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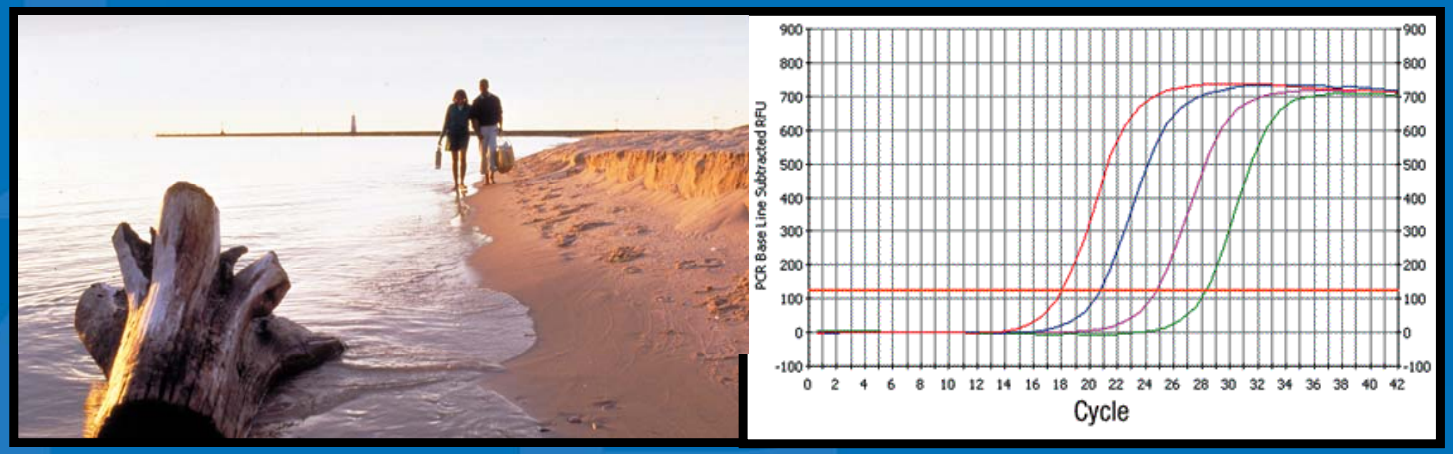
Quantitative Microbial Source Tracking in the Tillamook Basin

Xiang Li, *M. Sivaganesan, C.A. Kelty, A. Zimmer-Faust, P. Clinton, J.R. Reichman, Y. Johnson, W. Matthews, S. Bailey, and O.C. Shanks*



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

Presentation Overview

1. Microbial Source Tracking Overview
2. Tillamook Field Study
3. Technology Transfer

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Fecal Pollution is a Nationwide Problem

- Fecal microbes are the most common biological contaminants in U.S. surface waters
- Public, economic and ecological health risks

Fecal Pollution in Surface Waters:

EPA Responsibilities

Protect and Restore Waters for Recreational Use

- Clean Water Act 1972

Risk Assessment of Beach Contaminants

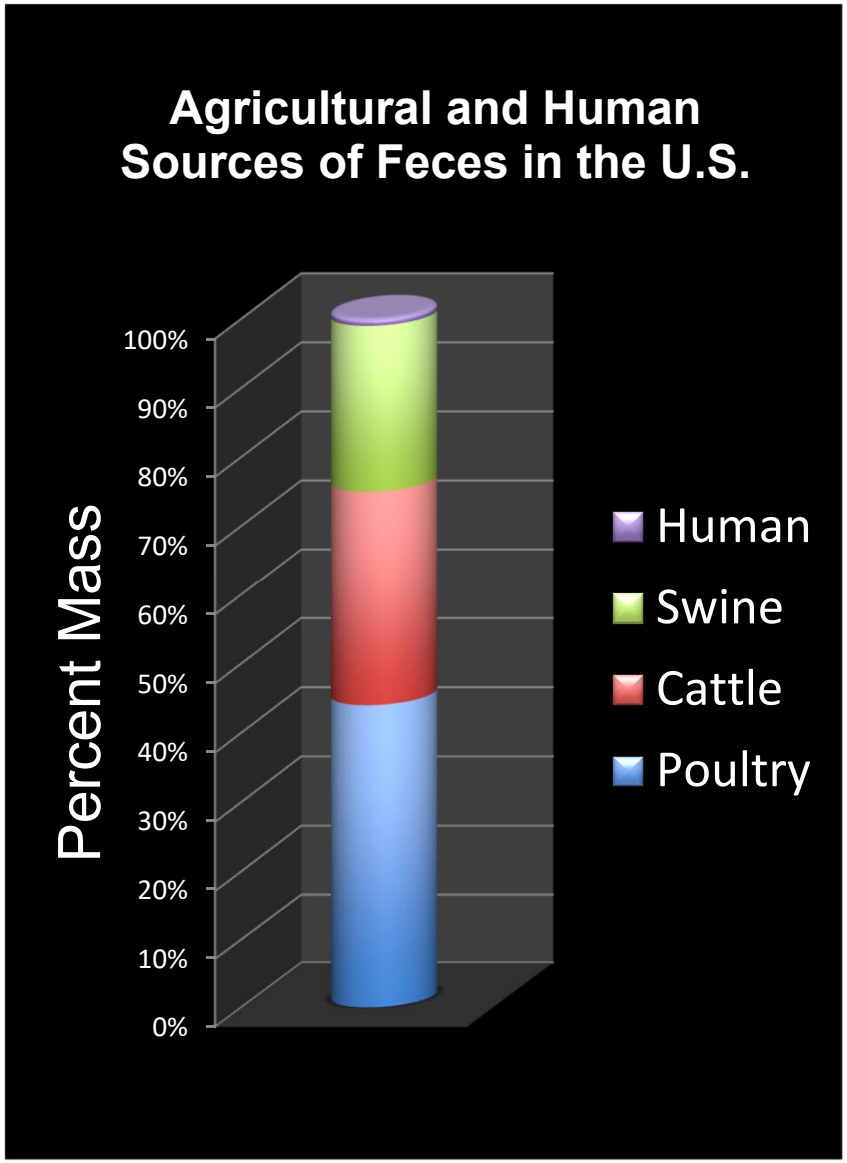
- BEACH Act (2000)
- Development of new or revised ambient water quality criteria (AWQC)

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

Source of Fecal Pollution is Important

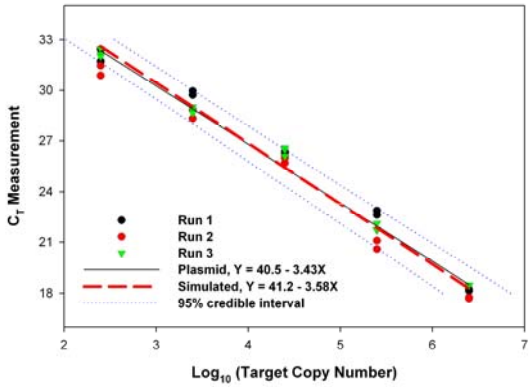
- Current Water Quality Criteria
  - Based on general fecal indicators
  - Measure total fecal pollution
  - Do not discriminate between sources
- Estimated $1x10^9$ tons of fecal material produced in U.S. each year
  - Human (0.01%)
  - Poultry
  - Cattle
  - Swine
- Animal sources require different management and remediation strategies



RL Kellogg, CH Lander, DC Moffitt, N Gollehon - NRCS and ERS GSA Publ. No. NPS00-0579. Washington, DC: USDA, 2000

Microbial Source Tracking:
Concept Review

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



Microbial Source Tracking:

Scientific Premise

Host-associated identifiers are expected to exist in different animal groups due to:

- **Gut conditions**
  - Temperature
  - Diet
  - Digestive physiology

- **Natural selection**
  - Space
  - Nutrients

Microbial Source Tracking:

Many Field Applications

- Identification of non-point pollution sources
- Impaired site prioritization for remediation
- Evaluation of a best management practices
- Bacterial Total Maximum Daily Load planning
- Nutrient discharge compliance monitoring tool
- Urban stormwater management support

Microbial Source Tracking Field Studies:

Significance and Rationale

- Single study design does not fit all possible applications
- Sampling strategy
  - -site selection
  - -sampling frequency
- Data interpretation
  - -ancillary data requirements
  - -additional data analysis procedures
- Resource logistics
  - -access to laboratory facilities
  - -local collaborations
- Need for real-world examples

Tillamook Microbial Source Tracking Project:

Background Information

- Important resource
  - Dairy, cheese-making and shellfish industries
  - Local human and wildlife populations
- Impact of fecal pollution
  - Economic loss
  - Poor conditions for recreational use
  - Endangerment of local wildlife
- Complex challenge
  - Multiple pollution sources
  - Limited resources for management and remediation

Tillamook Microbial Source Tracking Project:

Success through Partnership

United States Environmental Protection Agency

Local Volunteers

Oregon Department of Agriculture

Oregon Department of Environmental Quality

State of Oregon Department of Environmental Quality

MST Field Study

EPA Office of Research & Development

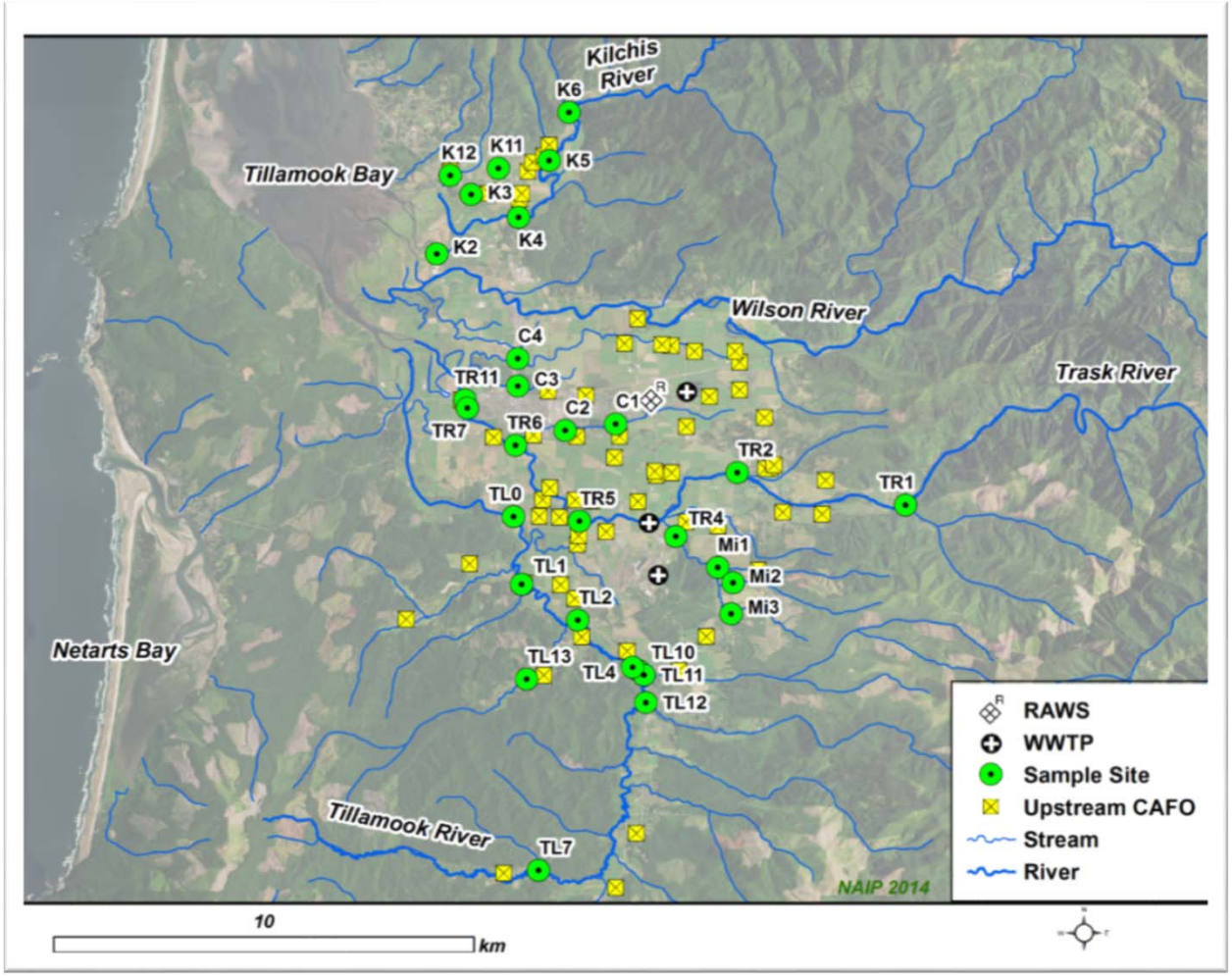
EPA Region 10

Tillamook Estuaries Partnership

Tillamook Estuaries Partnership

Tillamook Microbial Source Tracking Project:

Sampling Approach



- Tillamook Basin (Oregon)
- Three river systems
  - *Tillamook*
  - *Trask*
  - *Kilchis*
- 29 sampling sites
- 12-month sampling period
- Bimonthly sampling
  - *696 total samples*

Tillamook Microbial Source Tracking Project:

Historical Water Quality Trends

United States Environmental Protection Agency

- State water quality definition (*E. coli* 406 MPN/100mL)
- Previous MST study
  - End-point PCR
  - Spatial and temporal trends in ruminant and human sources

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 2006, p. 5537–5546 Vol. 72, No. 8
0099-2240/06/$08.00+0 doi:10.1128/AEM.03059-05

Basin-Wide Analysis of the Dynamics of Fecal Contamination and Fecal Source Identification in Tillamook Bay, Oregon

Orin C. Shanks,[1] Christopher Nietch,[1] Michael Simonich,[2] Melissa Younger,[2] Don Reynolds,[3] and Katharine G. Field[2]*

- Historical *E. coli* exceedance trends

River System	Site	n	*E. coli* ≥ 406 MPN/100mL		
			% ± SE	Trend	p-value
Kilchis	K12	230	28.3 ± 3.0		
	K3	227	34.4 ± 3.2	↓	0.01
	K6	229	0.4 ± 0.4		
	K5	234	0.4 ± 0.4		
	K4	234	2.1 ± 0.9	↓	0.06
	K2	229	1.3 ± 0.8		
Trask	C4	234	12.4 ± 2.2		
	C3	225	69.3 ± 3.1	↓	< 0.0001
	C1	172	61.6 ± 3.7		
	C2	175	58.3 ± 3.7		
	TR1	237	0.8 ± 0.6		
	TR2	237	3.4 ± 1.2		
	TR5	237	5.1 ± 1.4		
	TR6	237	59.5 ± 3.2	↓	< 0.0001
	TR7	237	7.2 ± 1.7		
	TR11	237	8.0 ± 1.8		
	Mi3	236	77.5 ± 2.7		
	Mi2	236	11.4 ± 2.1		
	Mi1	237	15.2 ± 2.3		
	TR4	237	28.3 ± 2.9		
Tillamook	TL7	230	37.0 ± 3.2	↓	0.07
	TL12	231	5.2 ± 1.5		
	TL11	231	14.3 ± 2.3	↓	0.03
	TL10	231	13.0 ± 2.2	↓	0.08
	TL4	231	11.7 ± 2.1		
	TL2	231	27.3 ± 2.9	↓	< 0.0001
	TL1	231	26.4 ± 2.9	↓	< 0.0001
	TL0	231	16.0 ± 2.4		
	TL13	231	12.6 ± 2.2		

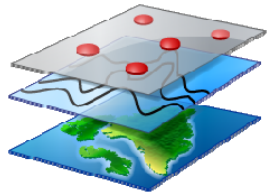
Tillamook Microbial Source Tracking Project:

Selected Methodologies

- Geographic Information System
- Weather Conditions
- Local Water Quality Metric
  - *E. coli* (IDEXX Colilert)
- MST Genetic Markers of Fecal Pollution
  - Human-associated (HF183/BacR287 and HumM2)
  - Ruminant (Rum2Bac)
  - Cattle (CowM2 and CowM3)
  - Dog (DG3 and DG37)
  - Avian (GFD)

Tillamook Microbial Source Tracking Project:

Land Use High Resolution Mapping



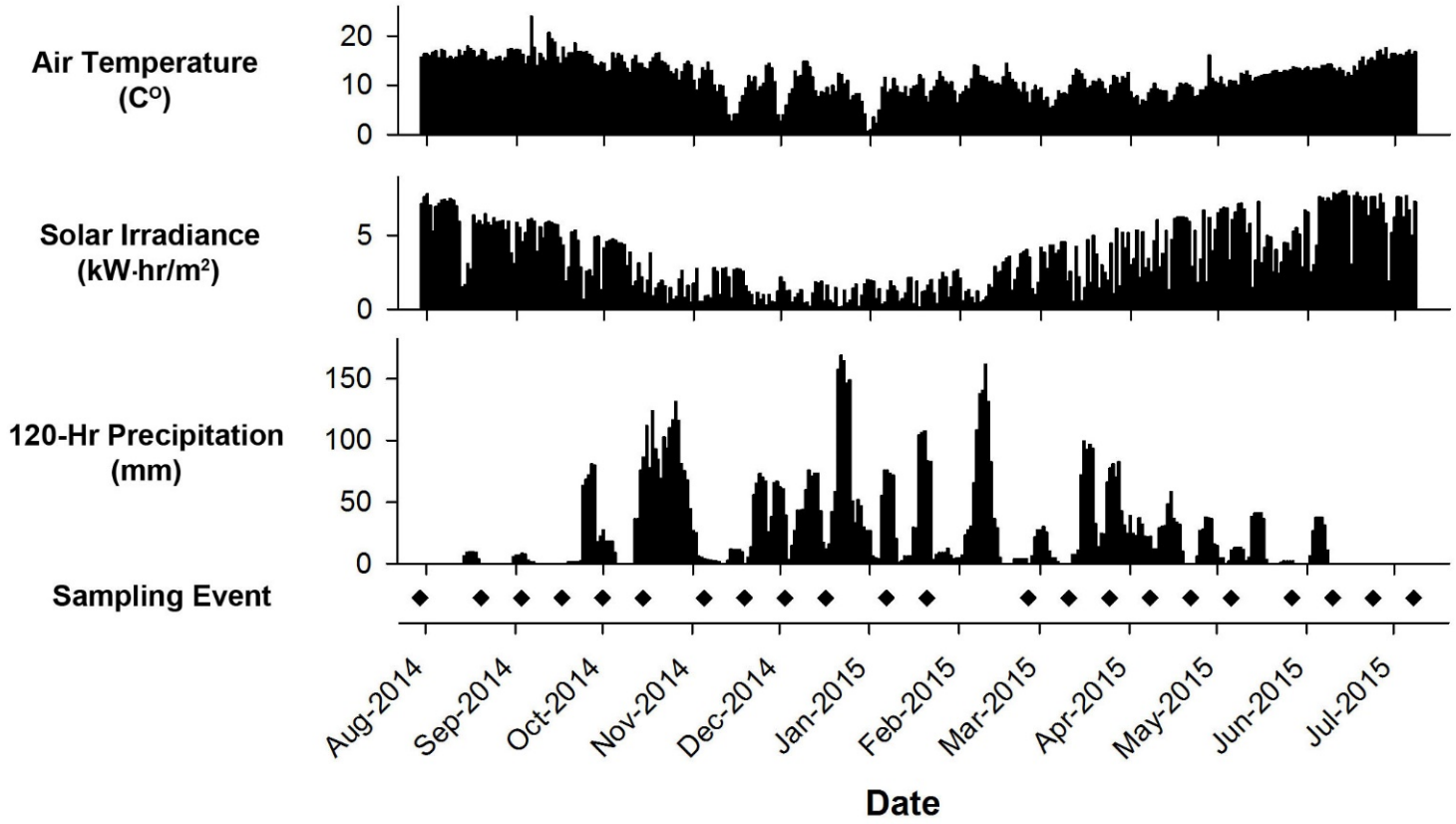
- Hydrology defined catchment area
- Cattle and human populations
- Percent non-sewer and crop land
- Investigate links to water quality data

Type	Parameter	Mean	Median	Std.	Min.	Max.
Land Use	Human Population	1051.6	533	1727.1	2	6395
	Permitted Cattle Population	2418.9	826	3472.7	0	12,371
	Non-sewer (%)	1.06	0.18	1.83	0.006	7.44
	Cropland (%)	1.97	0.53	4.89	0.08	25.7

Tillamook Microbial Source Tracking Project:

Weather Conditions over Study Period

- Multiple rain events over study period
- Seasonal trends in all parameters

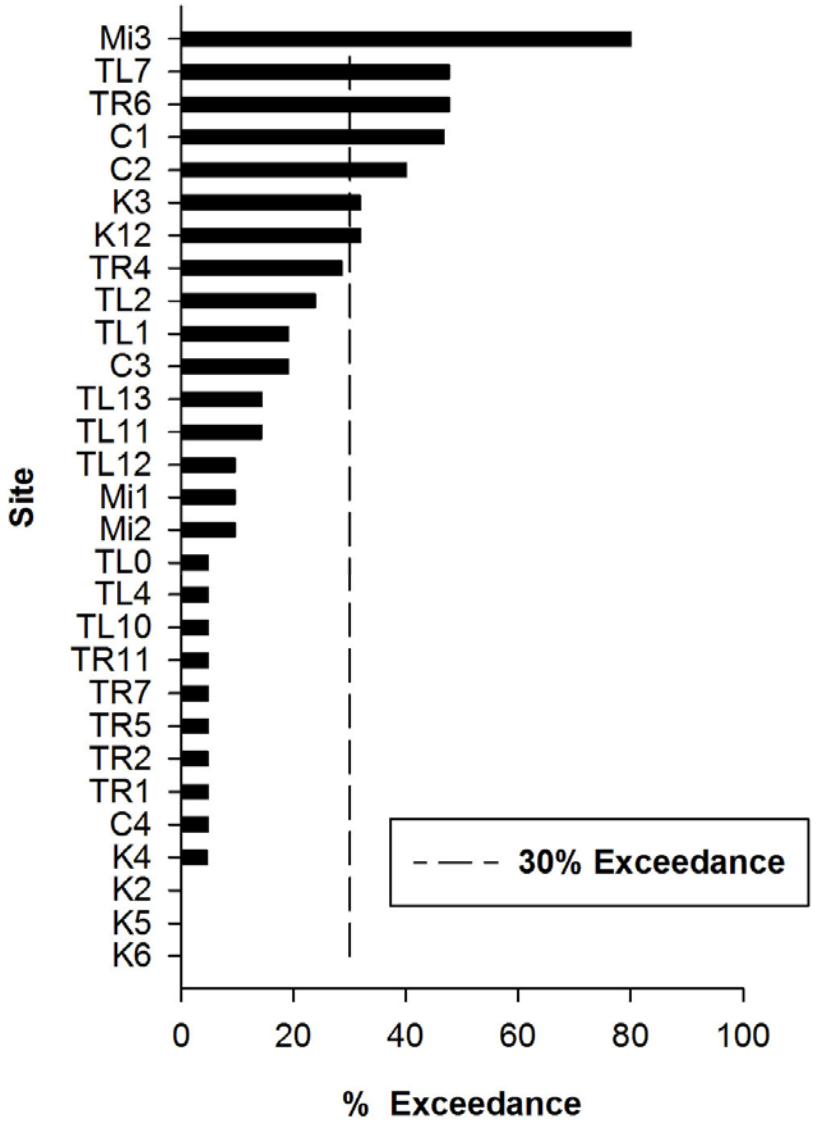


Type	Parameter	Mean	Median	Std.	Min.	Max.
Weather Condition	120-hr precipitation (mm)	27.3	9.5	36.6	0	168.9
	72-hr precipitation (mm)	16.4	3.8	25.9	0	144.5
	24-hr precipitation (mm)	5.5	0.1	12.3	0	101.1
	Solar Irradiance (kW-hr/m²)	3.5	2.9	2.3	0.1	8
	Air Temperature (°C)	11.9	12	3.6	0.7	23.9

Tillamook Microbial Source Tracking Project:

The Water Quality Management Perspective

- Single-Day Maximum of 406 MPN/100mL



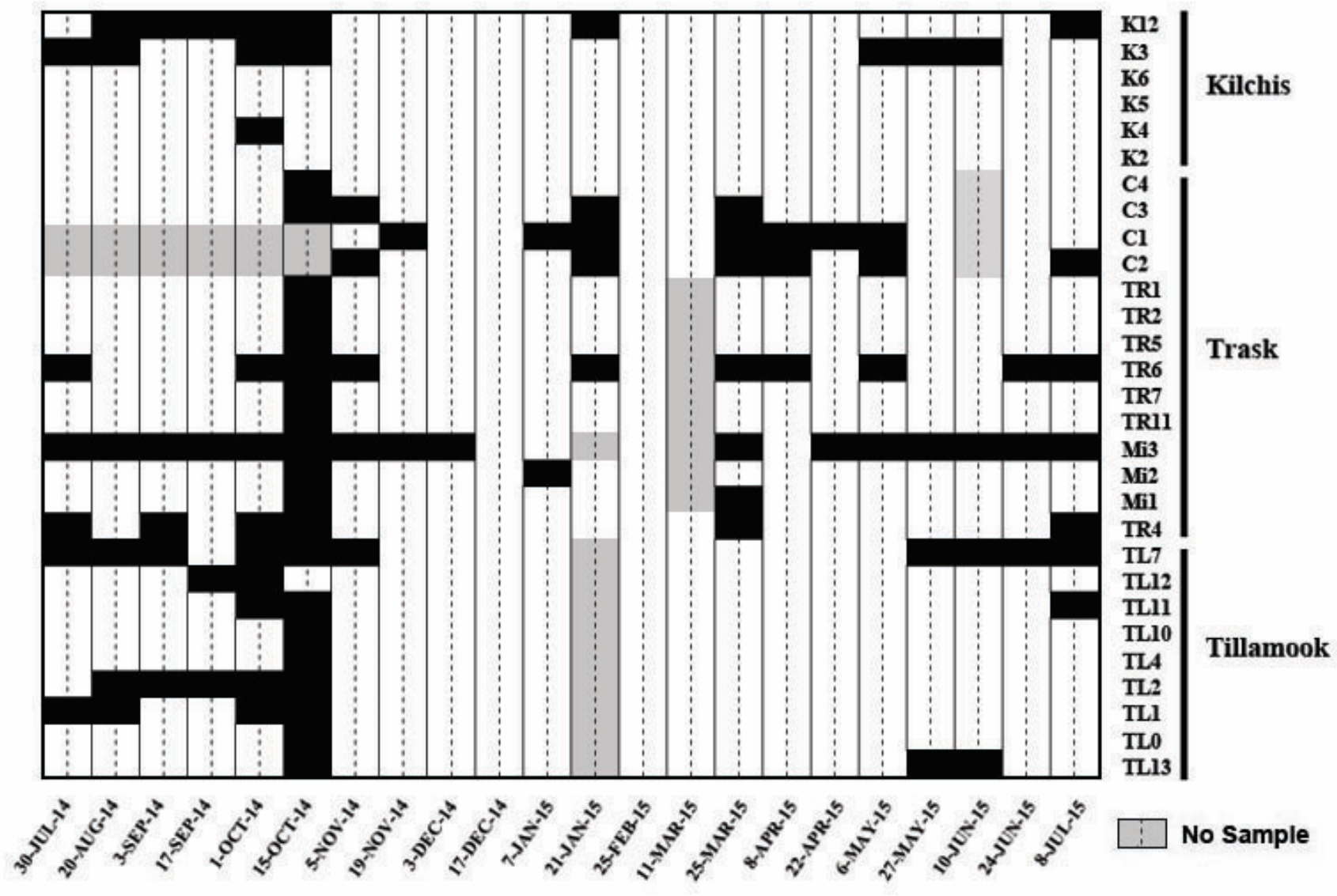
- 14.5% Exceedance (n=87 of 602)
- 7 sites with > 30%
  - Mi3, TL7, TR6, C1, C2, K3, and K12
- 3 sites with 0%
  - K6, K5, and K2

Tillamook Microbial Source Tracking Project:

The Water Quality Management Perspective

United States Environmental Protection Agency

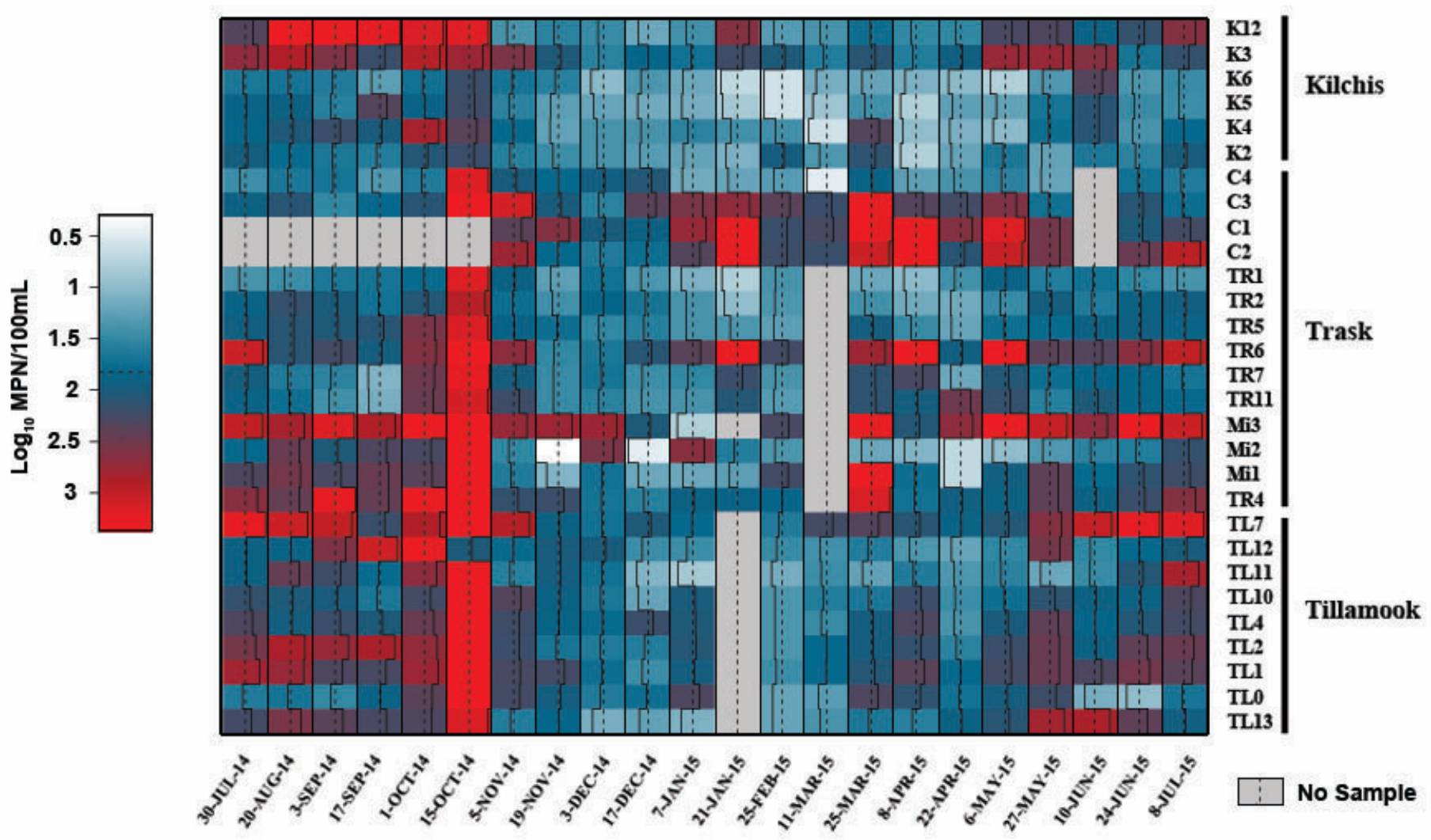
- Single-Day Maximum of 406 MPN/100mL



Tillamook Microbial Source Tracking Project:

E. coli Spatial and Temporal Trends

United States Environmental Protection Agency

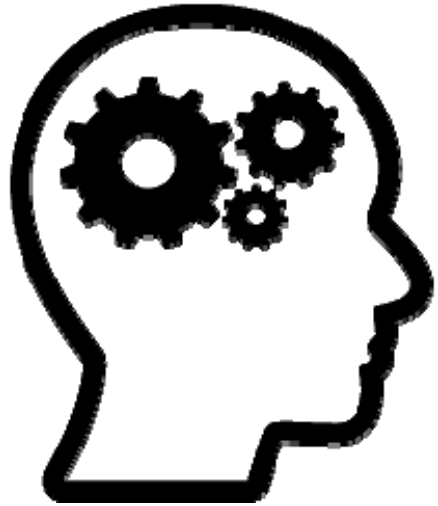


- Ubiquitous across all sites tested
- Evident temporal and spatial trends

Tillamook Microbial Source Tracking Project:

qPCR MST Data Interpretation Considerations

- Each MST genetic marker is a discrete measurement
  - Different bacterial species
  - Different DNA sequence
- Genetic marker occurrence can vary by:
  - Animal diet, age, and health
  - Geographic region
  - Cohabitation behaviors
- Incompatibilities between cultivated *E. coli* and MST genetic markers
  - *E. coli* = live cells that can be cultivated in lab
  - MST genetic markers = any live or dead cell and free-DNA
  - Live cells and genetic material respond differently to environmental stressors
- Inconsistencies between *E. coli* and MST measurements <u>prevent accurate source apportionment</u>
- Recommend independent analysis of each water quality measurement data set



Tillamook Microbial Source Tracking Project:

qPCR Performance in Study Area

United States Environmental Protection Agency

- Pollution source reference collection (n=114)
- Determine sensitivity and specificity in Tillamook study area

Source	n
Adult Cattle	32
Juvenile Cattle	19
Dog	11
Sewage	4
Wildlife Birds	15
Chickens	11
Elk	11
Horse	11

Ruminant and Cattle

Assay	Sensitivity		Specificity
	Adult	*Juvenile*	
Rum2Bac	93%	0%	100%
CowM2	36.5%	0%	99.4%
CowM3	82.3%	0%	100%

Avian

Assay	Sensitivity		Specificity
	Wildlife	*Chicken*	
GFD	24.4%	0%	100%

Human

Assay	Sensitivity	Specificity
HumM2	100%	99.1%
HF183	100%	99.4%

Dog

Assay	Sensitivity	Specificity
DG3	97%	100%
DG37	36.4%	100%

Key Observations:

- High specificity for all assays
- Age and diet important factors
- Avian method limitations
- DG3 superior host distribution

Tillamook Microbial Source Tracking Project:

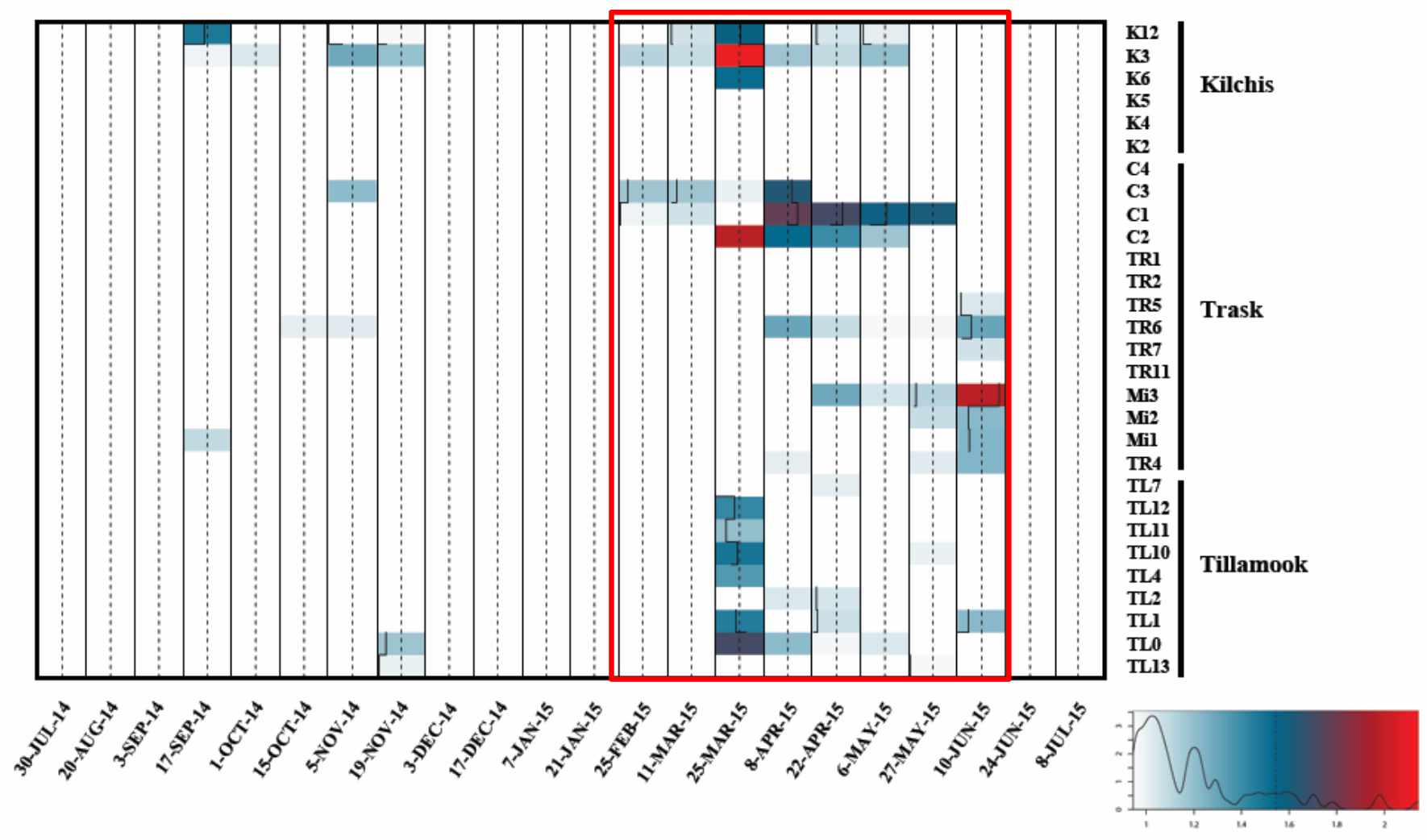
qPCR Data Quality Controls and Data Acceptance Criteria

- Standard curve performance
  - Outlier removal
  - Amplification efficiency (*E*)
  - Correlation coefficient (R^2)
- Amplification inhibition testing
  - Instrument run proficiency test
  - Internal amplification control with every sample
- Contamination screening
  - Field blanks
  - Method extraction blanks
  - No template controls
- Optimal DNA recovery monitoring
  - Batch proficiency test
  - Sample processing control with every sample

Tillamook Microbial Source Tracking Project:

Spatial and Temporal Trends in Avian Pollution

United States Environmental Protection Agency

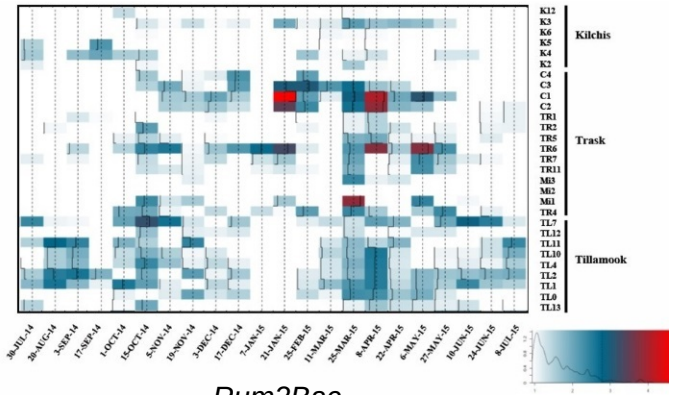


Potential bird migration water quality impact

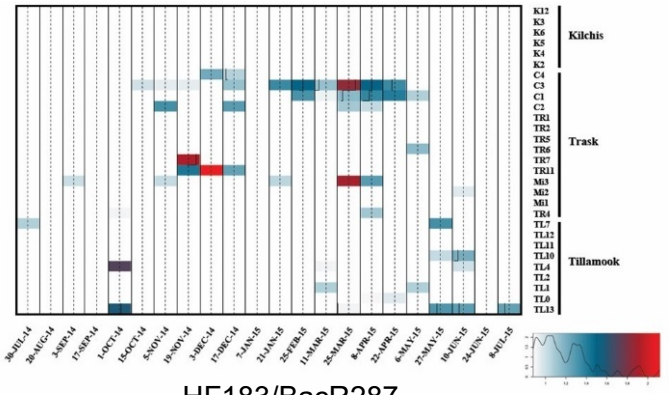
Tillamook Microbial Source Tracking Project:

Spatial and Temporal Trends in Ruminant, Cattle, Human and Dog Sources

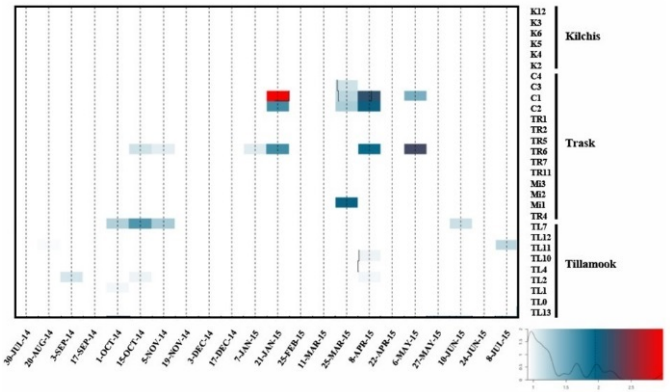
- Spatial trends
  - *Land use*
  - *Waste management practices*
- Temporal trends
  - *Weather conditions*
  - *Agricultural practices*
  - *Wildlife activities*
- Varies by assay



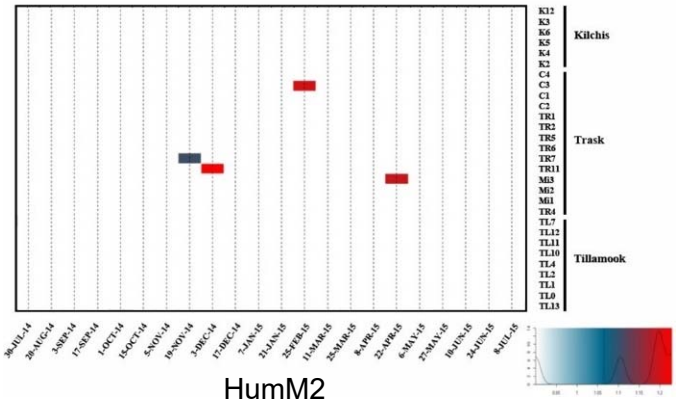
Rum2Bac



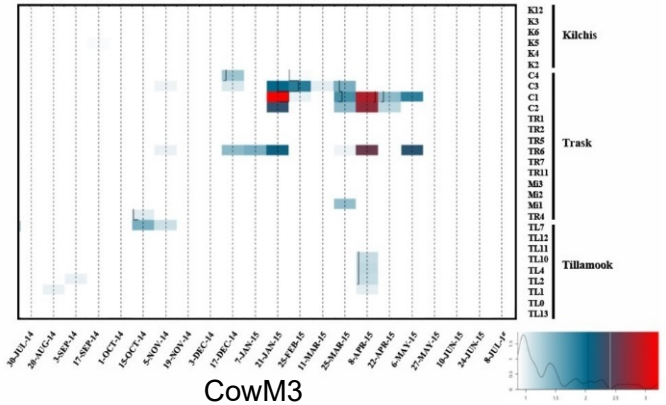
HF183/BacR287



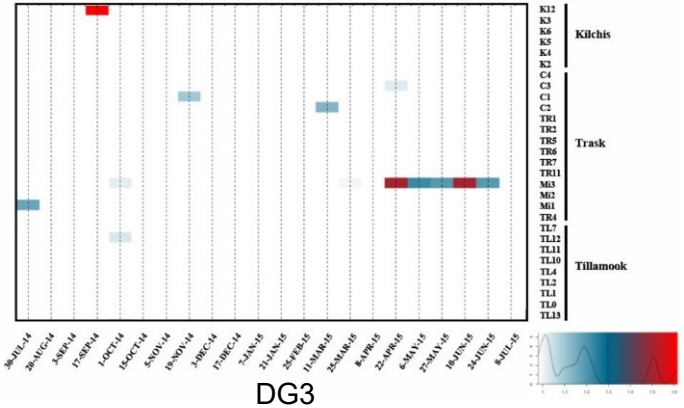
CowM2



HumM2



CowM3

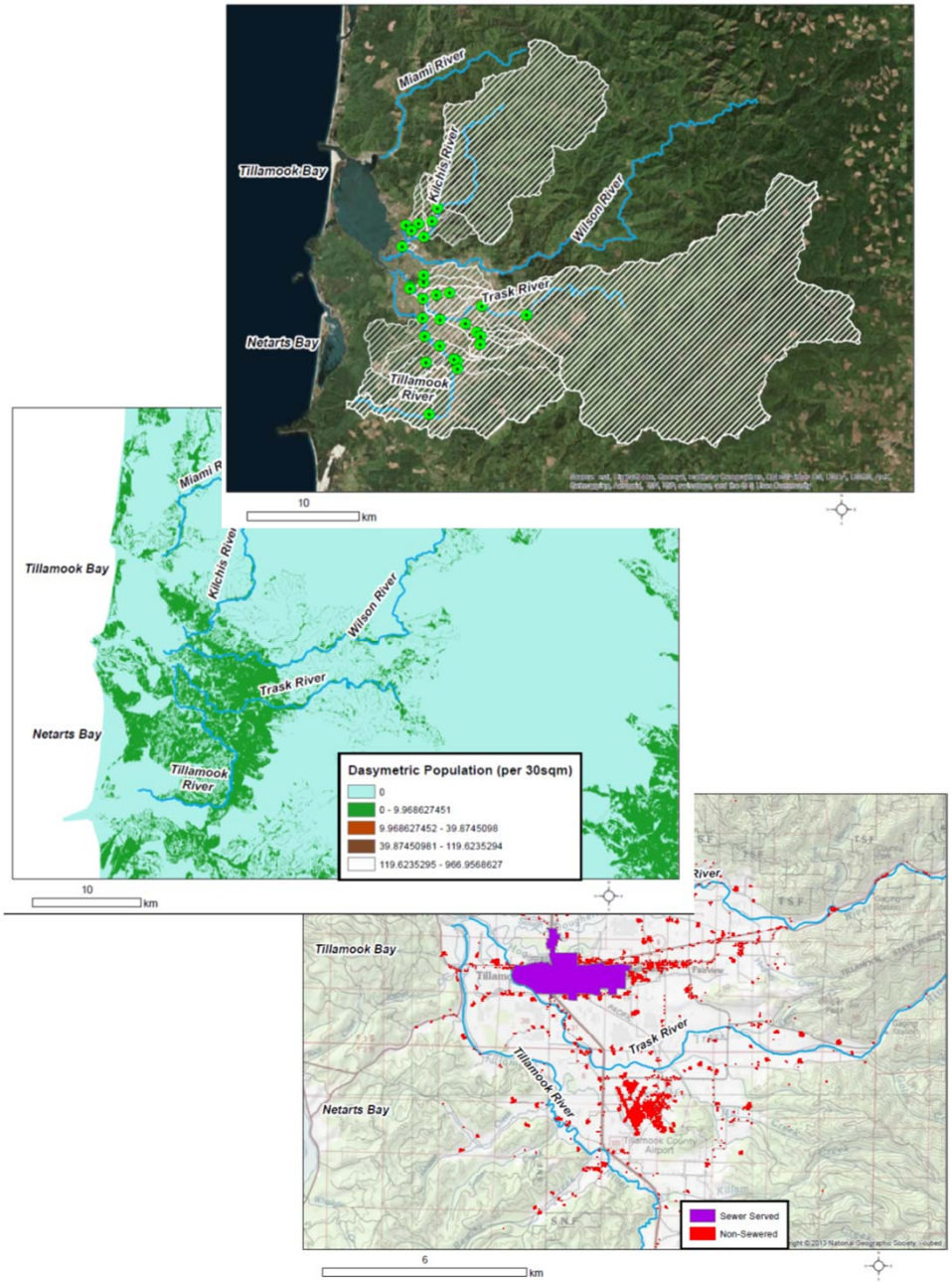


DG3

Tillamook Microbial Source Tracking Project:

Land Use and Water Quality Trends

- Identify pollution trends by fecal pollution metric and land use information
- Percent non-sewer most influential parameter
  - *E. coli* (+, R^2=0.23, p=0.081)
  - HF183/BacR287 (+, R^2=0.32, p=0.009)
  - GFD (+, R^2=0.31, p=0.014)
- Ruminant pollution closely linked to maximum number of permitted cattle
  - Rum2Bac (+, R^2=0.50, p=0.001)



Tillamook Microbial Source Tracking Project:

Weather Conditions and Water Quality Trends

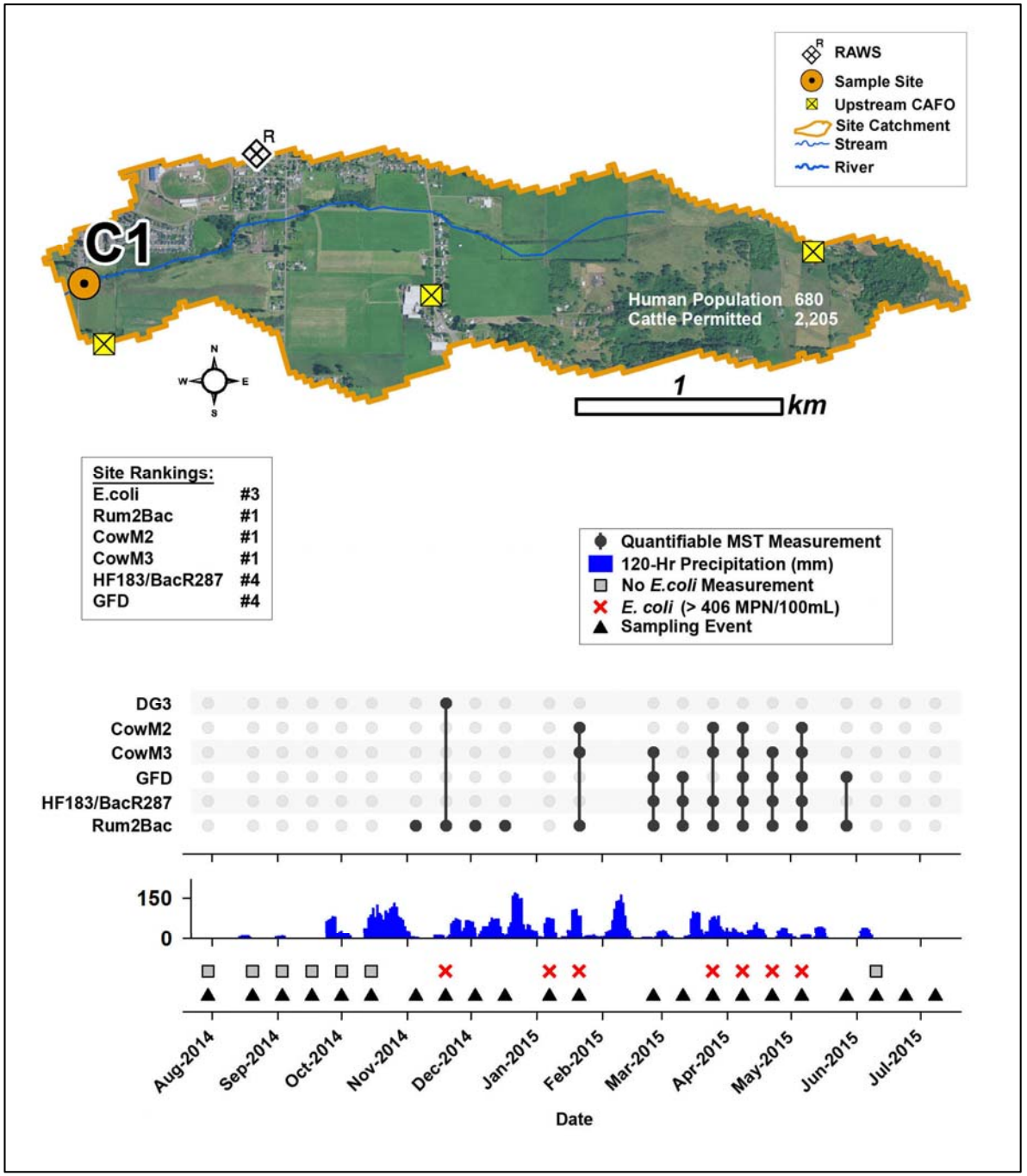
Weather Parameter	Count	Fecal Pollution Metric				
		E. coli	Rum2Bac	CowM3	HF183/BacR287	GFD
24-Hr Precipitation	208	**0.003**	**<0.0001**	0.247	0.455	0.167
72-Hr Precipitation	160	0.134	**<0.0001**	0.157	0.354	0.114
120-Hr Precipitation	207	0.184	**<0.0001**	0.193	0.381	0.499
Solar Irradiance	322	**<0.0001**	**0.002**	0.127	0.151	0.218
Air Temperature	294	**<0.0001**	**<0.0001**	**0.005**	**0.033**	0.170

- *E. coli* levels significantly associated with 24-Hr precipitation, solar irradiance, and air temperature
- Ruminant pollution concentration closely linked to weather conditions
- Avian pollution not linked to weather conditions

Tillamook Microbial Source Tracking in Action:

C1 Site Profile

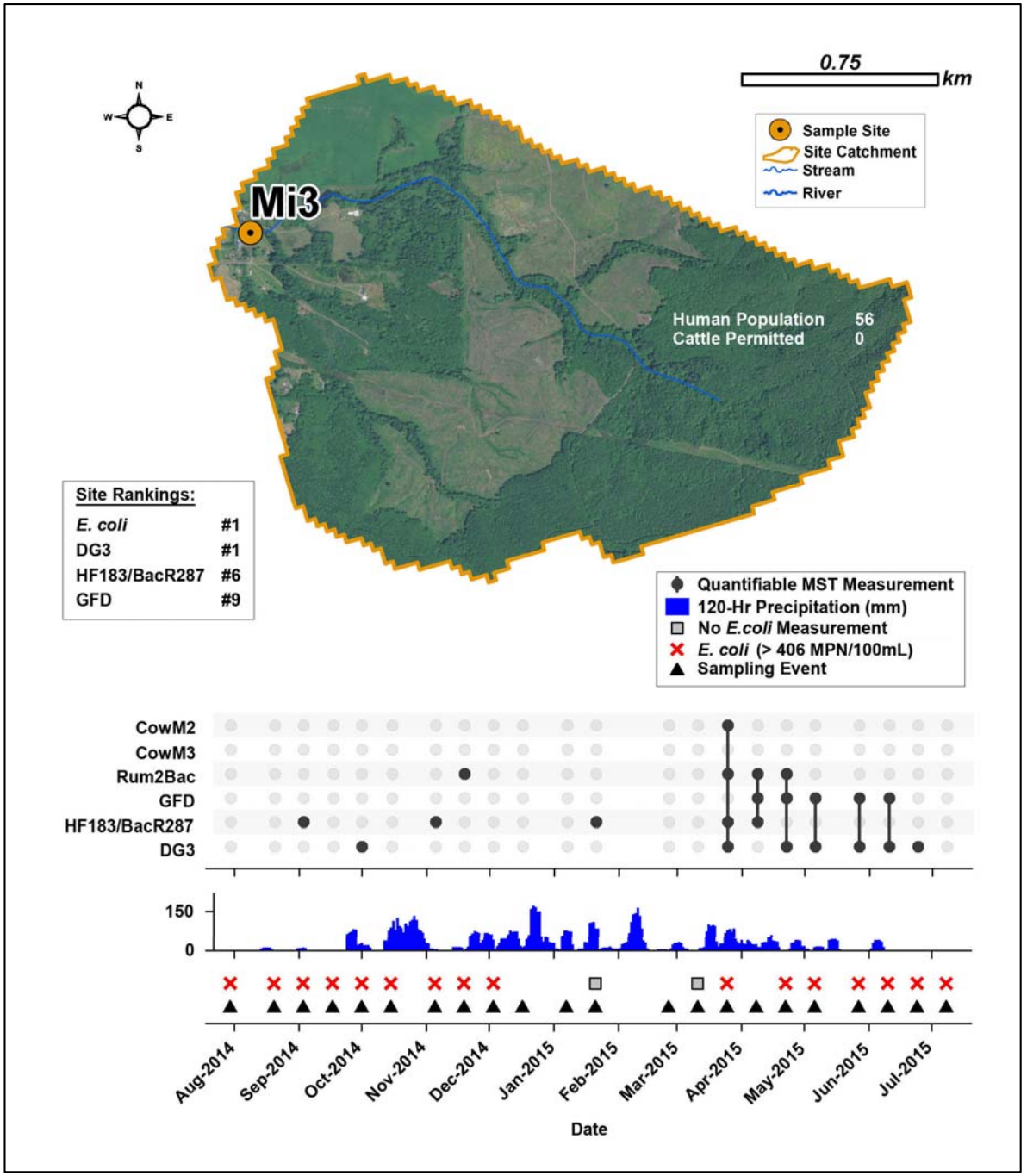
- Trask River System
- *E. coli* exceedance (46.7%)
- Possible bird migration impact
- Human impact during spring
- Consistent ruminant impact
- Management recommendations
  - Sanitary survey in Spring
  - Prioritize by fecal source



Tillamook Microbial Source Tracking in Action:

Mi3 Site Profile

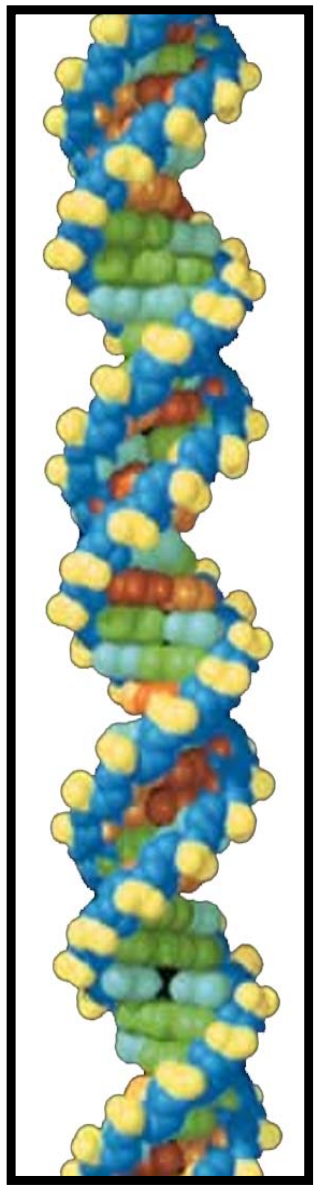
- Trask River System
- *E. coli* exceedance (80%)
- Seasonal dog pollution, target local breeding facility
- Possible bird migration impact
- Human impact during wet season
- Ruminant in spring, likely AFO
- Management recommendations
  - Sanitary survey in Spring
  - Target AFO, septic system, and dog facility



Tillamook Microbial Source Tracking Project:

Conclusions

- Full-scale field study implementing quantitative MST
- Evident temporal, spatial, weather, and animal source pollution patterns
  - Watershed level
  - Site level
- Quantitative MST enhances water quality management



Microbial Source Tracking with qPCR:

Technology Transfer

- Rapidly growing interest
- Many potential applications
- Need to transition from research method to management tool

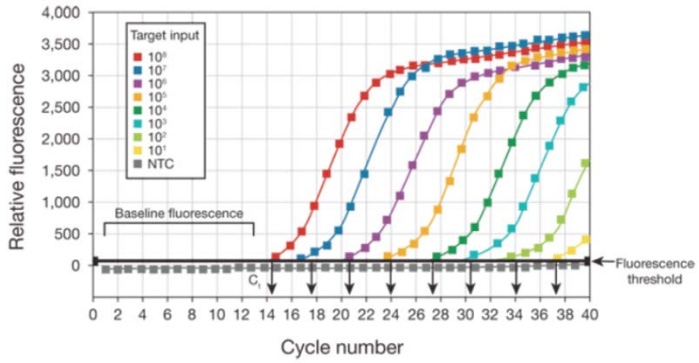
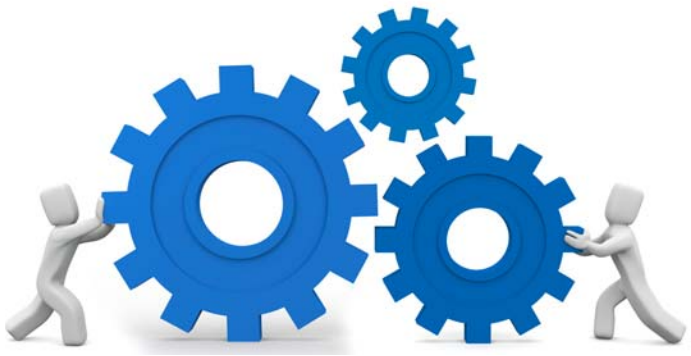


Figure 1. Relative fluorescence vs. cycle number. Amplification plots are created when the fluorescent signal from each sample is plotted against cycle number; therefore, amplification plots represent the accumulation of product over the duration of the real-time PCR experiment. The samples used to create the plots in this figure are a dilution series of the target DNA sequence.

Technology Transfer:
Building an Implementation Took kit



- Publication of two EPA Methods (pending EPA OW review)
- Automated data analysis tool
- Proficiency test procedure
- Development of certified reference material
- Training opportunities

Technology Transfer:

Field Studies for all Microbial Source Tracking Applications

- TMDL support (Tillamook Study)
- Identification of septic system pollution (East Fork Study)
- Recreational water site prioritization (Great Lakes)
- Urban stormwater management (Washington DC)

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QUESTIONS?