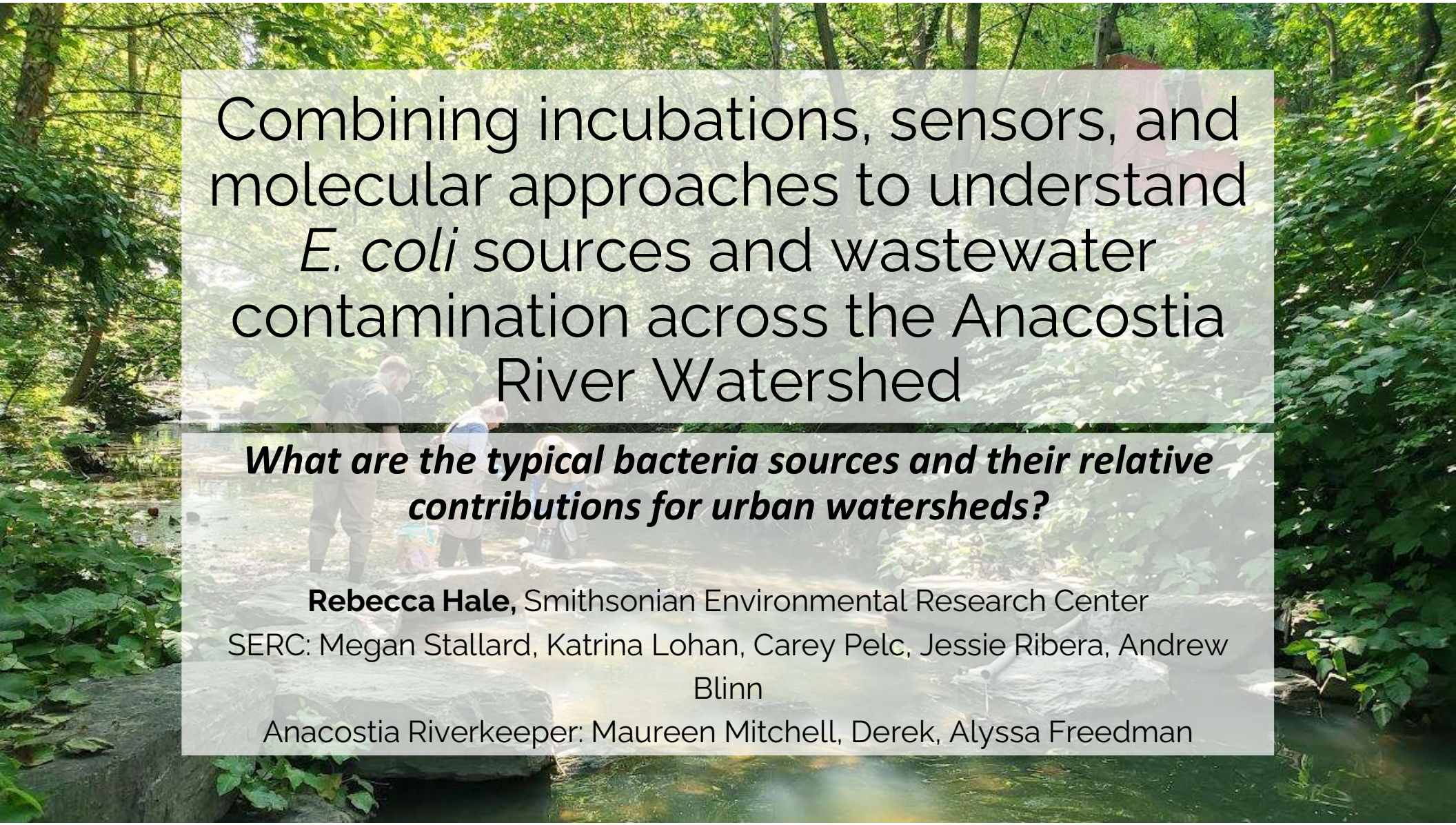


Combining incubations, sensors, and molecular approaches to understand *E. coli* sources and wastewater contamination across the Anacostia River Watershed

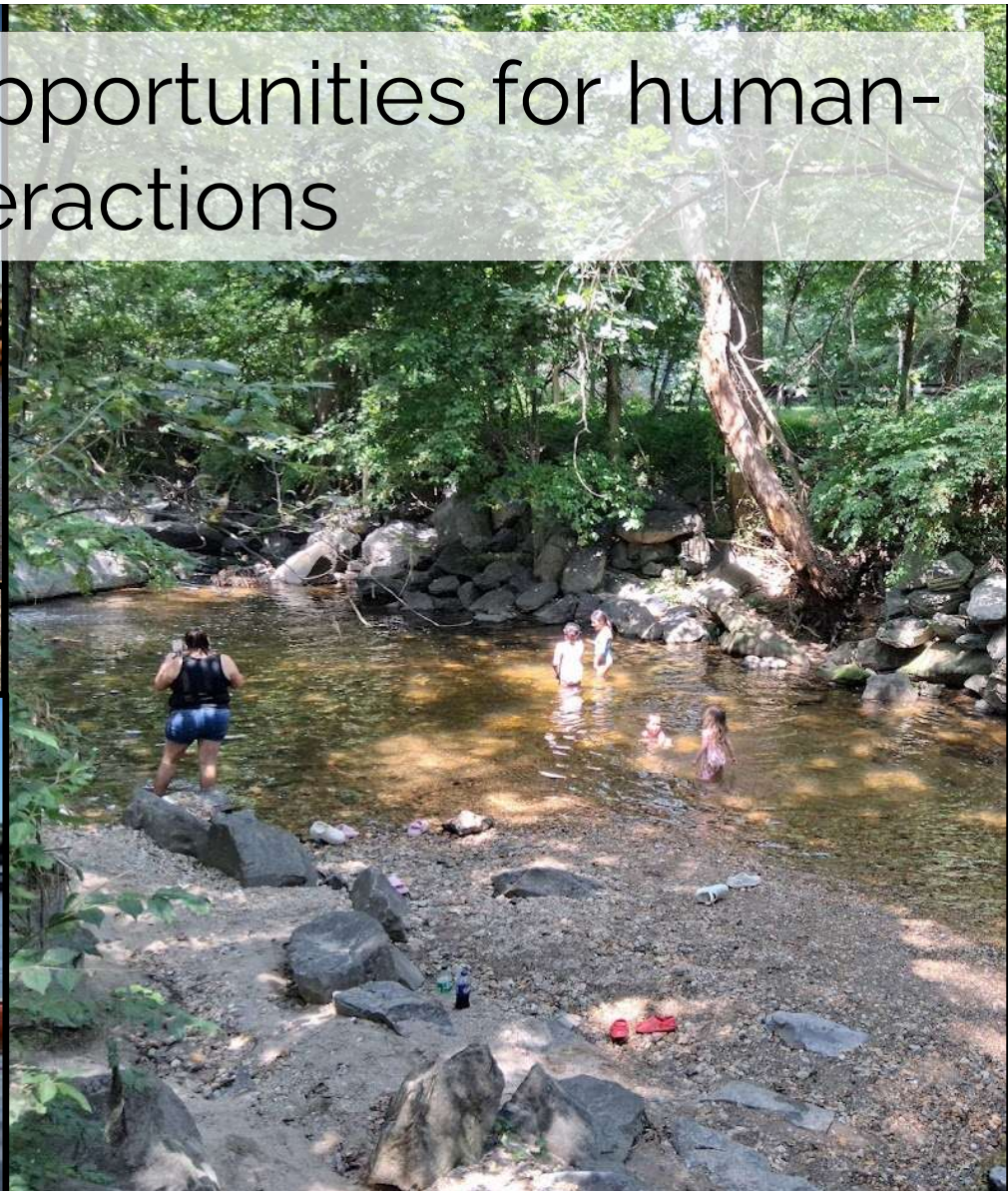
What are the typical bacteria sources and their relative contributions for urban watersheds?

Rebecca Hale, Smithsonian Environmental Research Center

SERC: Megan Stallard, Katrina Lohan, Carey Pelc, Jessie Ribera, Andrew Blinn

Anacostia Riverkeeper: Maureen Mitchell, Derek, Alyssa Freedman

Urban streams provide opportunities for human-nature interactions



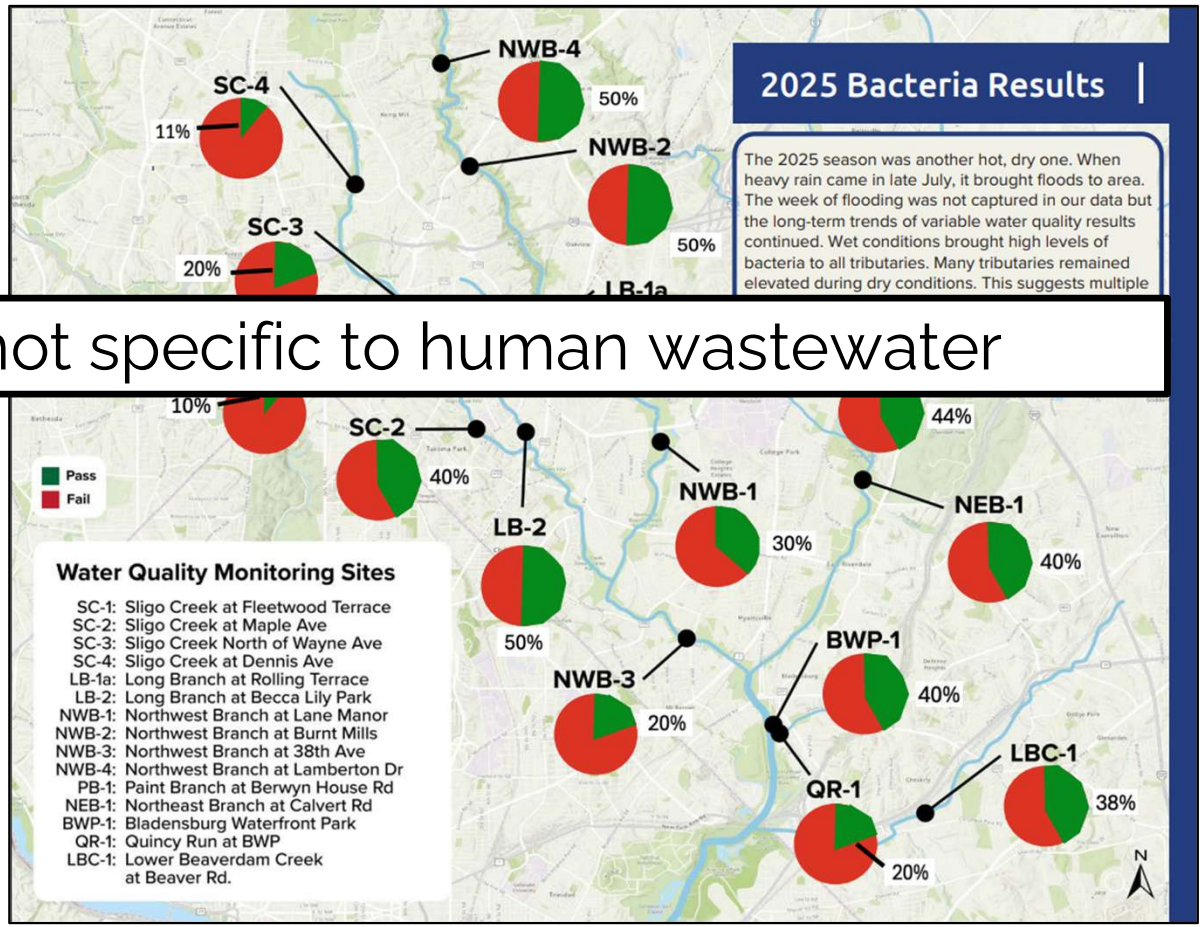
But urban streams are often….gross

Fecal indicator bacteria are used to assess recreational risk

E. coli bacteria

EPA standard: 410 mpn/100ml

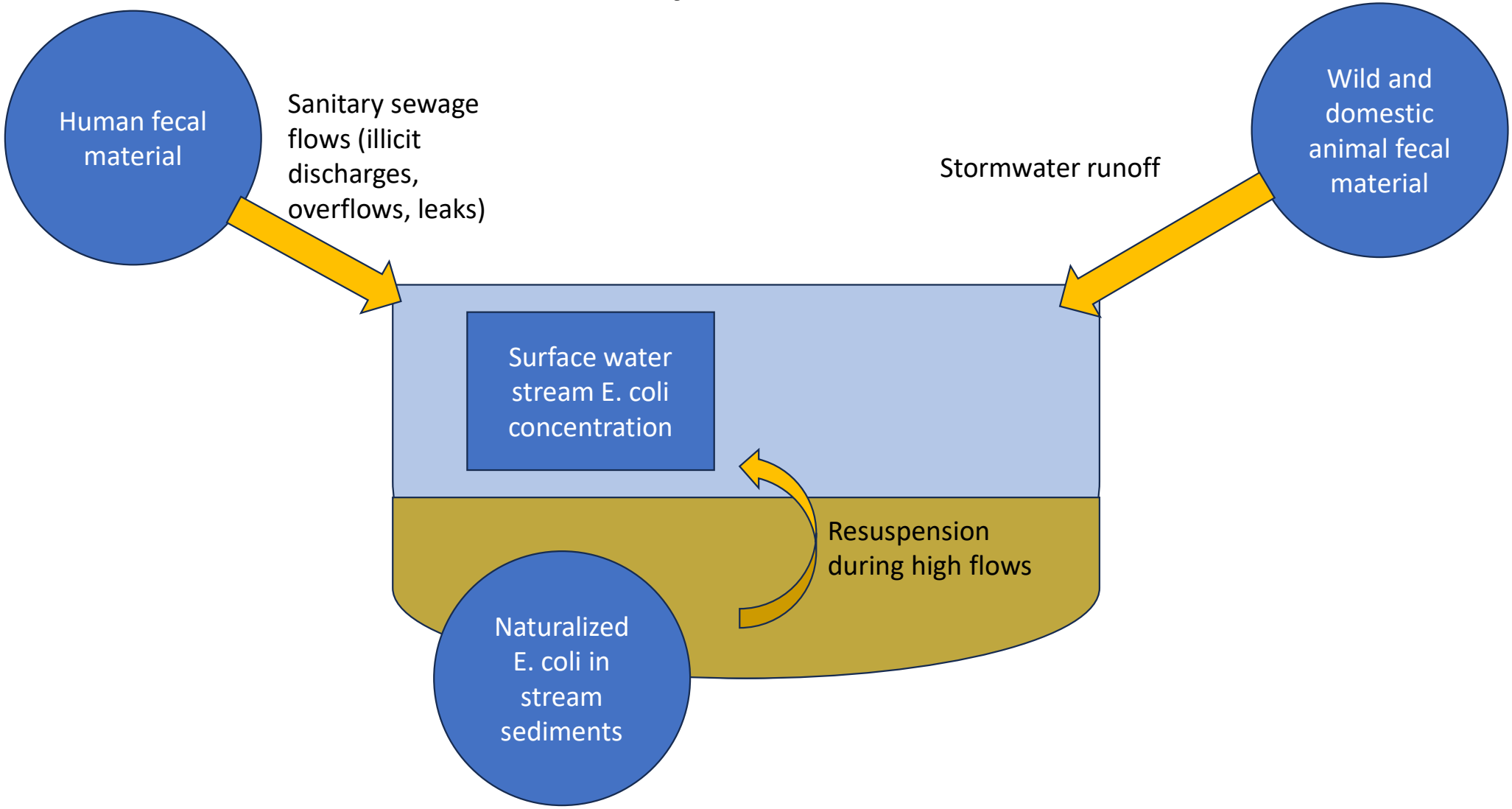
Across the Anacostia watershed, many streams are not safe



Anacostia River Keeper 2025 Maryland Report

Challenge: *E. coli* are not specific to human wastewater

Q1: What are the dominant sources of E. coli *across the Anacostia Watershed?*



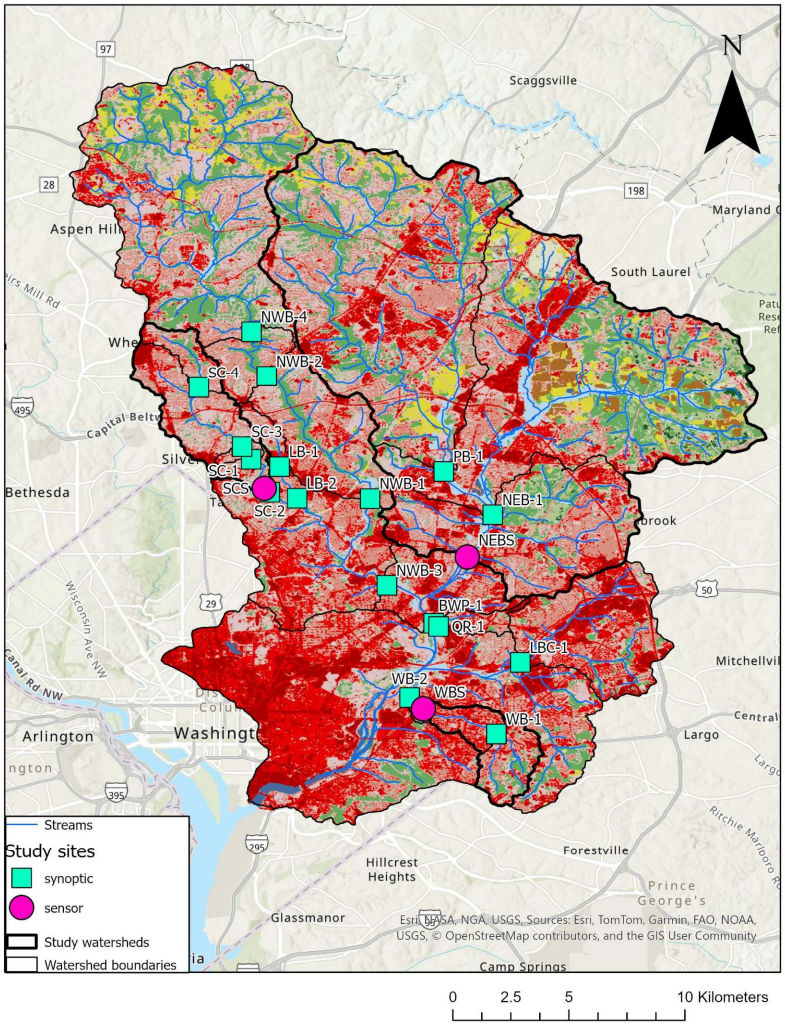
Q1: What are the dominant sources of E. coli *across the Anacostia Watershed?*

H1: Dominant sources of *E. coli* contamination will vary among streams depending on land cover and stormwater and wastewater infrastructure.

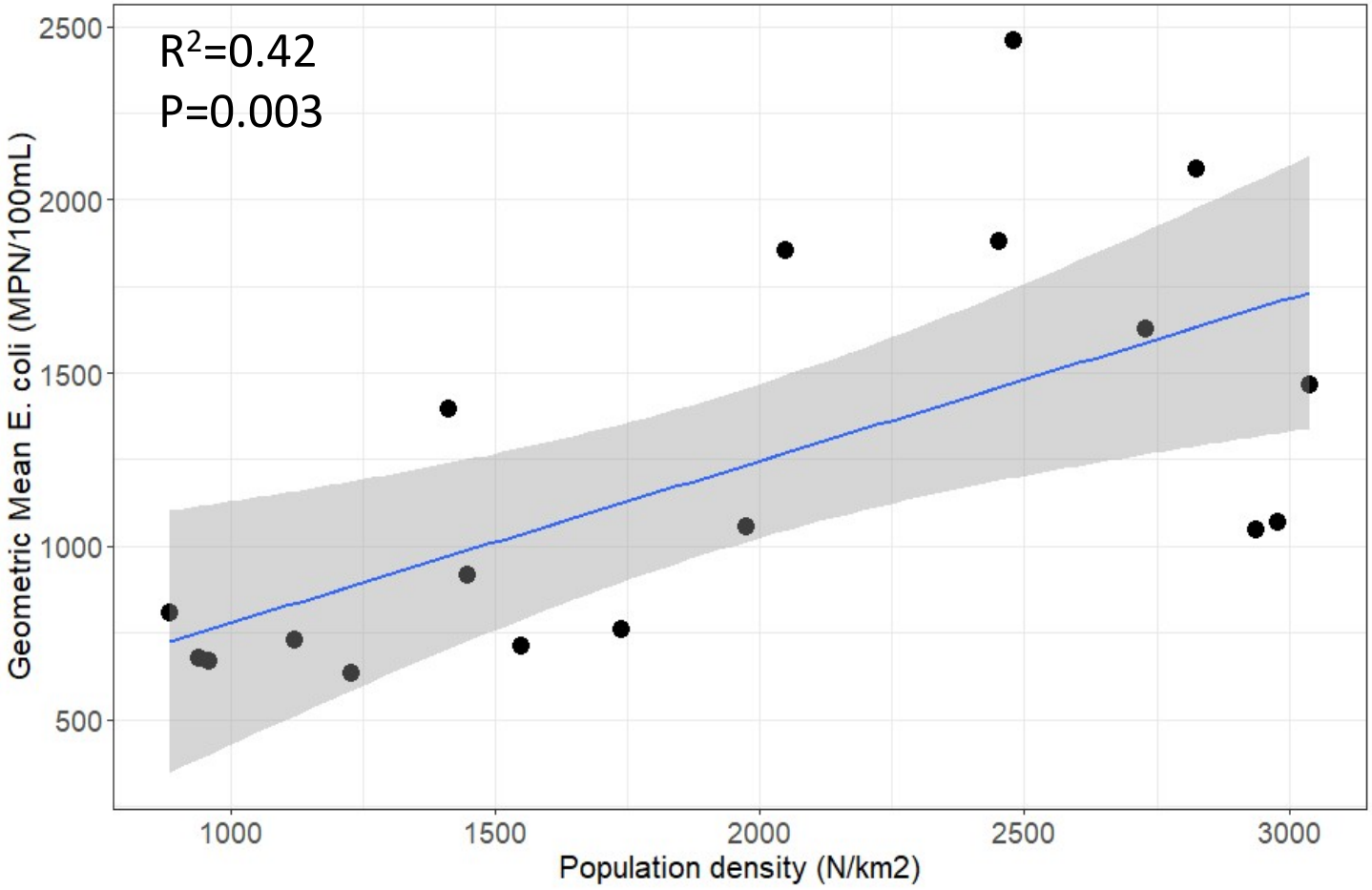
- more human and canine *E. coli* in watersheds with greater human population densities
- more wildlife-sourced *E. coli* in watersheds with greater forest cover

Approach:

1. Synoptic sampling: 20 locations 5 times
    1. Impervious cover: 19-49%
    2. Forest cover: 4-28%
    3. Population density: 890-4400 ppl/km2
2. Bacteria:
    1. Traditional incubation methods (IDEXX)
    2. Molecular source tracking (MST)



Mean *E. coli* concentrations associated with population density



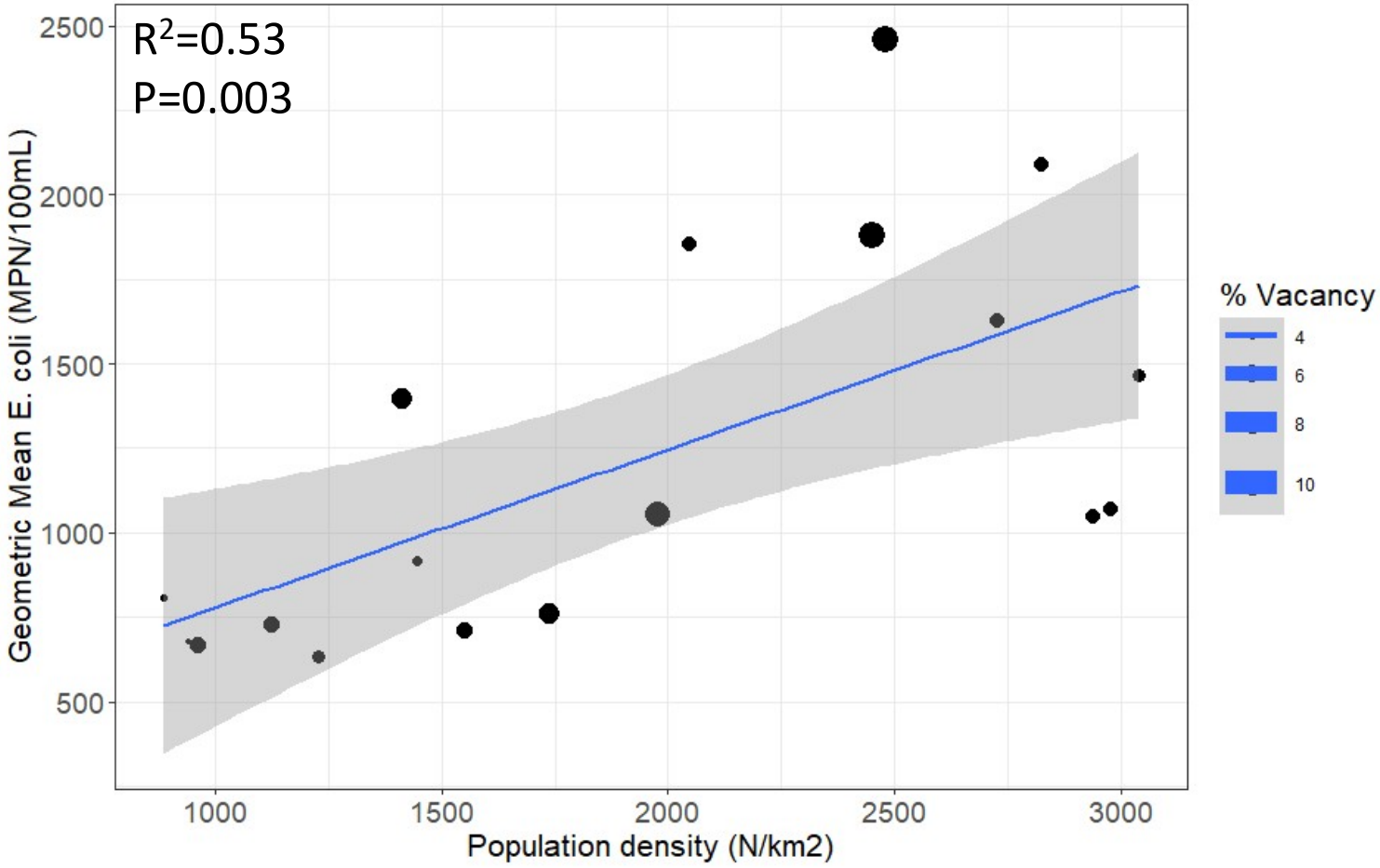
Equivalent predictors:

Housing density (+)

Tree cover over impervious (+)

Forest cover (-)

Mean *E. coli* concentrations associated with population density



Equivalent predictors:

Housing density (+)

Tree cover over impervious (+)

Forest cover (-)

Housing vacancy may be a proxy for infrastructure condition.

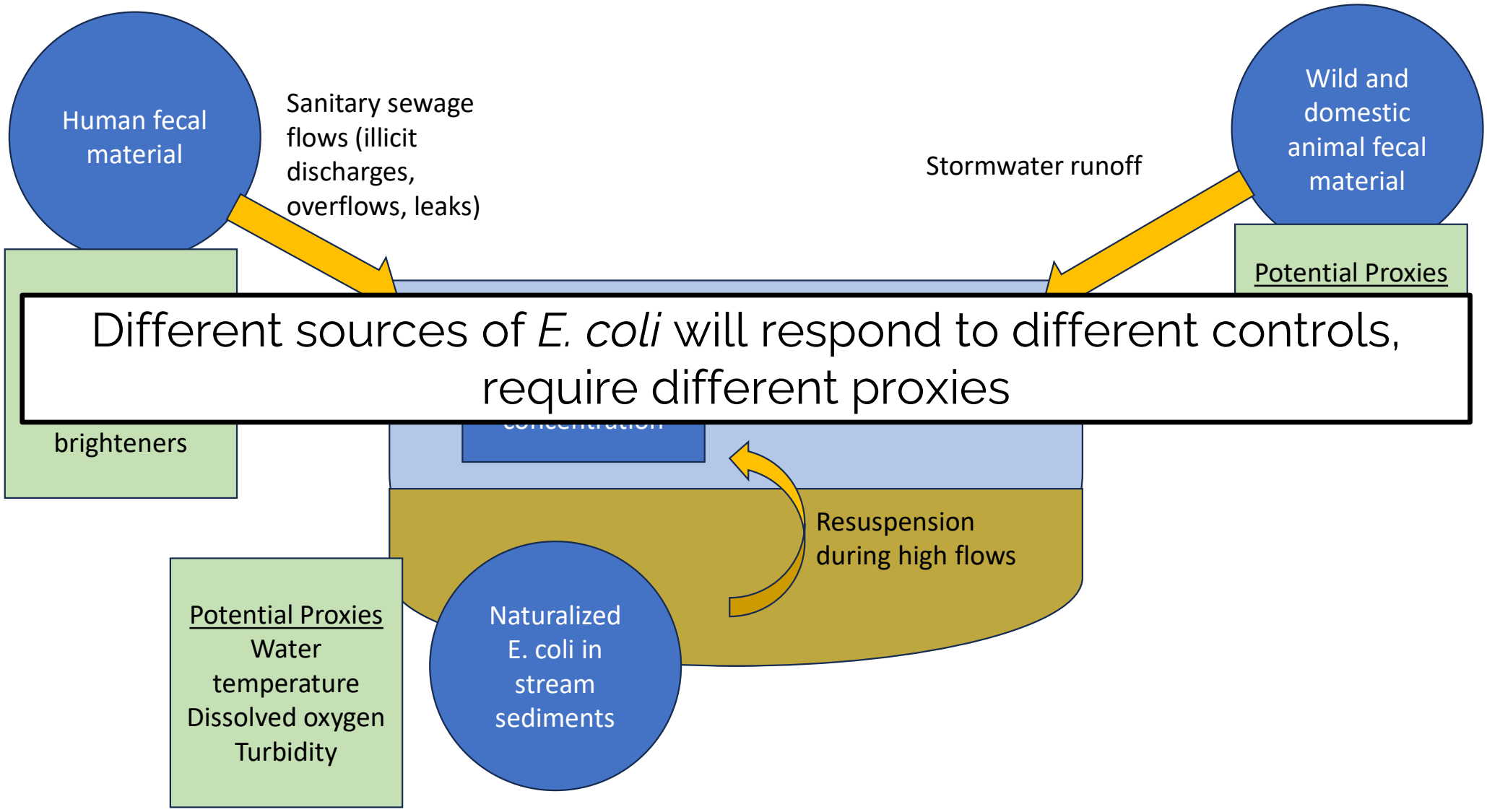
Microbial Source Tracking: what are sources of fecal bacteria?

Targets	DG3	HF183	GFD	RumBac

Digital droplet PCR technology

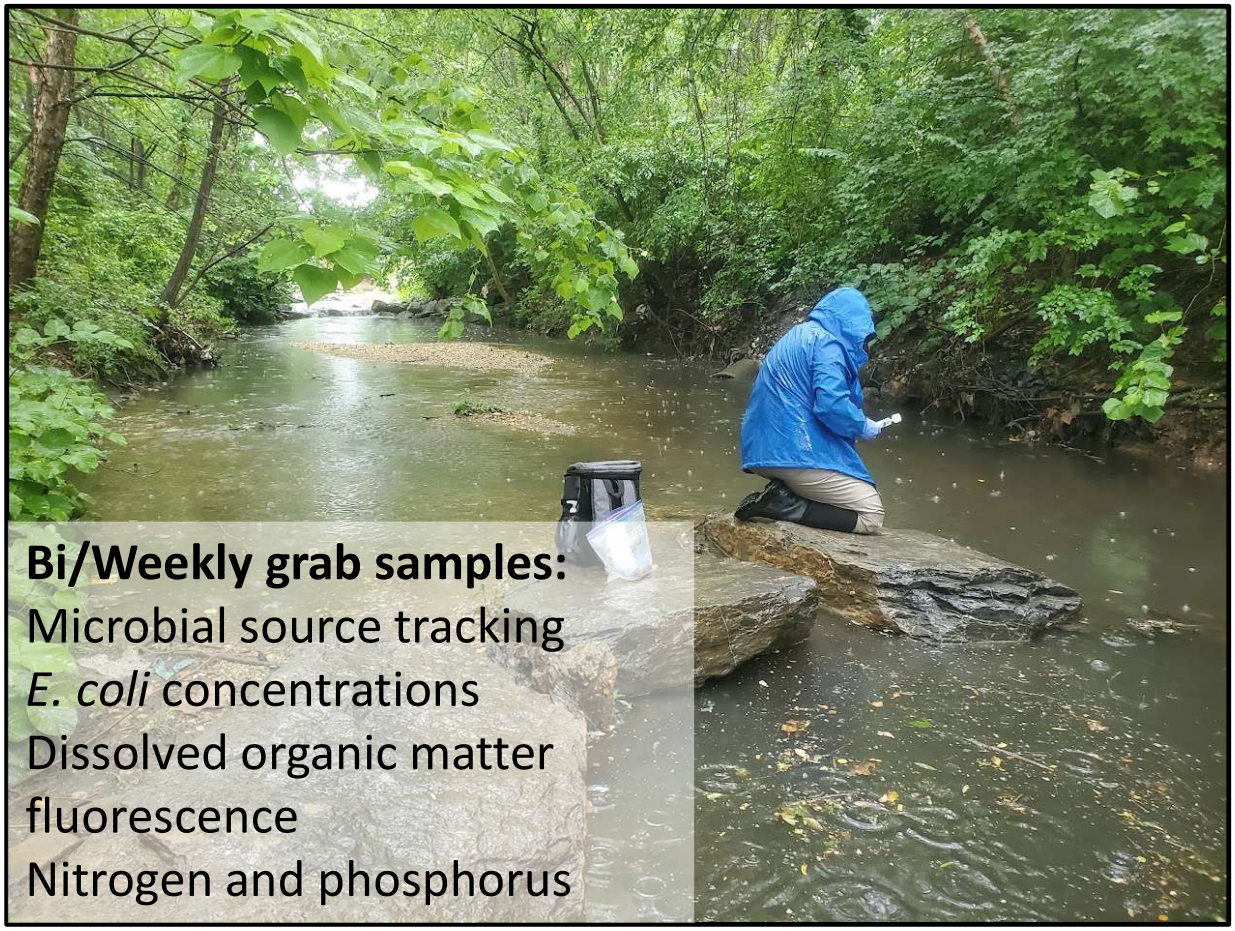
- Abundance of host-specific bacteria targets
- Expect to have completed end of summer

Q2: What sensor parameters are associated with E. coli *concentrations?*



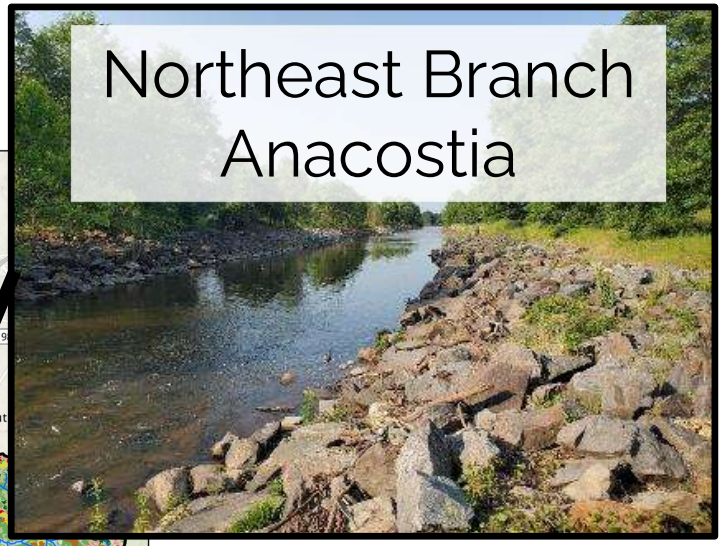
Q2: What sensor parameters are associated with E. coli *concentrations?*

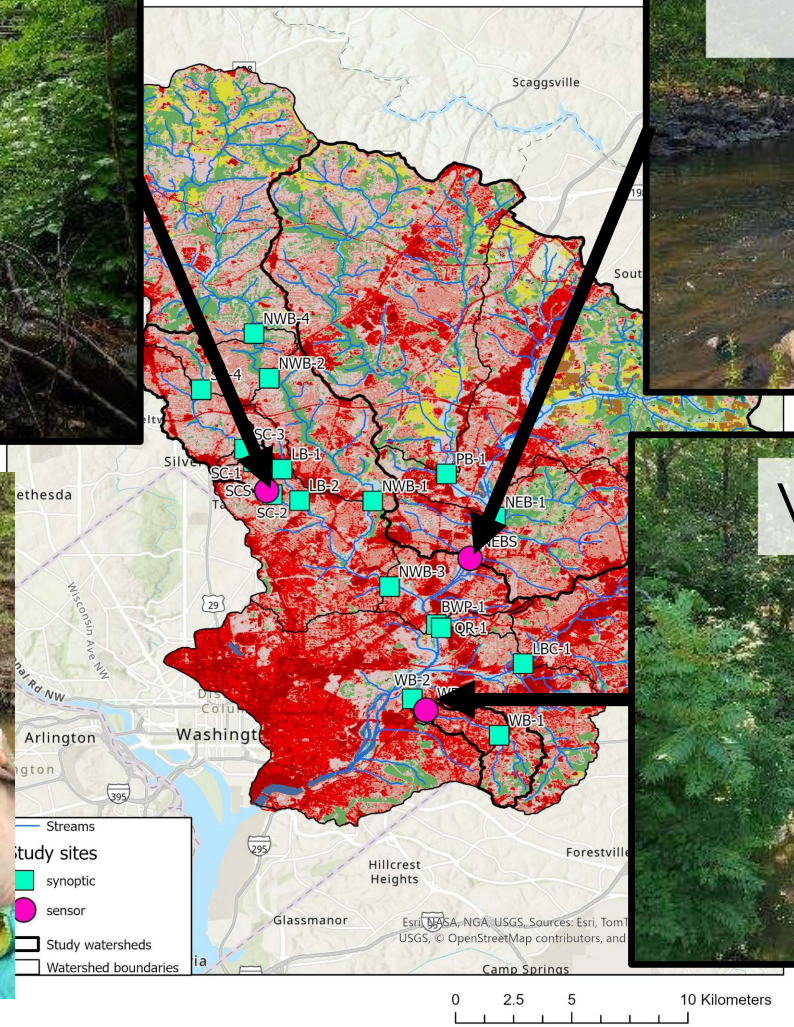
***In situ* sensors:**
Tryptophan-like fluorescence
Optical brighteners
Colored dissolved organic matter
Turbidity
Conductivity
Dissolved oxygen

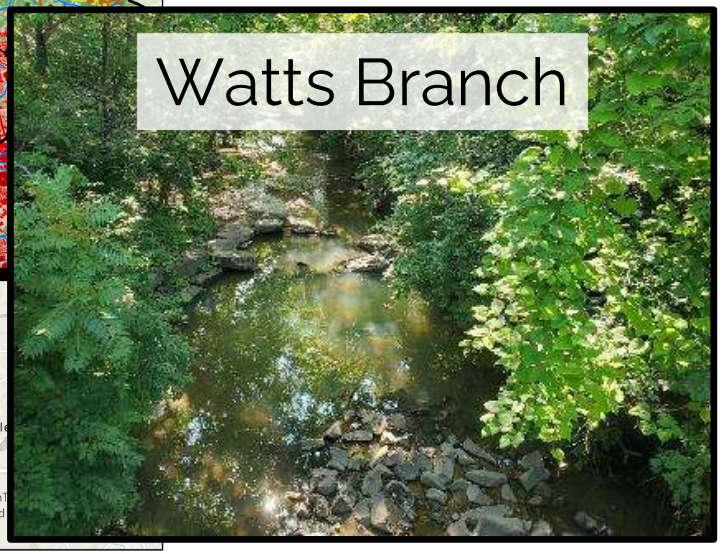


Bi/Weekly grab samples:
Microbial source tracking
E. coli concentrations
Dissolved organic matter fluorescence
Nitrogen and phosphorus

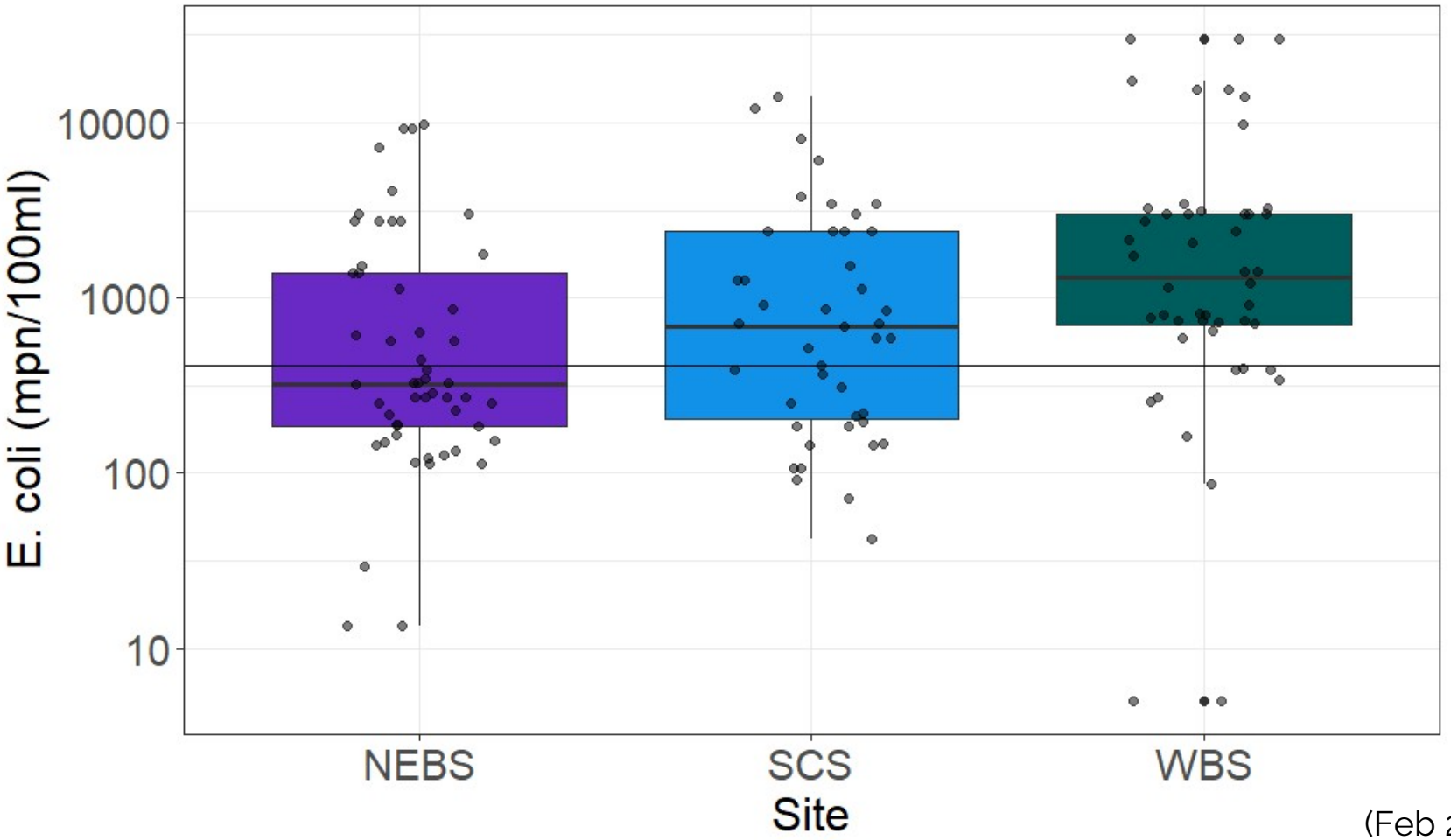

Sligo Creek


Northeast Branch
Anacostia


Scaggsville
NWB-4
NWB-2
SC-3
LB-1
SC-1
SCS
SC-2
LB-2
NWB-1
PB-1
NEB-1
NEBS
NWB-3
BWP-1
QR-1
LBC-1
WB-2
WB-1
Arlington
Washingt
Hillcrest
Heights
Glassmanor
Forestville
Camp Springs
Streams
Study sites
synoptic
sensor
Study watersheds
Watershed boundaries
0
2.5
5
10 Kilometers

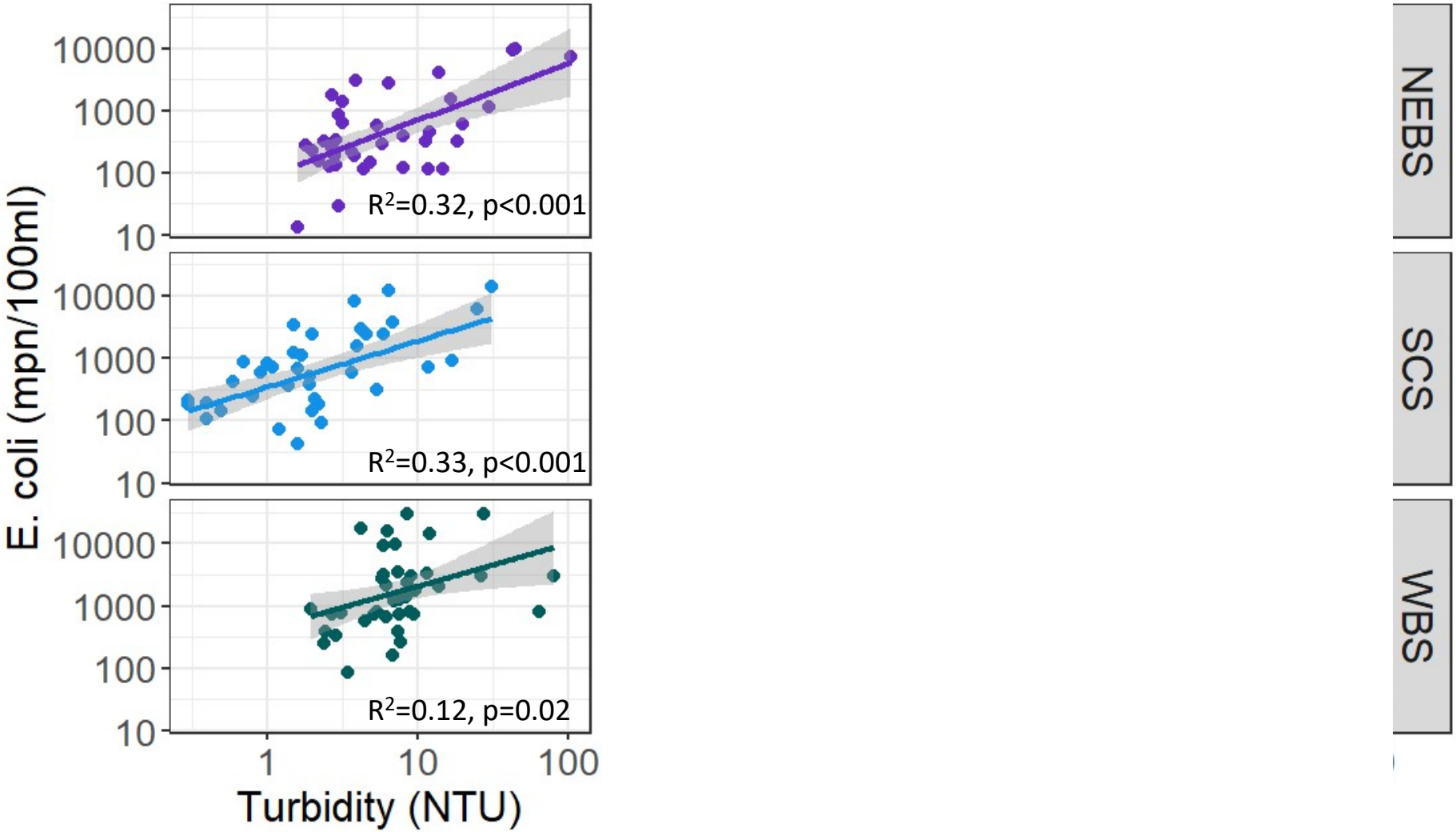

Watts Branch

E. coli concentrations span 4 orders of magnitude within sites

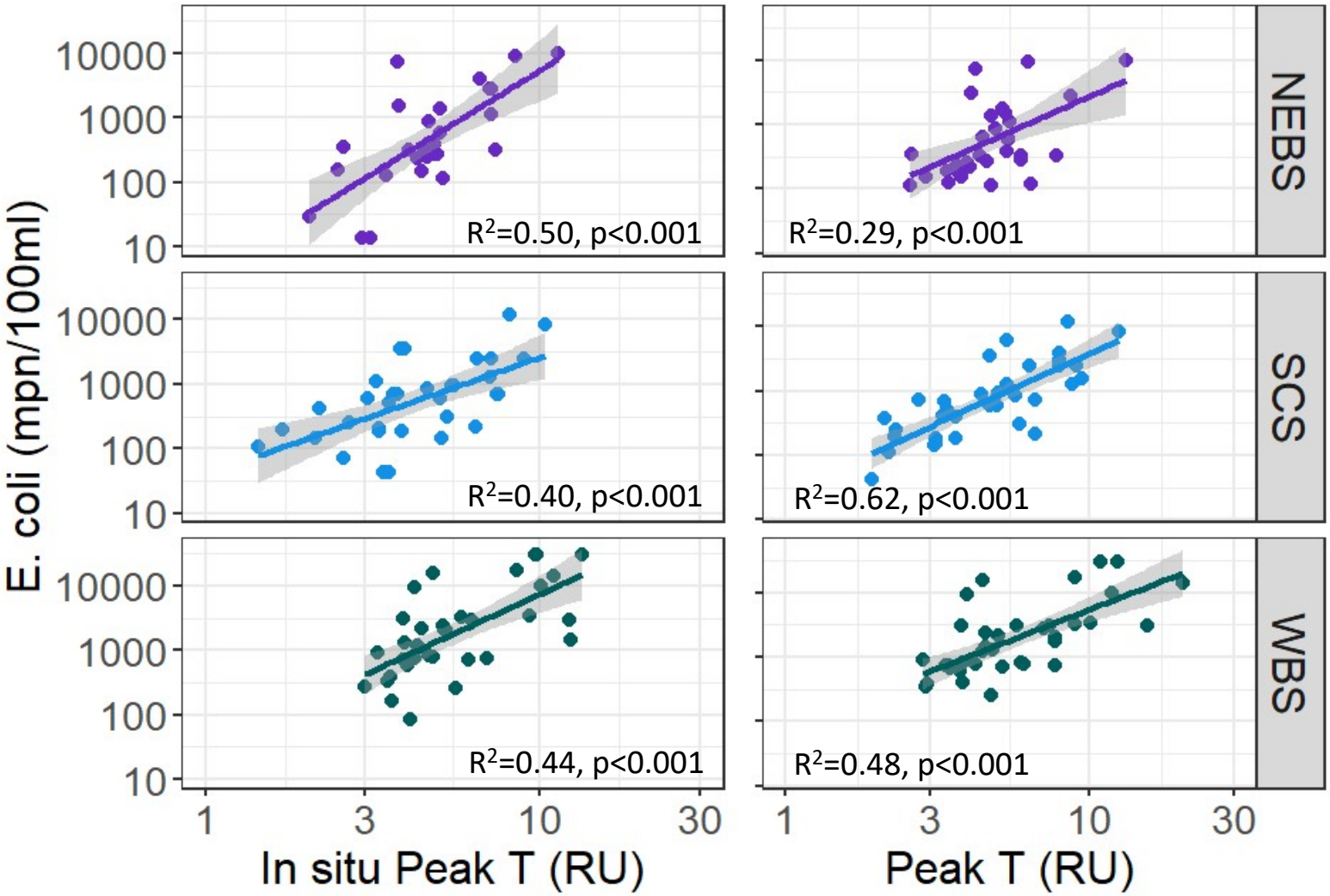


(Feb 2025-April 2026)

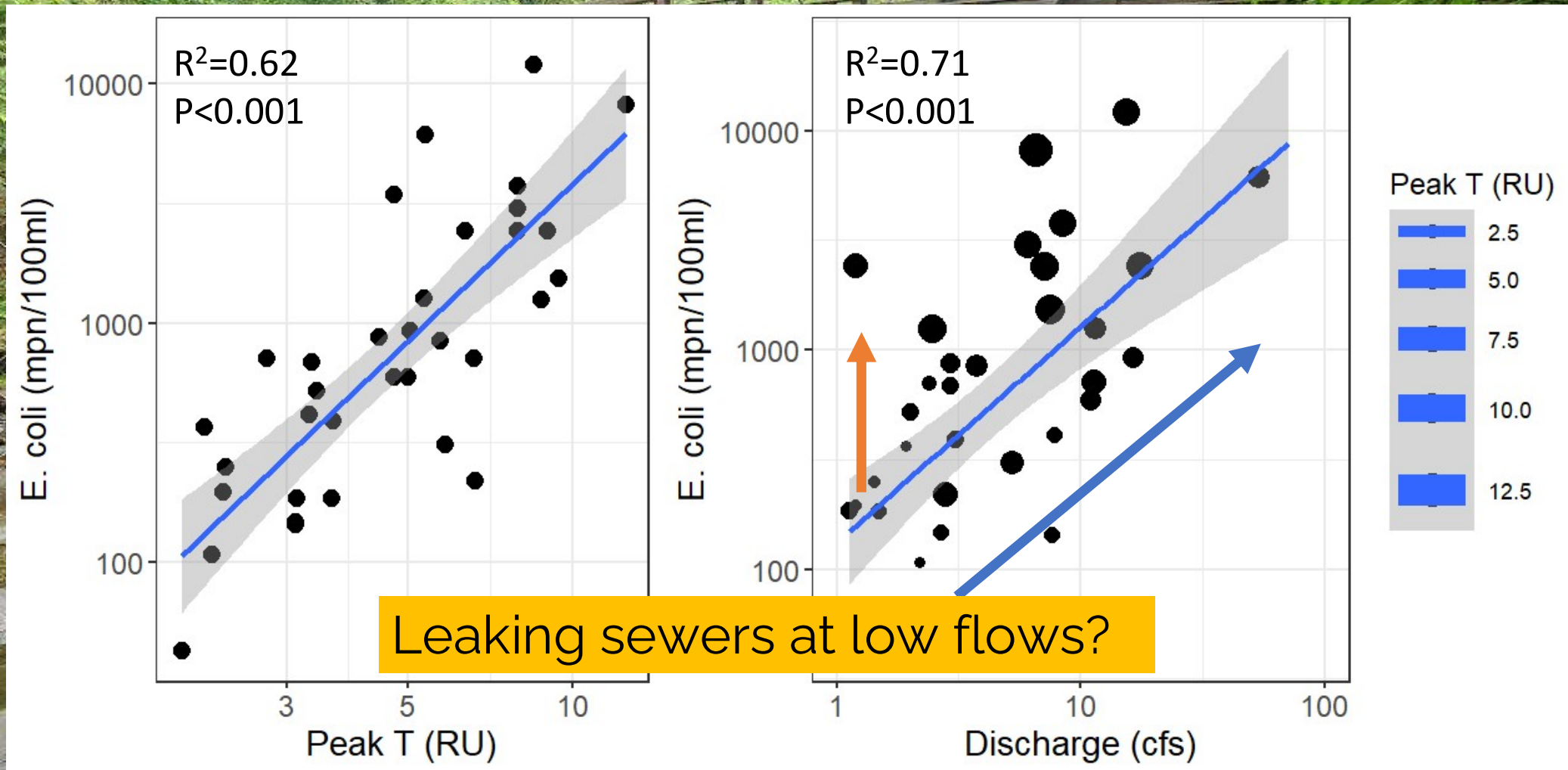
E. coli concentrations are correlated with stormwater proxies



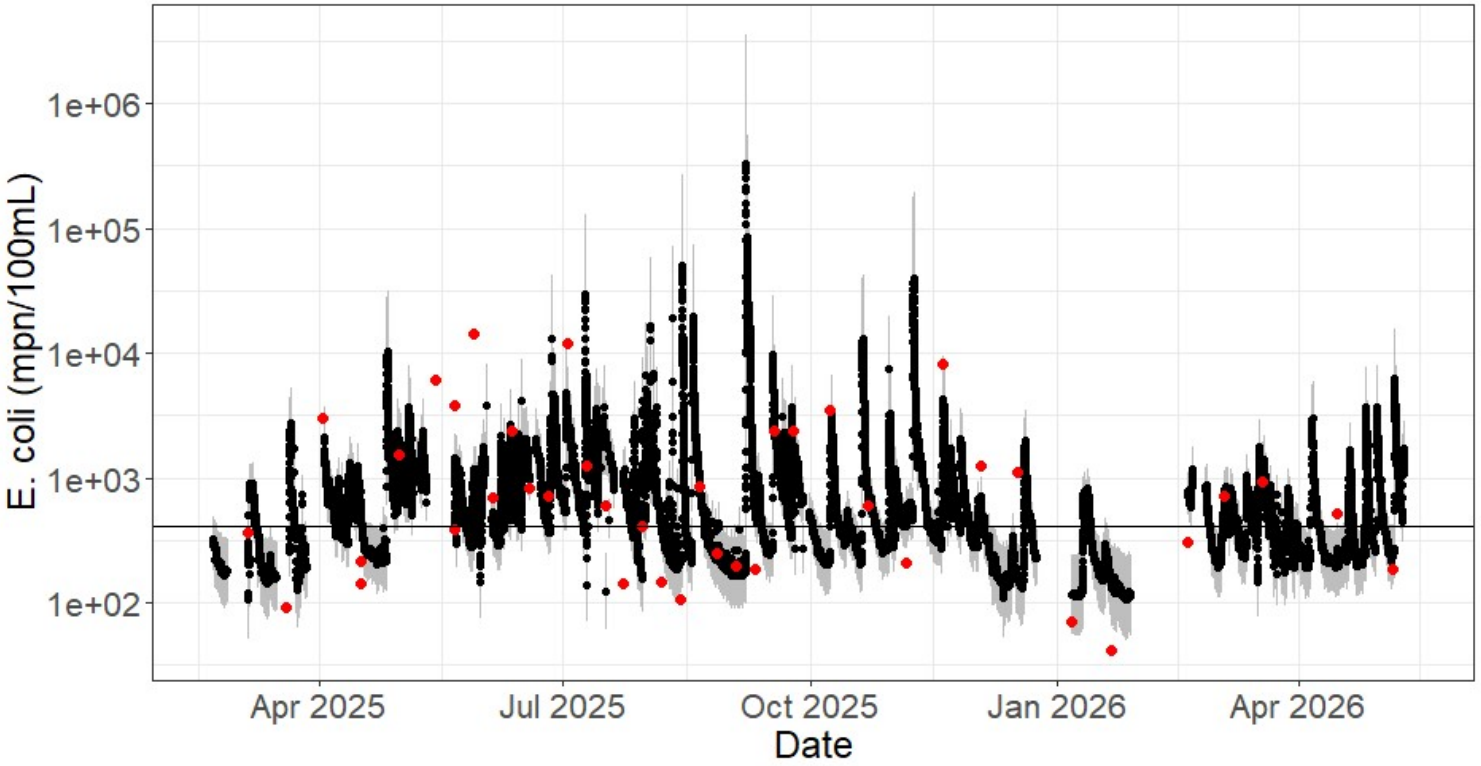
The best single predictor is tryptophan-like fluorescence



Wastewater proxies interact with flow at Sligo Creek

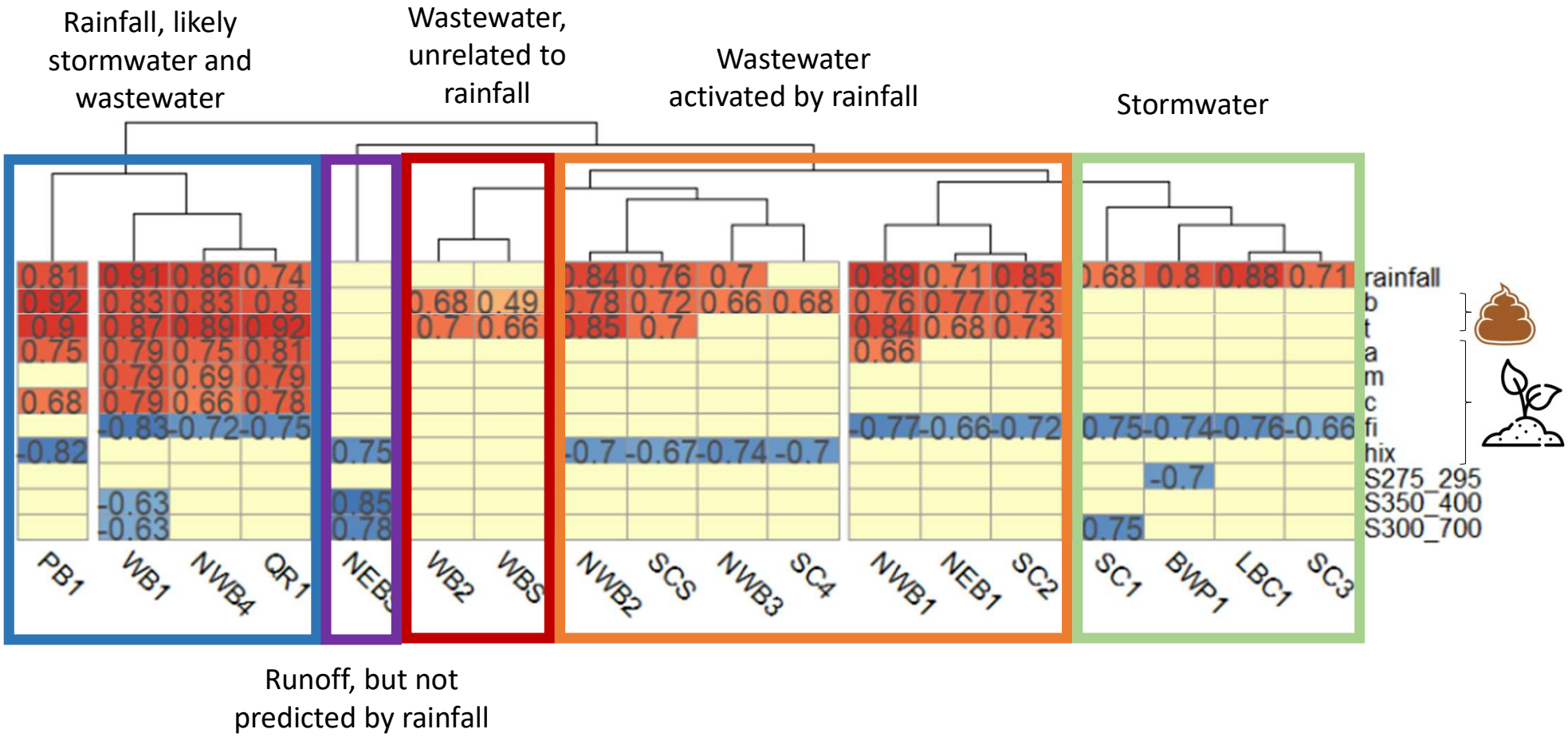


Goal: predictive models of *E. coli* for each site

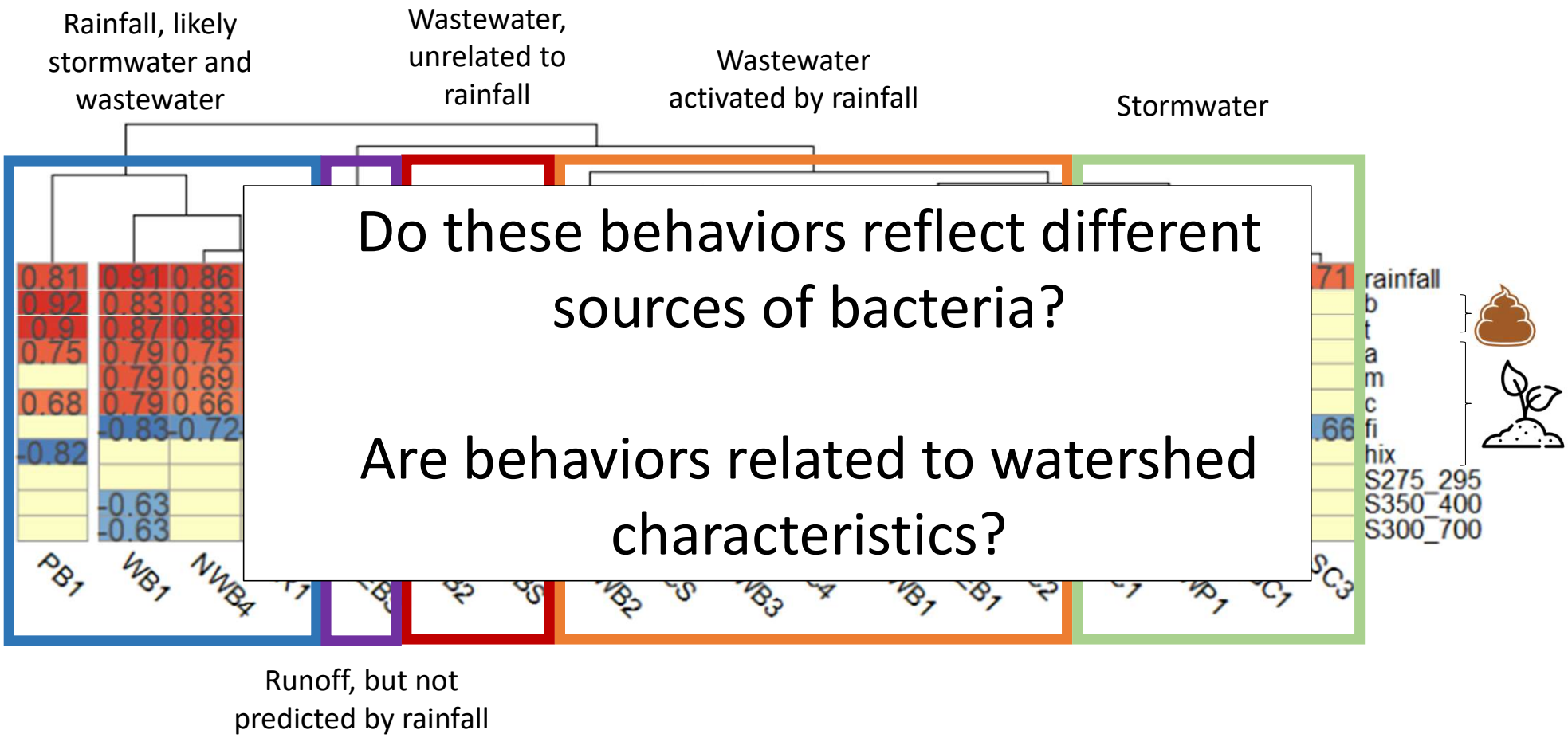


Estimated (black) and observed (red) E. coli concentrations for Sligo Creek, based on CDOM sensor data.

Controls on *E. coli* vary but there are general patterns



Controls on *E. coli* vary but there are general patterns



Do these behaviors reflect different sources of bacteria?

Are behaviors related to watershed characteristics?

E. coli concentrations consistently associated with tryptophan-like fluorescence

Next steps:

- Do sensor inferences align with microbial source tracking results?
- Is tryptophan a consistent predictor of *E. coli* and/or MST across broader sample of watersheds?
- Are watershed characteristics predictive of *E. coli* sources and proxies?

Goal this next year: Combine multiple methods to identify key assessment tools for managers. What information is needed to understand, manage, and improve system?

Funding & Acknowledgments

This research was funded through the Pooled Monitoring Initiative (PMI), Restoration Research Program.

Award #23852 | Smithsonian Institution | 2025-2027

The Pooled Monitoring Initiative funds applied science to advance restoration research for cumulative impacts, comparative effectiveness of stormwater practices, pollutants of emerging concern, and trade-offs with new research questions added annually.

WEBSITE: https://cbtrust.org/grants/restoration-research/

Translation

What are the take home points?
What does this mean for me?

Translation Slides by Morgan Corey

Maryland DNR, Watershed and Climate Services

What does this mean for me?

- *E. coli* levels vary across Anacostia watersheds, making it hard for community members to anticipate safe conditions to fish, wade, and paddle
- Contamination comes from different sources with higher *E. coli* levels in populated areas pointing to human & pet waste as a major pollution source
- No one size fits all approach → Site-specific solutions based on the source of pollution
- Make it easy for residents to check WQ status & share risks
- Better anticipating wastewater pollution is needed to understand sources of pollutants and reduce bacteria levels in the Anacostia watershed
- Repairing old infrastructure is key to prevent urban wastewater pollution

What does this mean for me?

What do I take from this if I am a practitioner:

- Contamination of streams comes from different sources in the Anacostia
- Solutions must be site-specific
- This research informs how to monitor WQ & estimate costs to monitor (e.g. install sensors for WQ information, use trypto & CDOM as indicators)

What do I take from this if I am a regulator:

- Human wastewater is a major stressor in highly populated areas
- Target sites with high *E. coli* levels (Watts Branch) for the most impact
- Better anticipating typical wastewater pollution sources is needed to reduce bacterial contamination in highly impacted urban streams


Thank you!
Questions?