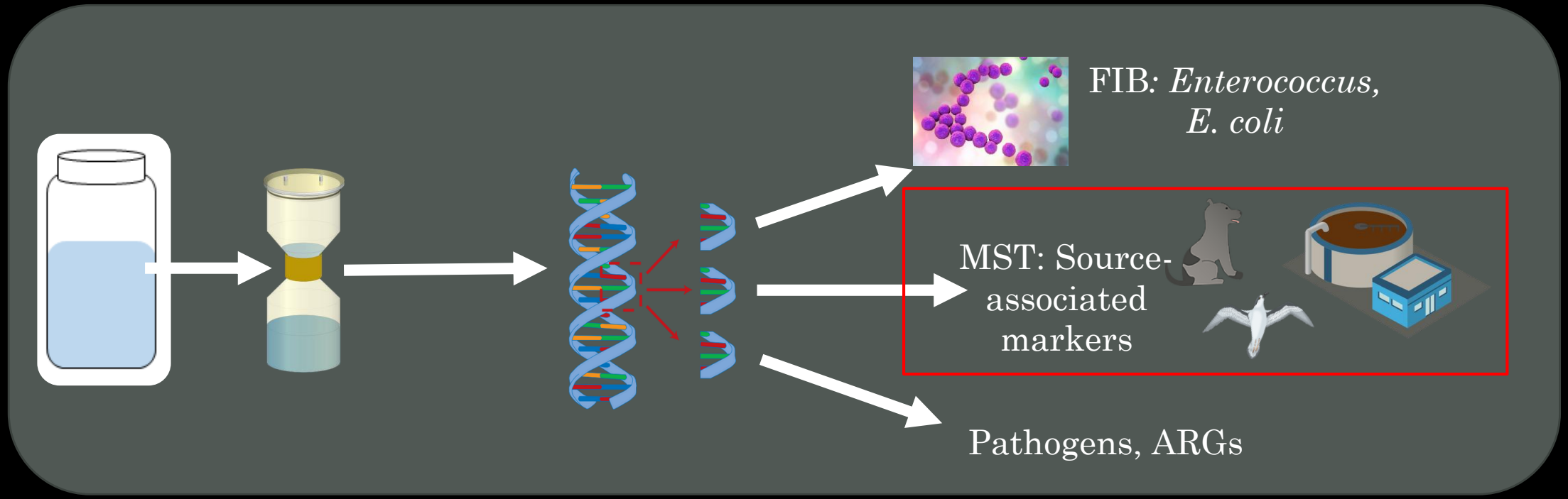


Demeter et al., 2023. FEMS Microbiology Reviews.

- **Impaired site prioritization for remediation**
- **Evaluation of best management practices**
- **Sewer overflow monitoring**
- **Beach closures and recreational water management**
- **Hot spot investigative tool**
- **Matrices:** freshwater, shellfish, sediment, stormwater, estuaries, seawater, groundwater

Molecular Methods for Water Quality Monitoring

- **Concept: Detection and quantification of RNA/DNA-based markers associated with different fecal sources by PCR-based method**



Sample collection/ Filtration

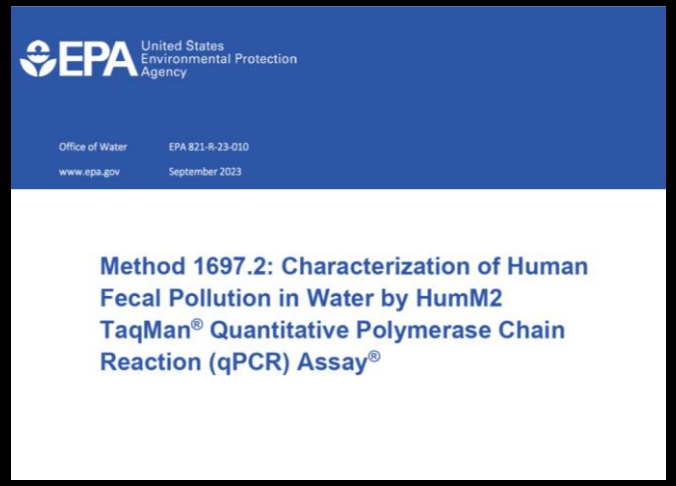
Nucleic Acid Extraction

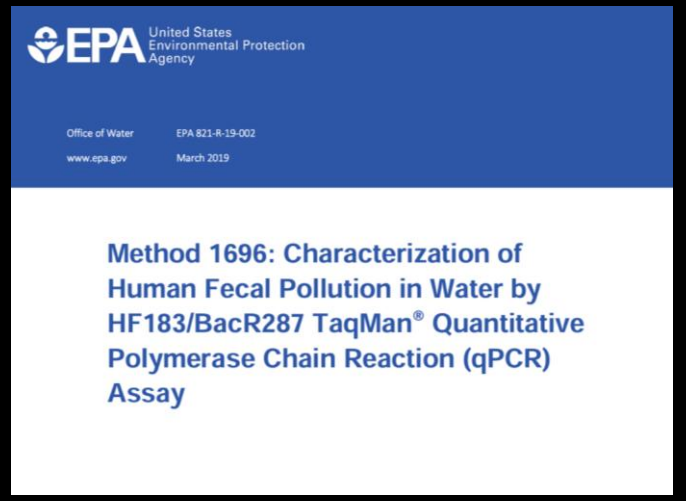
Detection/ Quantification

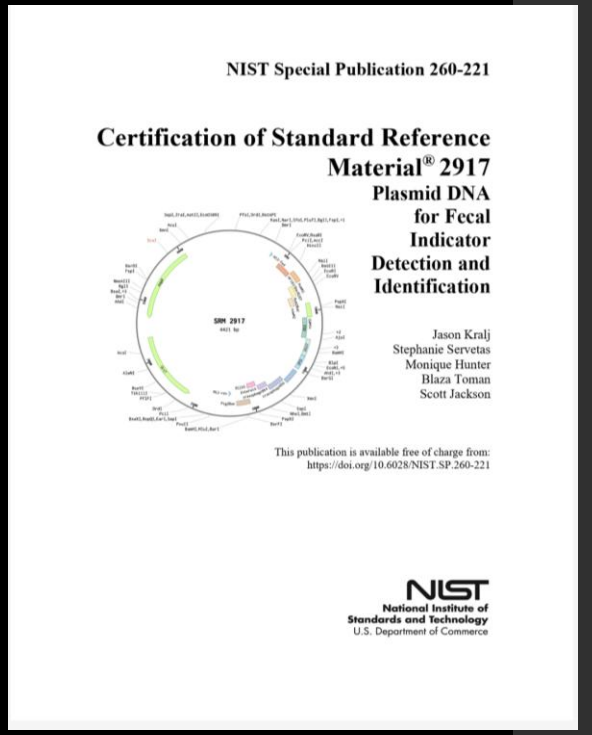
National Implementation - EPA

1. **EPA developed methods:**
   - HF183 human marker
   - HumM2 human marker
   - Certified reference materials
   - Methods include guidance on laboratory considerations, data analysis, and quality control

2. **EPA licensed assays (based on qPCR):**
   - Cow: CowM2/CowM3
   - Dog: CG3/DG37
   - Human: HF183/HumM2, crAssphage (viral human marker)

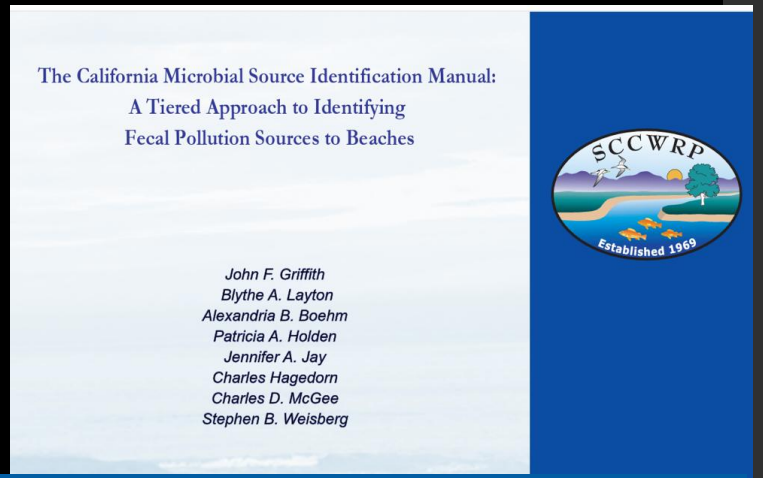




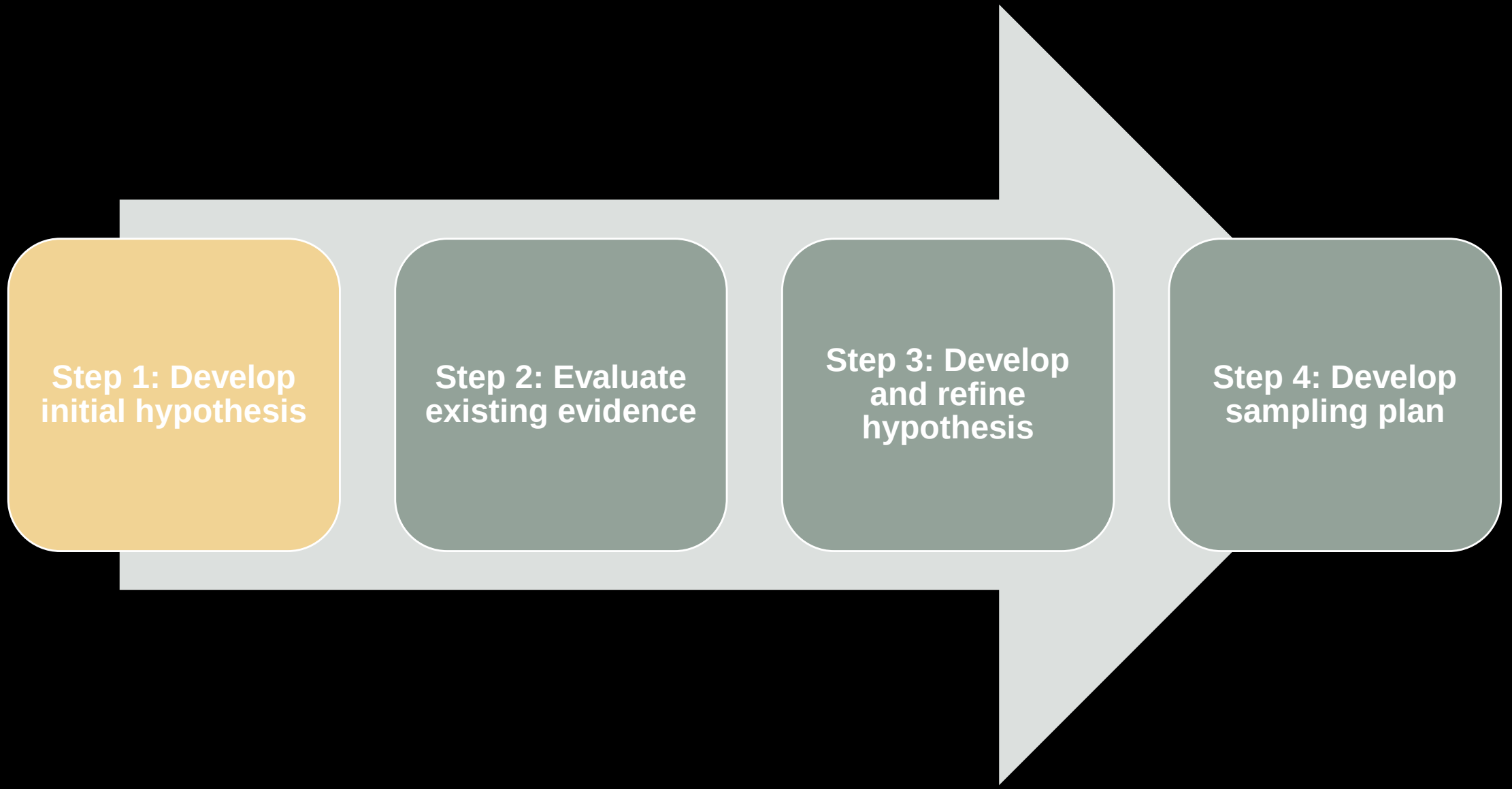


Implementation at State Level

- **California:**
  - California Microbial Source Identification Manual
  - CA approved ddPCR for rapid beach monitoring
  - Funded work to explore risk-based thresholds
- **Florida:**
  - St. Petersburg Guide to Source Tracking
- **Michigan**
  - Utilized qPCR for beach water quality monitoring since 2015



Study Design Considerations



Study Design Considerations

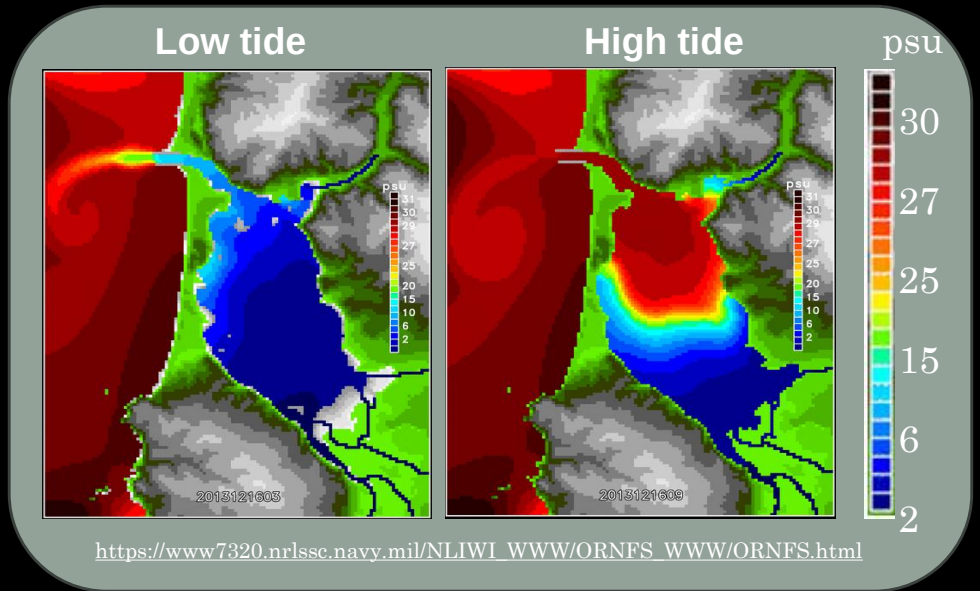
Step 2: Evaluate existing evidence

- Physical/env conditions (e.g., soil type, vegetation)
- Climatic data (e.g., precip, temp, wind direction/speed, UV)
- Hydrologic data, including modeling efforts

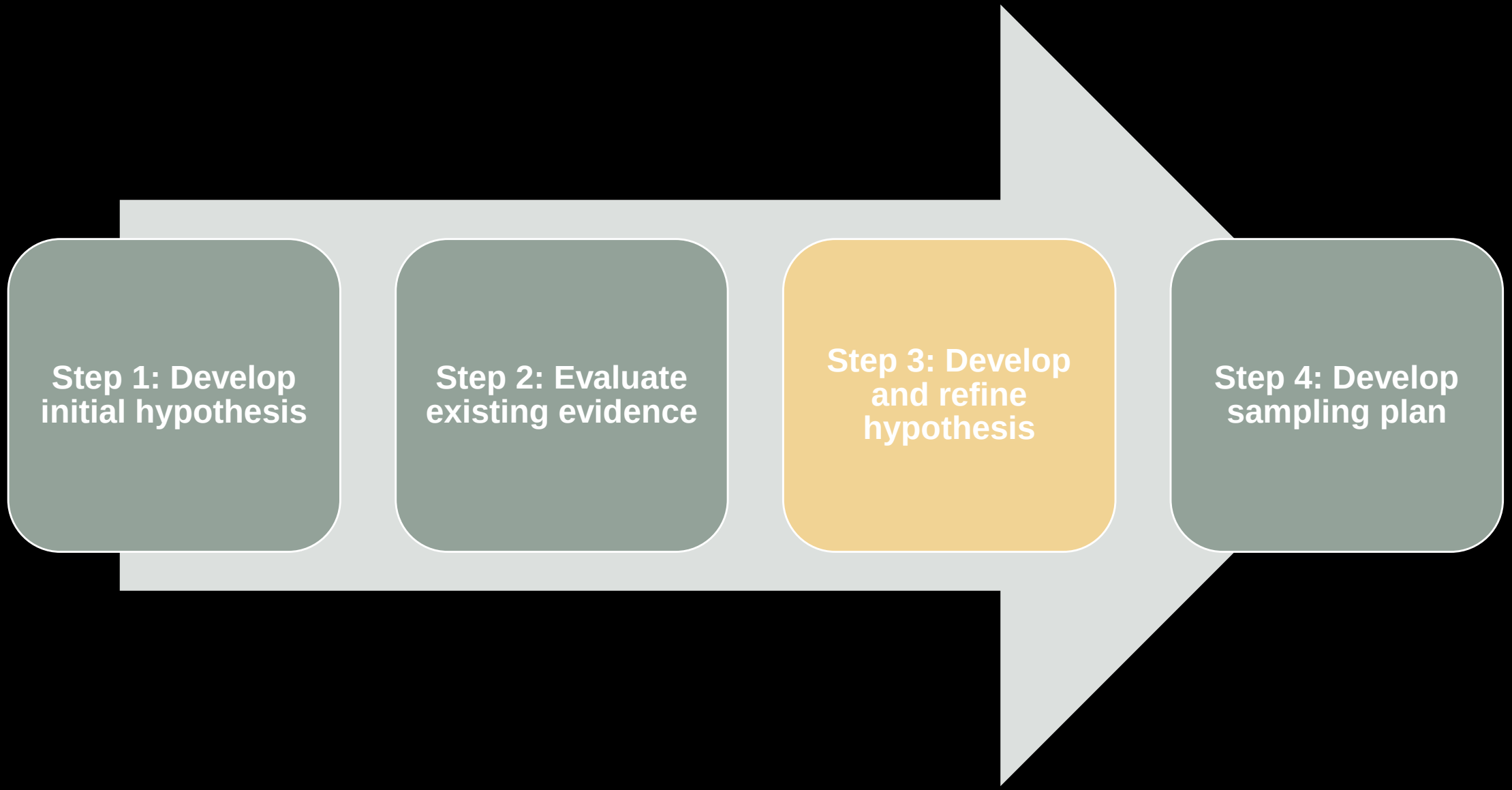
Fate & transport

- Land use data, including important features
- Historical FIB/water quality data
- Real-time/sonde data for analytes like salinity/temp/*E. coli*
- Visual reconnaissance and surveys

Source type and magnitude



Study Design Considerations



Study Design Considerations

Step 4: Develop sampling plan

When

- Consider conditions (environmental, climatic, and hydrological) associated with high bacteria levels
- Conditions associated with high risk
- Times of expected source activity

Where

- Locations of known or expected sources or areas with high bacteria levels; bracket potential hotspots
- Include reference or “upstream” conditions
- Consider potential dilution and mixing of sources

What

- Consider # tests per sample – need statistical significance
- Consider markers available and potential duplication for important sources
- Consider volume needed to allow for replication

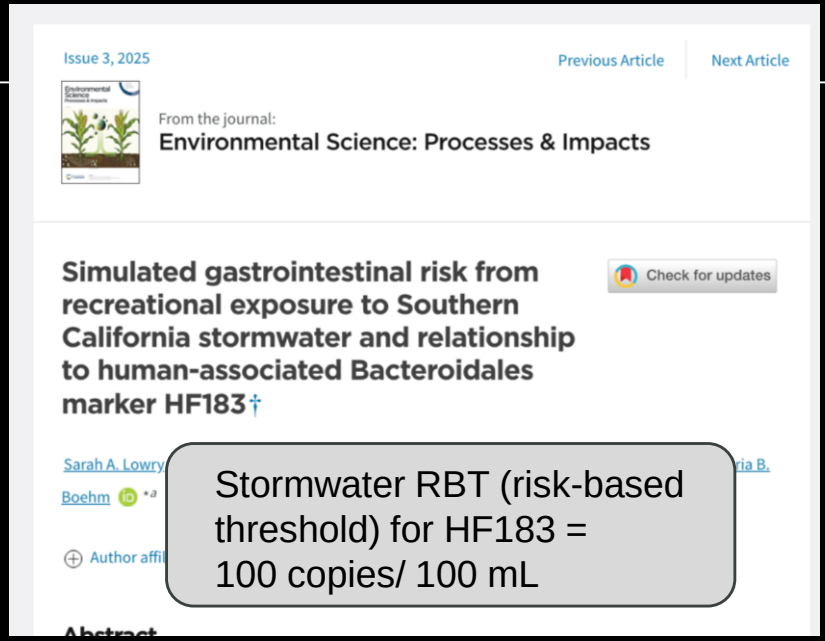
Data Analysis

- **Current marker-based methods allow for quantification of genetic markers associated with different sources**
- **Limitations and challenges exist in robust interpretation of the data:**
  1. No established risk-based thresholds for MST markers
  2. Frequent low or non-detections
  3. Environmental persistence and decay influence signal strength

Data Analysis

No established thresholds from MST markers

- Compare relative concentrations to identify hot spots or track trends
- Evaluate concentration in relation to FIB measurements, i.e., what sources are present at problematic sites
- Leverage ongoing research on risk-based thresholds for benchmarking


Issue 3, 2025 Previous Article Next Article

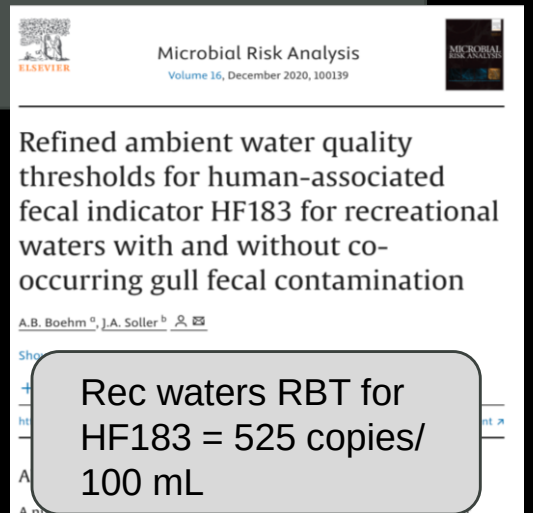
From the journal:
Environmental Science: Processes & Impacts

Simulated gastrointestinal risk from recreational exposure to Southern California stormwater and relationship to human-associated Bacteroidales marker HF183†

Check for updates

Sarah A. Lowry Boehm

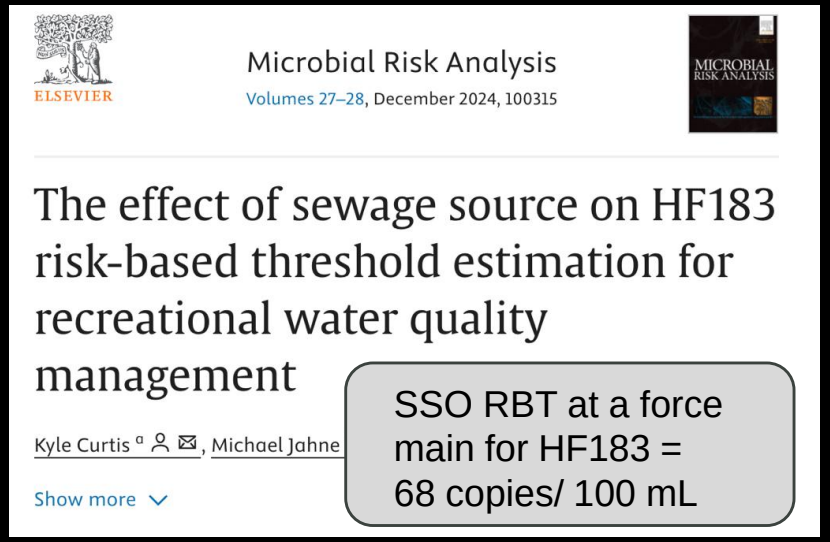
Stormwater RBT (risk-based threshold) for HF183 = 100 copies/ 100 mL


Microbial Risk Analysis
Volume 16, December 2020, 100139

Refined ambient water quality thresholds for human-associated fecal indicator HF183 for recreational waters with and without co-occurring gull fecal contamination

A.B. Boehm, J.A. Soller

Rec waters RBT for HF183 = 525 copies/ 100 mL


Microbial Risk Analysis
Volumes 27–28, December 2024, 100315

The effect of sewage source on HF183 risk-based threshold estimation for recreational water quality management

Kyle Curtis, Michael Jahne

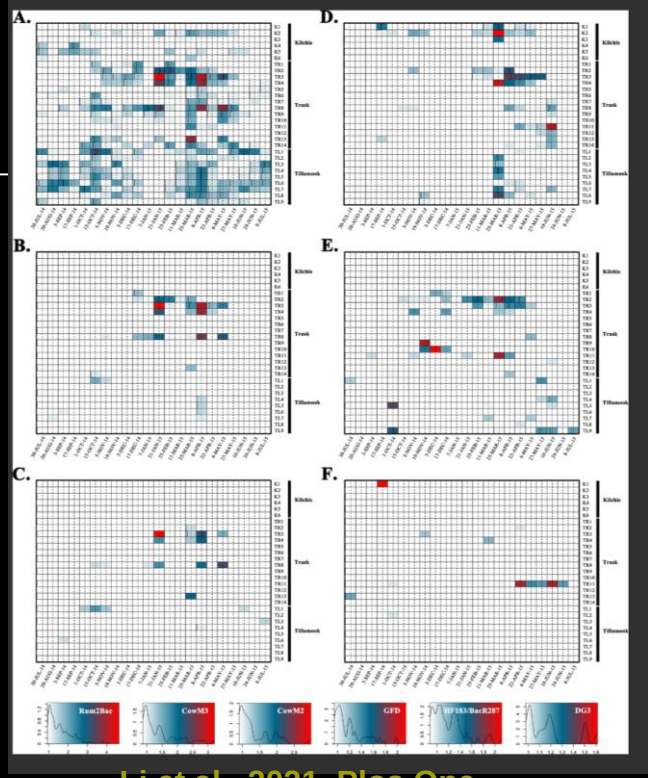
Show more

SSO RBT at a force main for HF183 = 68 copies/ 100 mL

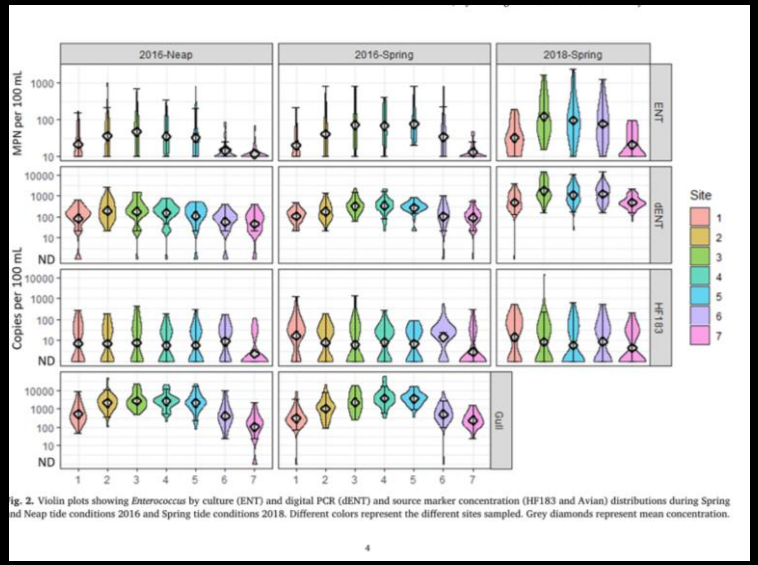
Data Analysis

High frequency of non/low-level detections

- Minimize false negatives: Optimize study design in terms of potential dilution of source, inadequate number of samples
- Visualize and evaluate frequency of detection in addition to concentration
- Clearly define and communicate limit of detection and lower limit of quantification for better comparison between different studies
- Box or violin plots can help to visualize data distribution



Li et al., 2021. Plos One.



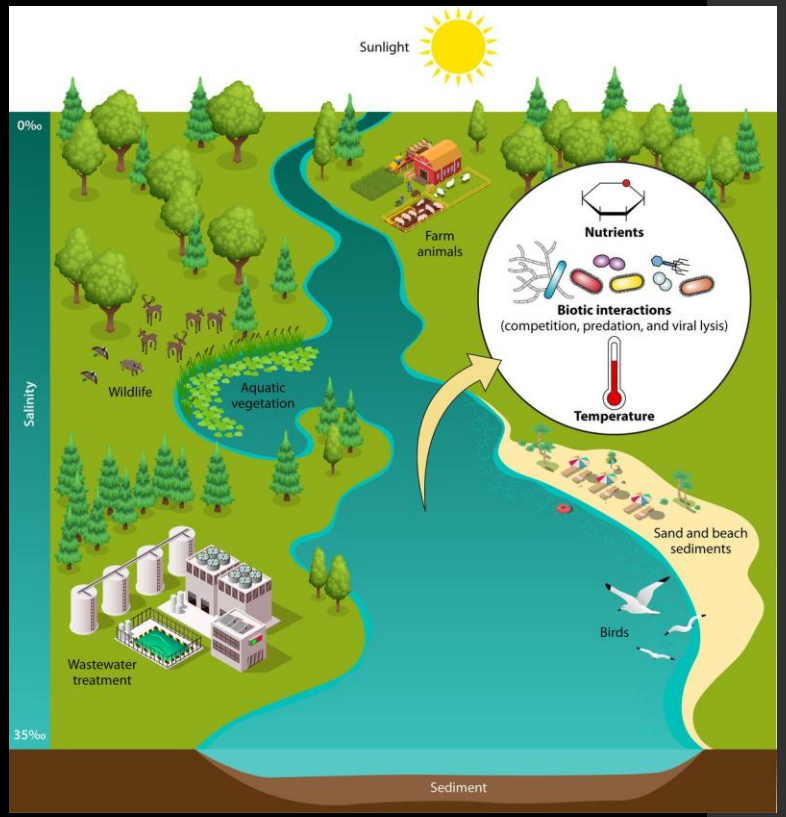
Zimmer-Faust et al., 2020. Marine Pollution Bulletin.

Data Analysis

Environmental persistence and decay can influence signal strength

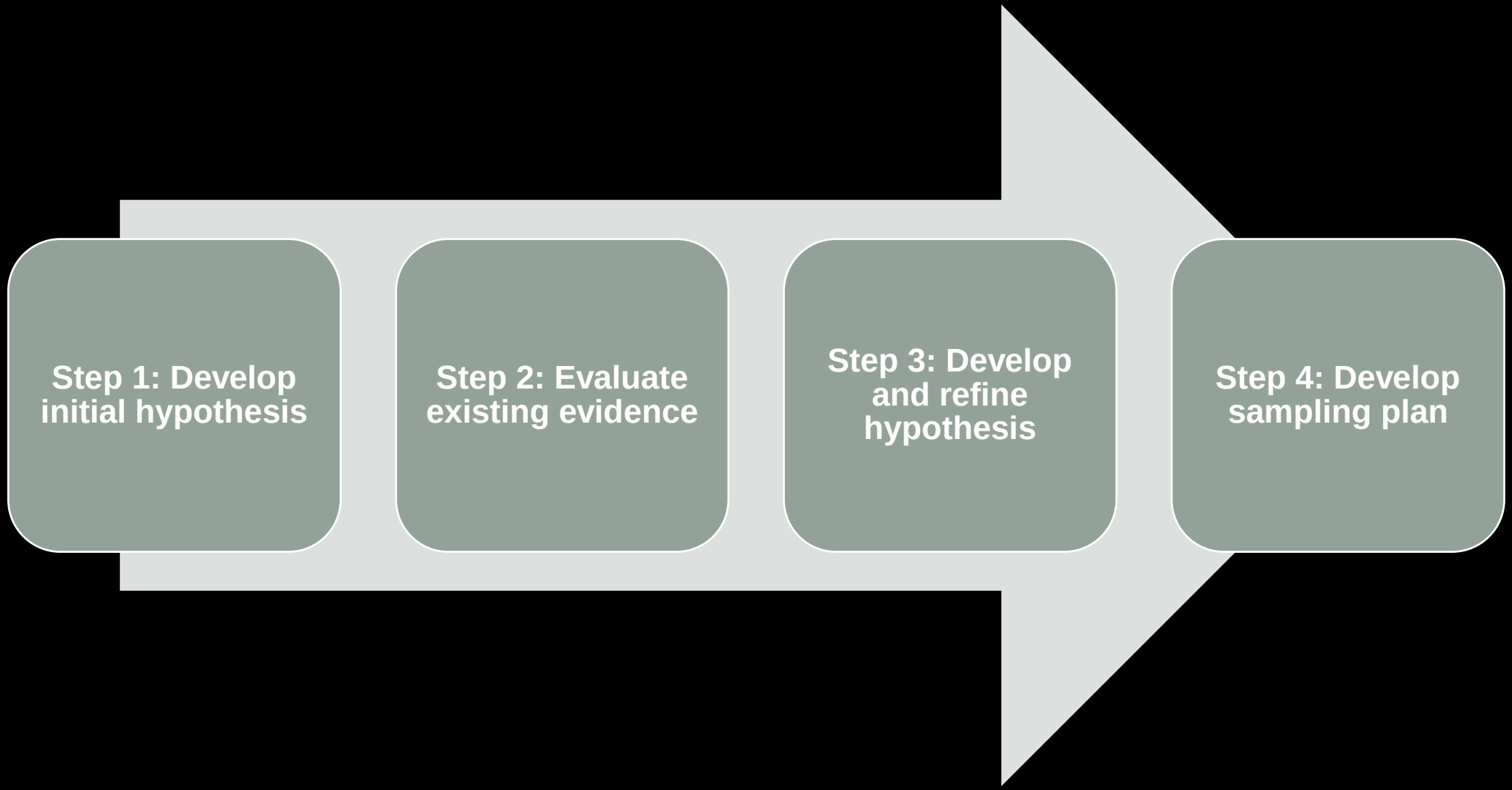
- Sample as close to the source as possible to minimize environmental decay
- Choose appropriate markers:
  - Viral vs. bacterial targets may degrade differently
  - Consider multiple markers; faster-degrading markers suggest recent contamination

** Many studies exist on marker decay and persistence



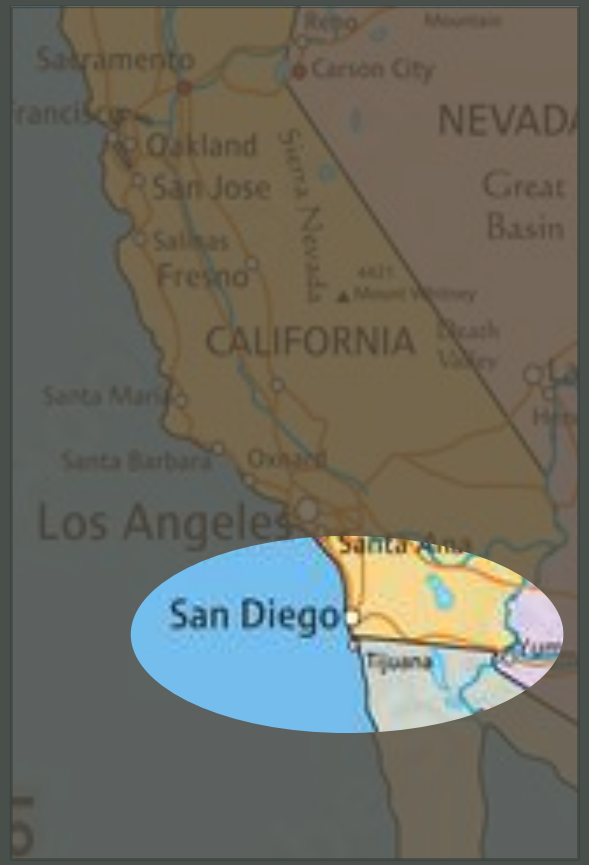
Korajkic et al., 2019. Microbiolgy and Molecular Biology Reviews.

Study Design Implementation



Highlighted Project

Tracking Nearshore Pollutant Dynamics along the Southwest U.S.-Mexico Border



Tracking IB's 'smelly water'

Ben McCue, a surfer and coastal program manager for the nonprofit group Wildcoast in Imperial Beach, calls the search for an annoying on-again, of-again scent "almost a reverse investigation." (/ Union-Tribune)

JULY 27, 2010, 10:00 PM

For the past decade, surfers and others have complained about an unusual odor wafting over the sand and waves of Imperial Beach.

It's often described as having a detergentlike quality, and it comes with shimmery bubbles in the surf zone. One scientific paper calls it "smelly water."

For just as long, the on-again-off-again scent has defied attempts to determine its source and answer questions about whether it poses dangers for beach users.

Aerial shot taken in winter

NEIGHBORHOOD NEWS | TIJUANA

0

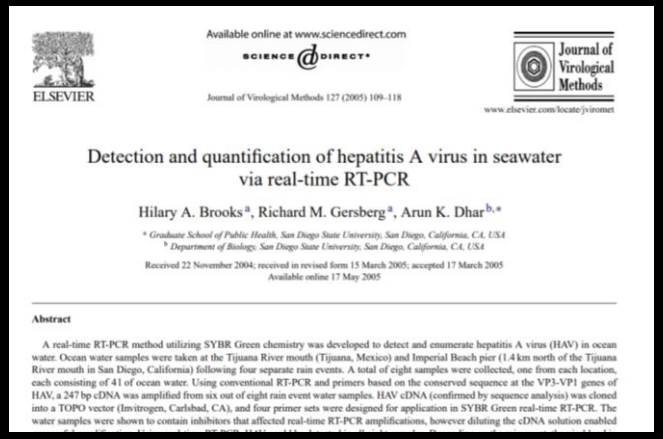
Video of Punta Bandera sewage explodes

Half million views

By Marty Graham, May 25, 2018

SADB WWTP

- Located 6 miles South of the U.S./Mexico border
- Treats to partial primary only
  - Constant source
  - 25 Million Gallons a Day
- Previous modeling efforts suggest water from Punta Bandera can travel North across border



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Detection and quantification of hepatitis A virus in seawater via real-time RT-PCR

Hilary A. Brooks [a], Richard M. Gersberg [a], Arun K. Dhar [b,*]

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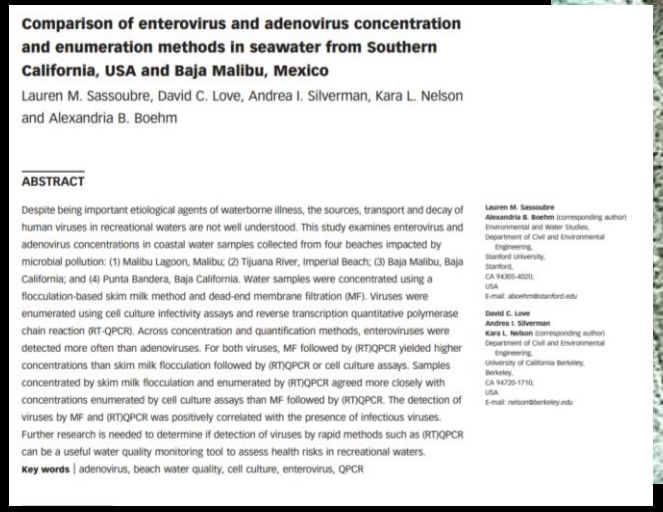
[b] Department of Biology, San Diego State University, San Diego, California, CA, USA

Received 22 November 2004; received in revised form 15 March 2005; accepted 17 March 2005
Available online 17 May 2005

Abstract

A real-time RT-PCR method utilizing SYBR Green chemistry was developed to detect and enumerate hepatitis A virus (HAV) in ocean water. Ocean water samples were taken at the Tijuana River mouth (Tijuana, Mexico) and Imperial Beach pier (1.4 km north of the Tijuana River mouth in San Diego, California) following four separate rain events. A total of eight samples were collected, one from each location, each consisting of 41 of ocean water. Using conventional RT-PCR and primers based on the conserved sequence at the VP3-VP1 genes of HAV, a 247 bp cDNA was amplified from six out of eight rain event water samples. HAV cDNA (confirmed by sequence analysis) was cloned into a TOPO vector (Invitrogen, Carlsbad, CA), and four primer sets were designed for application in SYBR Green real-time RT-PCR. The water samples were shown to contain inhibitors that affected real-time RT-PCR amplifications, however diluting the cDNA solution enabled



Comparison of enterovirus and adenovirus concentration and enumeration methods in seawater from Southern California, USA and Baja Malibu, Mexico

Lauren M. Sassoubre, David C. Love, Andrea I. Silverman, Kara L. Nelson and Alexandria B. Boehm

ABSTRACT

Despite being important etiological agents of waterborne illness, the sources, transport and decay of human viruses in recreational waters are not well understood. This study examines enterovirus and adenovirus concentrations in coastal water samples collected from four beaches impacted by microbial pollution: (1) Malibu Lagoon, Malibu; (2) Tijuana River, Imperial Beach; (3) Baja Malibu, Baja California; and (4) Punta Bandera, Baja California. Water samples were concentrated using a flocculation-based skim milk method and dead-end membrane filtration (MF). Viruses were enumerated using cell culture infectivity assays and reverse transcription quantitative polymerase chain reaction (RT-QPCR). Across concentration and quantification methods, enteroviruses were detected more often than adenoviruses. For both viruses, MF followed by (RT)QPCR yielded higher concentrations than skim milk flocculation followed by (RT)QPCR or cell culture assays. Samples concentrated by skim milk flocculation and enumerated by (RT)QPCR agreed more closely with concentrations enumerated by cell culture assays than MF followed by (RT)QPCR. The detection of viruses by MF and (RT)QPCR was positively correlated with the presence of infectious viruses. Further research is needed to determine if detection of viruses by rapid methods such as (RT)QPCR can be a useful water quality monitoring tool to assess health risks in recreational waters.

Key words | adenovirus, beach water quality, cell culture, enterovirus, QPCR

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Objectives:

- Quantify the extent and impact of transboundary pollution from the San Antonio de los Buenos Wastewater Treatment Plant.

Hypothesis:

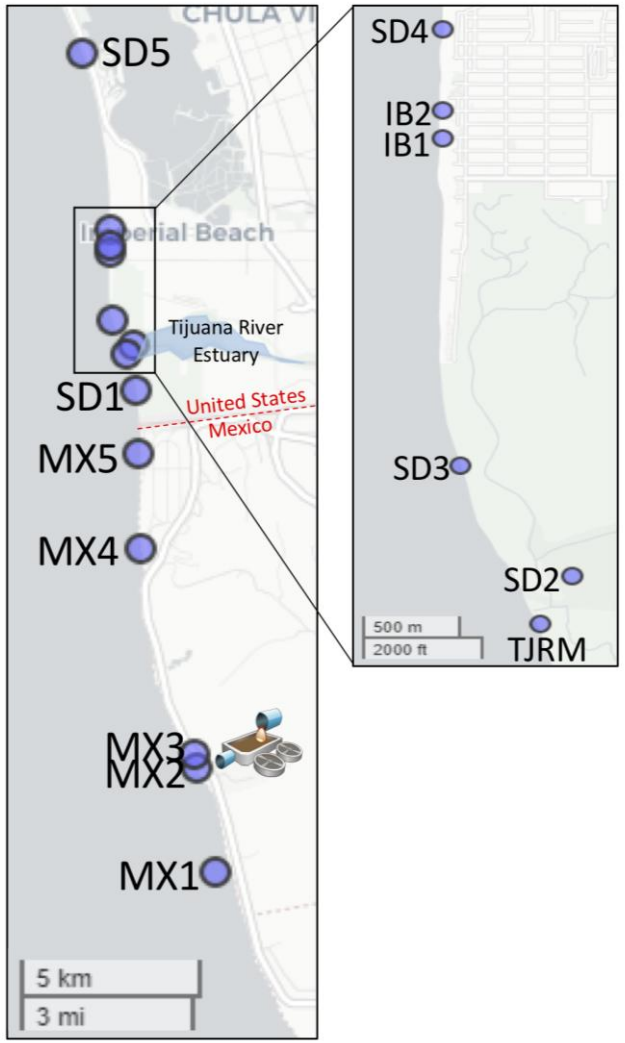
- Wastewater from the SADB plant is transported upcoast and contributes to beach closures at San Diego beaches

Scope:

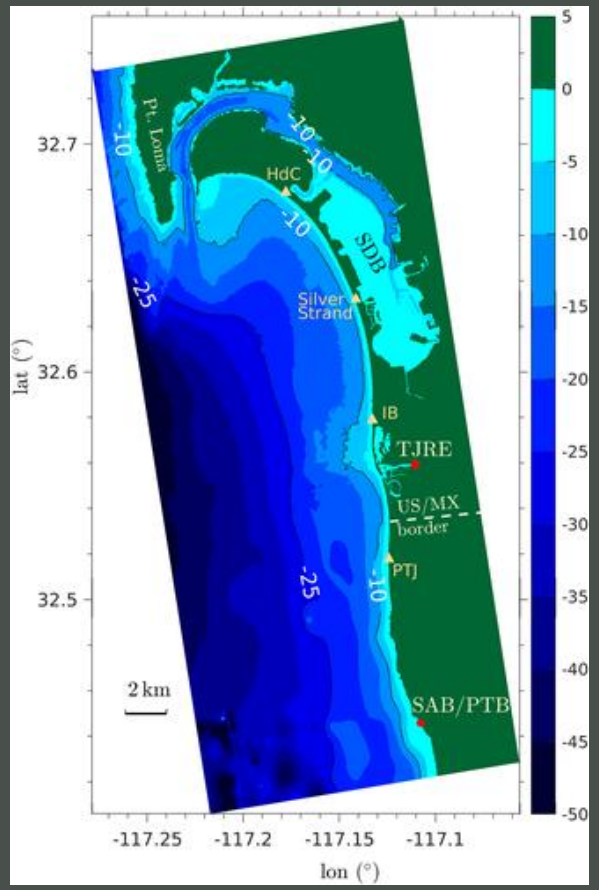
- Binational coastal zone near the U.S.–Mexico border, focused on the area surrounding the San Antonio de los Buenos WWTP.

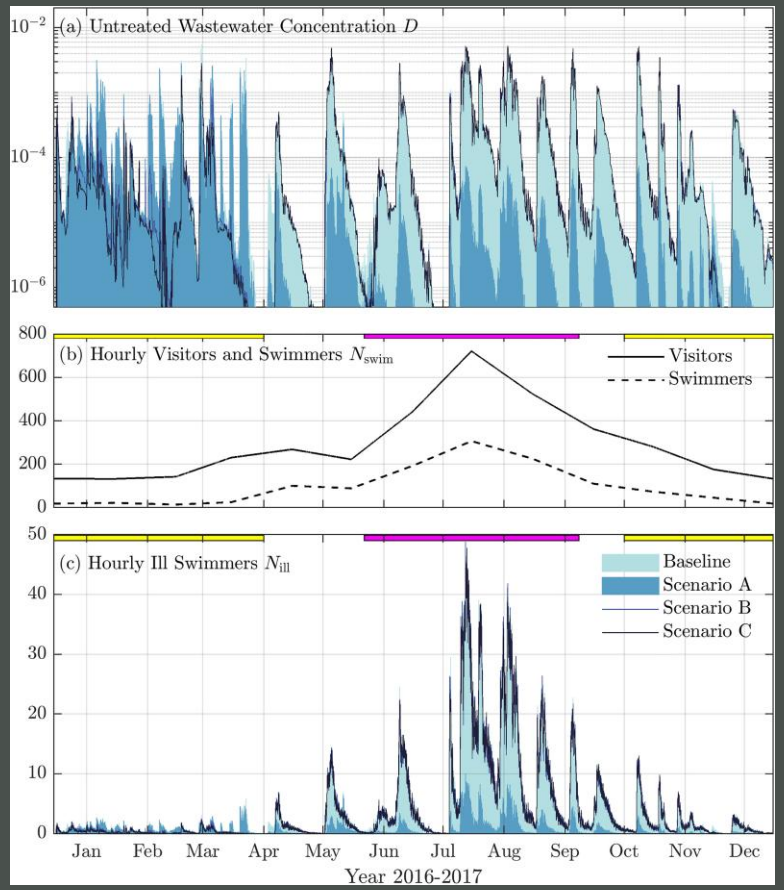
Develop Initial Hypothesis → Evaluate evidence → Refine Hypothesis → Develop Sampling Plan → Execution

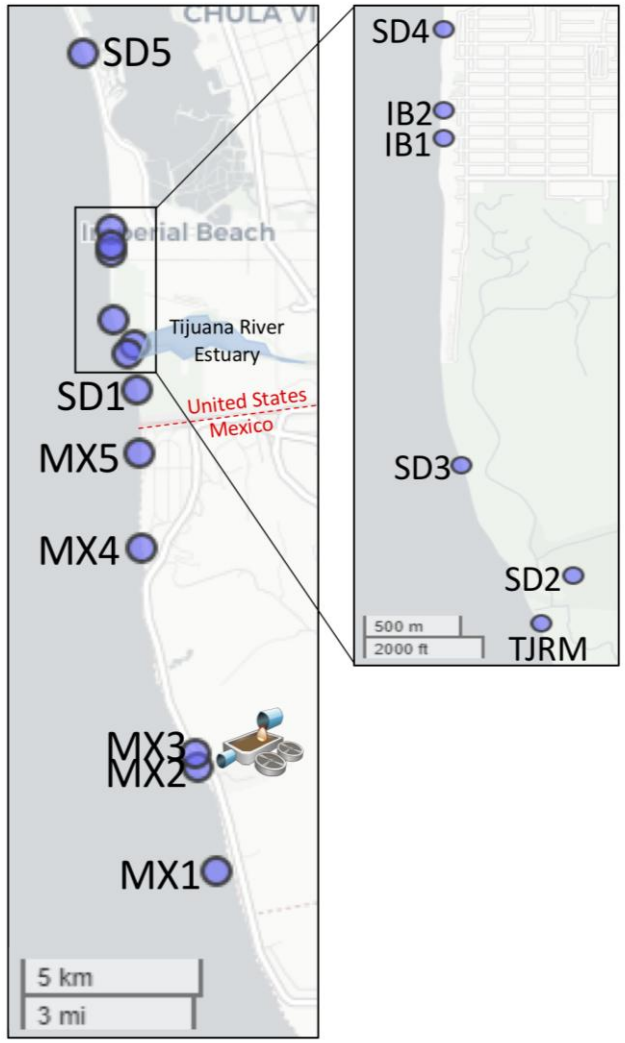
Develop Initial Hypothesis | Evaluate evidence | Refine Hypothesis | Develop Sampling Plan | Execution



UC San Diego | SCRIPPS INSTITUTION OF OCEANOGRAPHY

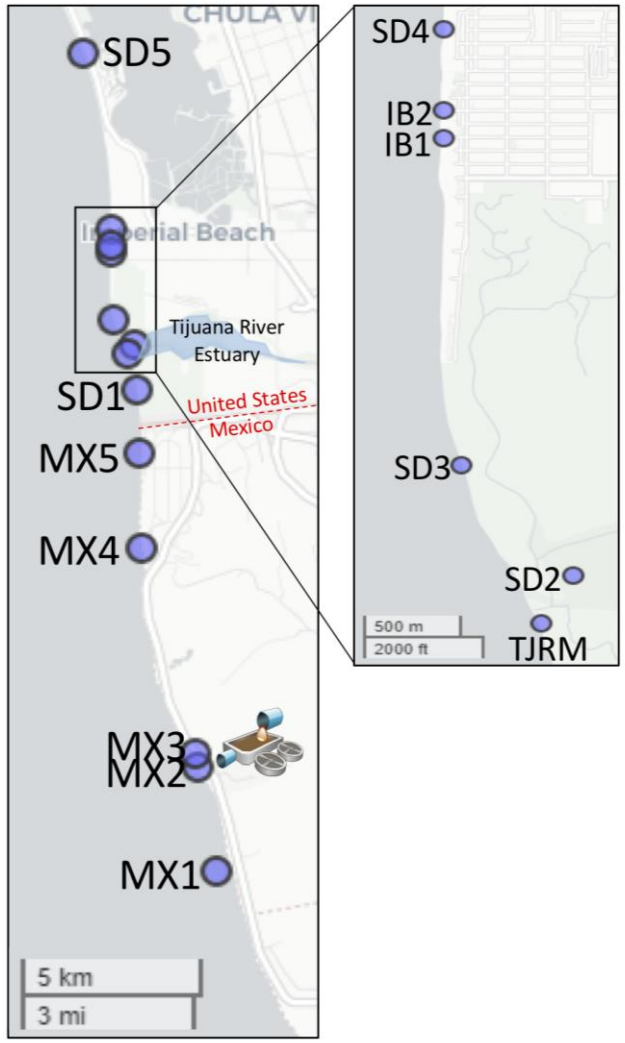






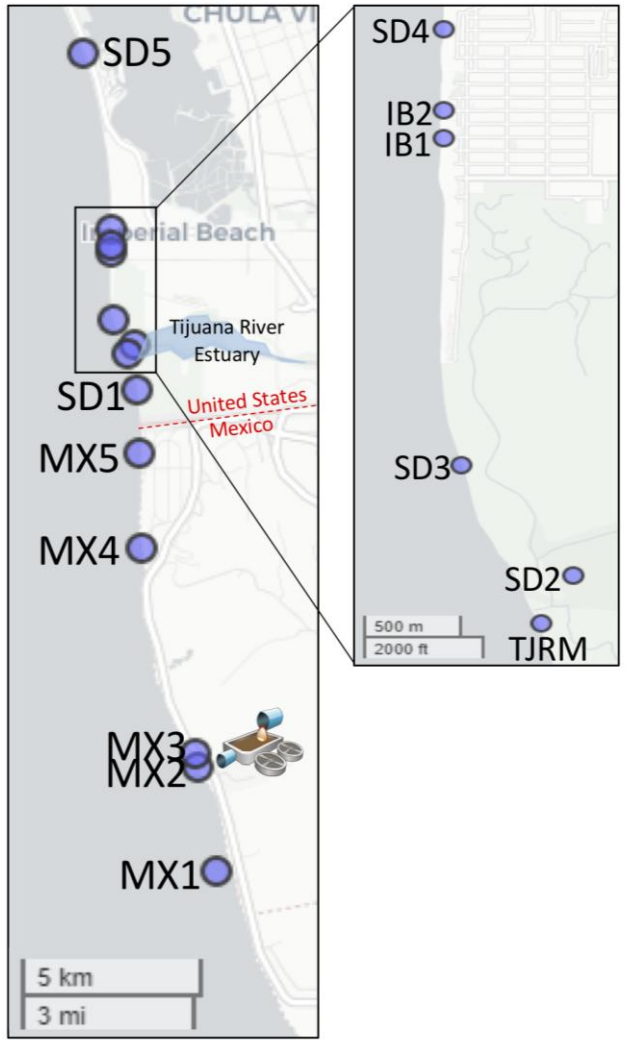
When and where to sample:

- Sample coastline between SADB WWTP outfall and Coronado
- Sampling conducted targeting specific conditions associated with northern transport of the SADB WTP effluent
- Target consecutive days during south swell conditions during dry weather (multiple days per event)

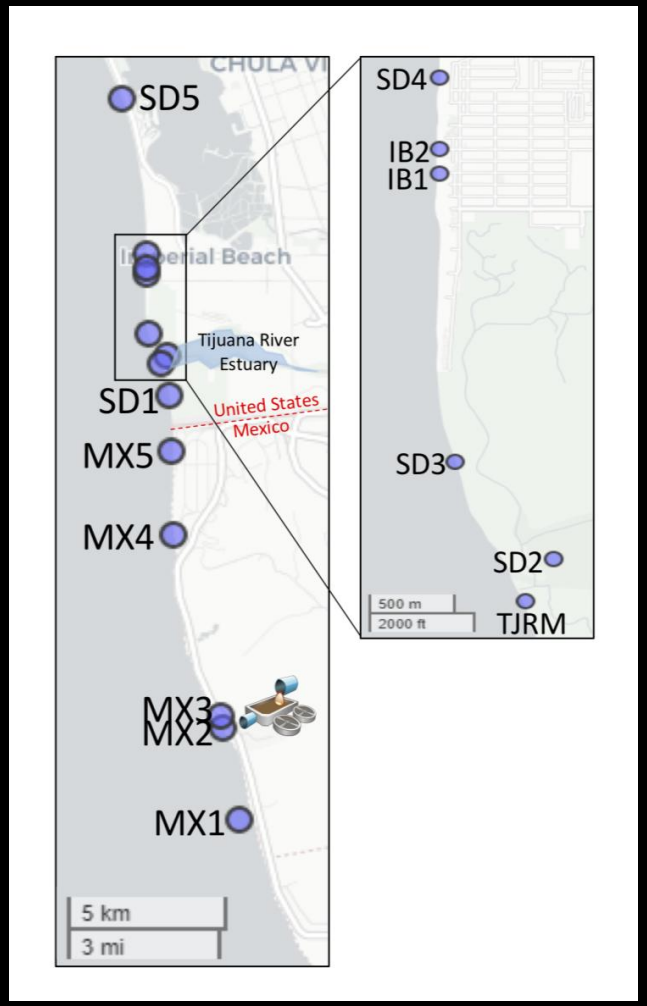


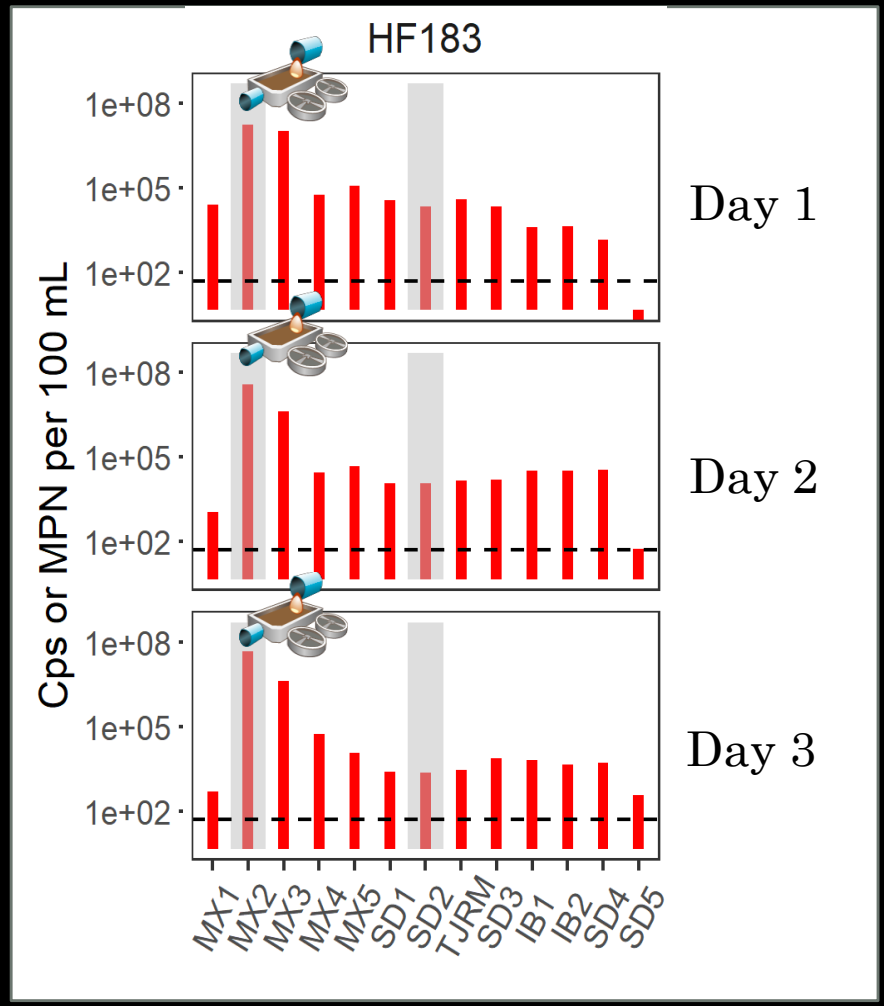
What to measure:

- Measured enterococci by IDEXX and digital droplet PCR
- Measure gradient in DNA-based human markers (HF183)and a secondary human marker by digital PCR from the Tijuana WTP
- Track DNA signature of the Tijuana WTP in the nearshore using microbial community sequencing-based technologies


SD5
Imperial Beach
Tijuana River Estuary
SD1
United States
Mexico
MX5
MX4
MX3
MX2
MX1
5 km
3 mi
SD4
IB2
IB1
SD3
SD2
500 m
2000 ft
TJRM

Quantify the extent and impact of transboundary pollution from the SADB WWTP: Human marker results during a strong south swell





Sites South → North

Lessons learned

- **Engage early –** Involve key stakeholders and collaborators from the start.
- **Leverage local capacity –** Partnering with groups like Imperial Beach lifeguards and Tijuana Waterkeeper expanded the monitoring reach of a tight budget.
- **Data builds trust and motivation –** MST results helped validate models and strengthened credibility with partners.
- Ocean conditions were key to explaining MST results.

Highlighted Project

Water Research
Volume 272, 15 March 2025, 122981

An integrated approach to coupled nutrient and microbial source tracking in an agricultural watershed ☆

Amity G. Zimmer-Faust [a 1], Cheryl A. Brown [a 1], Orin C. Shanks [b], William Rugh [c], T Chris Mochon Collura [a], Hilmar A. Stecher [c]

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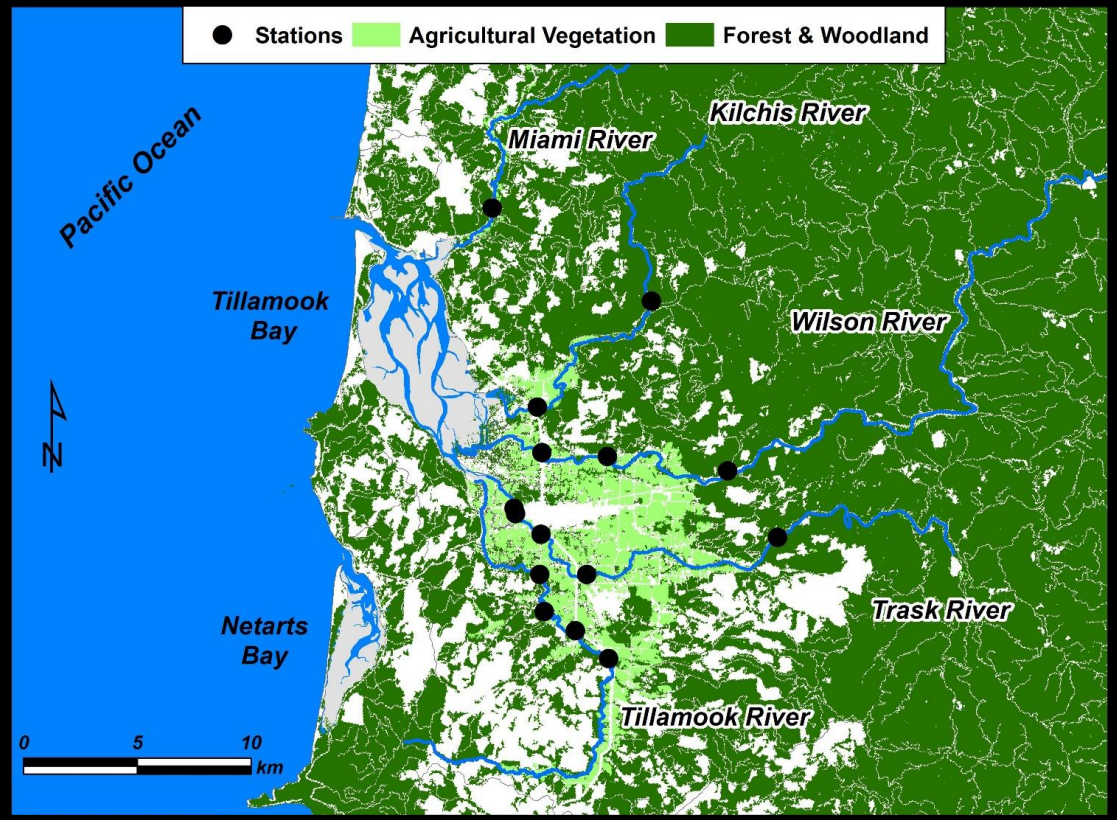
https://doi.org/10.1016/j.watres.2024.122981 Get rights and content

Highlights

- We tracked nitrogen and fecal bacteria in Tillamook Bay Watershed, Oregon (USA).
- Nitrogen and fecal bacteria entered surface waters as co-pollutants.
- Primary sources were agricultural inputs followed by human waste.
- Microbial source tracking was a useful tool to contextualize nitrogen sources.



- National Estuary Partnership Site
- 3rd largest Estuary in Oregon
- History of impairments: Sediments, nutrients, dissolved oxygen (DO) and temperature, and bacteria

Objectives:

- Quantify sources of watershed nitrogen and fecal bacteria to receiving waters in Tillamook Bay watershed.

Hypothesis:

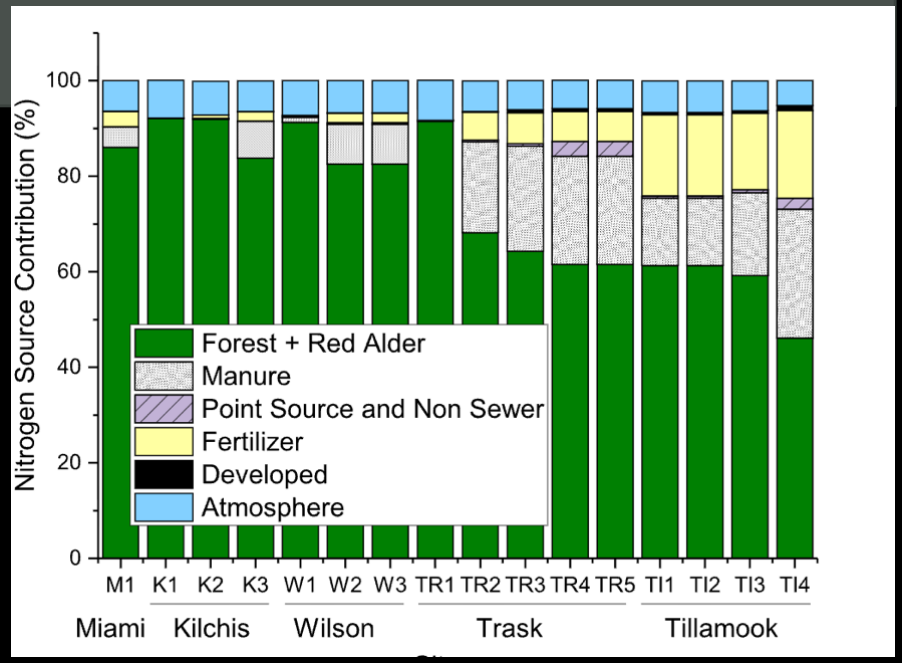
- Primary source of elevated fecal bacteria and nitrate in the Tillamook watershed is agricultural inputs.

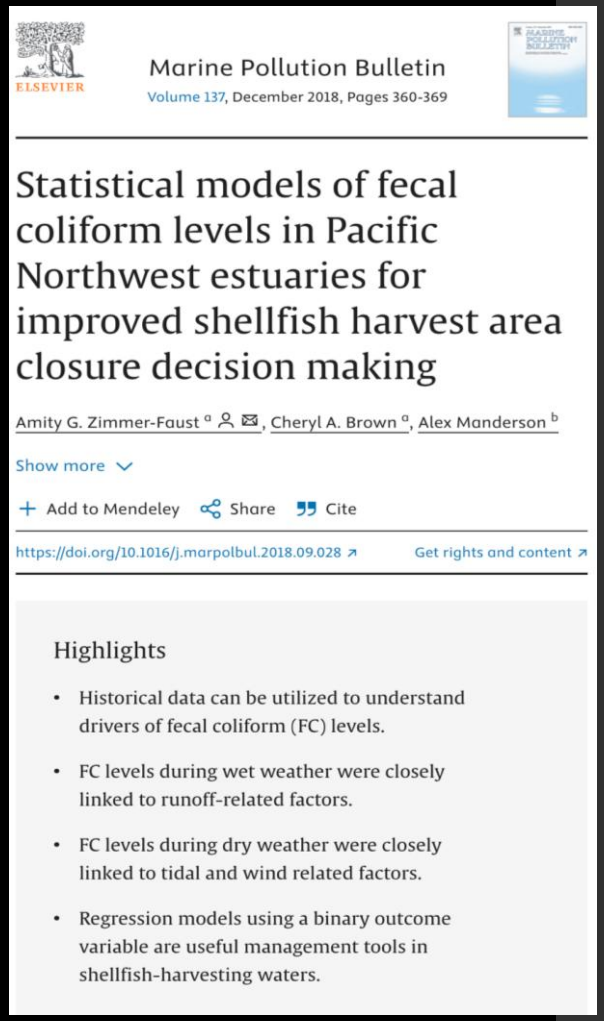
Scope:

- Gradient along the five major tributaries within Tilllamook Bay watershed.

Develop Initial Hypothesis → Evaluate evidence → Refine Hypothesis → Develop Sampling Plan → Execution

- **Analysis of land use data and relevant GIS layers:**
  - Location of point sources, CAFOs/AFOs, forest, non-sewered areas (assumed septic)
- **Historical FIB review:** spatial/temporal trends linked to environmental drivers





ELSEVIER

Marine Pollution Bulletin

Volume 137, December 2018, Pages 360-369

Statistical models of fecal coliform levels in Pacific Northwest estuaries for improved shellfish harvest area closure decision making

Amity G. Zimmer-Faust [a], Cheryl A. Brown [a], Alex Manderson [b]

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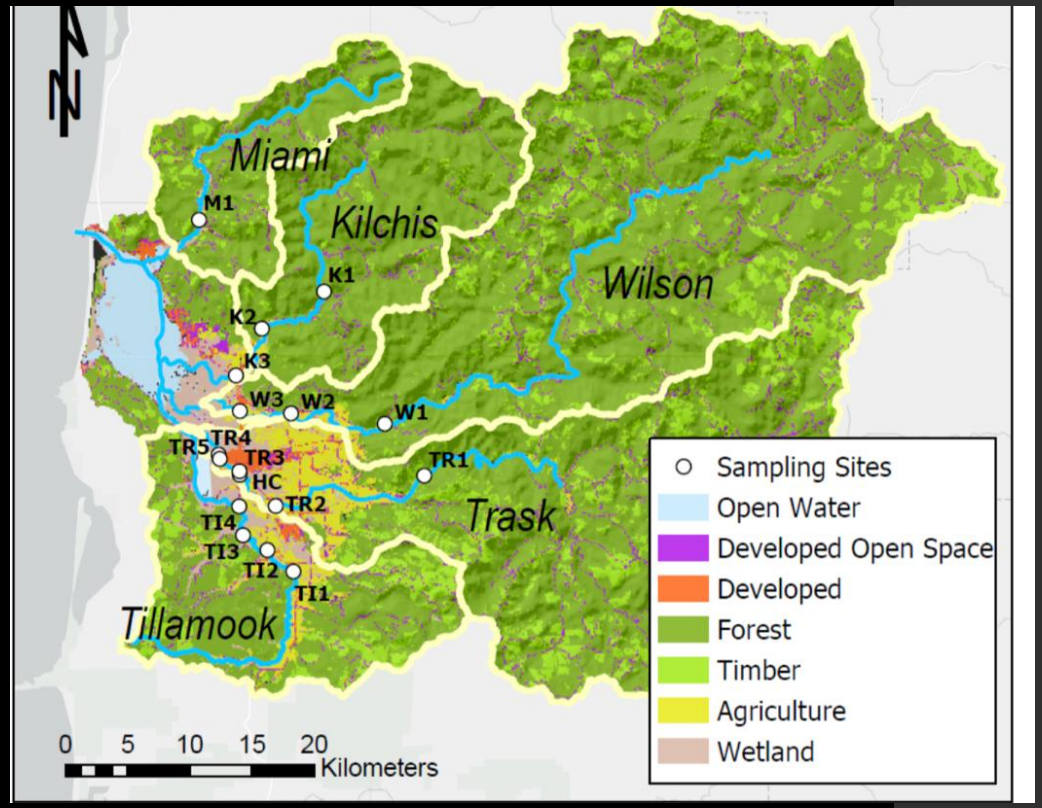
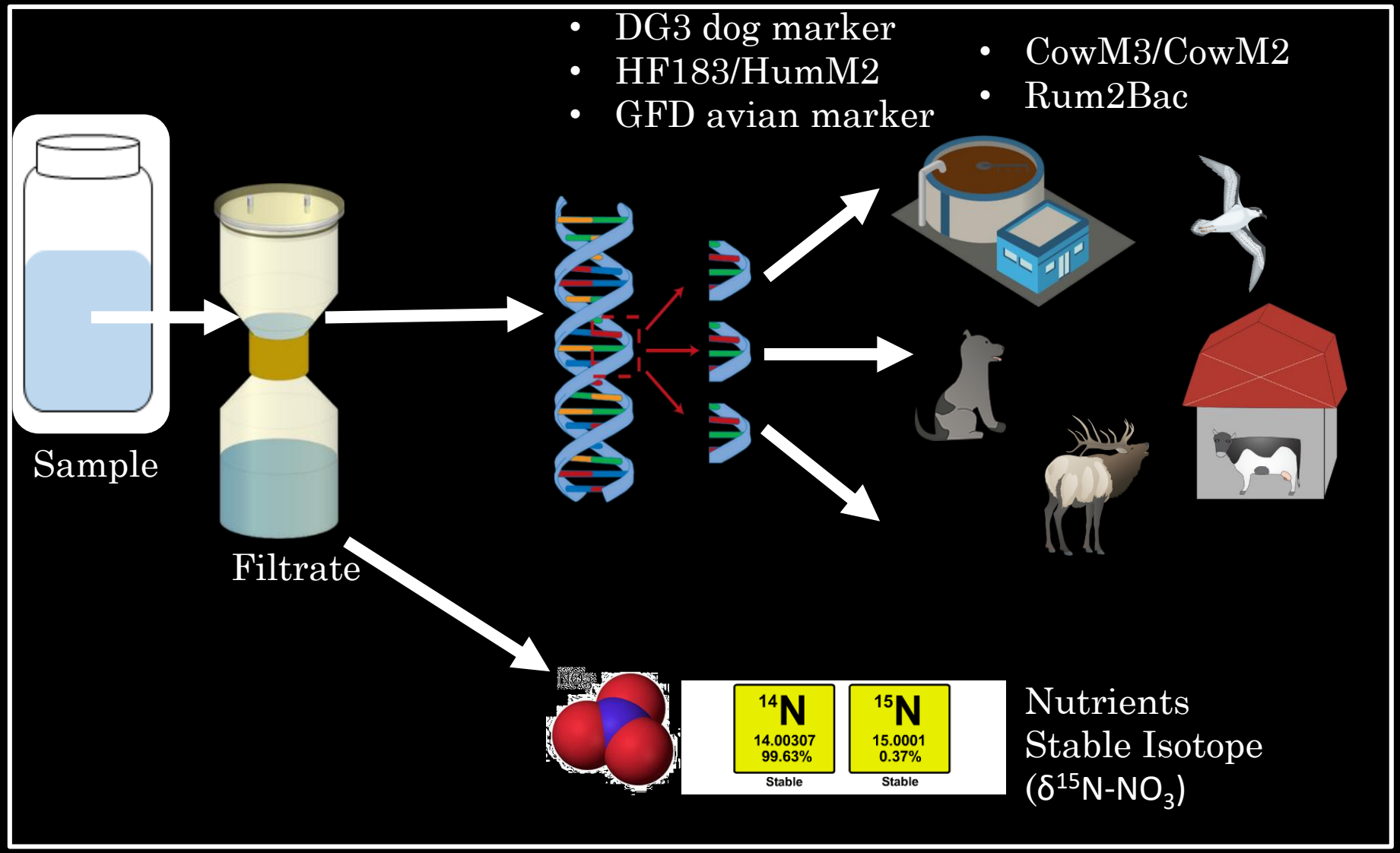
https://doi.org/10.1016/j.marpolbul.2018.09.028 Get rights and content

Highlights

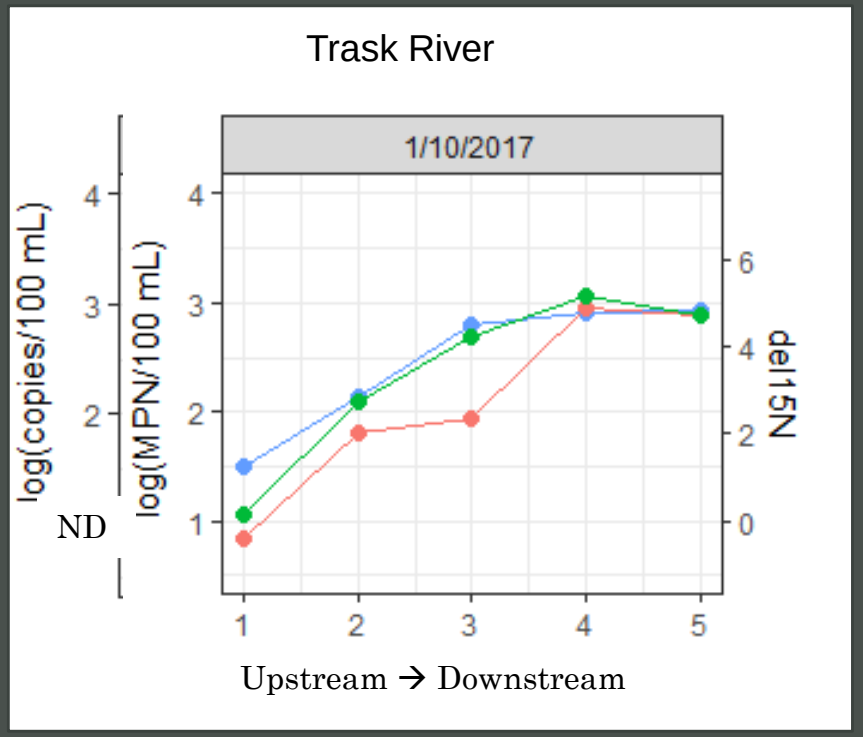
- Historical data can be utilized to understand drivers of fecal coliform (FC) levels.
- FC levels during wet weather were closely linked to runoff-related factors.
- FC levels during dry weather were closely linked to tidal and wind related factors.
- Regression models using a binary outcome variable are useful management tools in shellfish-harvesting waters.

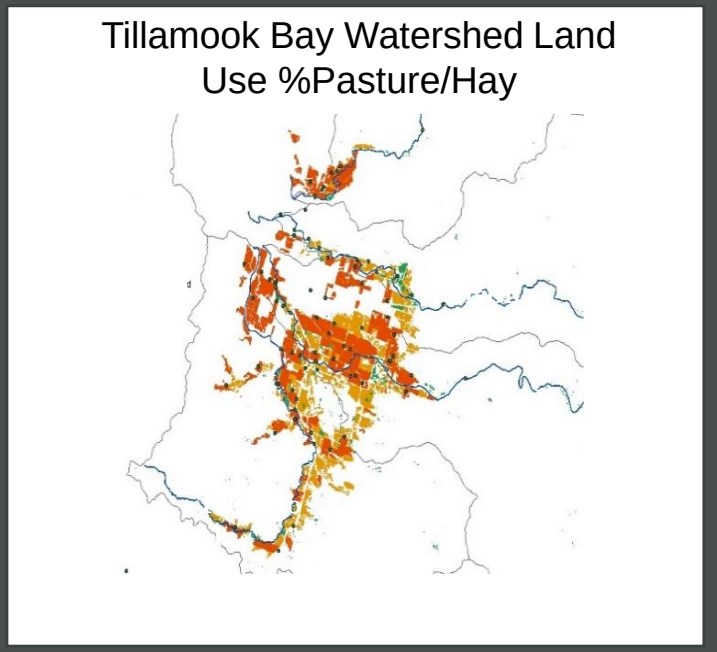
- Study coverage:
  - 15 tributary stations in 5 tributaries (Miami, Kilchis, Wilson, Trask and Tillamook)
  - Stations transitioned from Forested to agriculture/developed (except Tillamook).
  - Areas of tidal influence taken into account
  - Sites represented existing monitoring locations for other efforts (




• DG3 dog marker
• HF183/HumM2
• GFD avian marker
• CowM3/CowM2
• Rum2Bac
Sample
Filtrate
14N
14.00307
99.63%
Stable
15N
15.0001
0.37%
Stable
Nutrients
Stable Isotope
(δ15N-NO3)

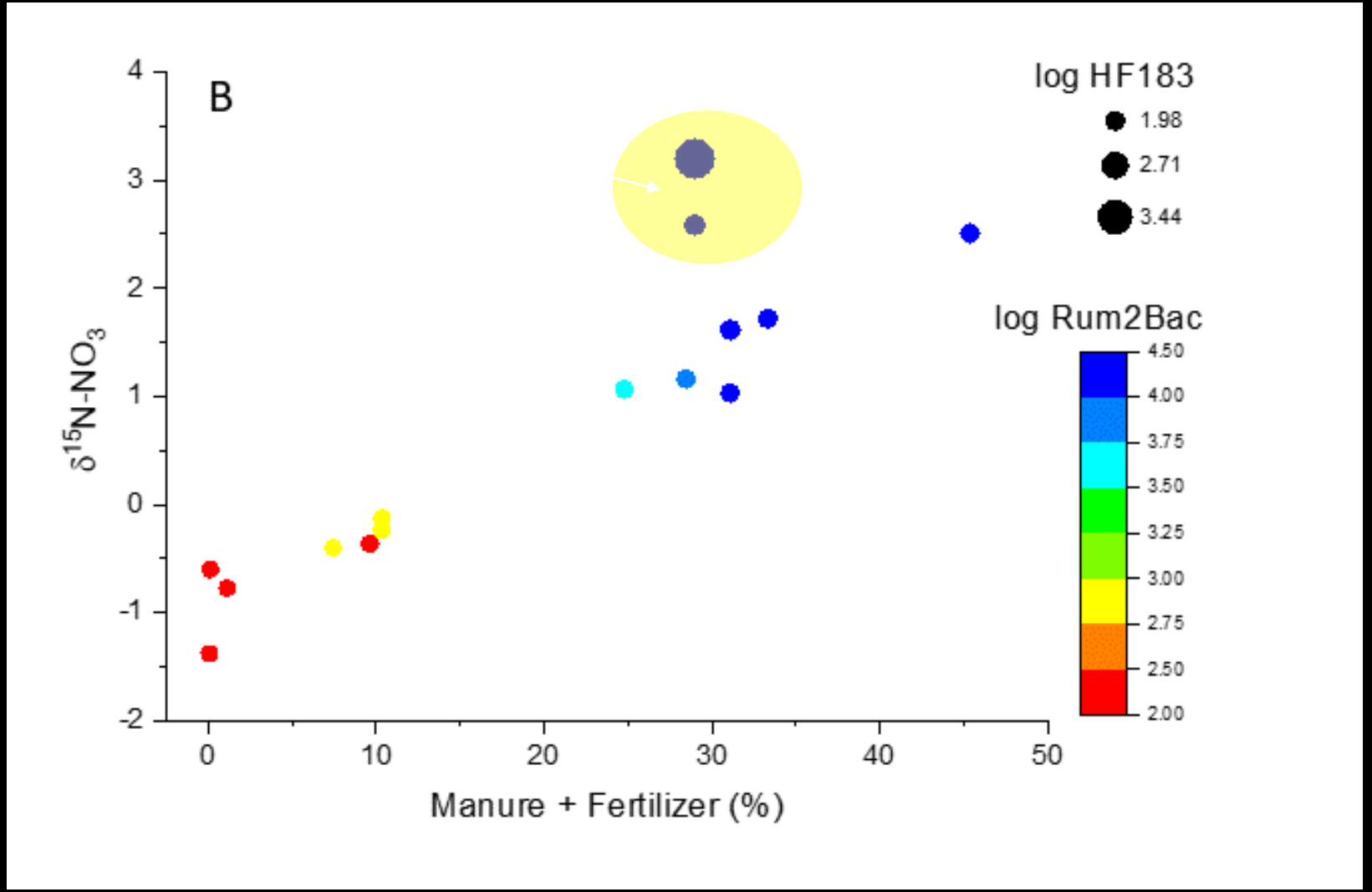
Co-occurrence of fecal bacteria & nitrogen sources





- Moving downriver along the Trask:
  - Increase in *E. coli* levels
  - Increase in Cow Marker levels
  - Increase in $\delta^{15}N\text{-}NO_3$ isotope
  - Increase in agricultural land use

Co-occurrence of fecal bacteria & nitrogen sources



Lessons learned

- **Apply spatial analysis –** Use GIS to delineate upstream catchments, summarize land use, and test relationships with MST results. This site-level context helps identify drivers of contamination.
- **Account for system dynamics –** In tidal or complex hydrologic systems, consider timing and environmental conditions when designing sampling strategies.
- **Build trust through data –** Sharing MST and water quality findings can support management decisions and strengthen trust with local stakeholders.
- Catchment-wide dynamics are important to consider for remediation of downstream water quality in estuarine watersheds.

Thank you!

Feel free to get in touch:
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Microbial Source Tracking: Origins, Technology and Application

Presentation to the **Puget Sound Microbial Source Tracking Workshop**

September 12, 2025

John F. Griffith

Southern California Coastal Water Research Project

Road Map

- **Origins of Microbial Source Tracking**
- 2003 MST Method Evaluation Study
- 2013 Source Identification Pilot Project
- California Source Identification Manual
- Which molecular MST method should I use?
- Differentiating Human Sources of Fecal Contamination

Early Days of Microbial Source Tracking

- Based on the idea that microbes evolve to become adapted to their specific host
- All early methods depended on comparing isolates from the environment with a library "known" isolates
  - Two main classes:
    - Phenotypic methods (antibiotic resistance analysis, carbon utilization)
    - Genetic methods adopted from the food safety sector (ribotyping, PFGE, box-PCR)
- By 2003, multiple methods had been published
  - *They all worked!*
  - Some were in use commercially
- None had been independently tested

Road Map

- Origins of Microbial Source Tracking
- **2003 MST Method Evaluation Study**
- 2013 Source Identification Pilot Project
- California Source Identification Manual
- Which molecular MST method should I use?
- Differentiating Human Sources of Fecal Contamination

2003 MST Method Evaluation

- Each researcher was provided “library” feces from five known sources
  - Human (11 Humans)
  - Regional “human” (sewage influent)
  - Dog (12 dogs)
  - Cow (12 cows)
  - Seagull (4 flocks)

- Between one and three of these known sources were placed in various combinations into 12 “blind” sterile water matrix samples
  - Researchers asked to identify the percentage of each source in each blind sample

- 21 researchers using 12 classes of methods analyzed these samples

- Twelve additional “blind” samples placed in complex matrices
  - Salt water
  - Humic acid
  - Only analyzed by a subset of researchers

Evaluation Criteria

- Ability to correctly identify the presence of human fecal material.
- Ability to correctly identify the absence of human fecal material.
- Ability to correctly identify the dominant source of fecal material contained in a sample
- Ability to accurately identify all sources of fecal material contained in a sample.
- Stability of response across the three matrices.

General Categories of Methods

- library-based Genotypic methods
  - Identify sources based on patterns in the genetic material of bacterial isolates
    - Ribotpyping, PFGE, BOX-PCR

- library-based Phenotypic methods
  - Identify sources based on traits of bacterial isolates
    - ARA, CSU (Biolog)

- Non-library based genetic methods
  - Identify genetic markers unique to fecal bacteria of targeted host species
    - Host-specific PCR, t-RFLP, toxin genes

- Human virus and F+ colipage
  - Target human enteric viruses or those that infect *E. coli*

Findings

- No method performed consistently well across all evaluation criteria
  - HS PCR (Hf183) was the only method able to reliably differentiate between human and non-human
    - Not quantitative

- False Positives were the biggest problem across all methods

- Quantitative methods frequently able to identify dominant source in samples
  - Often identified large fractions of sources not present
  - Results were not always repeatable between replicates

- Among quantitative methods, genotypic generally fared better than phenotypic

- Human virus and F+ coliphage methods performed well at identifying the presence of human fecal material
  - Only when the human source was sewage

Road Map

- Origins of Microbial Source Tracking
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- **2013 Source Identification Pilot Project**
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- Which molecular MST method should I use?
- Differentiating Human Sources of Fecal Contamination

2013 Method Evaluation Study

- The 2003 MST Method Evaluation Study completely changed the source tracking research landscape
  - Over subsequent years, library-based MST methods were largely abandoned

- Researchers turned their attention to developing new host-specific assays using qPCR
  - >50 source specific methods for fecal identification developed
  - proliferation of multiple competing methods and assays caused confusion among managers about which they should use.

- Once again, an independent method evaluation study was needed

Method Evaluation Approach

- Challenge the methods with 64 blind samples
  - Some combination of 12 different fecal source types
- 41 MST methods evaluated
  - 27 participating laboratories
- Most methods run by multiple labs to assess method repeatability
  - Its not just whether the method developers can do it
  - We needed to know whether the method is transferable to others

Sources

- Human
  - Individuals, sewage, septage
- Dog
- Gull
- Cattle
- Pig
- Horse
- Geese
- Deer
- Pigeon
- Chicken


Sacramento
Nevada
Sierra Nevada Mountains
Stanford
San Jose
California
Monterey, CA
Las Vegas
Bakersfield, CA
UCSB
UCLA
Los Angeles
SCCWRP
San Diego
Legend
Human Source
Septage
Sewage Source
Dog
Deer
Goose
Pigeon
Gull
Chicken
Horse
Pig
Cow
128 mi
© 2011 Europa Technologies
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
© 2011 Google
© 2011 INEGI
35°19'32.69" N 119°03'44.73" W elev 1014 ft
Google earth
Eye alt 519.48 mi


HANALEI SURF

Sample creation

- Feces, sewage or septage from each individual sample (varied by source) combined with artificial water
- Stirred to mix (liquids) or homogenized in laboratory blender (solids)
  - Screened through 300 micron mesh to remove largest particles
- *Enterococcus* measured by qPCR
  - Intended to inform amount of diluent needed to standardize levels of *Enterococcus*
- Equal volumes of each sample combined to create composite solution
- Solutions used as is, mixed or diluted

Logistics

- > 6,000 individual filters or water samples created
- 7 days of near continuous work
- Distribution
  - 7 US states
  - 6 European countries


DuPont
Tyvek


For Laboratory Use Only
FRAGILE
THIS SIDE UP!
50 Vials


HL-214
E

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- Murulee Byappanahalli, USGS
- Theng Theng Fong, Tetra Tech
- Maurice Larenas, Source Molecular

Classes of methods

- Presence/ Absence
  - Detect single source
  - Provide binary answer

- Quantitative
  - Detect single source
  - Provides information on concentration of source in sample

- Community
  - Detect multiple sources
  - May provide some information about relative concentration in sample

Evaluation Criteria

- Correctly identify presence/absence of a host source?
- Correctly identify the dominant source?
  - Relative contribution from each source?
- How repeatable are the assays?

Summary

	Human	Cow	Dog	Gull	Pig	Horse
Binary	HF183endpt, HF183SYBR	CF193 CowM2 CowM3 Rum2bac	BacCan	Gull2EndPt Gull2SYBR LeeSeaGull	PF163 mtPigDNA Phylochip Bac TRFLP	HoF597 Phylochip Bac TRFLP
Quant.	HF183Taqman BacH	BacR Rum2bac BacCow*	BacCan	LeeSeaGull	pig2bac	n.a.

In addition, all community methods were excellent for deer, and phylochip was excellent for chicken.

Outcome

- Identified methods that were both specific and sensitive for five key fecal sources:
  - Human
  - Dog
  - Pig
  - Cow
  - Gull

- Consensus among scientific community
  - Almost every key scientist in the field participated
  - Brought them together to help develop the conclusions
  - Water Research dedicated an entire journal issue to the study
  - Achieved a level of consensus that is rare in science

Road Map

- Origins of Microbial Source Tracking
- 2003 MST Method Evaluation Study
- 2013 Source Identification Pilot Project
- **California Source Identification Manual**
- Which molecular MST method should I use?
- Differentiating Human Sources of Fecal Contamination

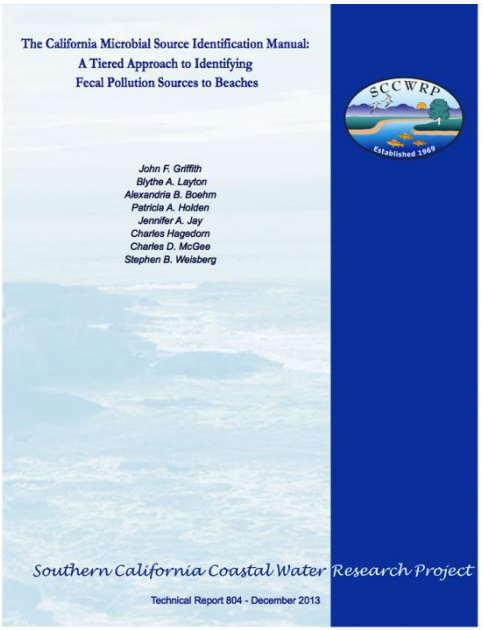
Source Identification manual

- 2013 MST Method Evaluation was part of a larger effort to provide guidance to those seeking to carry out MST studies.
  - Test drove the methods: Conducted demonstration projects at 3 beaches
  - Technology transfer: Trained local labs to perform the methods

- Captured what we learned into a written guidance document
  - How does a beach manager get started?

- Extensive outside review
  - California Clean Beach Task Force
  - State Water Board Beach Water Quality Workgroup
  - US EPA

- A tiered approach
  - Hypothesis-driven
  - Start with less costly methods to localize and refine the problem
  - Use more expensive methods in a focused manner



Road Map

- Origins of Microbial Source Tracking
- 2003 MST Method Evaluation Study
- 2013 Source Identification Pilot Project
- California Source Identification Manual
- **What's the difference between molecular MST methods?**
- Differentiating between Human Sources of Fecal Contamination

Molecular MST Methods

- There are multiple gene amplification methods that can be used for microbial source track applications

- qPCR is by far the most common
  - All quantitative methods in the 2013 MST method evaluation study used qPCR
  - EPA-approved quantitative MST methods use qCPR

- digital PCR uses existing qPCR primer/probe sets
  - More sensitive and less prone to inhibition from environmental constituents
  - BioRad droplet digital PCR (ddPCR) is the most common variant
  - Qiagen and Thermo also offer chip-based dPCR
  - All can generally use the same primers and probe as qPCR assays

Other Molecular Methods for MST

- Isothermal methods
  - Loop-mediated isothermal amplification (LAMP) < 250nt
  - Nucleic acid sequenced based amplification (NASBA) <150 nt
  - Transcription mediated amplification (TMA) < 150 nt

- Clustered regularly interspaced short palindromic repeat (CRISPR)
  - Highly sensitive and quantitative when digitized
  - Multiple medical diagnostic tests on the market
  - Just beginning to be explored for MST in food safety sector
  - High potential for environmental MST
  - CrAss phages may be an opportune target, but a wider array of gene sequences from *Bacteroides* will likely be necessary

Road Map

- Origins of Microbial Source Tracking
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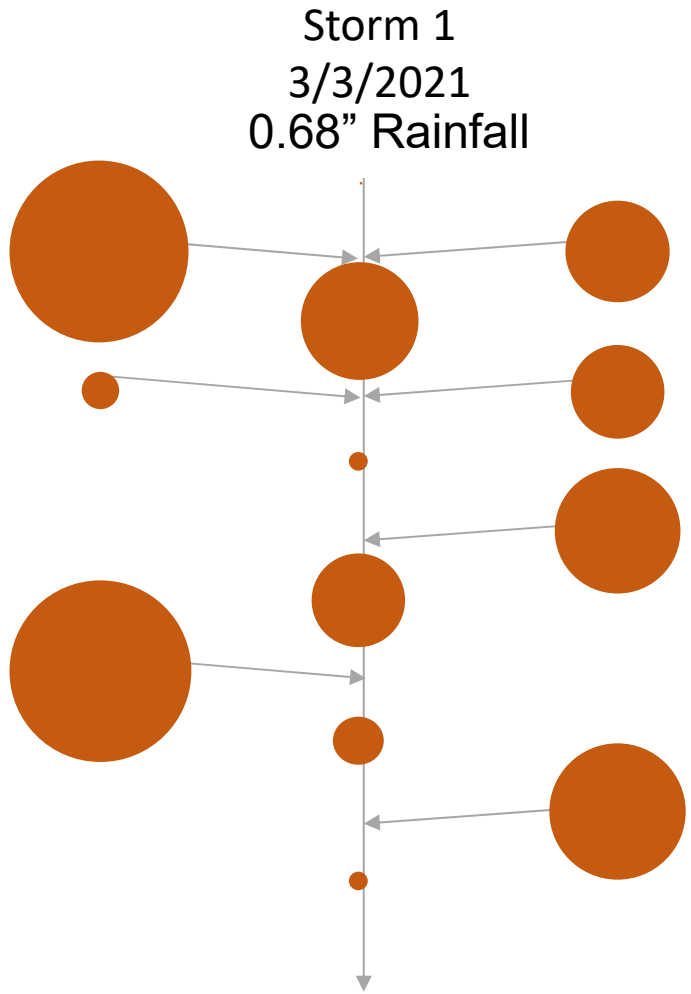
- 2013 Source Identification Pilot Project
- California Source Identification Manual
- What’s the difference between molecular MST methods?
- **Differentiating between Human Sources of Fecal Contamination**

Biofilm Microbial Community Source Tracking Background

- Human markers in stormwater don't distinguish between different human sources
- Sewer community biofilms are a potential way to track sanitary sewer signal in stormwater
- Goal was to develop and test this tool in the San Diego River Watershed



Biofilm Microbial Community Source Tracking Tool Questions

- Are biofilm microbial communities different between sanitary sewer and storm drains?
- How sensitive and persistent are biofilm community signals?
- Do we see these signals in wet weather samples?

Biofilm Microbial Community Source Tracking Tool Questions

- Are biofilm microbial communities different between sanitary sewer and storm drains?

  Yes!

- How sensitive and persistent are biofilm community signals?

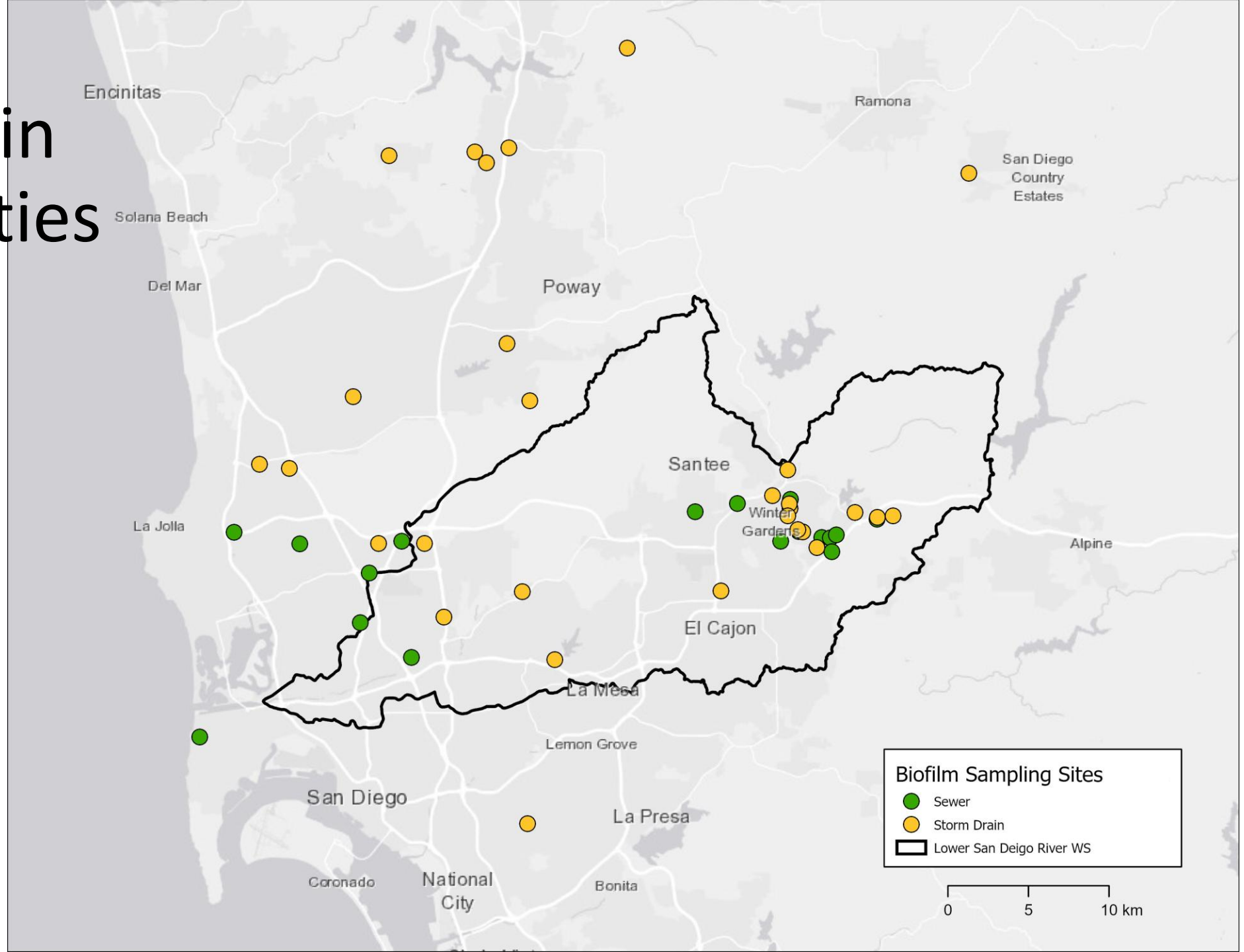
  Persistent across years and seasons

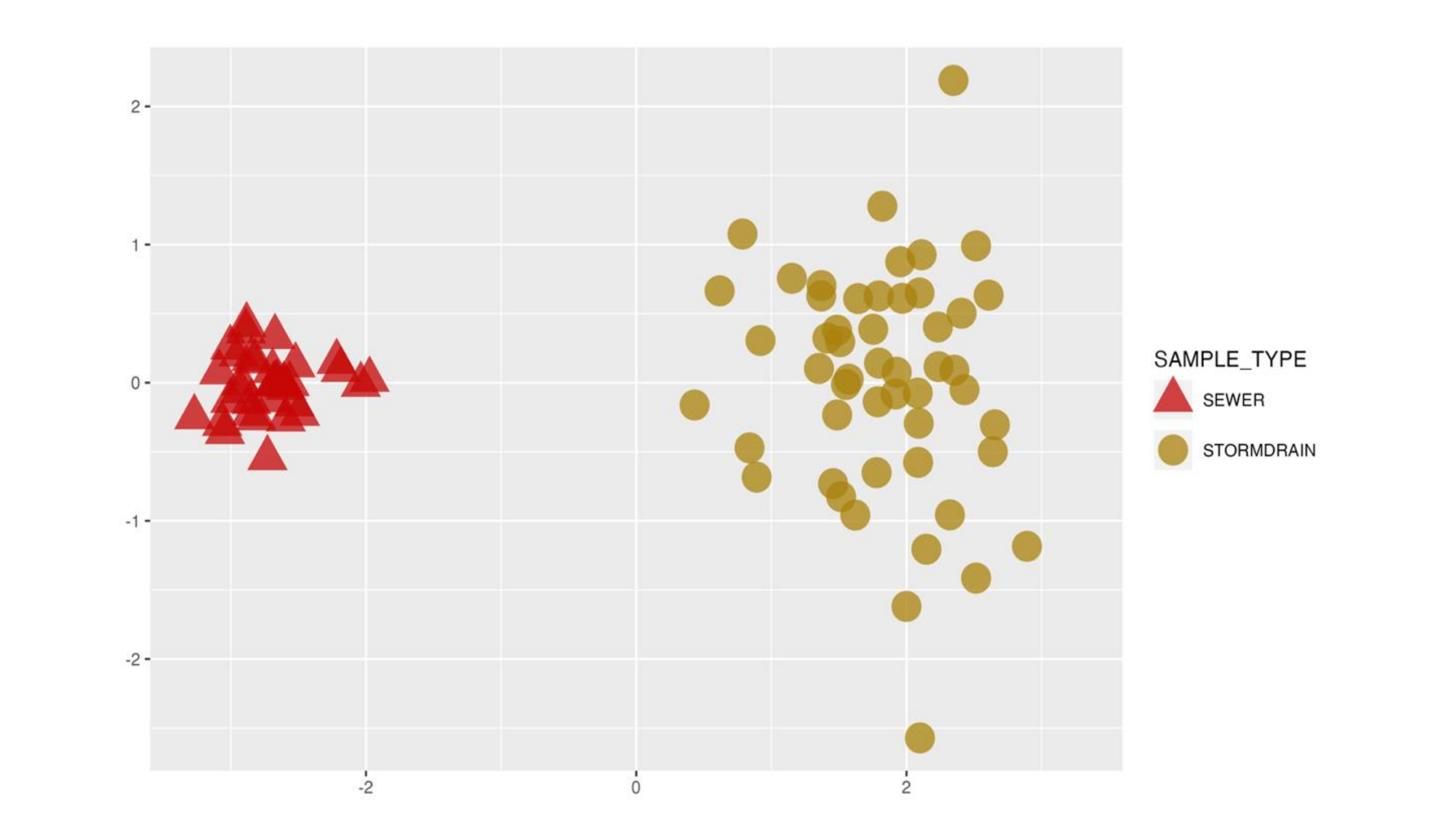
  Sensitive enough to apply to stormwater

- Do we see these signals in wet weather samples?

  Yes, a widespread, dilute signal

Sewer vs storm drain communities




2
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-2
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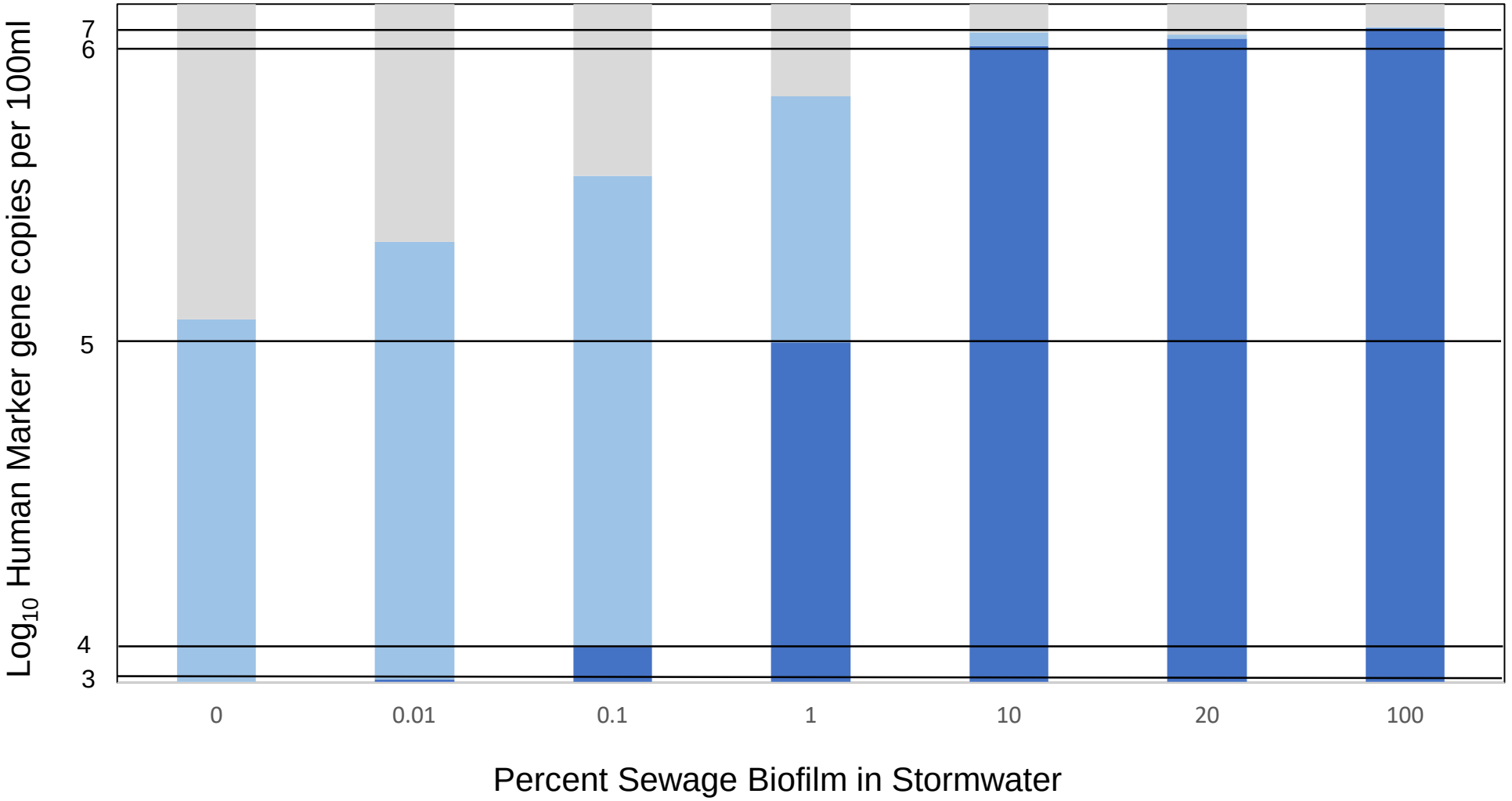
2
SAMPLE_TYPE
SEWER
STORMDRAIN

Sensitive Detection of Sewage Biofilm in Stormwater - Sourcetracker



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- Do we see these signals in wet weather samples?

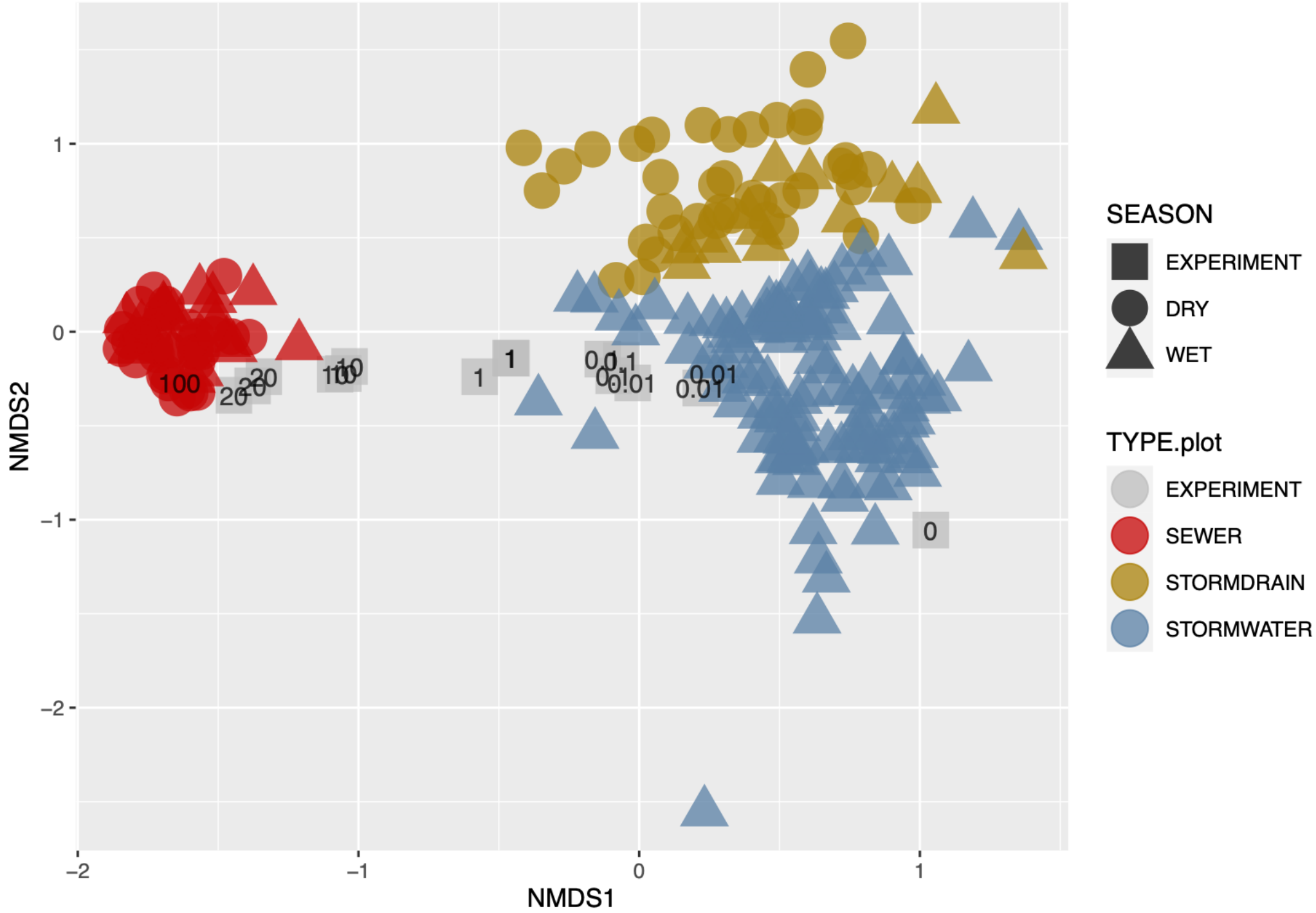
  Yes, a widespread, dilute signal

Wet Weather Biofilm Screening Approach

- Previous 13 sites along mainstem and major tributaries
- Sampled 3 storms using grab sampling
- Measured HF183 and microbial community biofilms
  - HF183 confirms there is a human source in stormwater
  - If sewer biofilm community signal is found, determine strength of the signal

Strength of Sewer Biofilm Signal

- Used sensitivity experiment to calibrate sewer biofilm community abundance to experimental dilution
- Categorized sewer biofilm proportion in experiment and stormwater samples using SourceTracker
- Compare proportion in stormwater samples to experimental samples to estimate strength of signal


NMDS2
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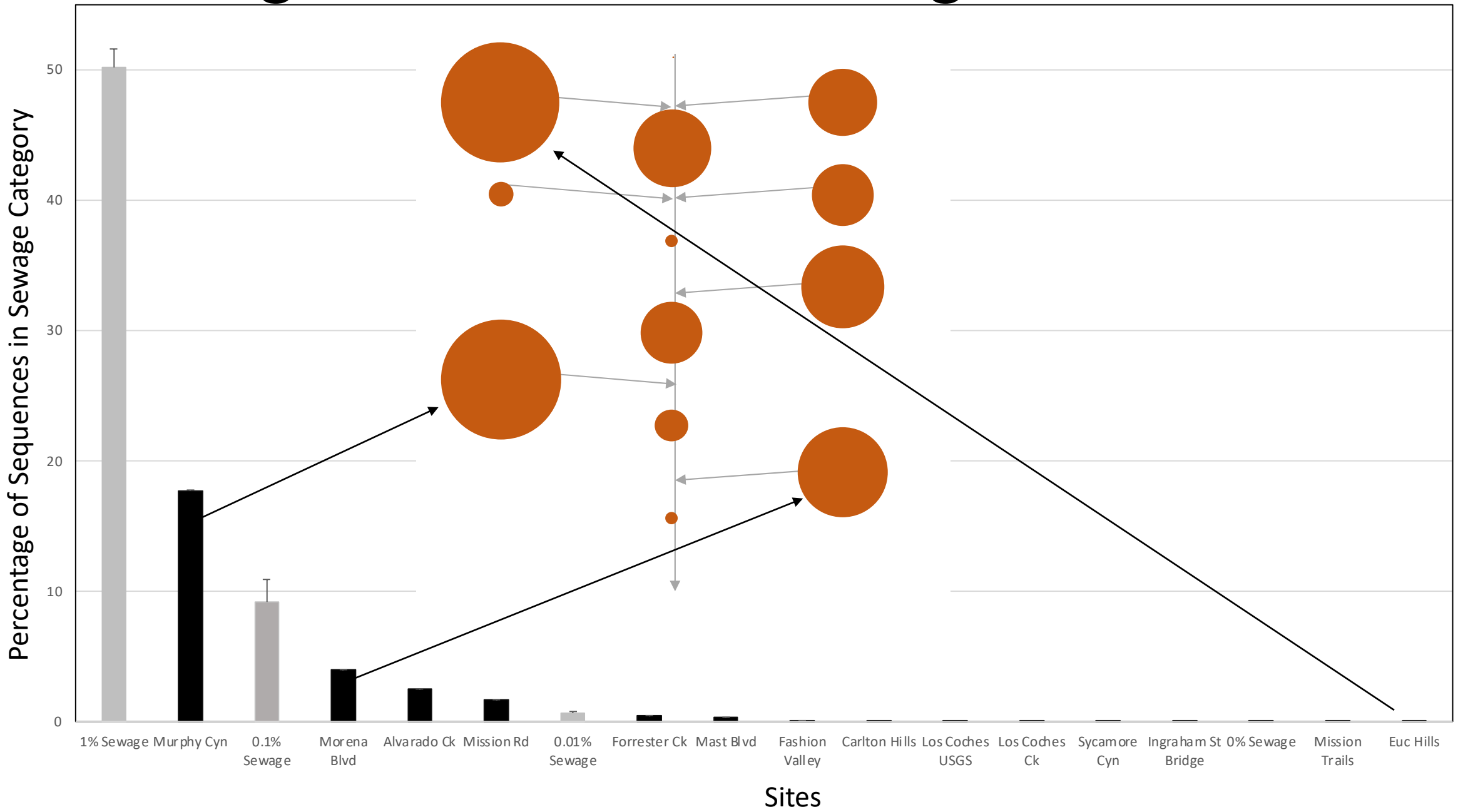
SEWER
STORMDRAIN
STORMWATER

Strength of Sewer Biofilm Signal - Storm 1



Wet Weather Biofilm Results Highlights

- Sewer biofilms detected in nearly all stormwater samples
- Sewer biofilm signal was dilute
  - < 0.01% to 1%
- Measured HF183 in storm samples at concentrations ranging from 10^1 - 10^4 /100ml

Citations

Griffith, J.F., Weisberg, S.B., and C.D.McGee. (2003) Evaluation of microbial source tracking methods using mixed fecal sources in aqueous test samples. Journal of Water and Health. 1: 141-151

Alexandria B. Boehm, Laurie C. Van De Werfhorst, John F. Griffith, Patricia A. Holden, Jenny A. Jay, Orin C. Shanks, Dan Wang, Stephen B. Weisberg (2013), Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. Water Research. 47: 6812-6828

Microbial Source Tracking Manual

http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/804_SIPP_MST_ManualPag.pdf

johng@sccwrp.org

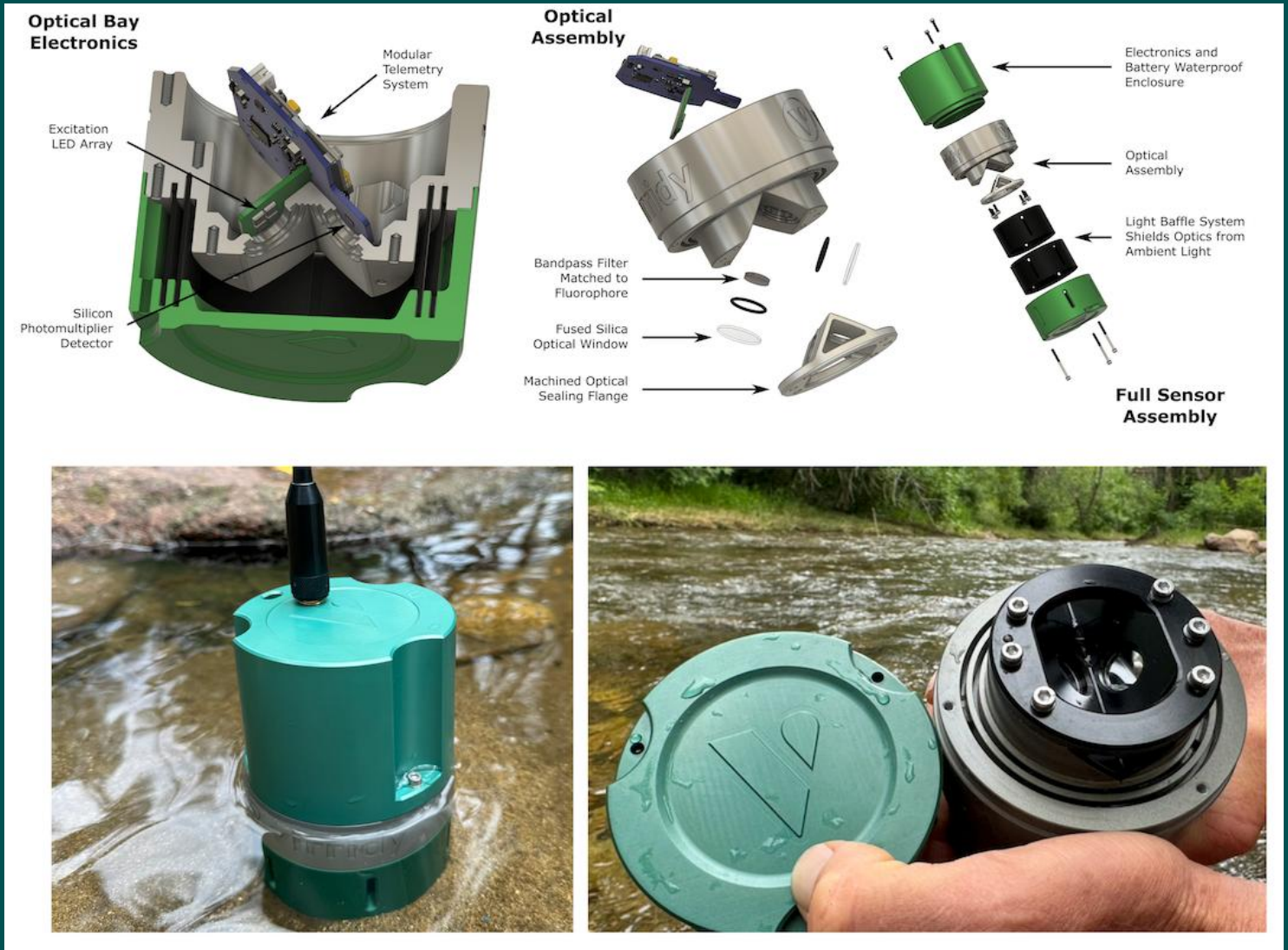
Puget Sound Microbial Source Tracking Workshop
September 12, 2025

The Virridy Lume

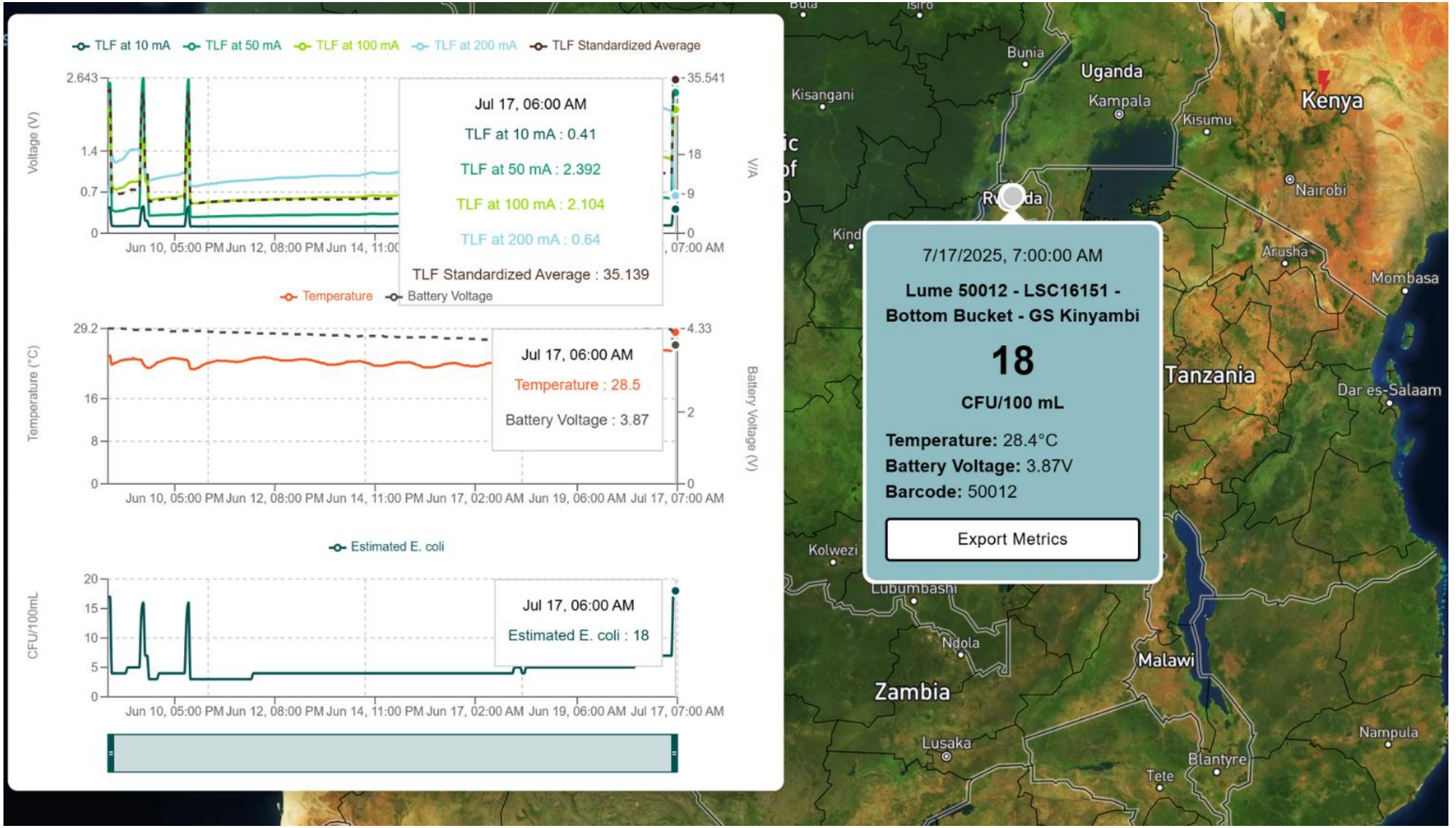
Virridy has developed the Lume™ for the measurement of fecal contamination risk in water.

This technology is the first to demonstrate a fully integrated in-situ, autonomous, internet-connected fecal risk sensor.

Puget Sound Microbial Source Tracking Workshop
September 12, 2025



Realtime E. Coli Monitoring



Virridy Presents the Lume

Microbial Monitoring Technology

- Virridy's **Lume** sensor uses tryptophan-like fluorescence as a proxy for microbial activity
- Readings are fused with machine learning analytical models to predict *E. coli* contamination/risk
- Validated and applied by University of Colorado Boulder Mortenson Center

Key features:

- Outputs quantification of *E. coli* not RFU
- Does not require regular calibration
- Is designed continuous in-situ use
- Retails for considerably lower cost

Lume Performance

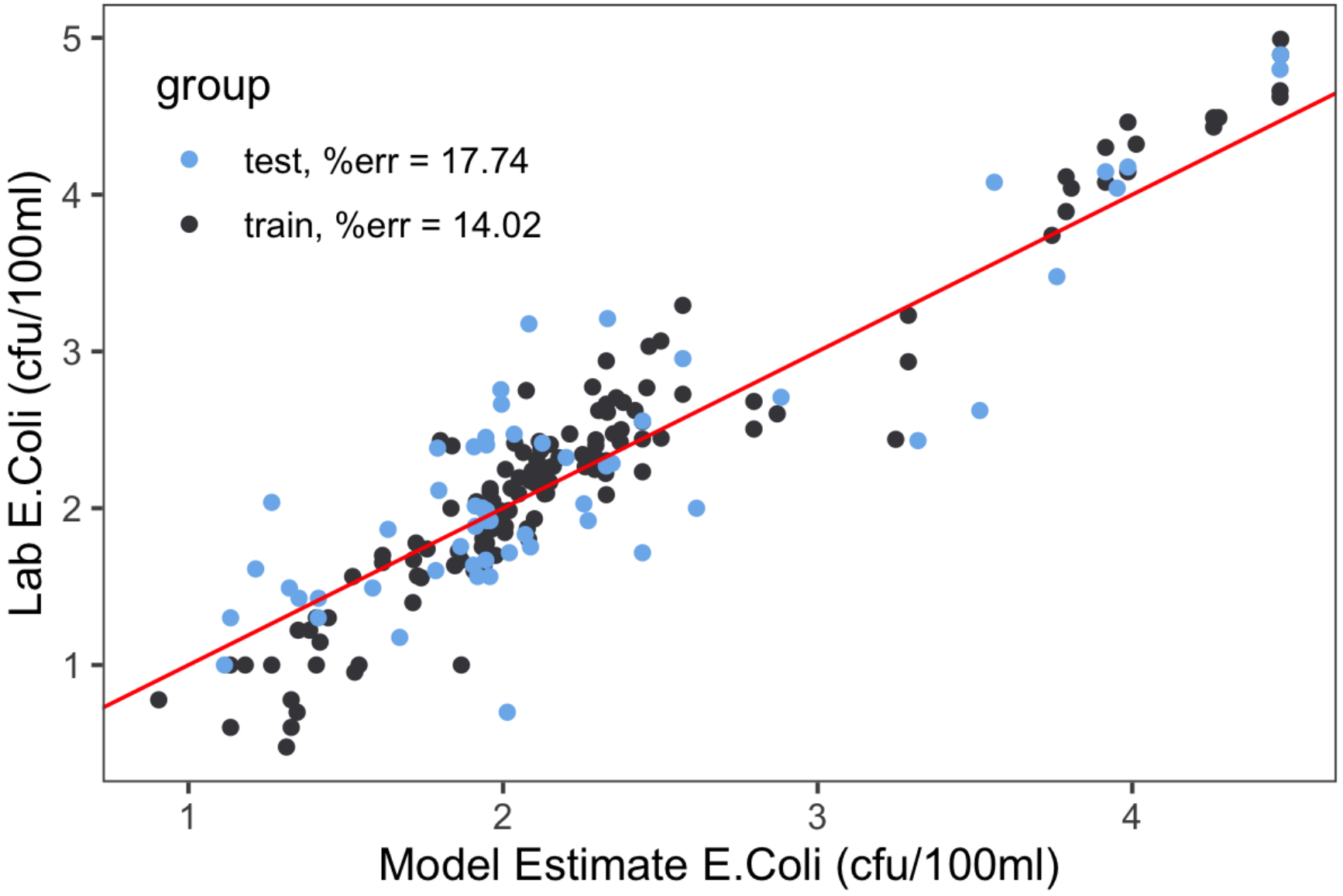


Figure 1. Model estimates vs. lab measurements; approaching lab/regulatory standards

Pilots and Applications

Freshwater Recreation

- Boulder Creek, *City of Boulder*, CO
- Calumet River, *H2NOW/Current*, Chicago, IL
- Marne and Seine, *City of Paris*, France
- Euclid Creek, *Cleveland Water Alliance*, Cleveland, OH

Brackish Water Recreation

- Manchester Bay, *Blues*, West Manchester, MA
- Tijuana River Estuary, *SDSU/EPA*, Sandiego, CA (future)

Rural Drinking Water

- Primary Schools, Lifestraw, Kigali, Rwanda
- Groundwater systems, Millennium Water Alliance, Northern Kenya

Virridy Presents the Lume

Puget Sound Microbial Source Tracking Workshop
September 12, 2025

Thanks!

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Q&A

MST in Skagit County

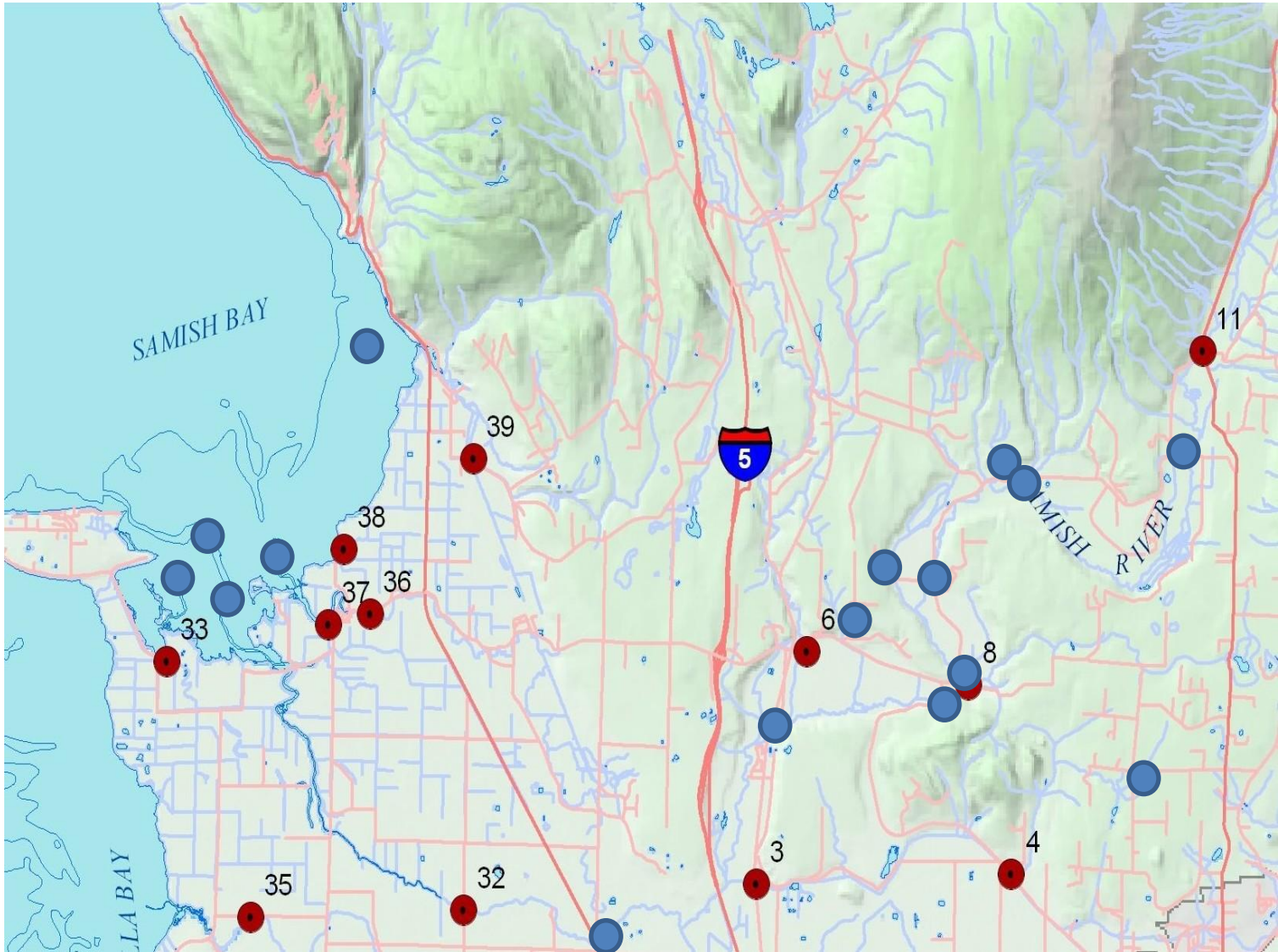
Karen DuBose
PIC Coordinator
Skagit County
September 12, 2025

Background

- 123 square mile watershed
- Known problems since ~2007
- 2010 downgrade of Samish Bay shellfish growing area

2010 Experience

- Sample sites had large catchments
- Ambient sampling
- Human, ruminant, gull, horse, dog, & bird markers



2010 Results

Found:

- Ruminant (58%)
- Avian (54%)
- Human (22%)
- Dog (one site)
- Other markers (57%)

Not found:

- Horse
- Gull

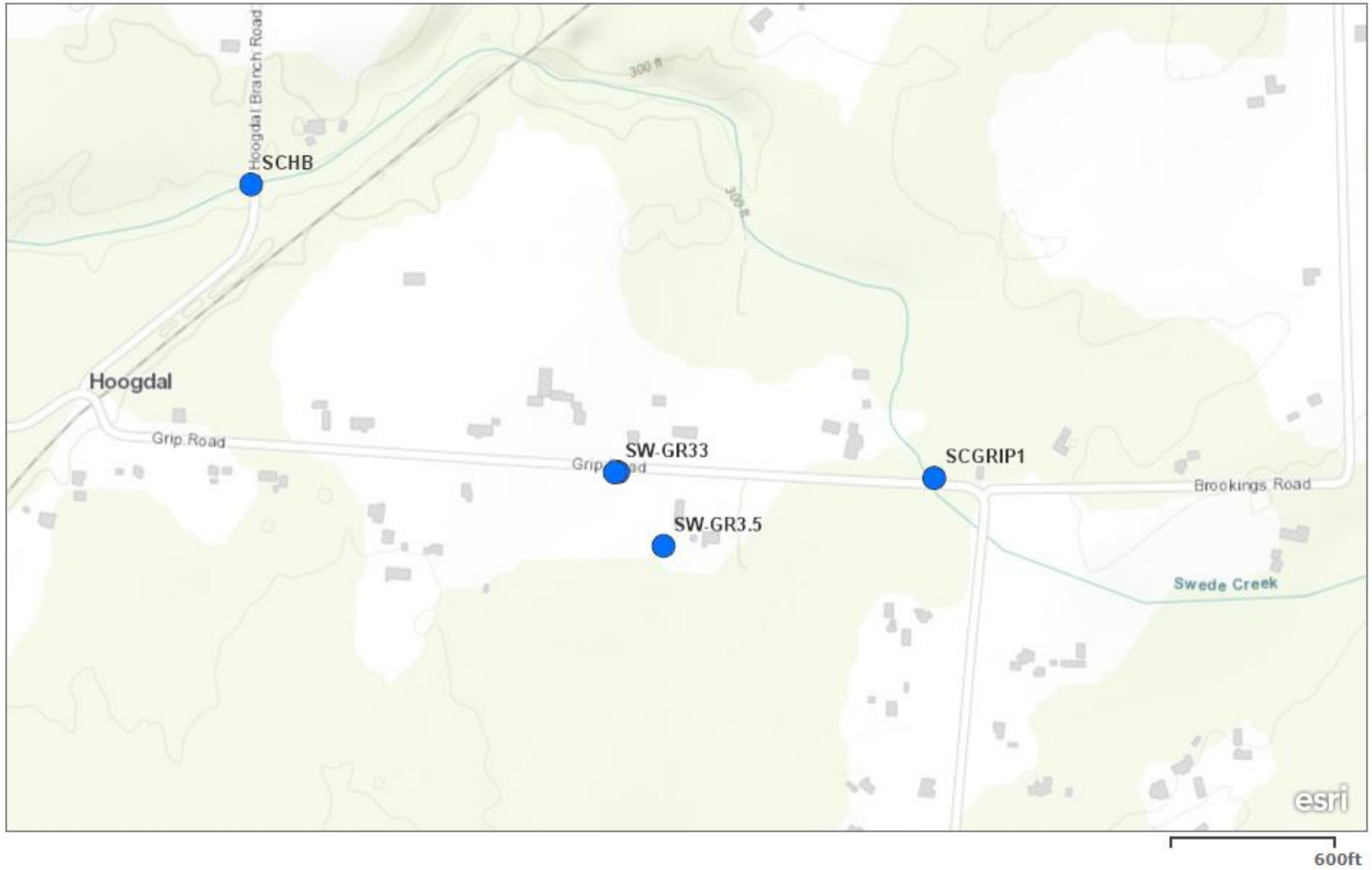
What we gained

Proof of cattle (ruminant) and human problems.

BUT…little to no insight on where problems were.

2019-21 Experience

- Focus on smaller areas
- Storm-focused
- Cow, ruminant, human, dog



2019-2021 Results

Found:

- Evidence to push for enforcement at a few sites
- Human & cattle markers scattered around
- Dog markers EVERYWHERE
- No markers in summer in Thomas Creek

Pros & Cons

Pro:

- Useful as a big picture tool
- Science that people seem to trust
- Results from small areas can help narrow down potential sources

Con:

- Expensive in labor & cost
- Storms can’t be scheduled
- Results often inconclusive

Microbial Source Tracking in King County

Puget Sound Microbial Source Tracking Workshop

September 12, 2025


Sampling outfall to Issaquah Creek

Program purpose

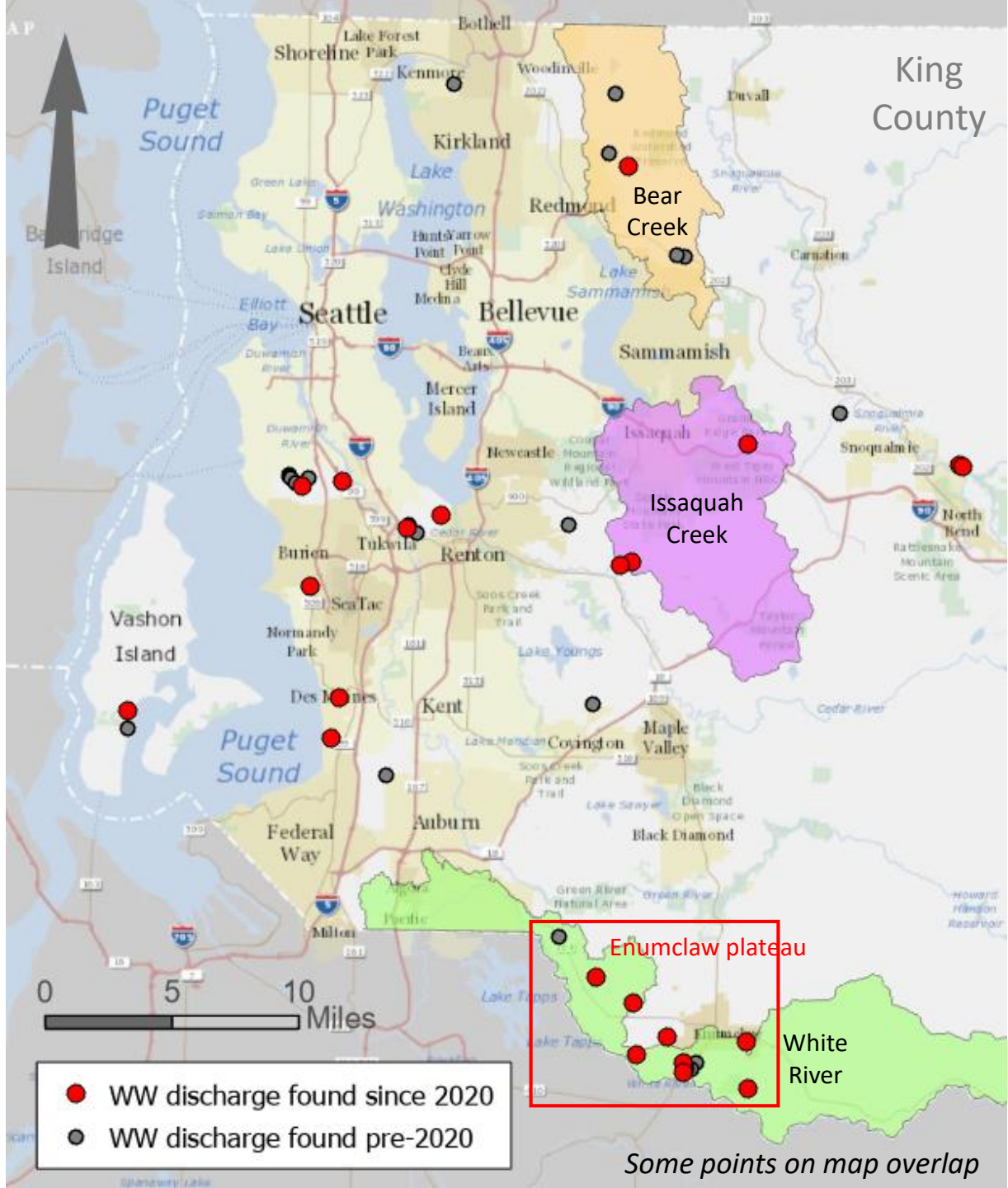
- King County Stormwater
  - Dept. of Natural Resources + Parks
- Illicit discharge detection and elimination (NPDES permit)
- Emphasis on bacterial discharges (impaired water bodies)
  - Emphasis on human fecal waste

Problems identified – proactive screening

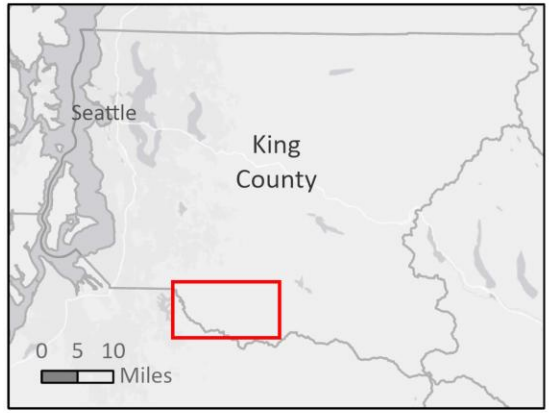
Since 2020, human wastewater discharges identified at:

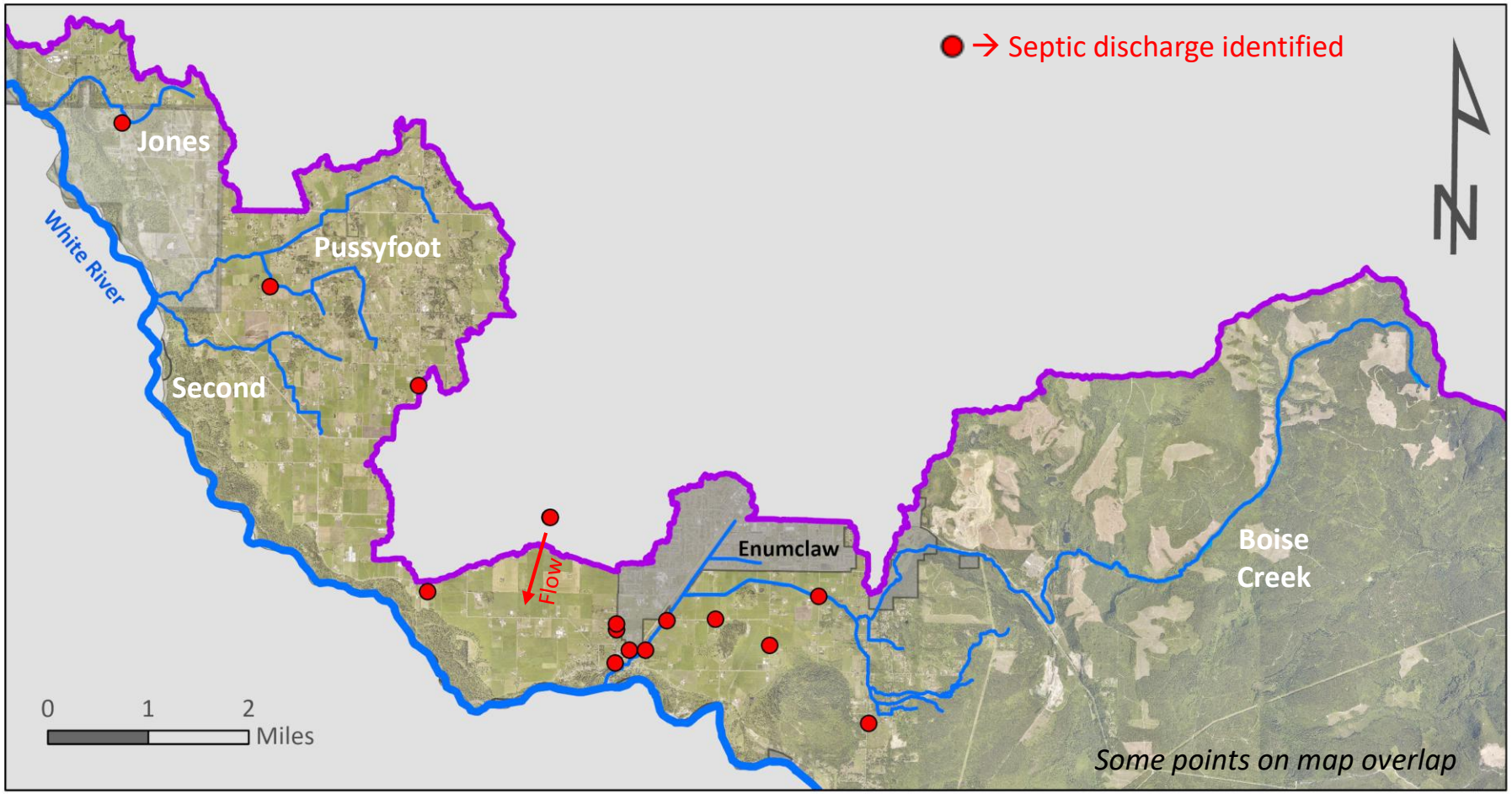
- 25 residences (mostly OSS, some sewage cross connections)
- 1 apartment complex
- 1 mobile home park

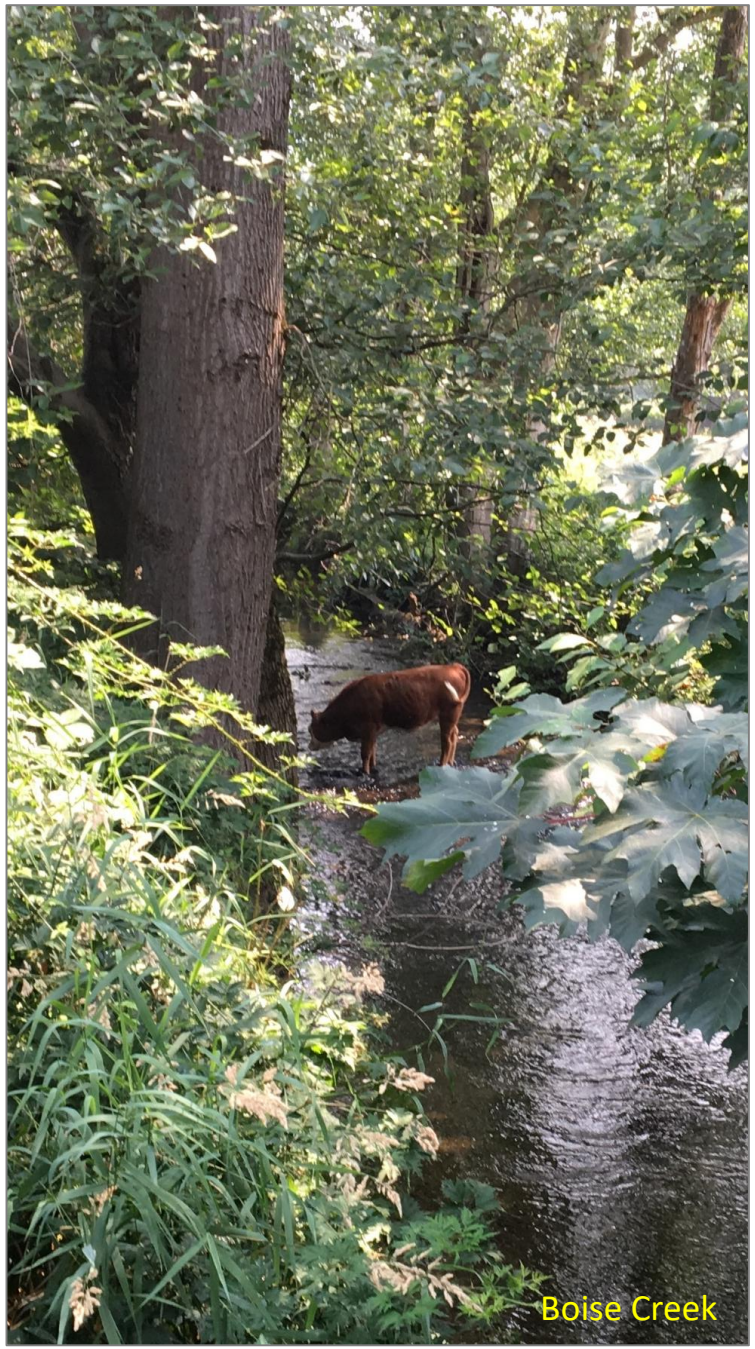
(Does not include discharges found based on tips from citizens and agency partners)

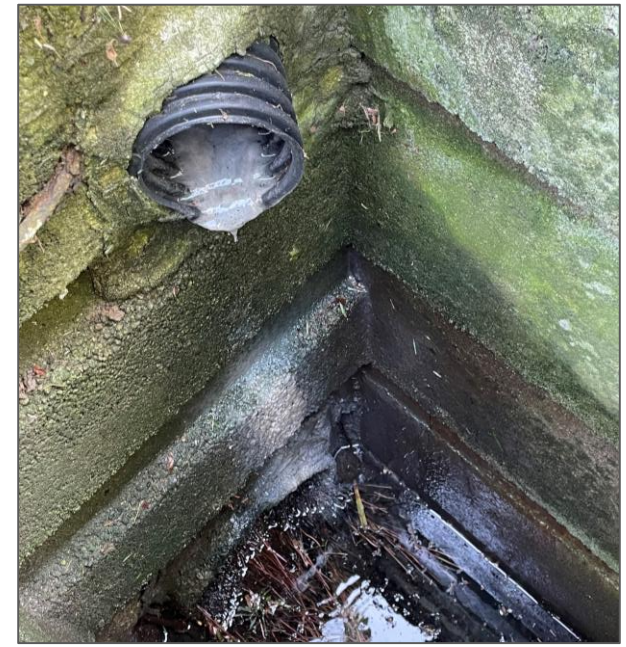


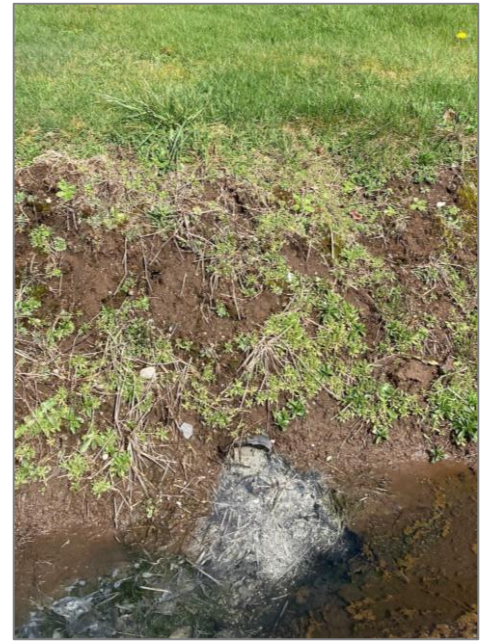
Enumclaw plateau/ White River

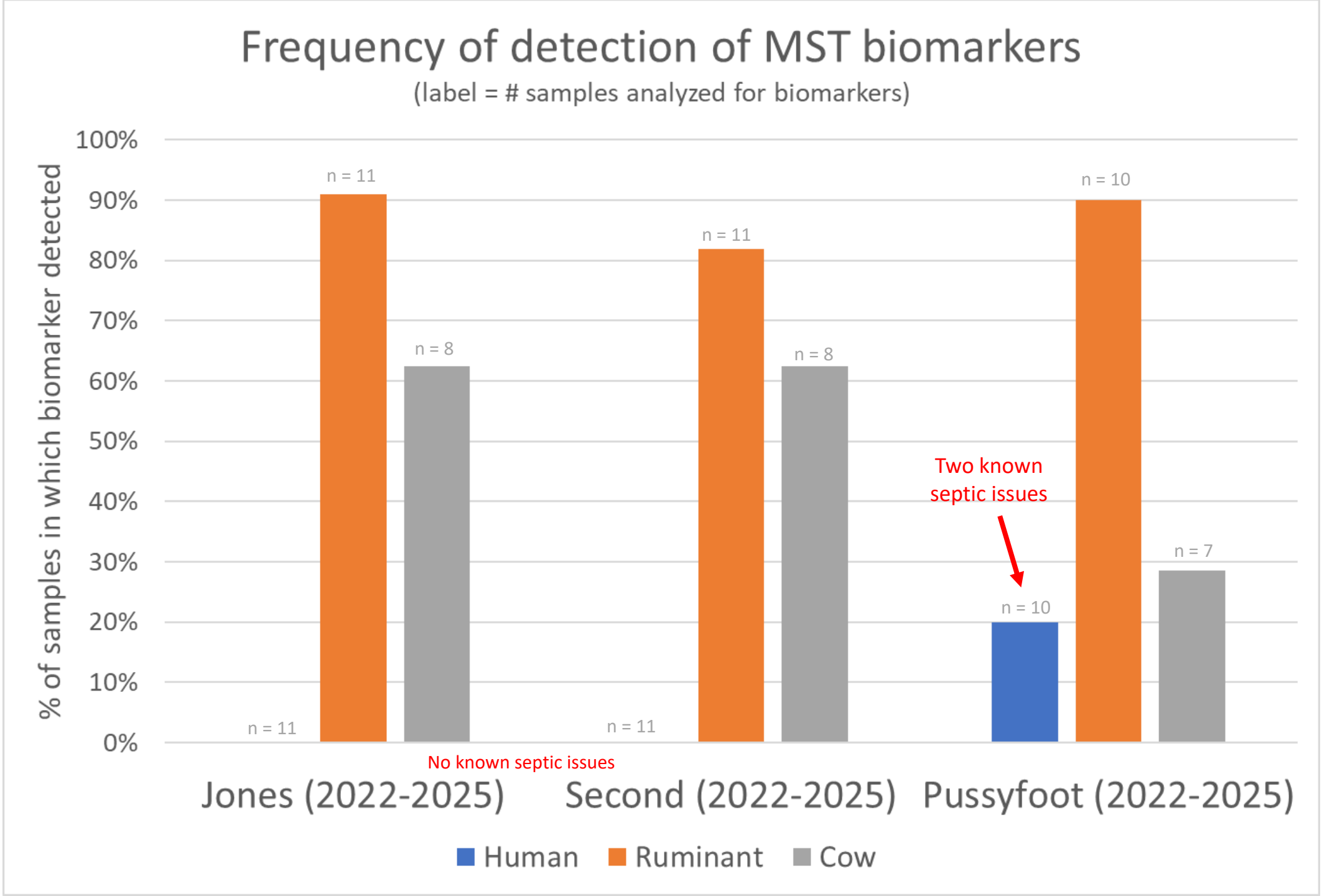





Boise Creek





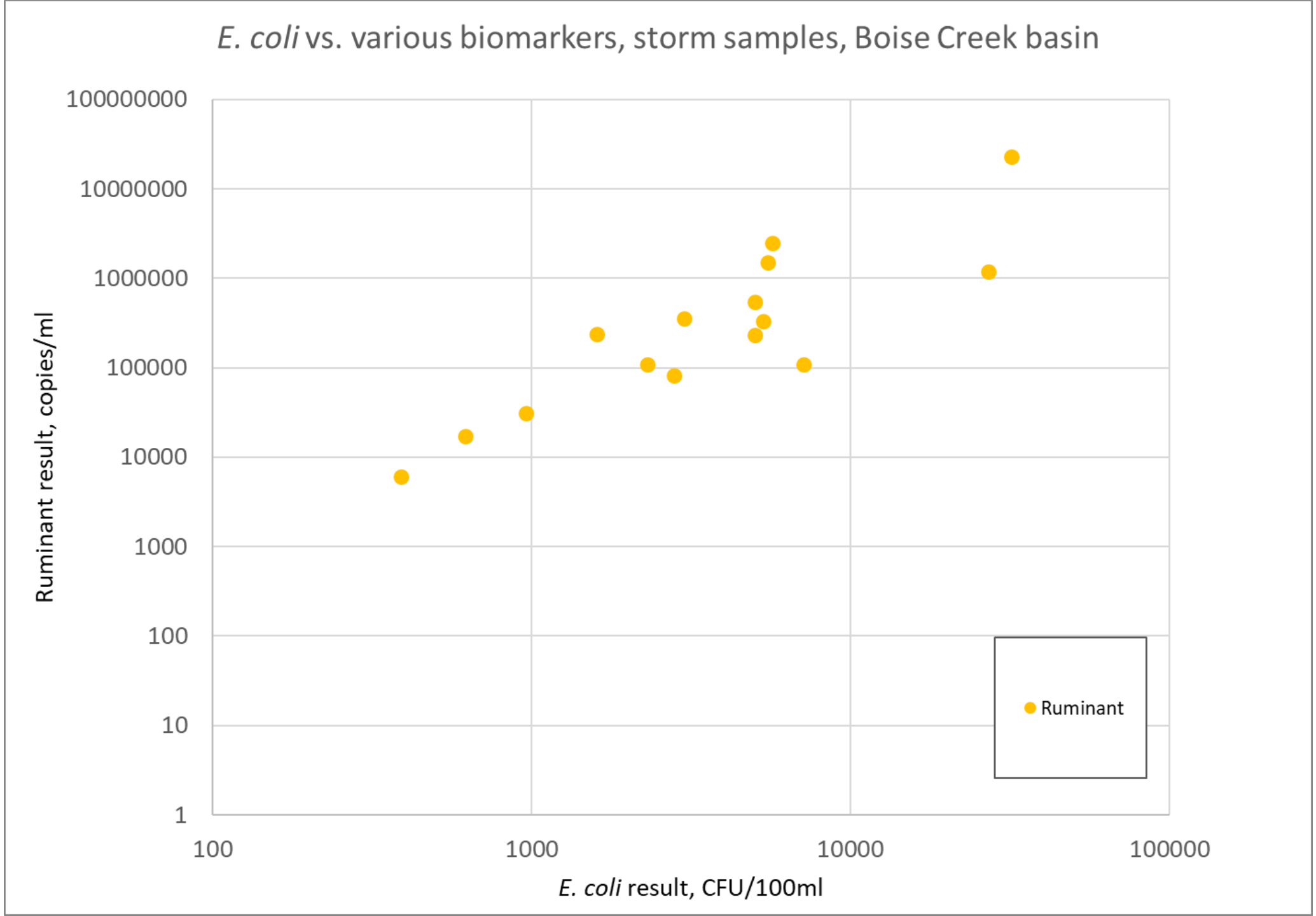

Frequency of detection of MST biomarkers
(label = # samples analyzed for biomarkers)
% of samples in which biomarker detected
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
n = 11
n = 11
n = 8
n = 11
n = 11
n = 8
n = 10
n = 10
n = 7
Two known septic issues
No known septic issues
Jones (2022-2025)
Second (2022-2025)
Pussyfoot (2022-2025)
Human
Ruminant
Cow

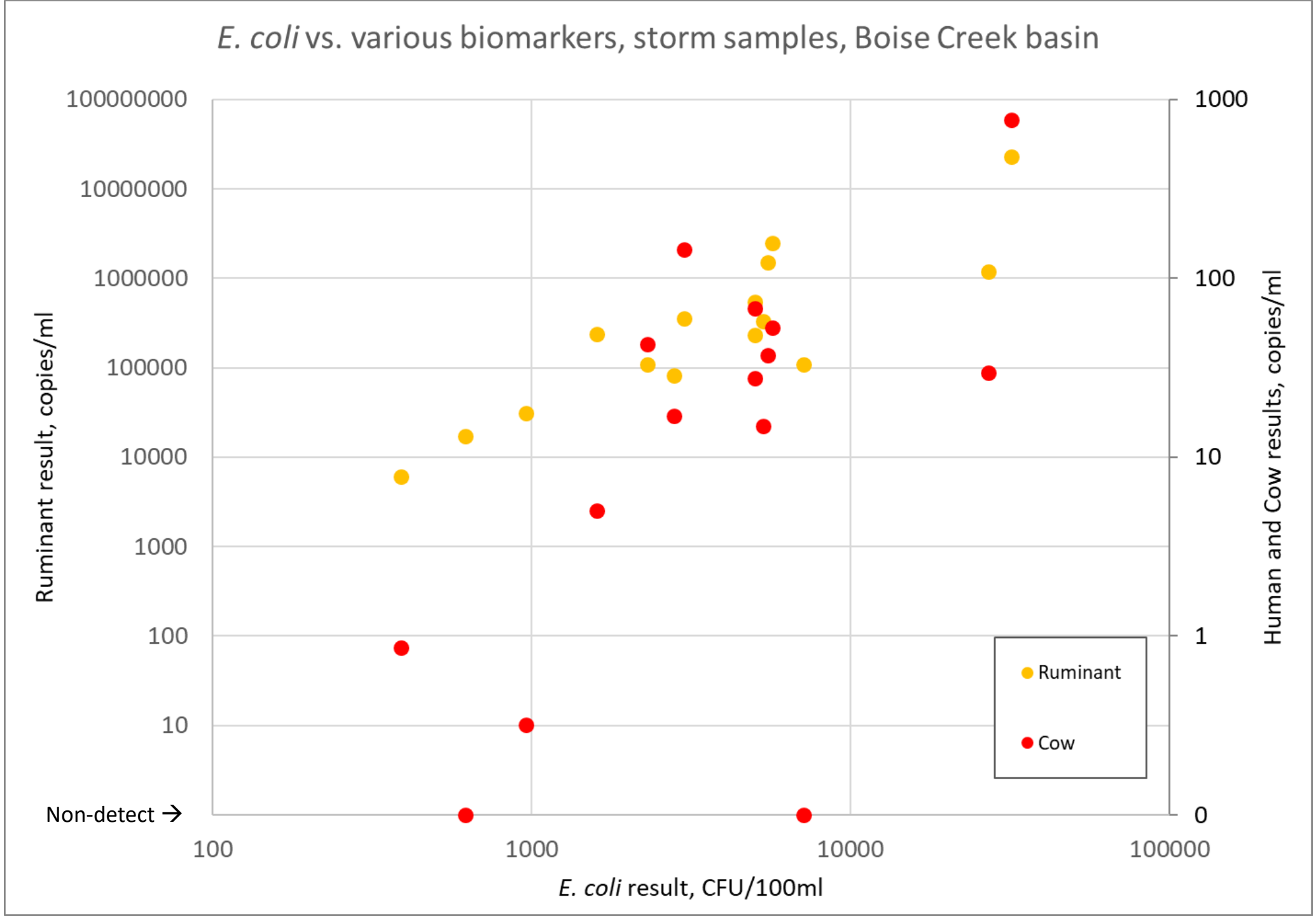
Ruminant marker in other stream basins (2019-2023)

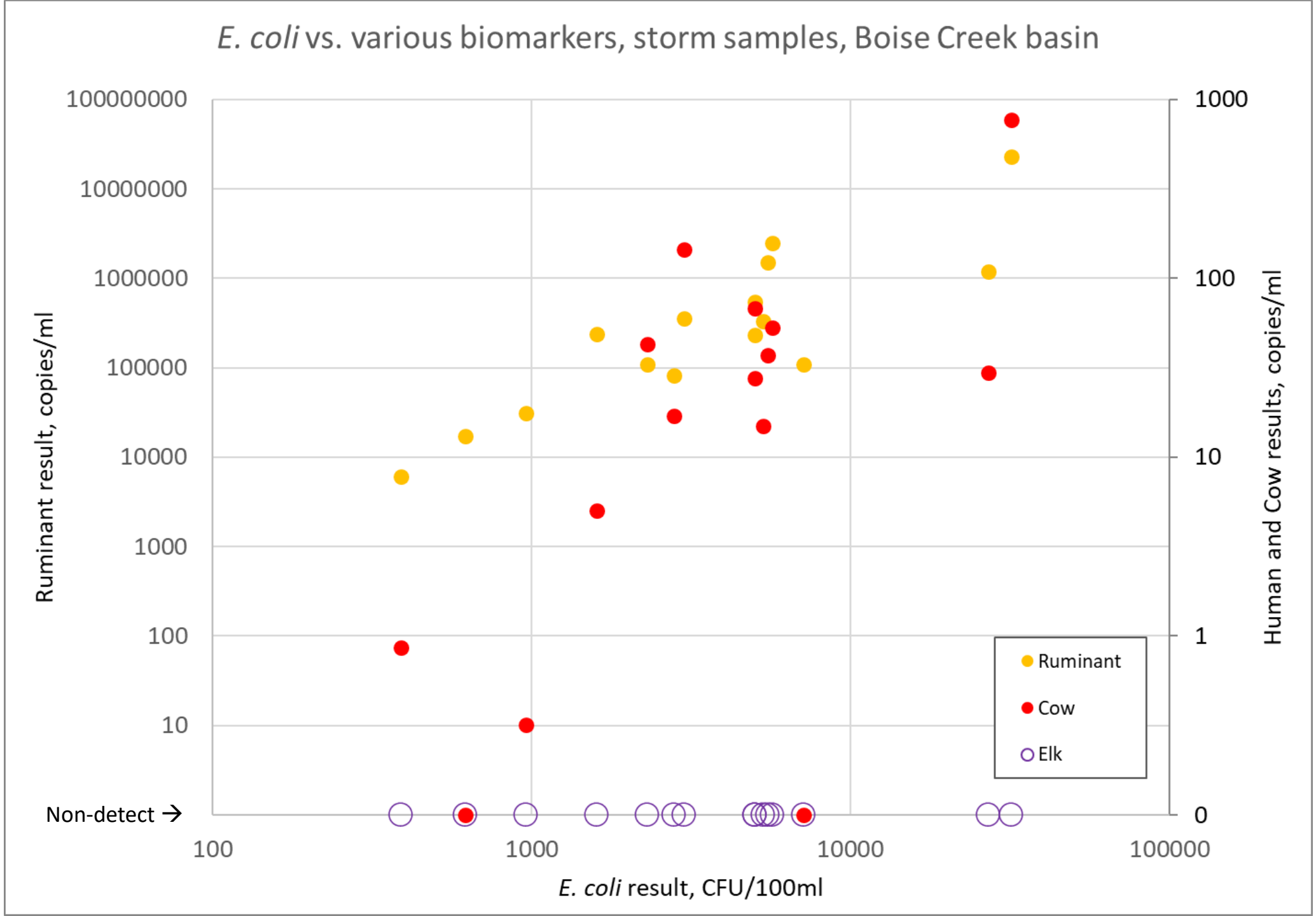
- Issaquah tributaries: detected in 1 of 18 samples
- Bear tributaries: detected in 0 of 63 samples

North Rainier Elk Herd

Herd of 30 elk in farm field near Boise Creek (Enumclaw plateau)


E. coli vs. various biomarkers, storm samples, Boise Creek basin
100000000
10000000
1000000
100000
10000
1000
100
10
1
Ruminant result, copies/ml
100
1000
10000
100000
E. coli result, CFU/100ml
Ruminant


E. coli vs. various biomarkers, storm samples, Boise Creek basin
Ruminant result, copies/ml
Human and Cow results, copies/ml
E. coli result, CFU/100ml
Non-detect →
100000000
10000000
1000000
100000
10000
1000
100
10
100
1000
10000
100000
1000
100
10
1
0
Ruminant
Cow


E. coli vs. various biomarkers, storm samples, Boise Creek basin
Ruminant result, copies/ml
Human and Cow results, copies/ml
E. coli result, CFU/100ml
100000000
10000000
1000000
100000
10000
1000
100
10
Non-detect →
1000
100
10
1
0
100
1000
10000
100000
Ruminant
Cow
Elk

Non-human biomarkers - KCEL

- Ruminant (Bacteroidales)
  - Developed in France, permission to use without legal restrictions
- Cow (two) (Bacteroidales)
  - Developed at EPA (Shanks *et al*.), KCEL has contract to use (only for County projects)
- Dog (two) (Bacteroidales)
  - Developed at EPA (Shanks *et al*.), KCEL has contract to use (only for County projects)
- Avian (*Helicobacter*)
  - KCEL found qPCR primer/probe set in literature and made modifications
  - No limitation on use (not an EPA-licensed assay)

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- Public Health – Seattle & King County
- Washington State Department of Ecology
  SWRO non-point WQ specialists
- Washington State Department of Agriculture
- Skyway Water and Sewer District
- King Conservation District
- City of Enumclaw
- King County WLRD and King County PIC program

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Thank you!

Whatcom County MST Study

Cynthia May, Water Quality Data Coordinator, Whatcom Conservation District

Puget Sound MST Workshop

September 12, 2025

Previous MST Efforts

- **1998 Ribotype Study**
  - Limited local reference library
  - Marine sites only
  - Spring season only
- **2006-07 Host Specific Polymerase Chain Reaction (HSPCR) Study**
  - Marine sites mostly
  - 6 months only
- **2016 PCR Study**
  - Full year
  - 7 freshwater, 1 marine
  - Very few biomarkers were detected, no local reference samples were collected to confirm method can detect sources in our local region
- **2019 Fecal Source Reference Catalog (Whole Genomic Sequencing)**
  - 29 species
  - First phase of method development = promising
  - Tool needs more development for confident source ID

Whatcom County, WA

23 sites

4 watersheds

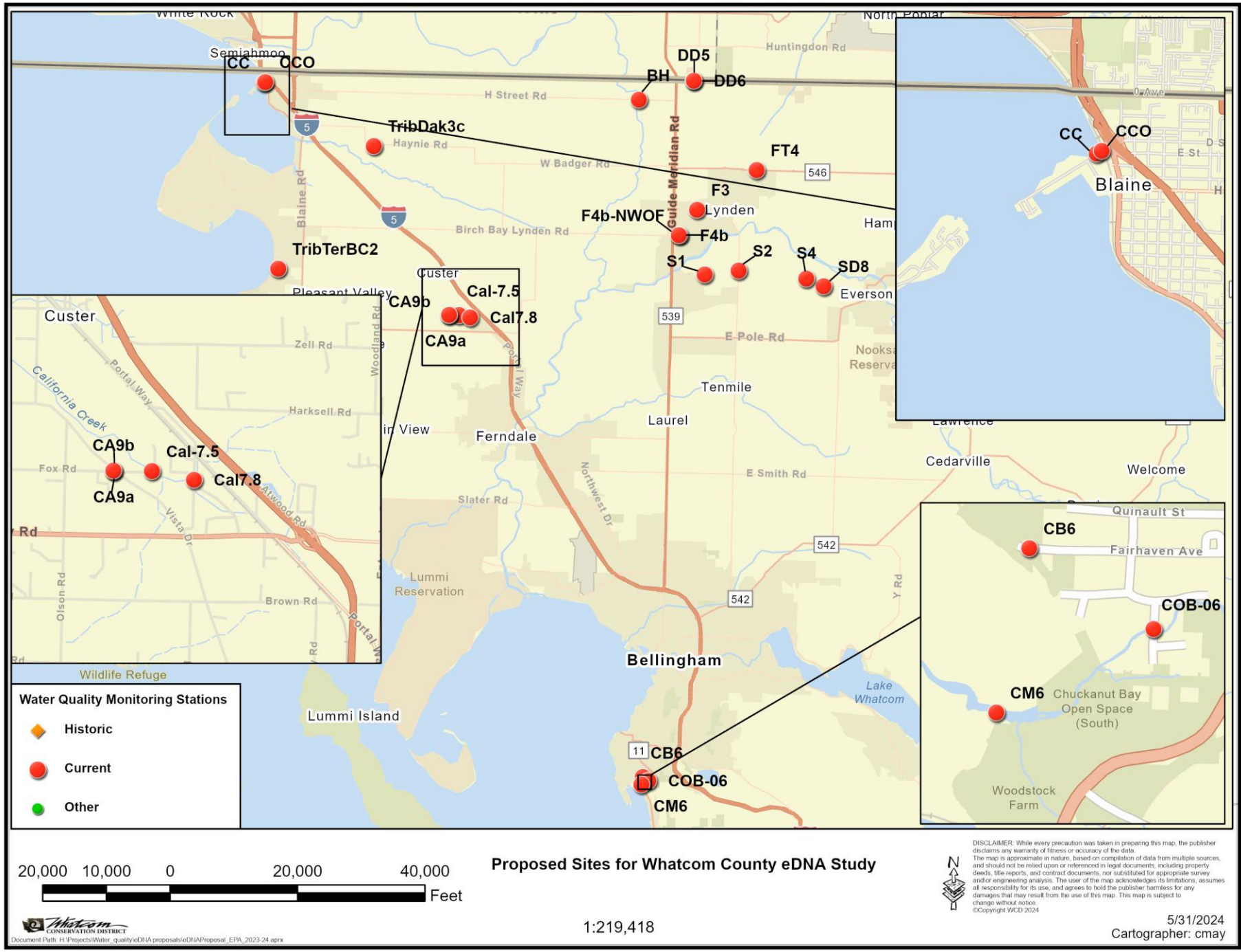
Biomarkers:

Human(2)

Cattle (2)

Non-cattle ruminant (1)

Dog (1)



Project Improvements

- **qPCR method**: faster, quantitative, widely validated
- **Seasonal sampling**: year-round with extra in high-count season
- **Site focus:** smaller drainages with suspected sources
- **Reference samples**: confirm EPA methods work for local strains

Anticipated Project Benefits

- **Identify** mystery sources
- **Track** DNA marker concentrations across time and space
- **Interpret** results when no markers are detected
- **Evaluate** biomarker performance

Management Actions

- Transboundary coordination
- Coordinated follow-up actions by source:
  - **Human**: septic system follow up
  - **Cattle**: dairy follow up
  - **Dog**: pet owner outreach
  - **Non-cattle ruminant**: goat/sheep owner follow up
  - **No markers detected**: homeowner outreach about wildlife

This project has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement PC-01J18001-6 to the Washington State Department of Health. The contents of this document do not necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

GEMSTONE

Genomic Ecological Microbial Source Tracking for Oceans, Nature and the Environment

BC Centre for Disease Control

Natalie Prystajecky (Microbiology)
Michael Lee (Epidemiology)
Lorraine McIntyre (Food Safety)

BC SHELLFISH GROWERS ASSOCIATION

Nico Prins (Executive Director)

Malahat

Andrew Sheriff (Fisheries Coordinator)

BC Centre for Disease Control

We gratefully acknowledge that we live and work on the traditional unceded territory of the Coast Salish Peoples, including the Squamish, Musqueam, Tsleil-Waututh First Nations and the Katzie First Nation.



BC SHELLFISH GROWERS ASSOCIATION

Malahat

Challenge: frequent closures without understanding the sources of pollution.

GEMSTONE OVERVIEW

Assay validation

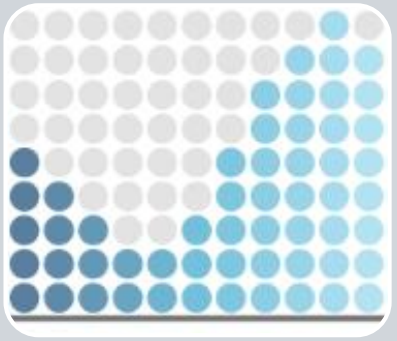
- MST
- Bacterial indicators
- Norovirus

Longitudinal Testing

- 1 year, 3 sites
- Biweekly and surge sampling

Geospatial data

- Climate
- Vessel
- Satellite



Data integration

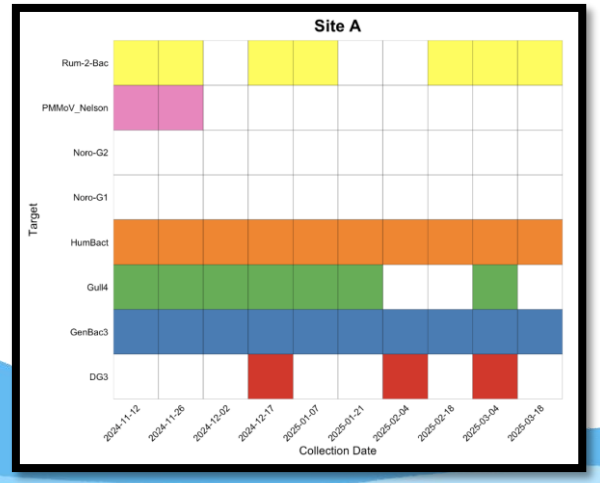
- Source prediction
- Risk prediction

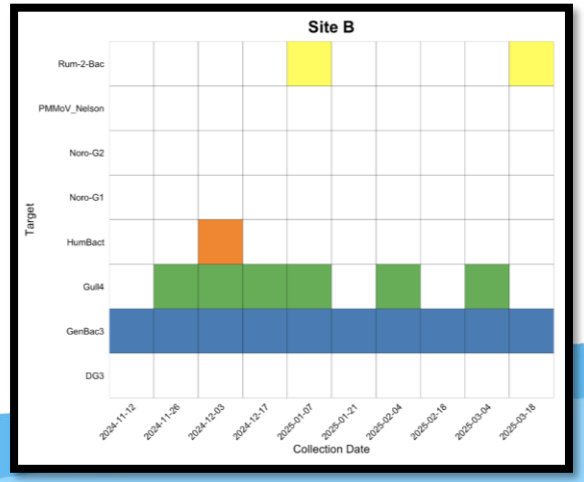
Knowledge Translation

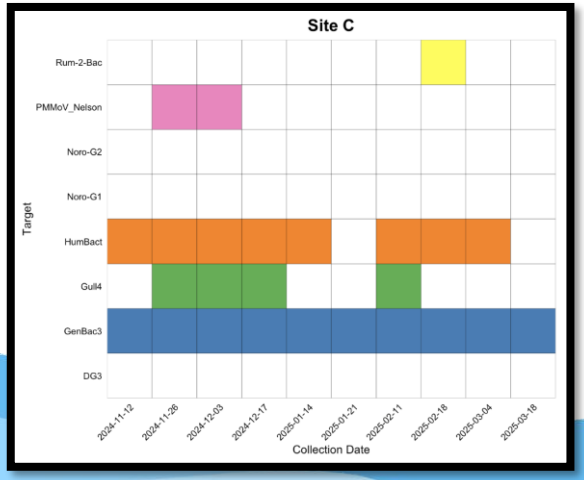
- Technical and non-technical reports
- Method training

Project Progress

- Steering committee established and have met 6 times to inform study plans
- Systematic lit review on applications use of MST and fecal indicators to identify and mitigate sources of fecal pollution in coastal environments
- Validation of 5 MST markers and norovirus assay
- Longitudinal and surge data nearing completion (80%)
- Geospatial data acquisition and data integration underway







Project Outcomes – Why We Are Doing This Work

- **Smarter, data-driven decisions** with genomic and geospatial tools that pinpoint fecal pollution sources and guide long-term management.
- **Stronger Indigenous food security** by improving safe access to subsistence and ceremonial shellfish harvesting.
- **Protected public health** by reducing risks of foodborne outbreaks and unsafe recreational waters.
- **Economic savings** through fewer and shorter shellfish harvest closures, preventing multimillion-dollar losses.

Acknowledgements

Research Team

- Mike Lee (UBC/BCCDC)
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- Christina Chan (UBC)
- Kuljeet Chattha (BCCDC)
- Carly Marshall (UBC)
- Michael Donoghue (UBC)
- GEMSTONE Steering Committee

Funders

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- BC Ministry of Environment and Climate Change
- Pacific Public Health Foundation
- BC Ministry of Health
- BC Ministry of Agriculture and Food
- Comox Valley Regional District
- Malahat Nation
- BC Shellfish Growers Association

Questions?
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