Use of molecular sewage indicator methods to reduce uncertainty in watershed remediation efforts and water contact recreation

CBT Pooled Monitoring Workshop

Maryland Department of the Environment
Baltimore, MD 21230
June 18, 2025

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Zooquatic, Baltimore DPW, Blue Water Baltimore, Healthy Harbor Initiative

Research Question and Hypotheses

- Question 5a: “Restoration at project scale”: Pollutants of emerging concern, Bacteria.
- *Original Hypotheses*
- **H1**) Combining MST and FIB methods will allow a qualitative assignment of the relative proportion of human versus non-human FIB in a given water sample.
- **H2**) Daily testing, using both standard FIB culture and PCR methods to detect human vs non-human fecal bacteria, will show that high FIB counts do not always correspond to high human MST (Bacteroides) signals.
- **H3**) Daily testing of water quality will provide knowledge about the duration and drivers of sewage-derived bacteria and other FIB in tidal water that could not be achieved with weekly testing.

Swimmable Harbor Goals

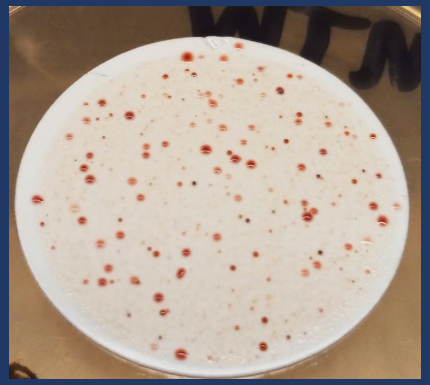
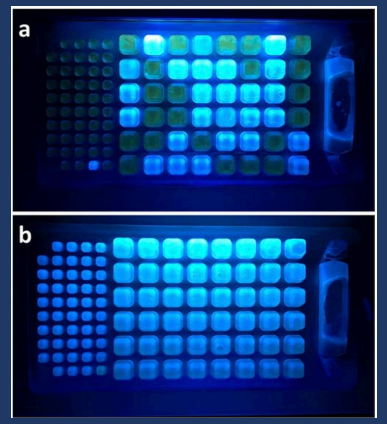
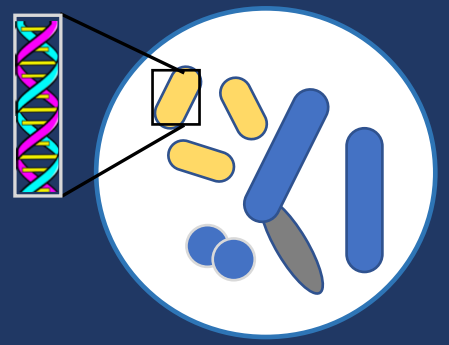
- Goal set in 2010 by the Waterfront Partnership
- Baltimore under EPA, DOJ sewage consent decree
- “Swimmable” = Fecal Indicator Bacteria below EPA threshold

The reimagined Middle Branch

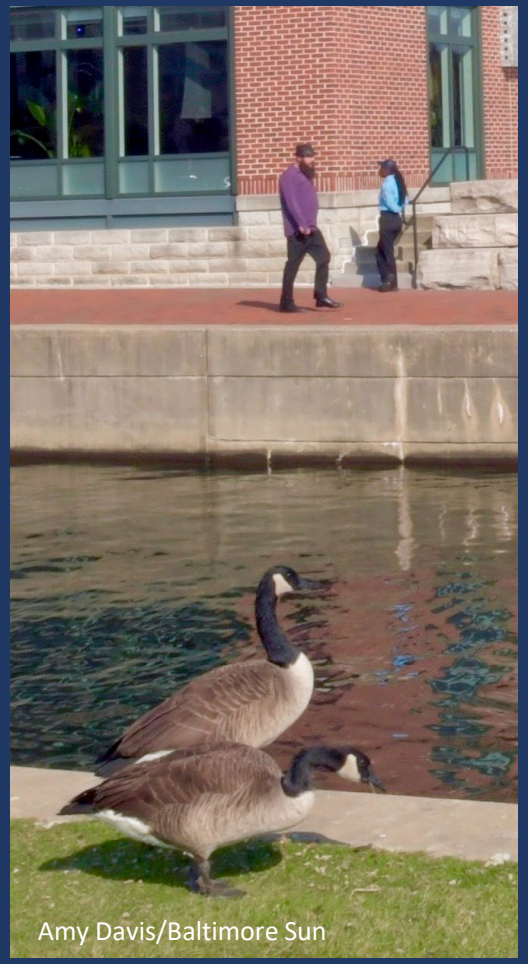
Baltimore Blue Way

Fecal Indicator Bacteria (FIB) can be measured many ways

e.g., Enterococcus

	M-entero agar	IDEXX	Species-specific qPCR
			
Minimum process time	24 hours	24 hours	6 hours
What is measured	Colony growth	Metabolic activity	Bacteria genomic DNA
Data output	CFU	MPN	Genome copy

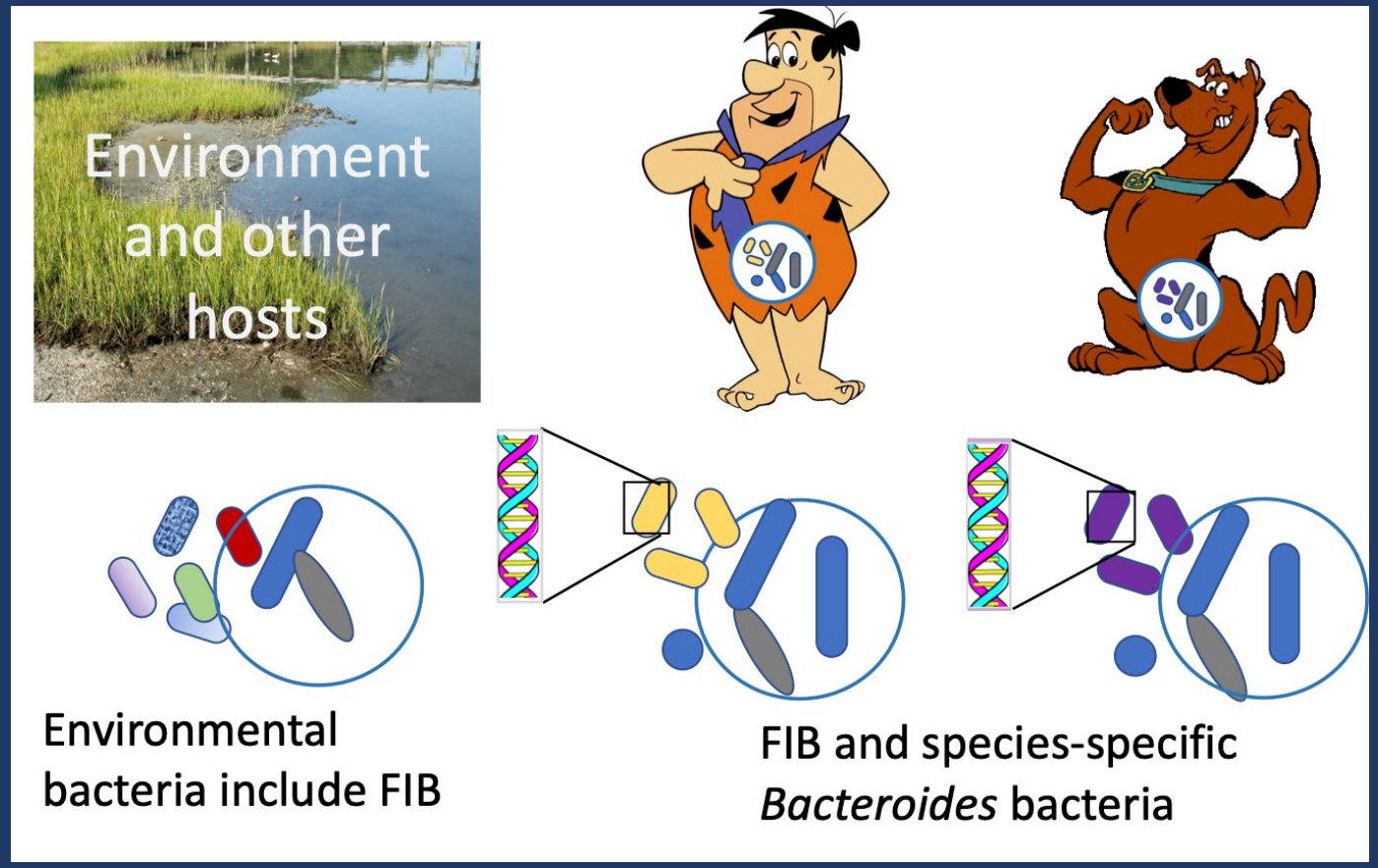
FIB have various possible origins



(Upper photo: Cladophora in Baltimore: https://eyesonthebay.dnr.maryland.gov/hab/news_062404.cfm)

Some fecal indicator species are more host-specific than others

E. coli
Enterococcus



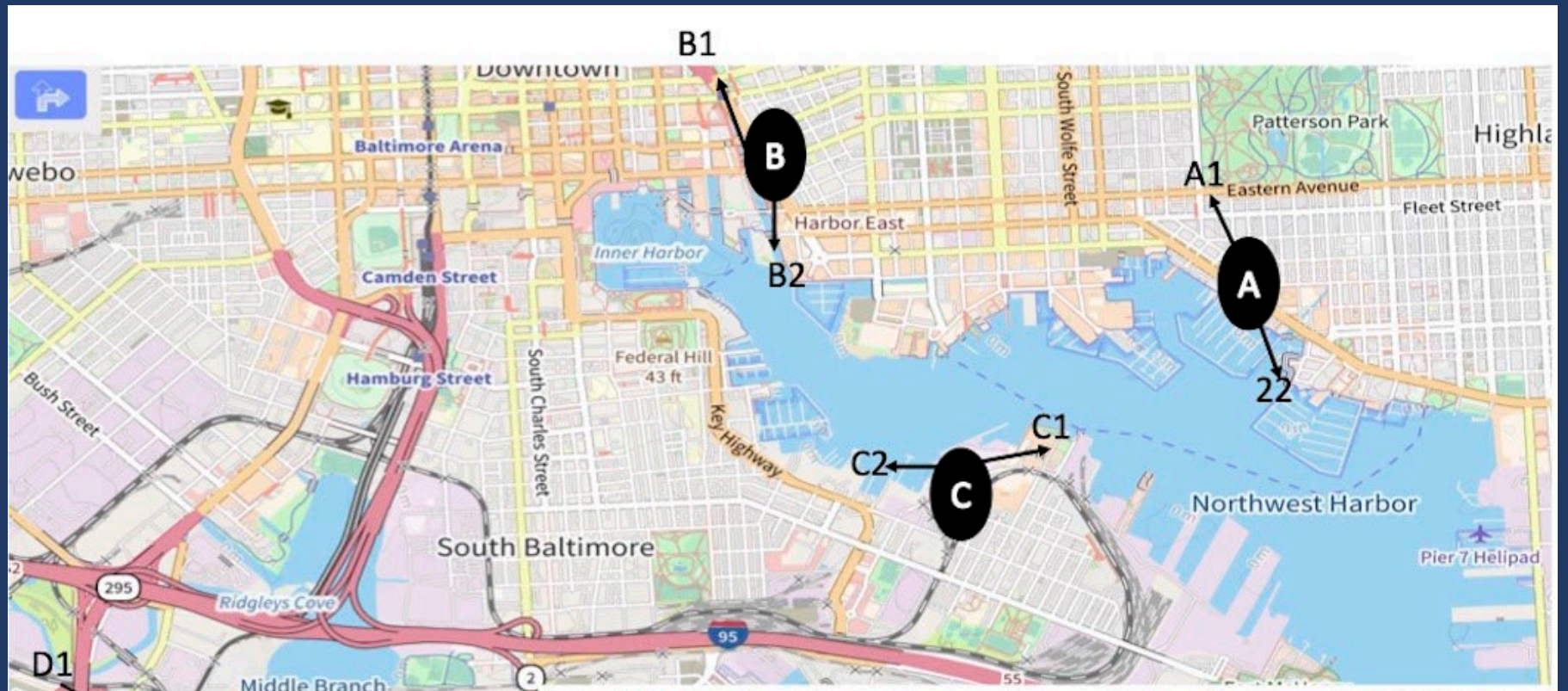
Lachnospiracae, humans
Others species for other hosts

Study design

Paired tidal and nontidal sites, four locations

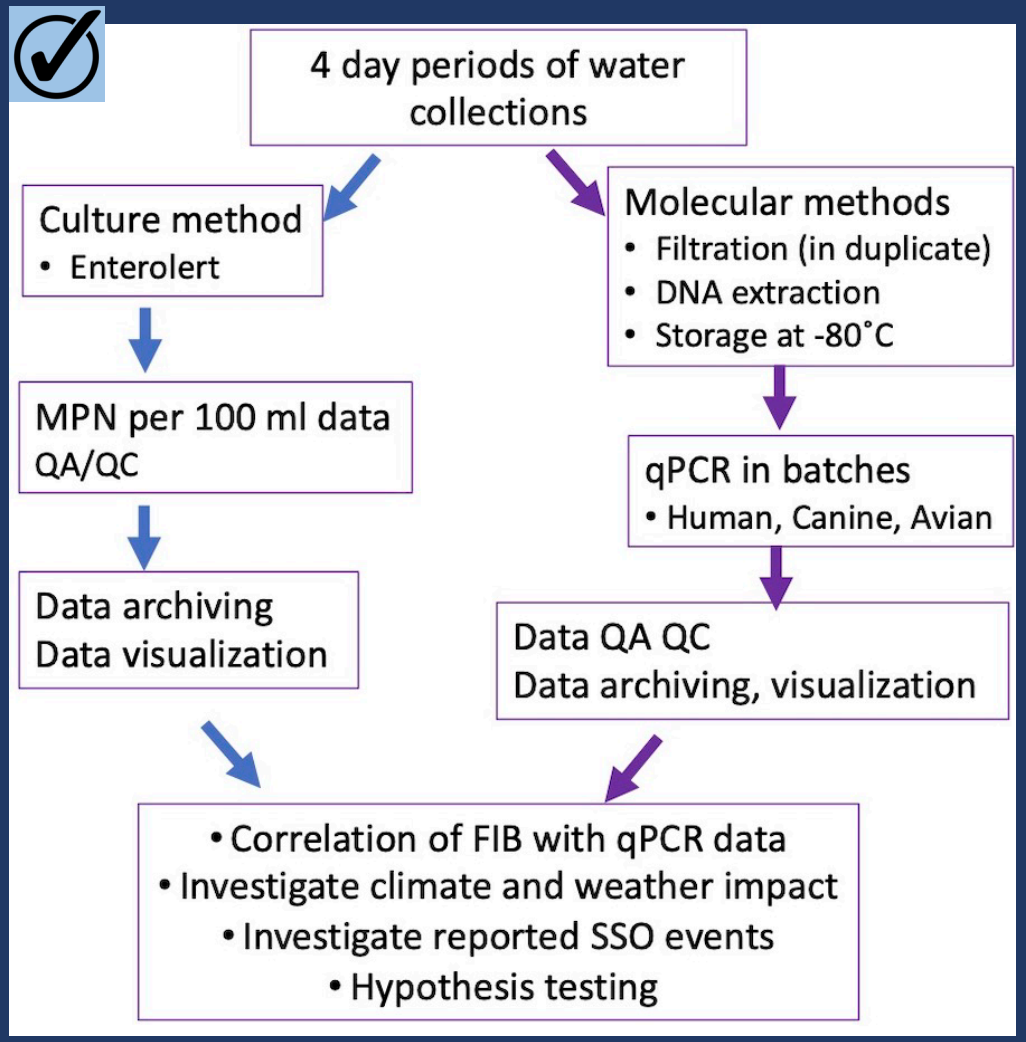
Four-day repeated samples at each location
-morning collection by 9 am

Three months in the recreational season
-July, Aug, Sept.



Map #	Station ID	Station Name	GPS Coordinates	COMAR Use (I - IV)
A1	Can-A1	Canton nontidal	39.282965, -76.579851	n.a.
A2	HC-A2	Harris Creek tidal	39.279116, -76.5804091	II
B1	JFP-B1	JF at Penn nontidal	39.3104185, -76.6197909	II
B2	JFTW-B2	JF @ Trashwheel tidal	39.28310709, -76.6035508	II
C1	UA-C1	Under Armor nontidal	39.275643, -76.590734	n.a.
C2	SC-C2	Sailing Center tidal	39.275063, -76.599843	II
D1	GF-D1	Gwynns Falls nontidal	39.2715712, -76.6487133	II
D2	MB-D2	Middle Branch tidal	39.256057, -76.621375	II

Study Design



Human, canine

QA/QC

Anticipated findings

Rivers and major outfalls will behave like point sources to the estuary

→ MPN and human MST will be higher in the river than in the receiving tidal water

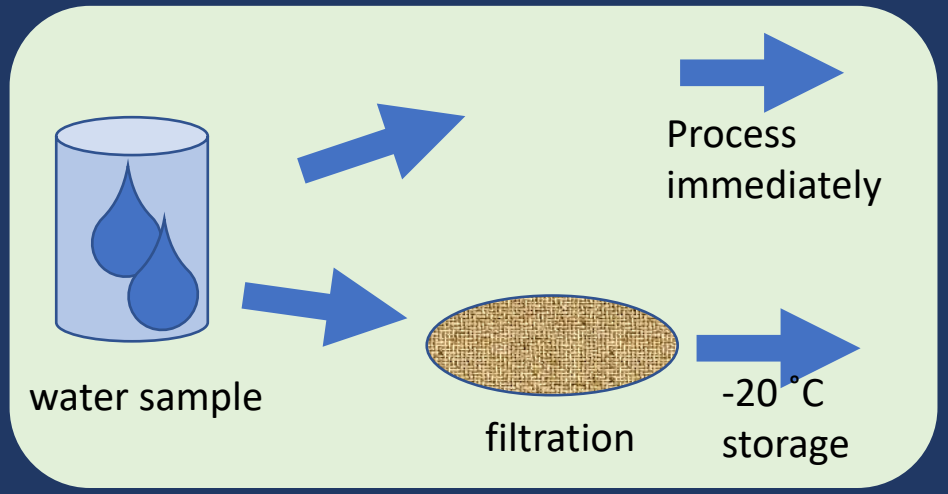
Tidal versus nontidal locations

→ Rainfall will be a driver of high MPN and MST signal

In the absence of rain, if there are non-human sources of enterococcus in the tidal water, then the upstream MST will be higher than in the tidal water, but Enterolert may be higher in the estuary.

Canine MST is hard to predict. Will not necessarily correlate with human MST or with Enterolert. Based on prior data, there may be high levels in direct harbor runoff.

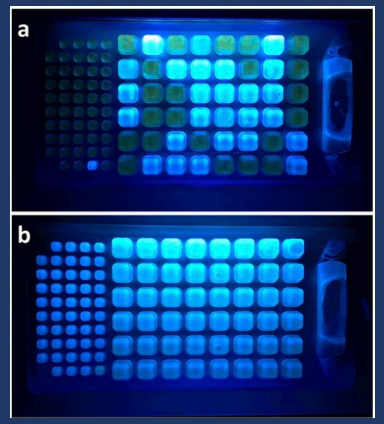
Analytical approaches



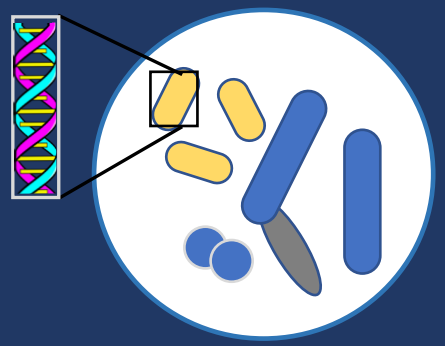
Enterolert dilution and culture → Most probable number

extract DNA → Count bacteria genomes by PCR

Enterolert



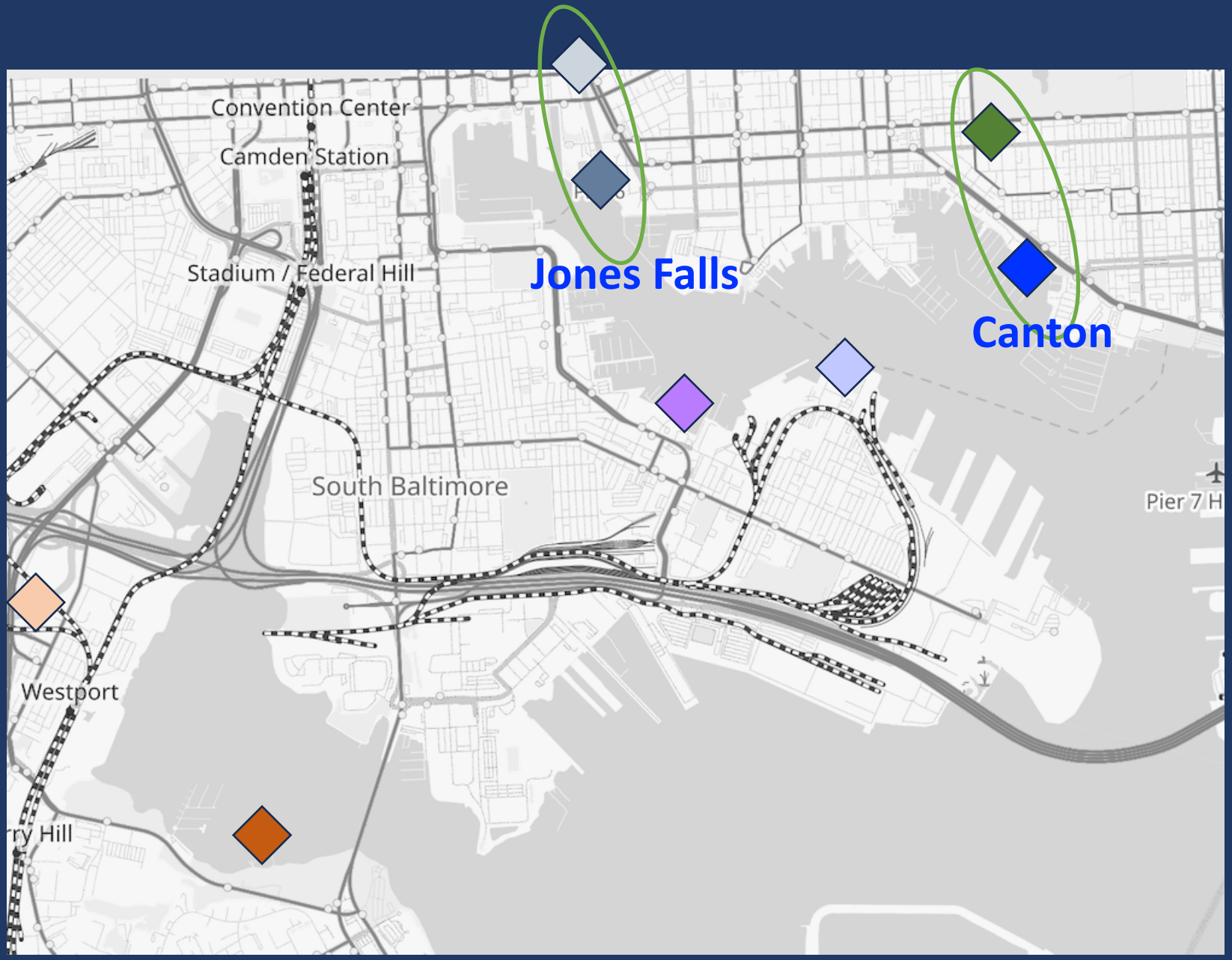
Human and Canine PCR



Visualization and inspection of results

- Compare paired locations: Tidal / Nontidal
- Compare qualitative trends of Enterolert vs qPCR
- Human and canine
- Rainfall effects

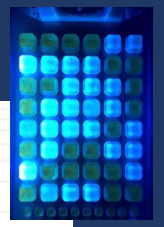
Jones Falls and Canton

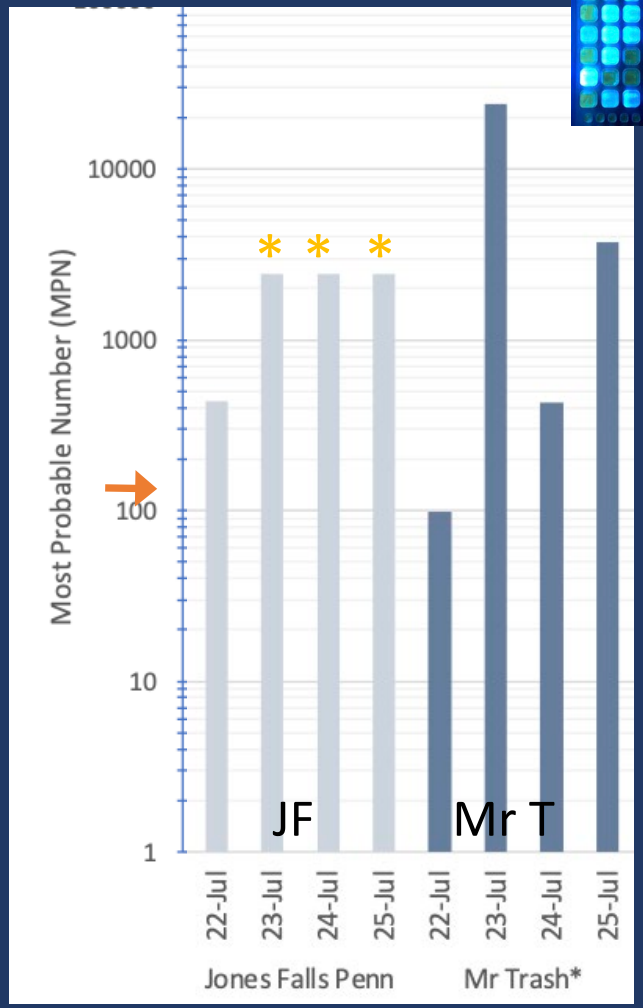


Jones Falls / Mr. Trash Wheel

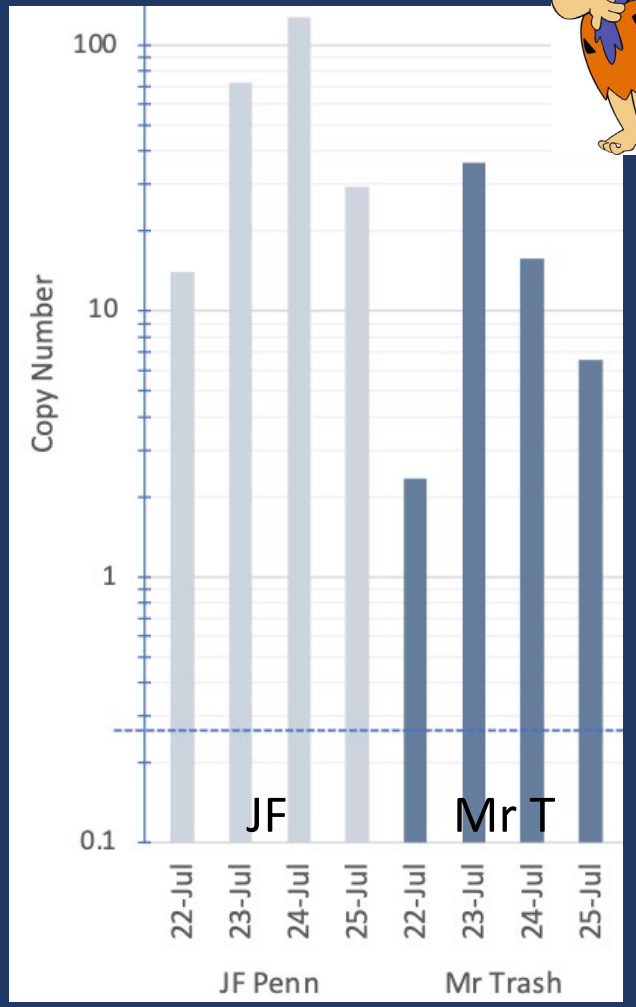
July

Enterolert

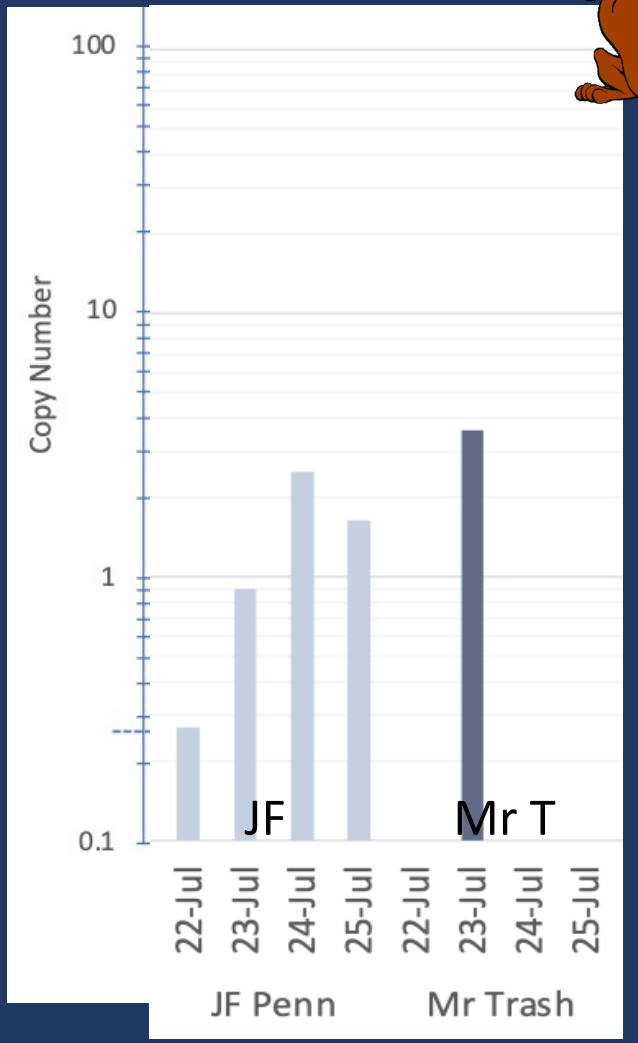




Human MST



Canine MST



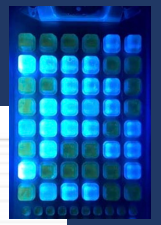
* Upper limit of Enterolert assay

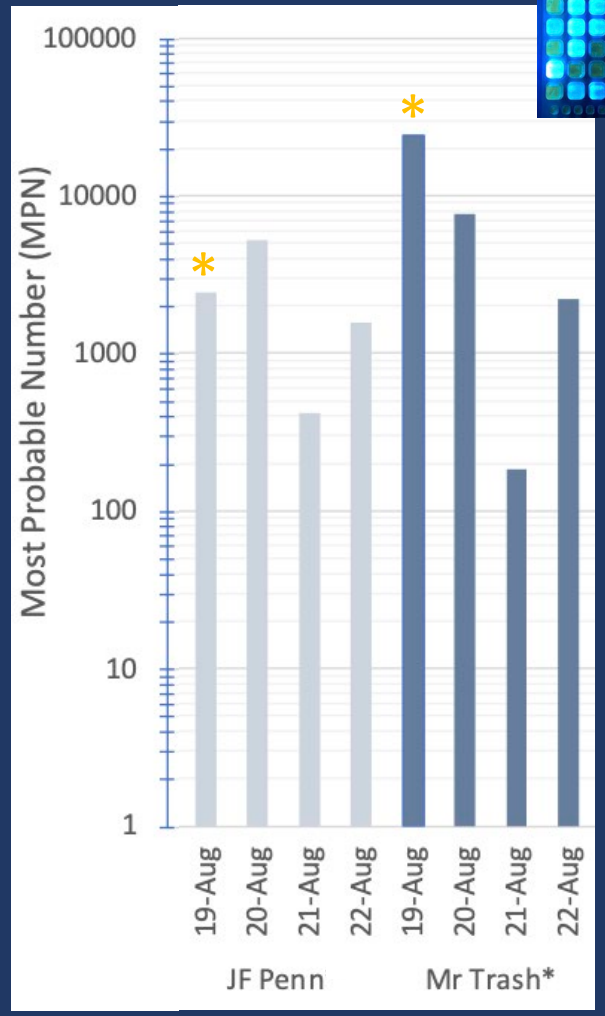
0.3 " rain 2 pm on July 22, trace at 11 pm July 23

Jones Falls / Mr. Trash Wheel

August

Enterolert





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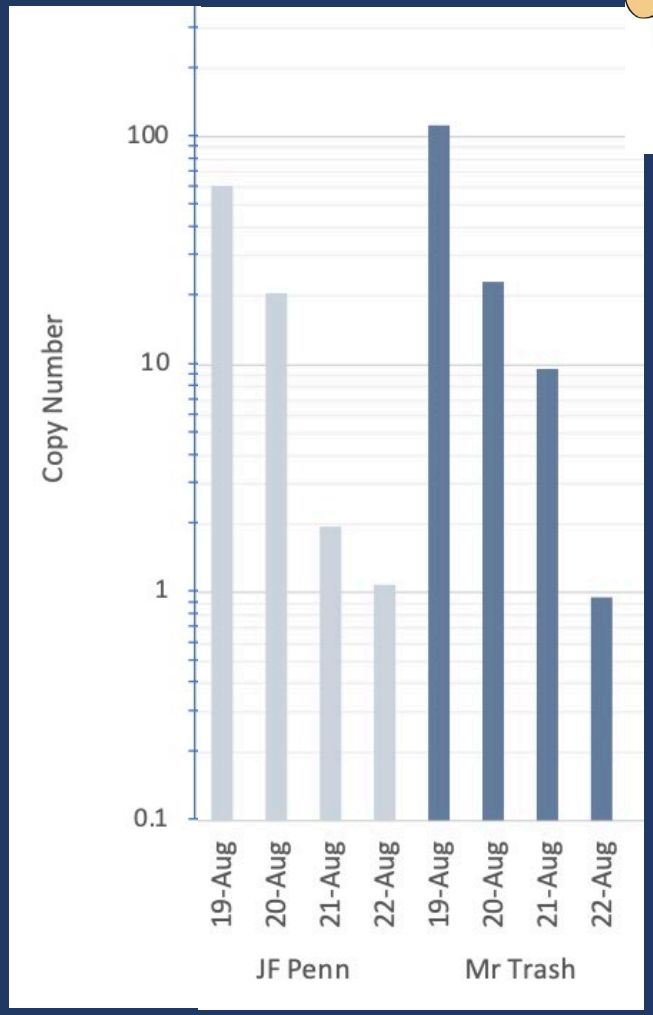
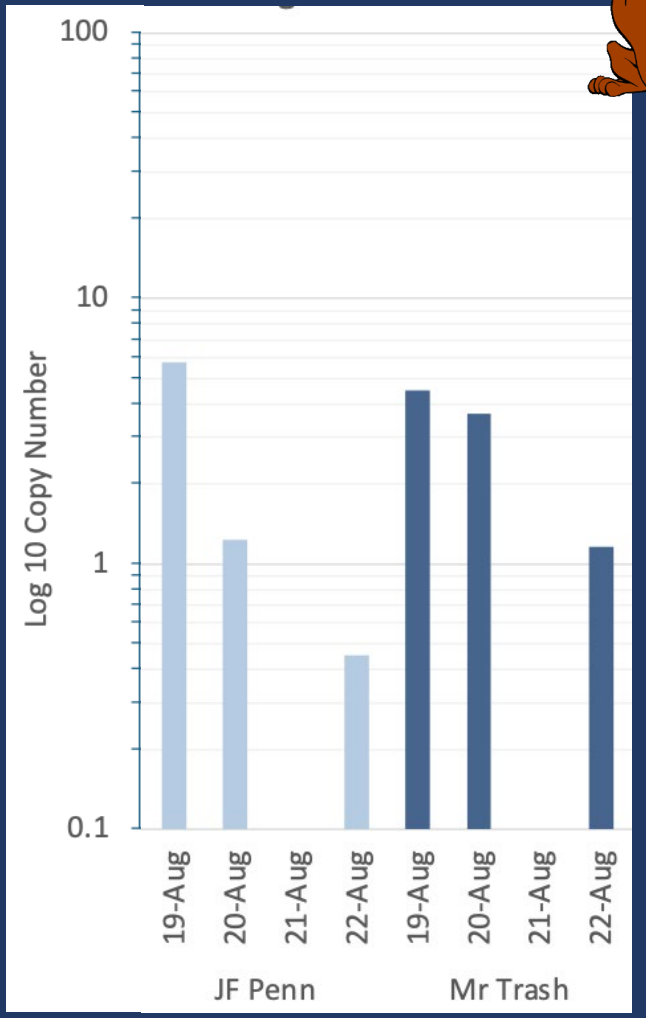


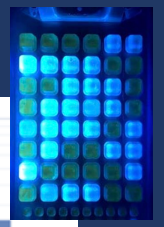
1.0 - 2.7 inches late on Aug. 18.

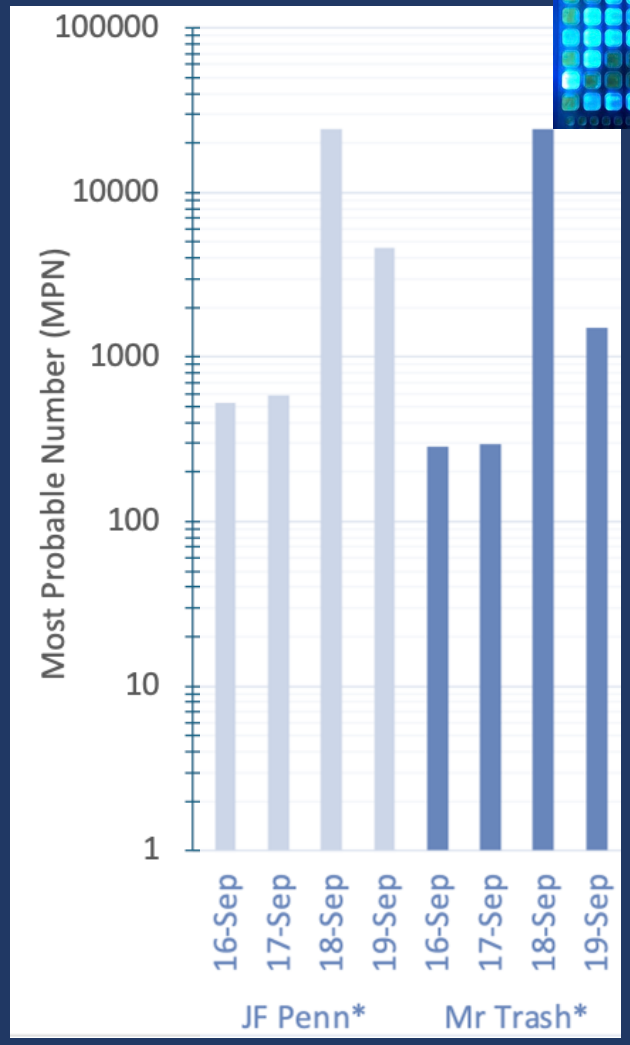
Canine MST



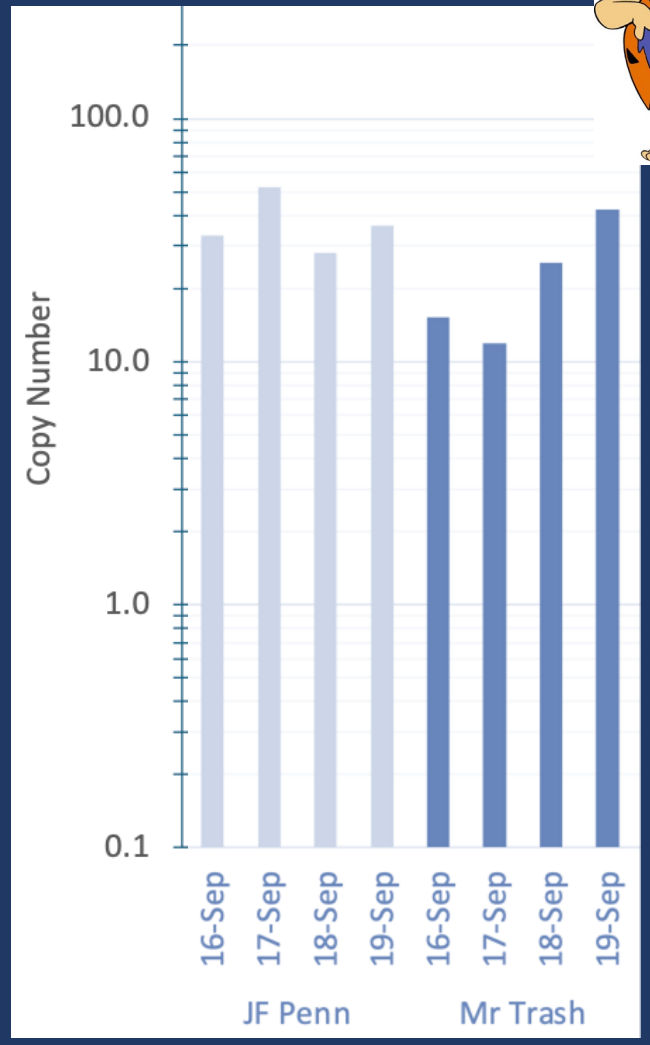
Jones Falls / Mr. Trash Wheel
September

Enterolert





Human MST



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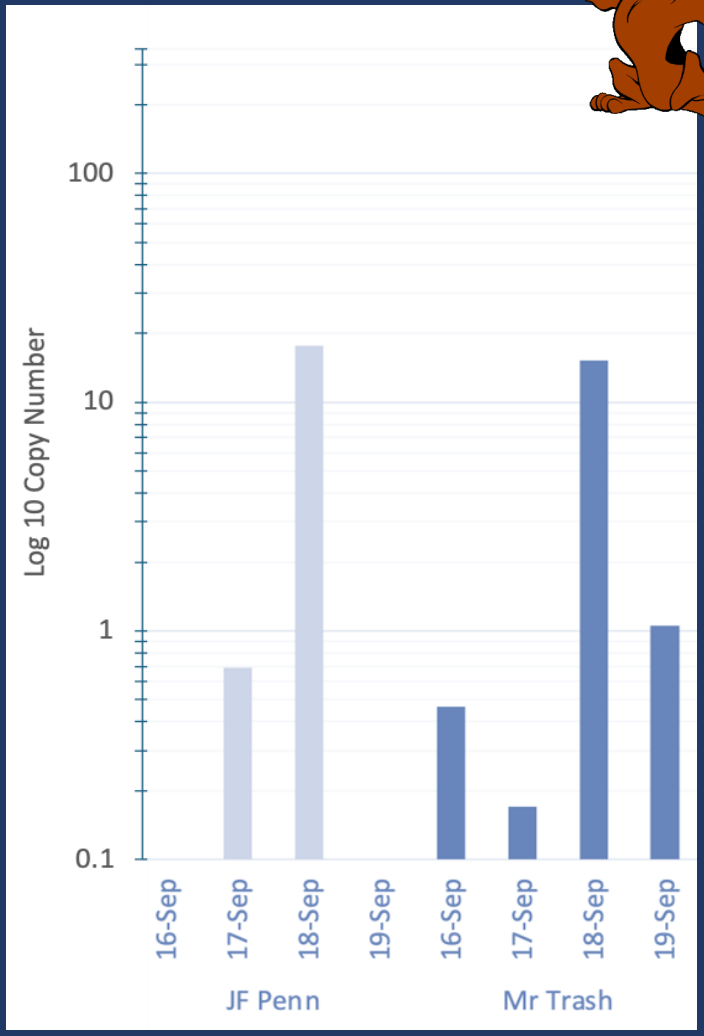
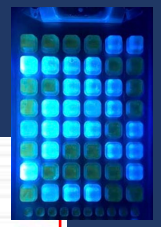
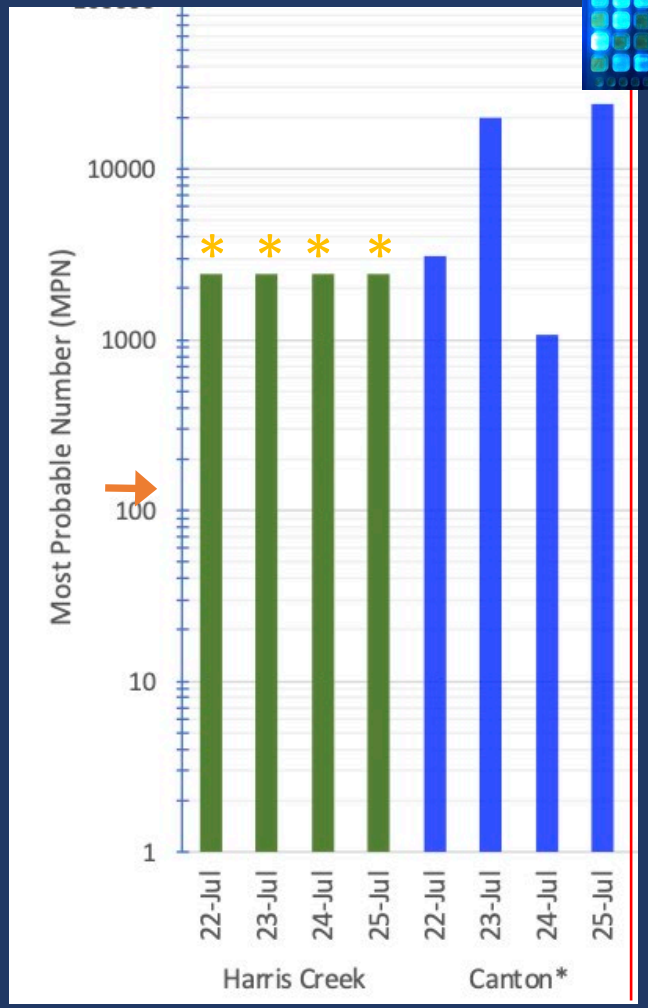


0.5“ rain 4 am - 8 am Sept 18

Harris Creek / Canton
July

Enterolert





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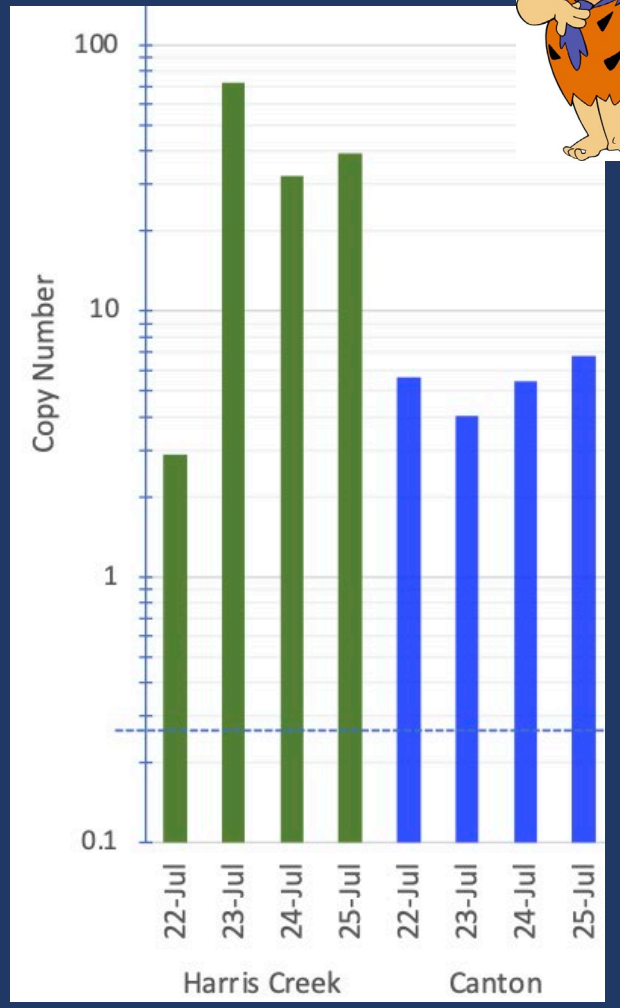
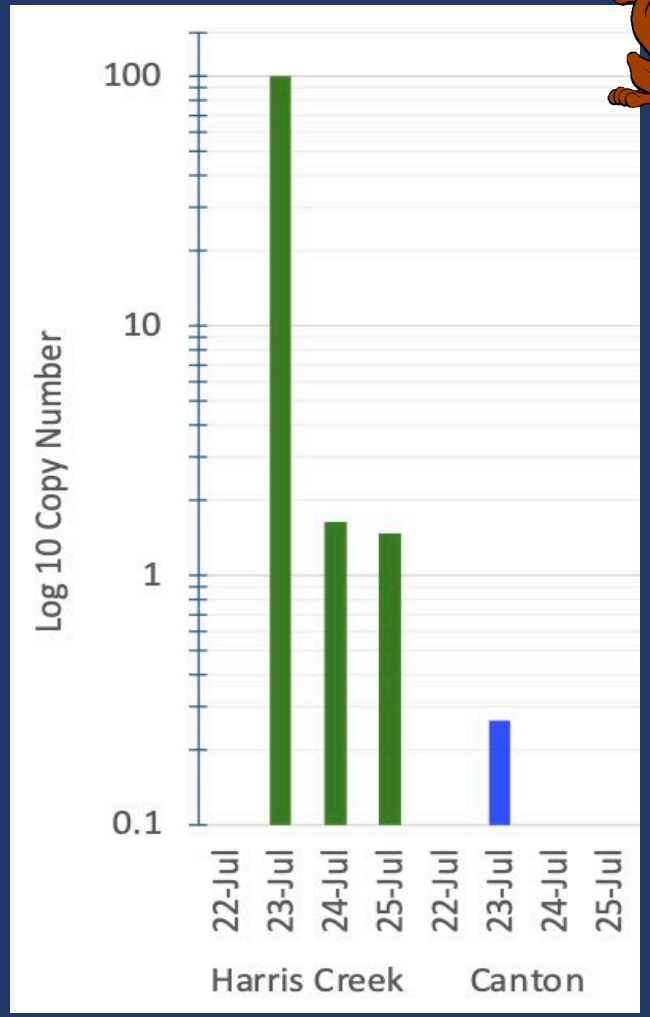


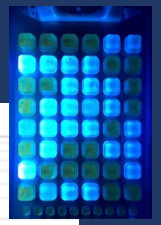
0.4 “ rain late on July 22

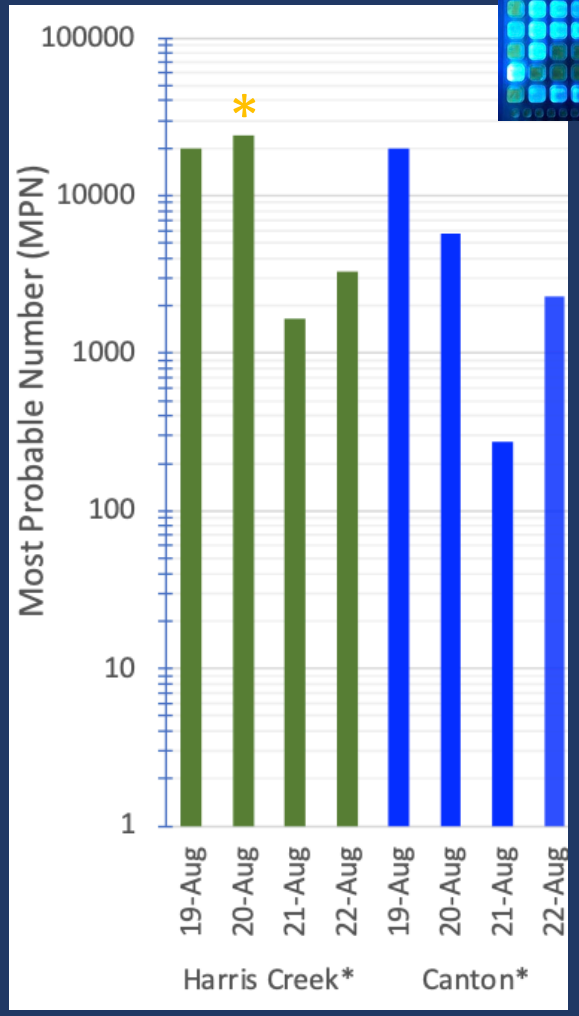
Canine MST



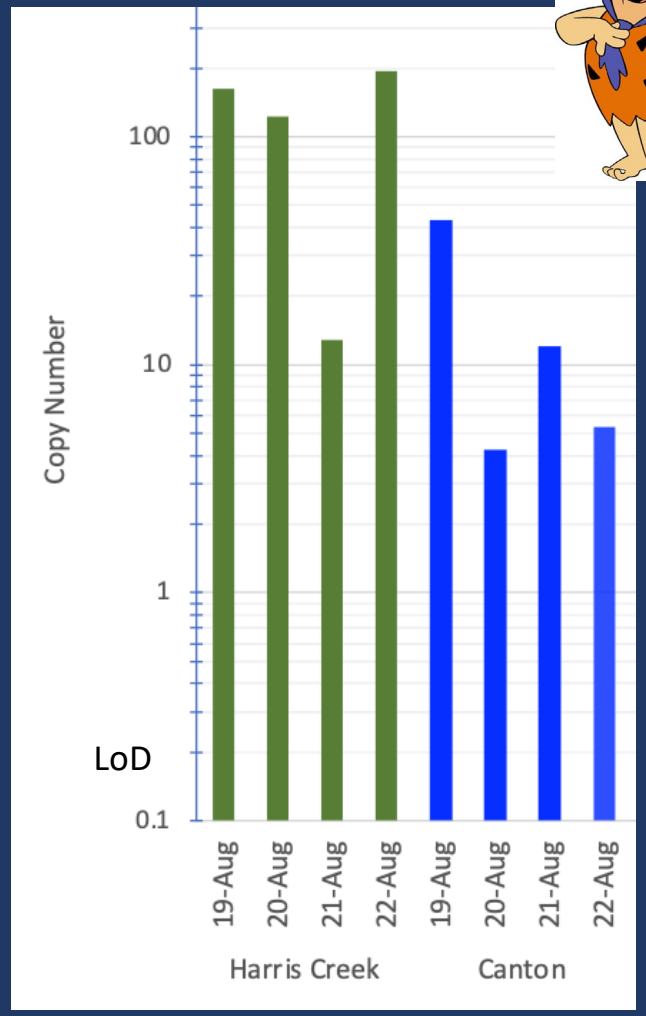
Harris Creek / Canton
August

Enterolert





Human MST



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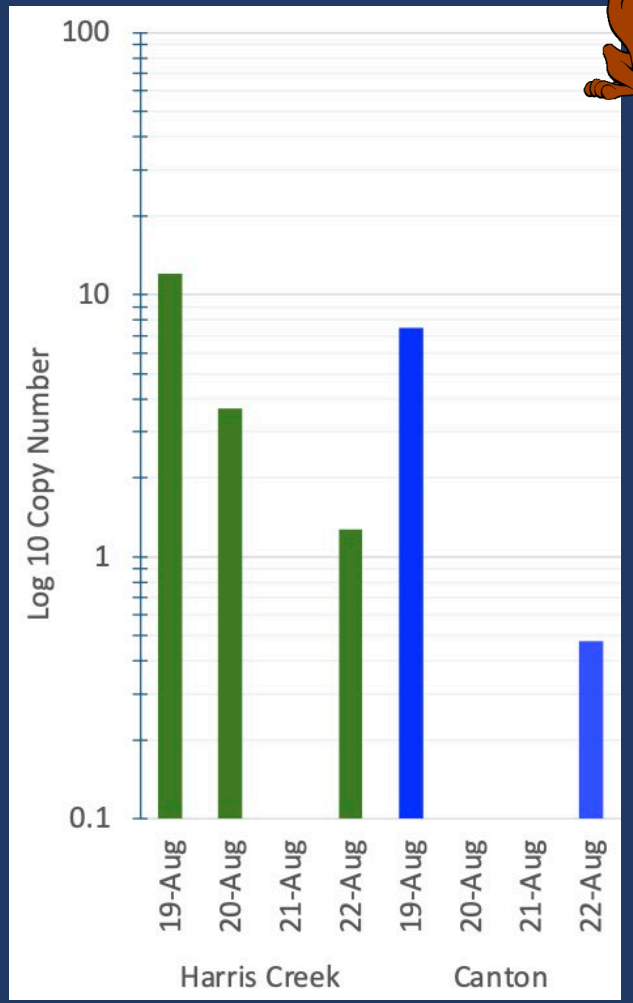

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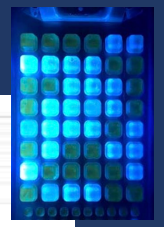
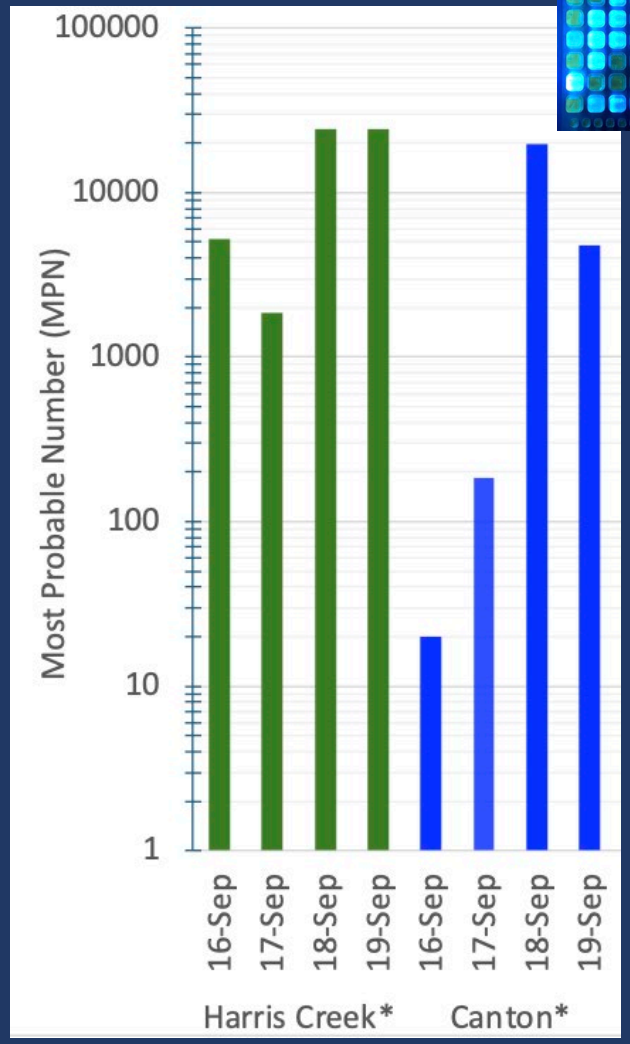
Rain late on Aug. 18.

Harris Creek / Canton
September

Enterolert





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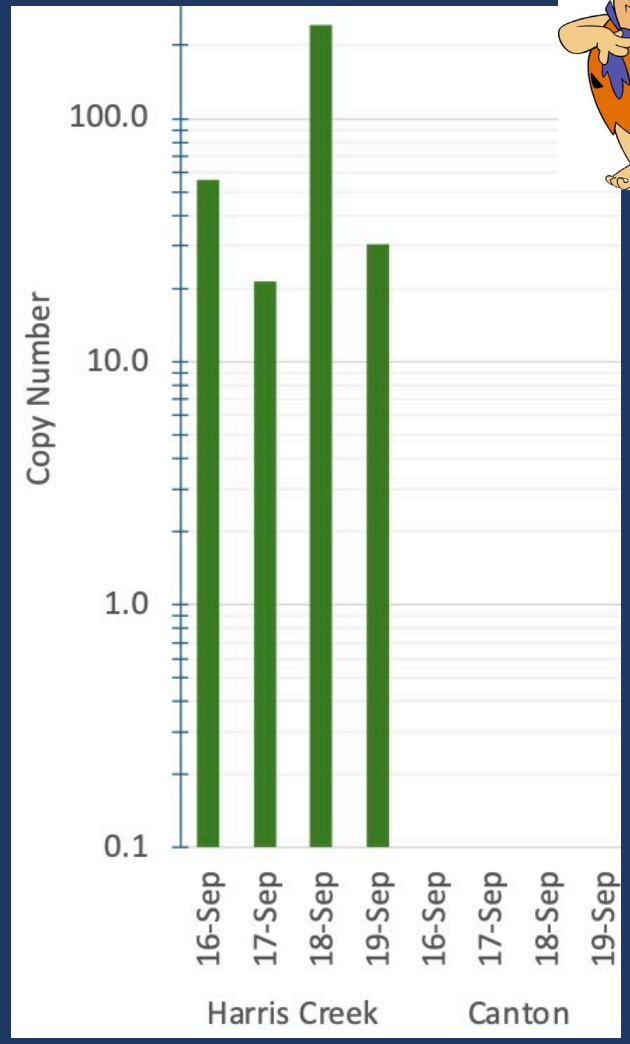

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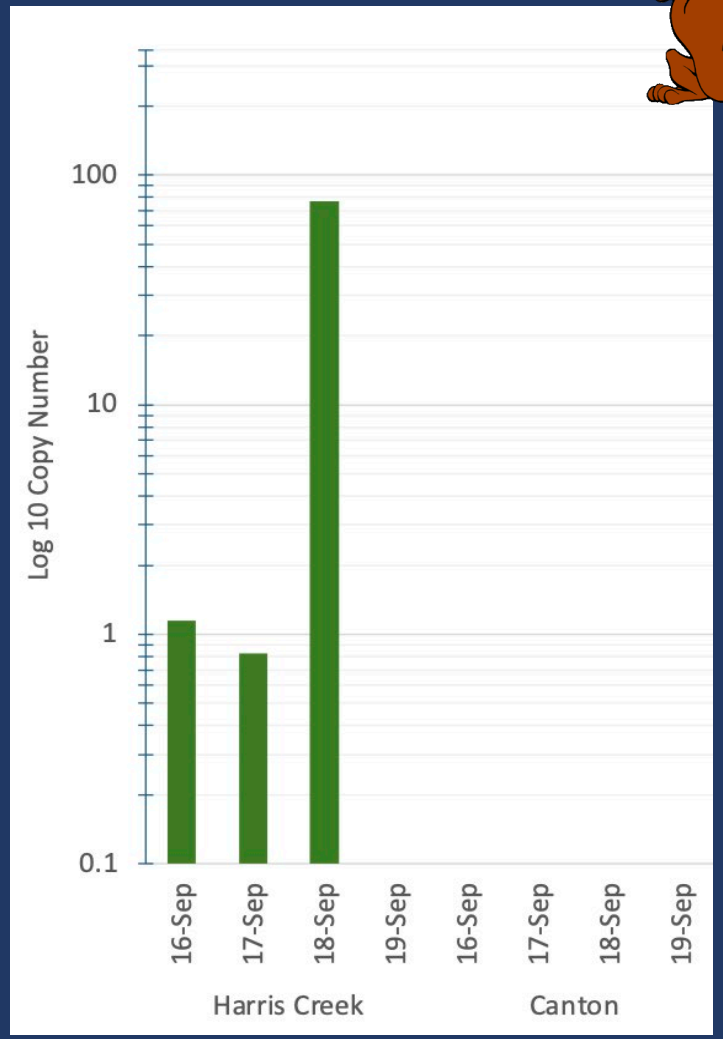
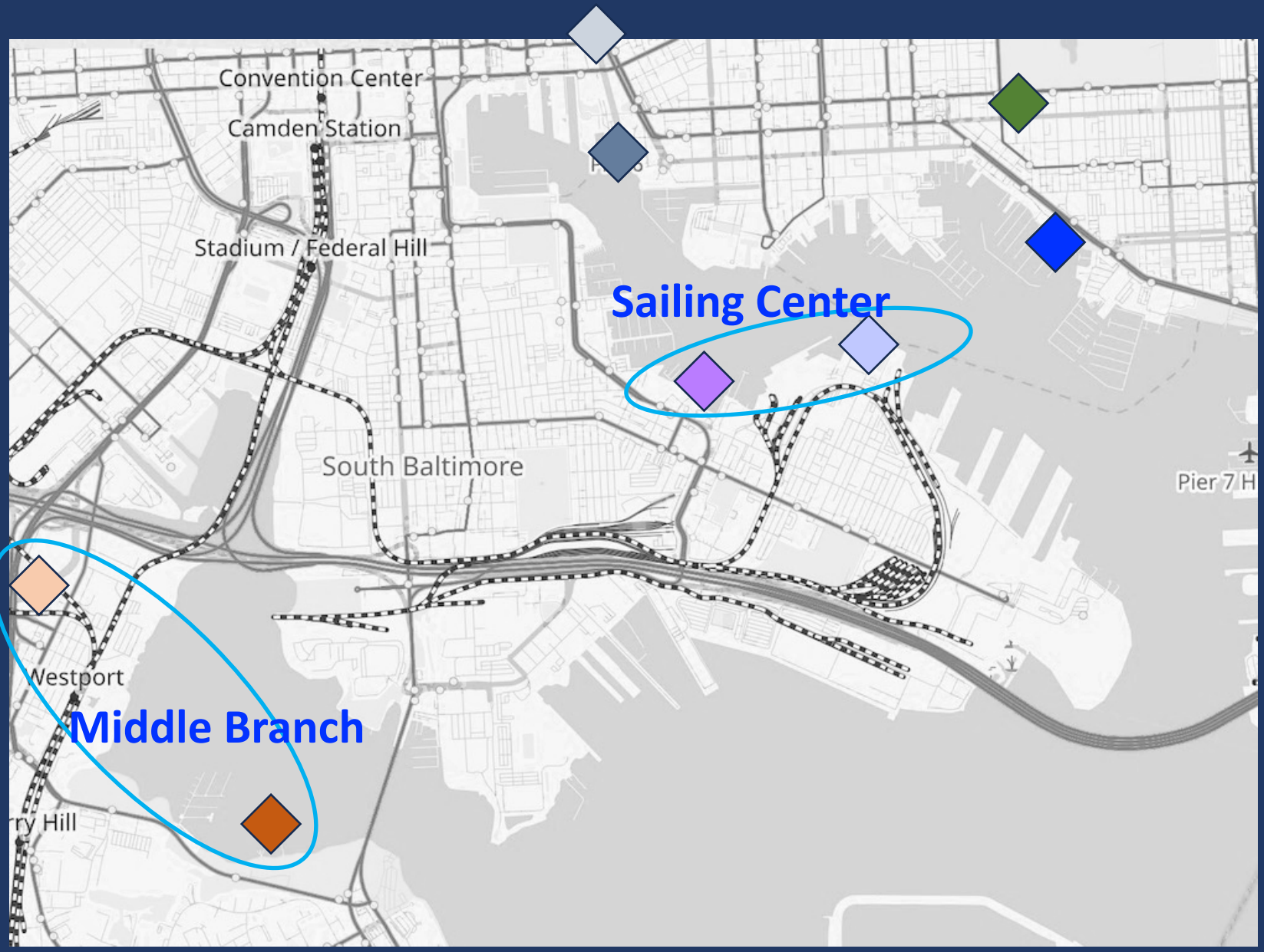


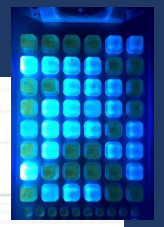
0.5“ rain 4 am - 8 am Sept 18

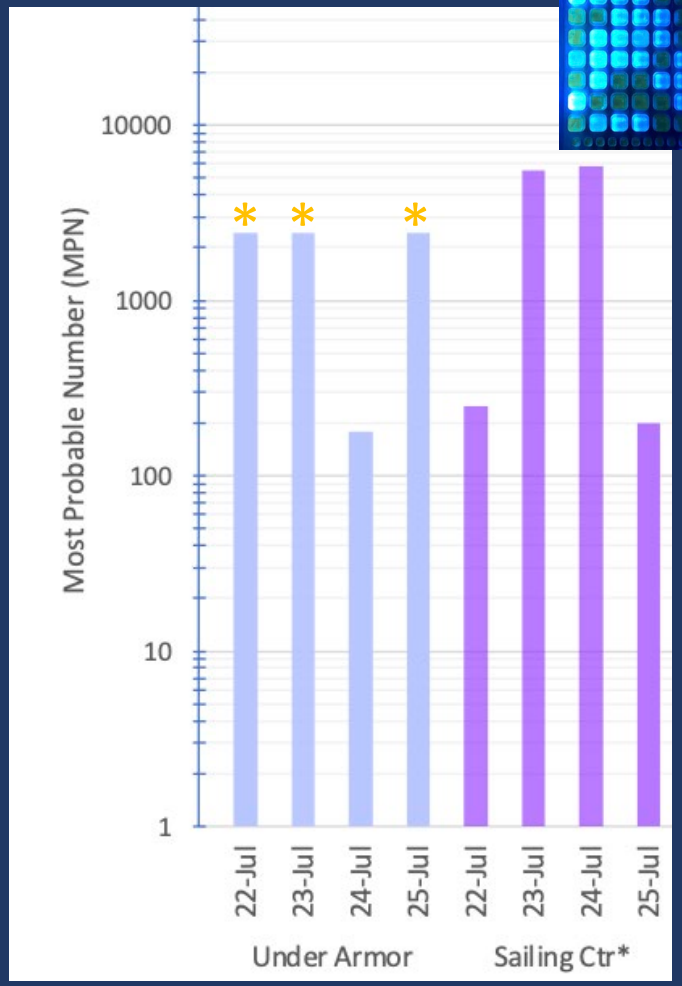
South shore of Inner Harbor and the Middle Branch



UA Drain / Sailing Center
July

Enterolert





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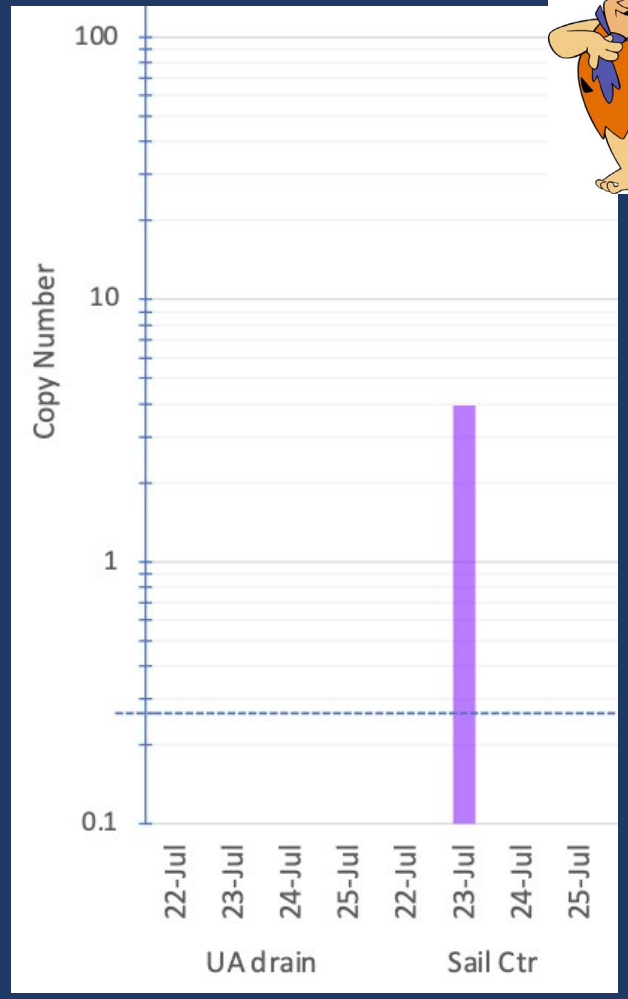

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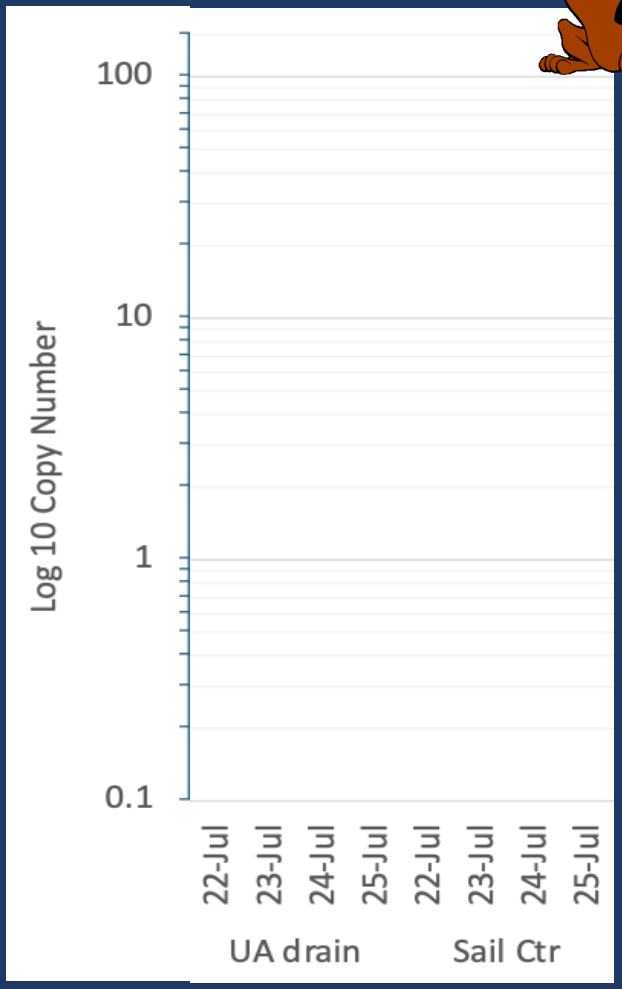
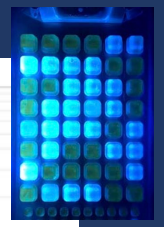
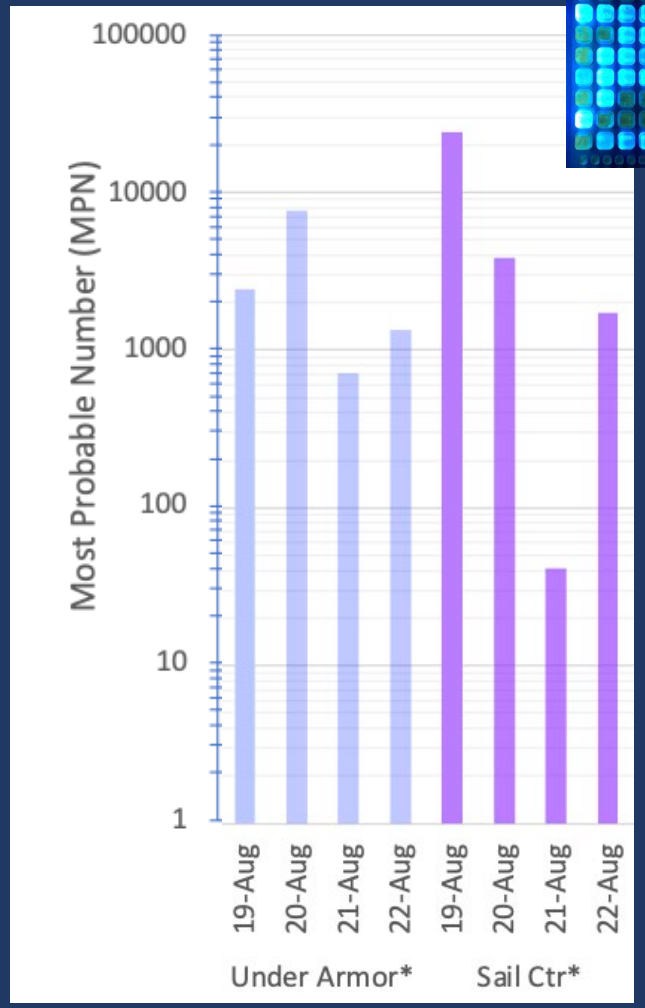


0.4 “ rain late on July 22

UA Drain / Sailing Center
August

Enterolert





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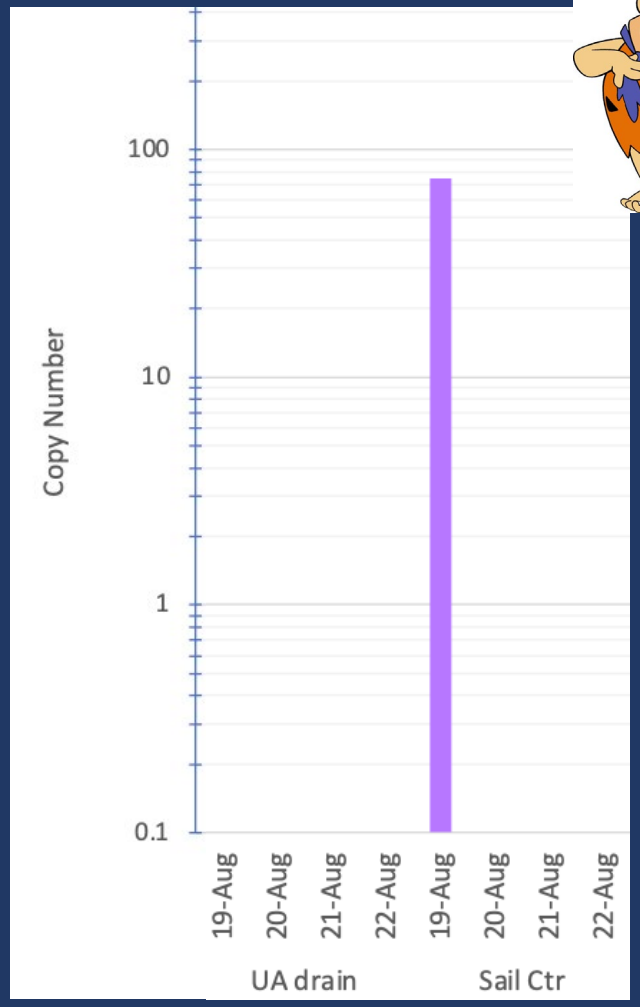

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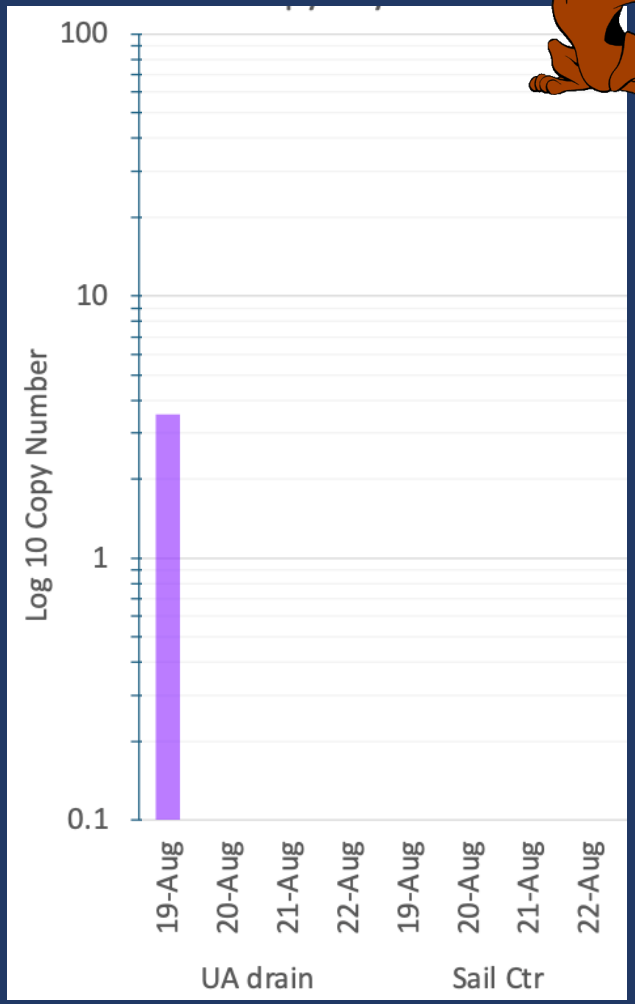
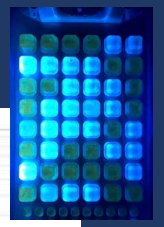
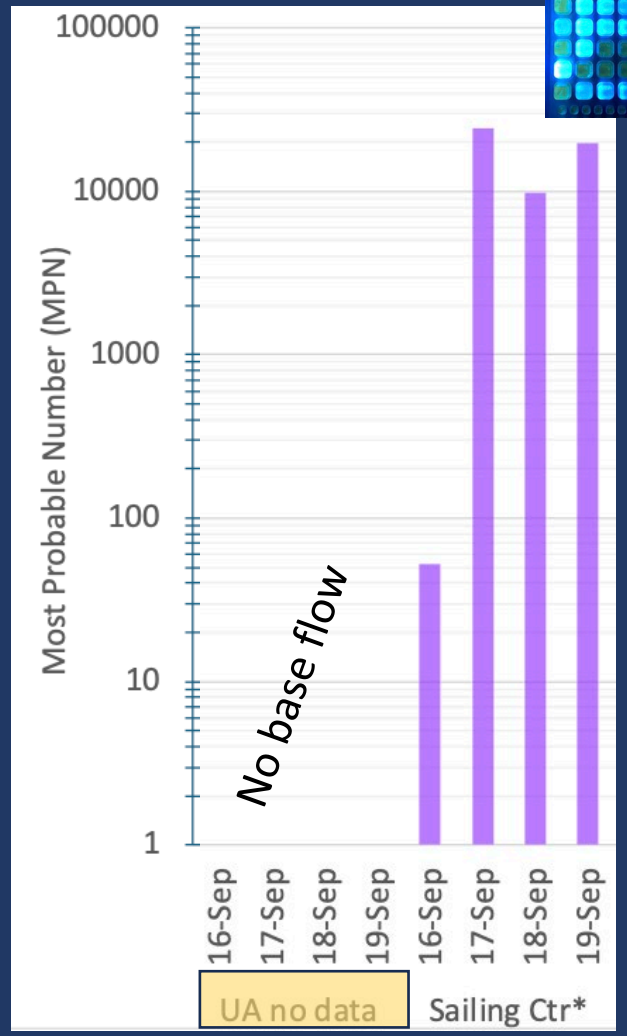


Rain late on Aug. 18

UA Drain / Sailing Center
September

Enterolert





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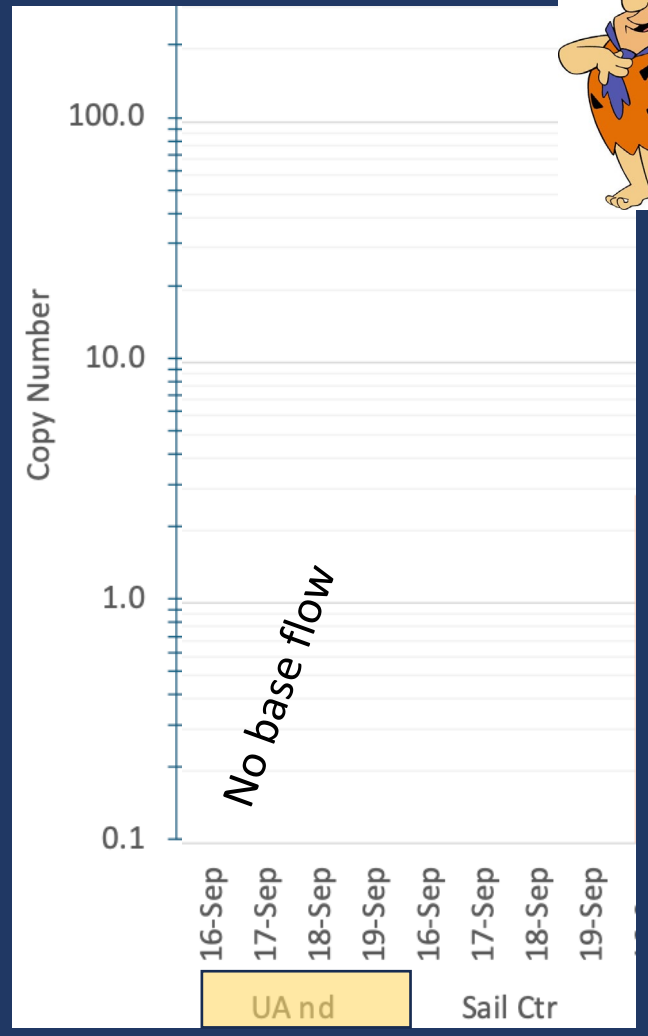

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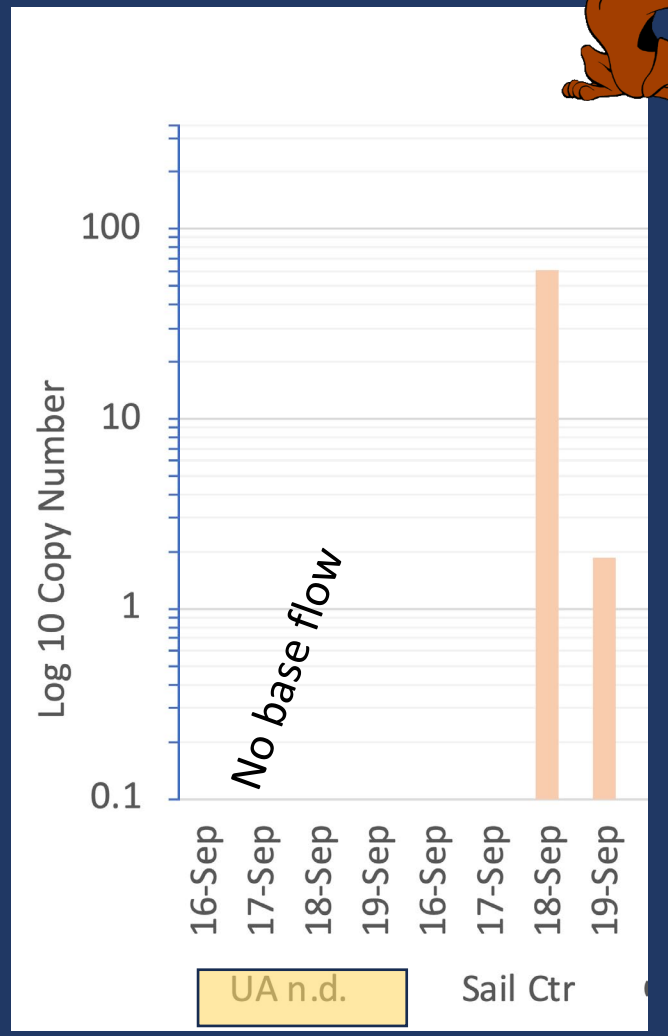
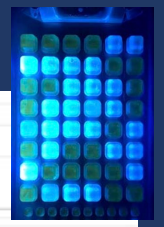
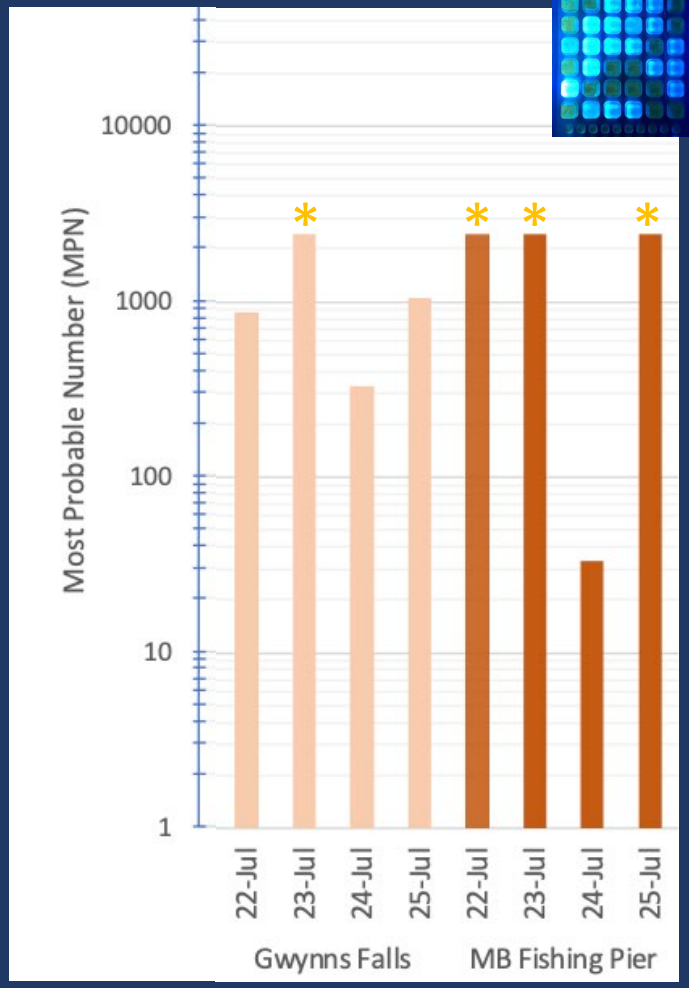


0.5“ rain 4 am - 8 am Sept 18

Gwynns / Fishing Pier
July

Enterolert





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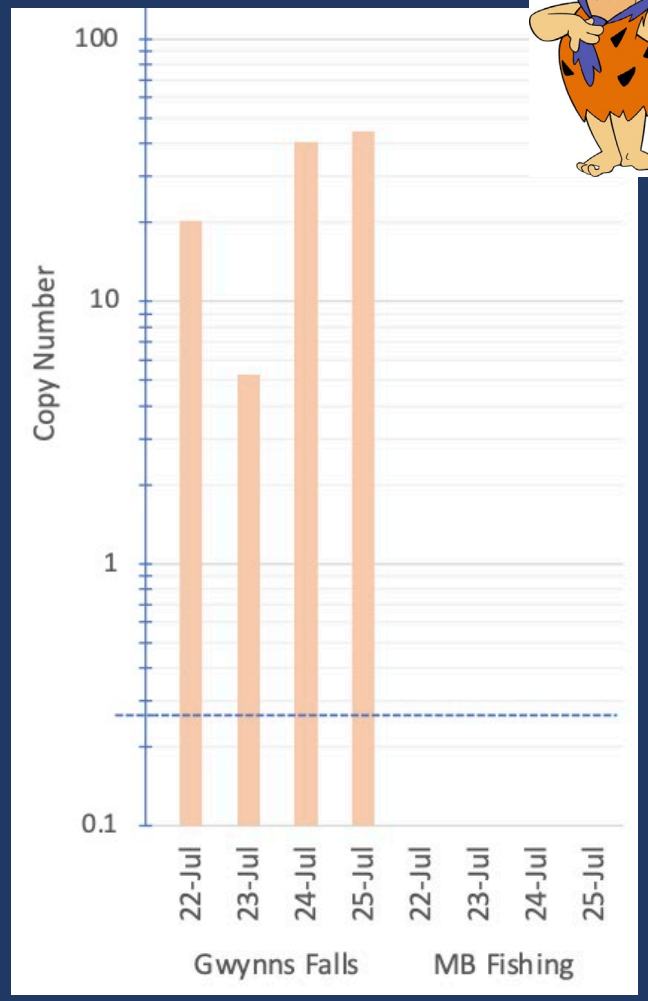
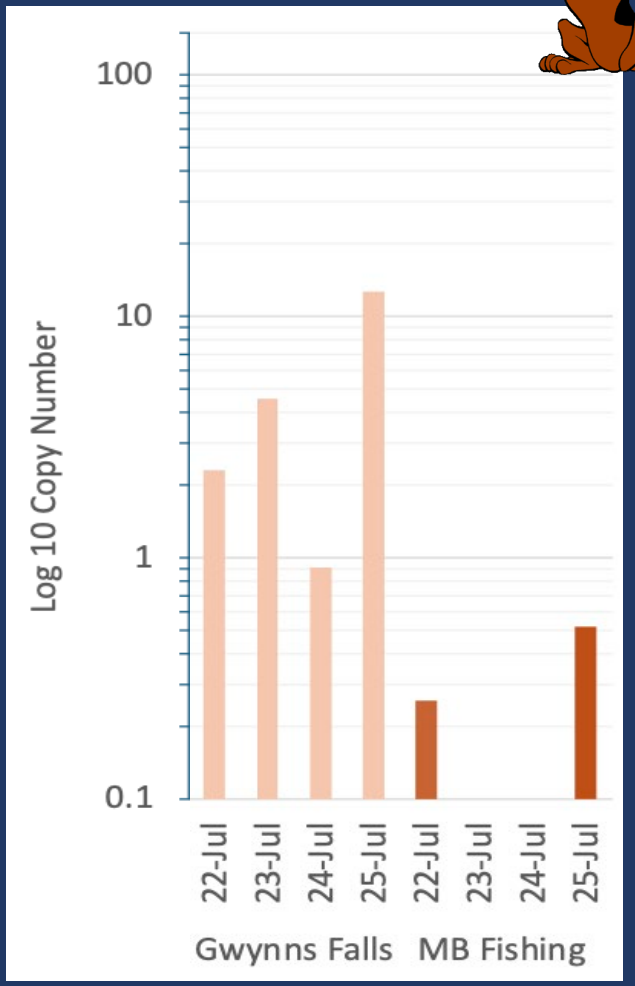


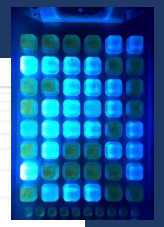
Canine MST

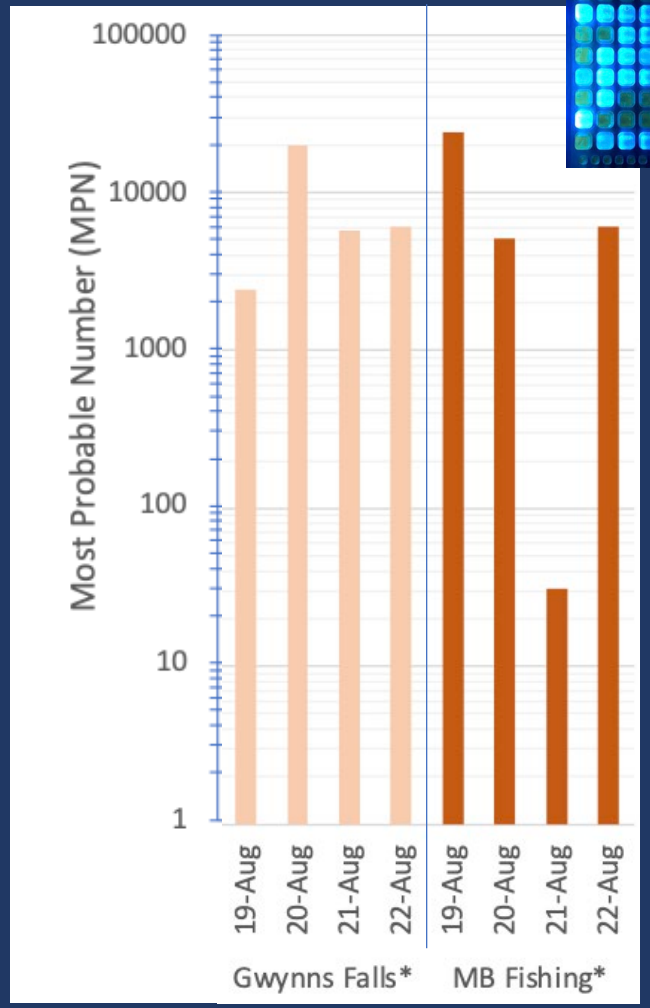


0.4 “ rain late on July 22

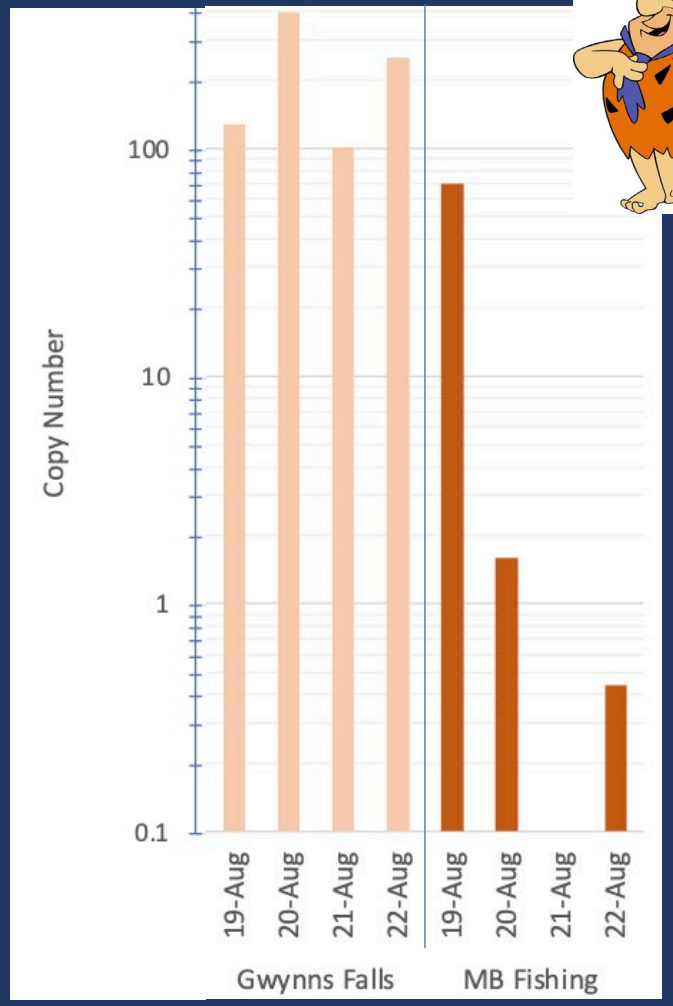
Gwynns / Fishing Pier
August

Enterolert

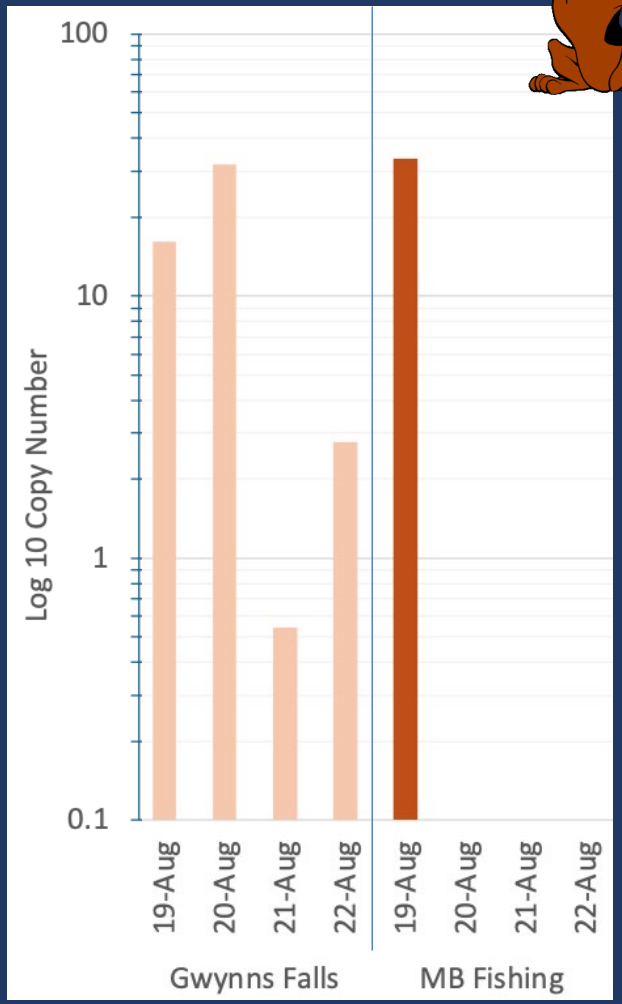




Human MST



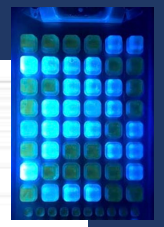
Canine MST

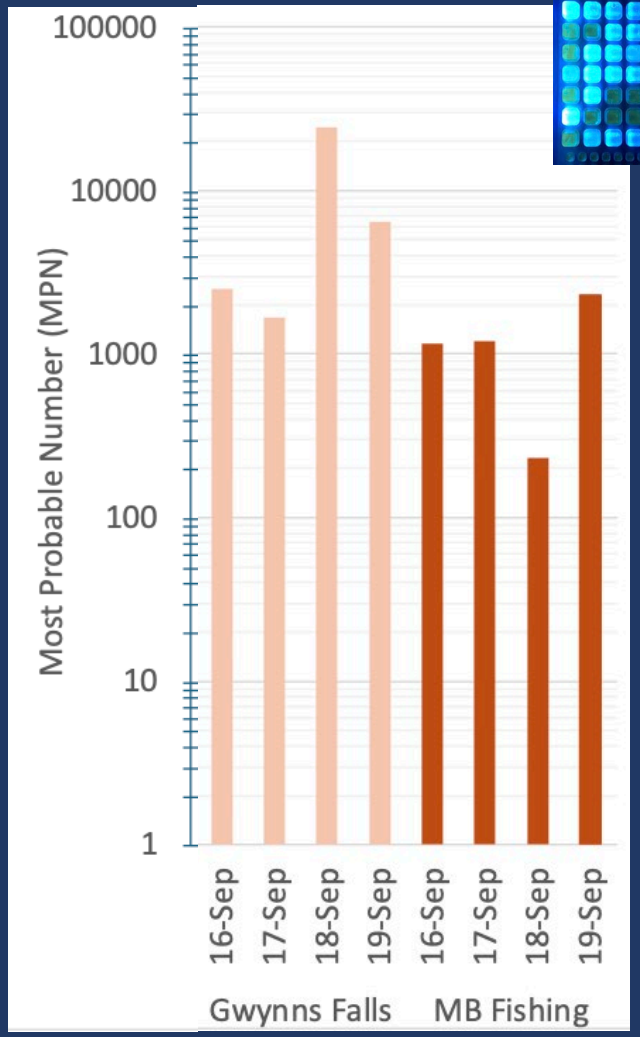


Rain late on Aug. 18

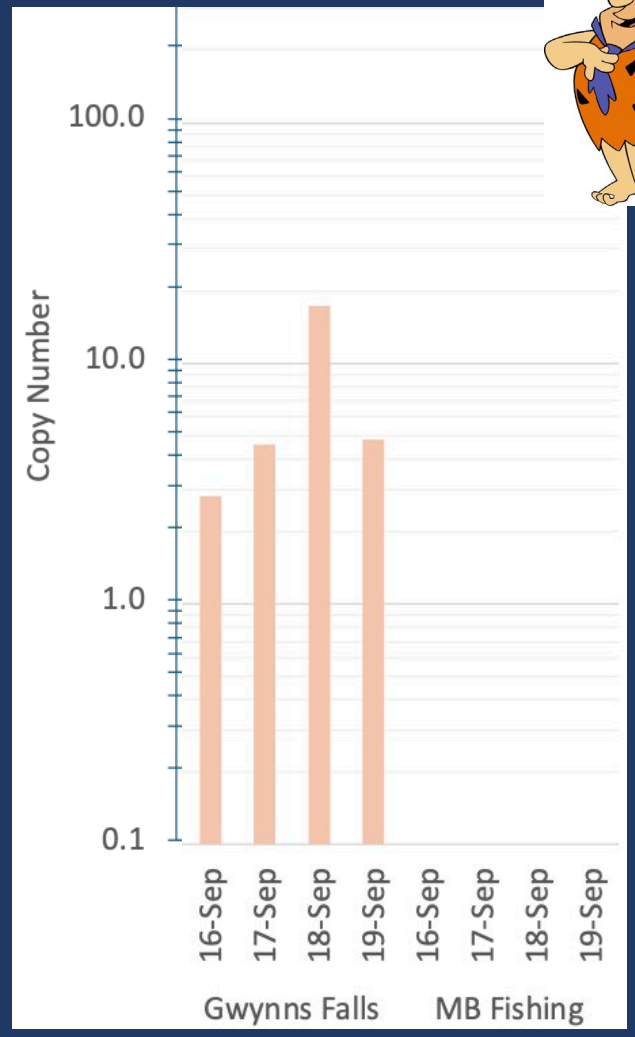
Gwynns / Fishing Pier
September

Enterolert

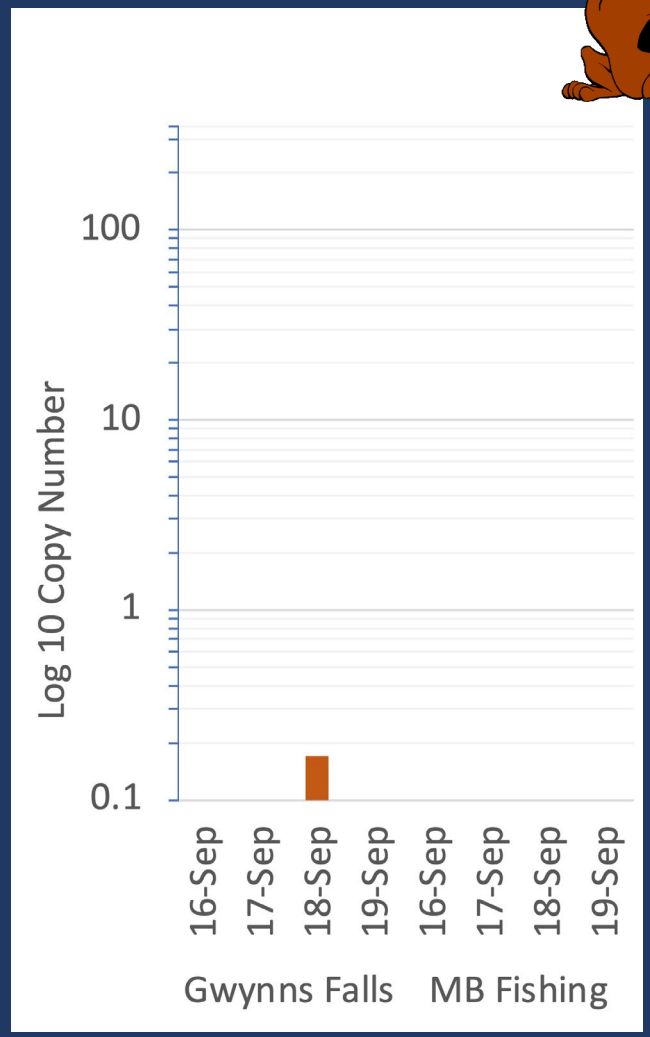




Human MST



Canine MST



0.5“ rain 4 am - 8 am Sept 18

Back to the hypotheses

- **H1**) Combining MST and FIB methods will allow a qualitative assignment of the relative proportion of human versus non-human FIB in a given water sample.

  Human MST marker did not always track with Enterolert

- **H2**) Daily testing, using both FIB culture and PCR methods to detect human vs non-human fecal bacteria, will show that high FIB counts do not always correspond to high human MST (Bacteroides) signals.

  This is true especially at the Sailing Center and Middle Branch

- **H3**) Daily testing of water quality will provide knowledge about the duration and drivers of sewage-derived bacteria and other FIB in tidal water that could not be achieved with weekly testing.

  The decay rate of MPN and MST signals can be seen to differ in August at the Sailing Center and Middle Branch

NEXT

Further study of archived samples

DNA-based methods allow archiving of water, filters or DNA

Re-investigation with the same targets

Re-investigation for new targets

- Total enterococcus
- Birds, other hosts
- Rats?

PCR methods have a wide range of assay quantification. 1 copy to 1 million.

Build local capacity

Engaged working group

- Non-profits
- Academia
- Municipal

Private lab

Technician training

Shared/pooled resources

Collections
Contract lab
Training and expertise

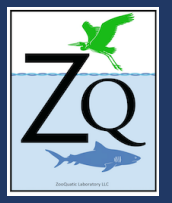
IMET
Institute of Marine & Environmental Technology

ZQ

Thanks to many

Mariah Mckenzie
Interns

Van Sturdevant, Joan White, Kim Grove



Brent Whitaker
Morgan Shapiro

Alice Volpitta, Sarah Holter, others

Allison Blood
Adam Lindquist

Translation: Use of Molecular Sewage Indicators

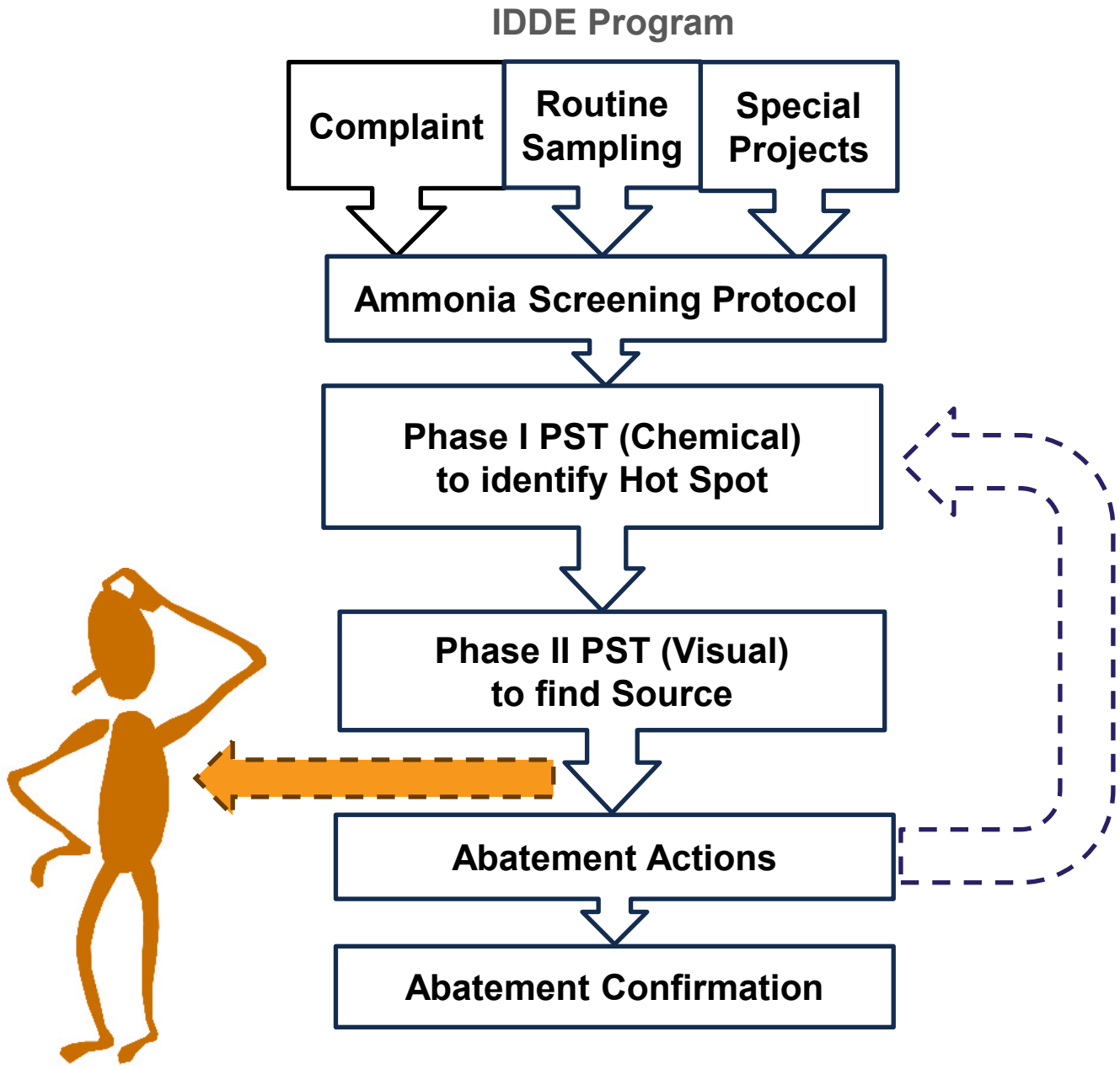
Chesapeake Bay Trust
Pooled Monitoring Program
2025 Annual Meeting

BALTIMORE CITY
DEPARTMENT OF PUBLIC WORKS

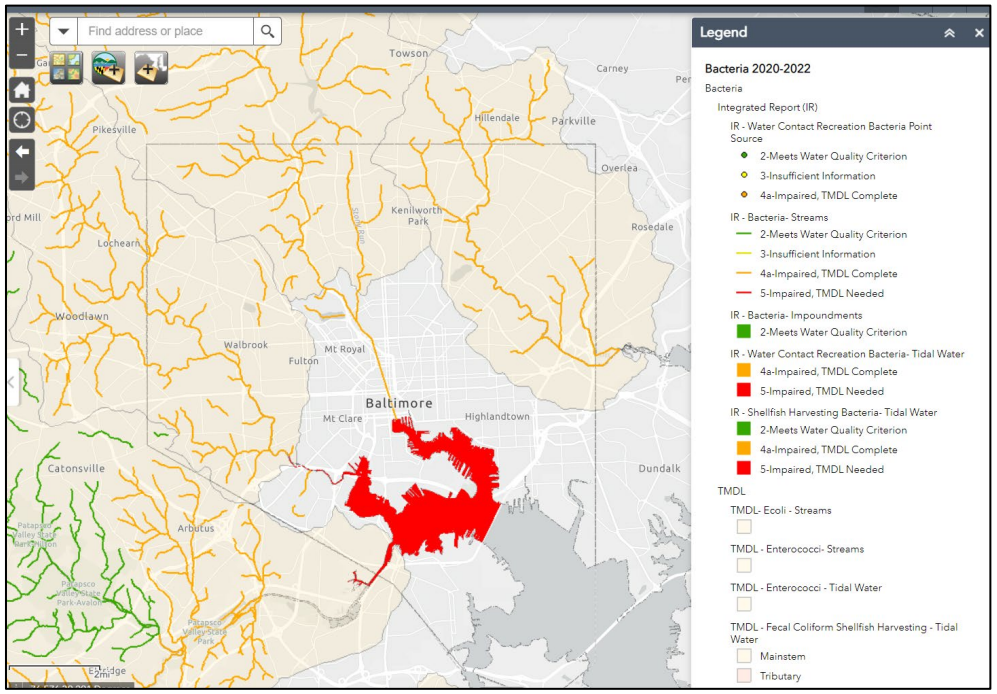
June 18, 2025

MST: Past Use

- Used when other investigation techniques have been exhausted.
- Human markers < 1%, discontinue investigation.
- FY 17 to 19: 14 of 20 PST investigations allowed to be discontinued.



Ultimate Goal: Swimmable Waters

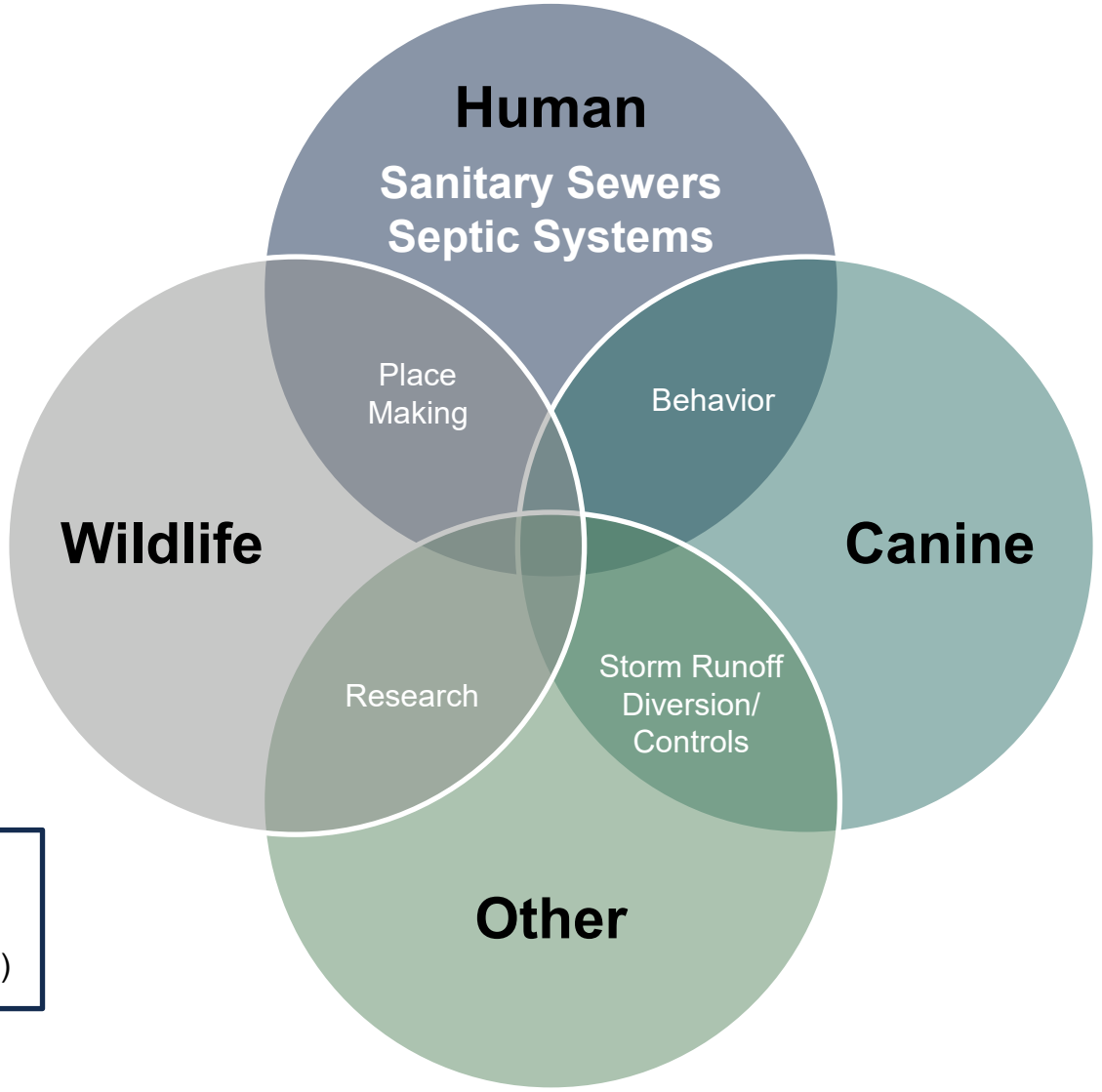


Source: Water Quality Assessments (IR) and TMDLs (state.md.us)

Enterococcus Criteria

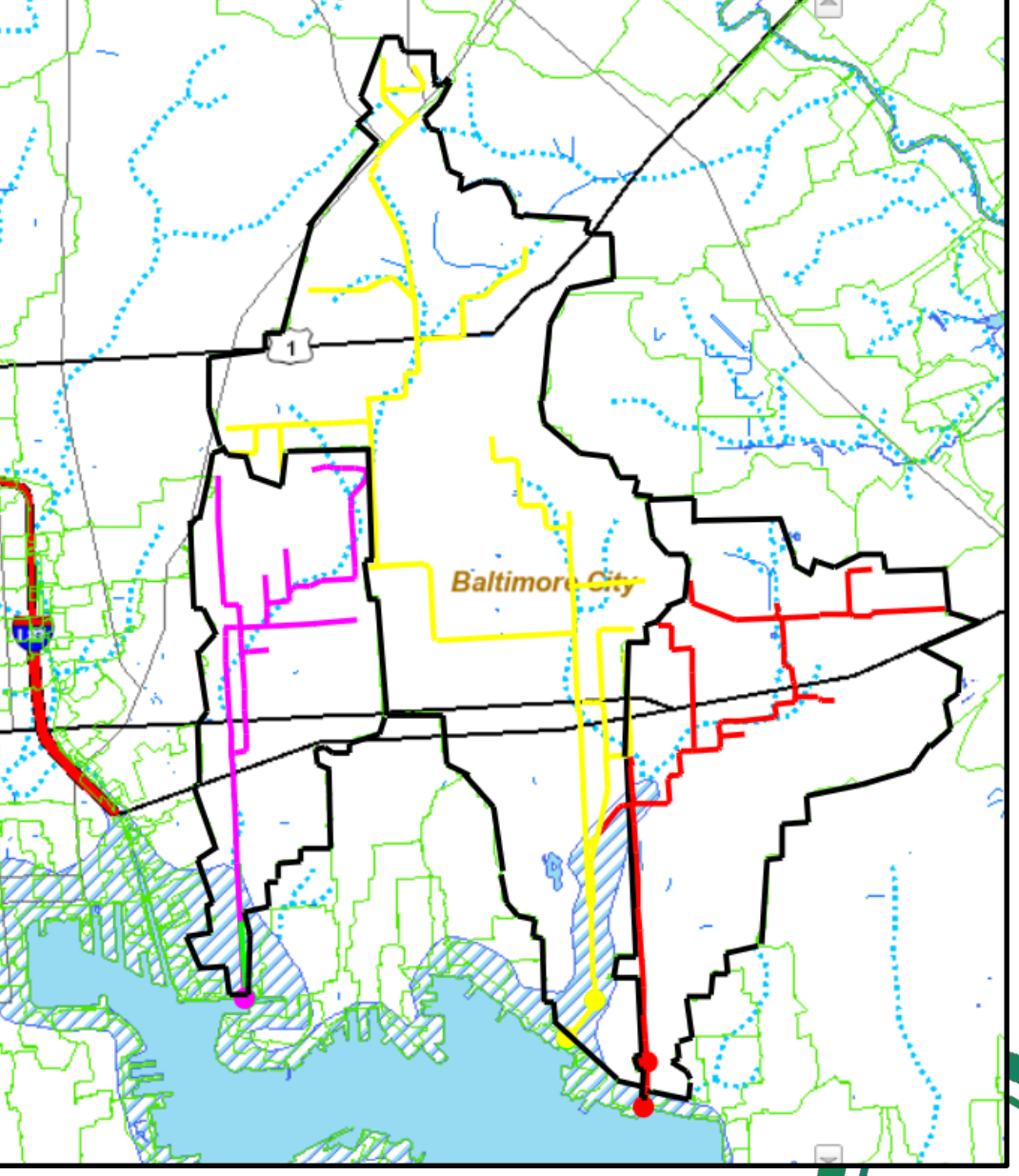
Geometric mean (GM) for 90+ days < **35** MPN / 100 ml

Less than 10% of single sample results > **130** MPN / 100 ml (STV)



Observations from this research

- Swimmable waters will not be achieved solely by fixing the sanitary sewer system.
- Canine sources are primarily conveyed by stormwater runoff.
- Human markers demonstrate a more complex fate-transport pathway.
- The other bacteria sources need to be identified.

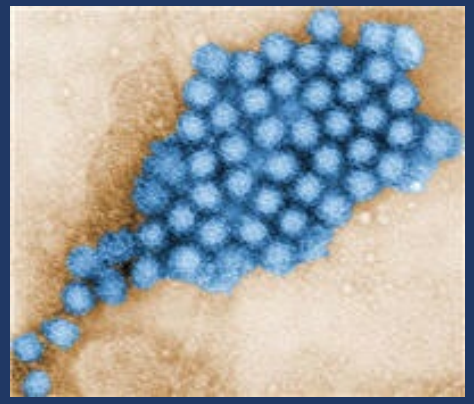


MST: Future

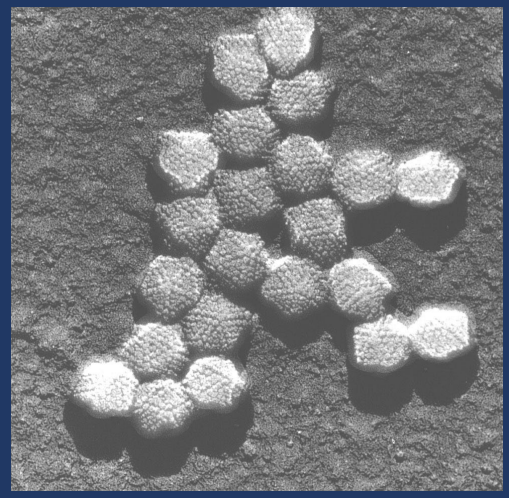
Historic stream conveyance

1. Same-day sampling of buried stream to determine spatial source trends.
2. Use more markers (birds, rats, deer).

Human pathogens common in sewage

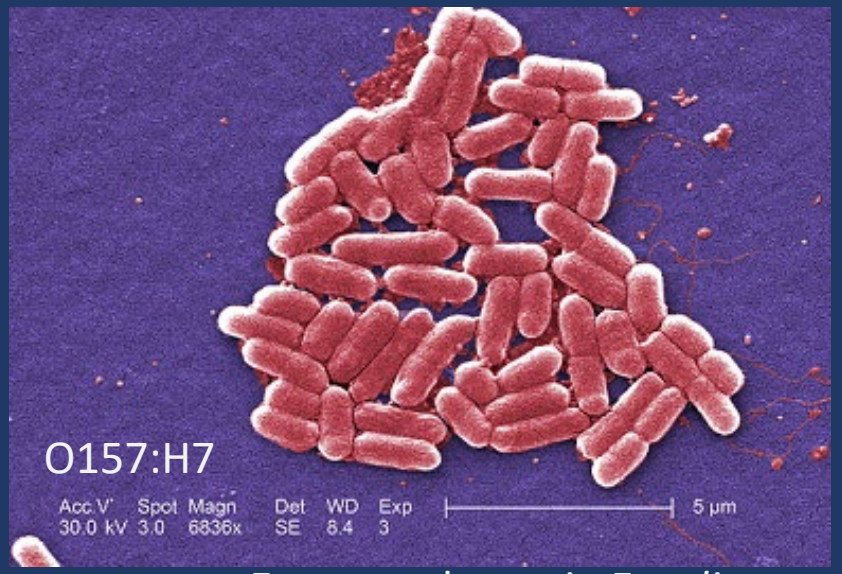


Norovirus



Adenovirus

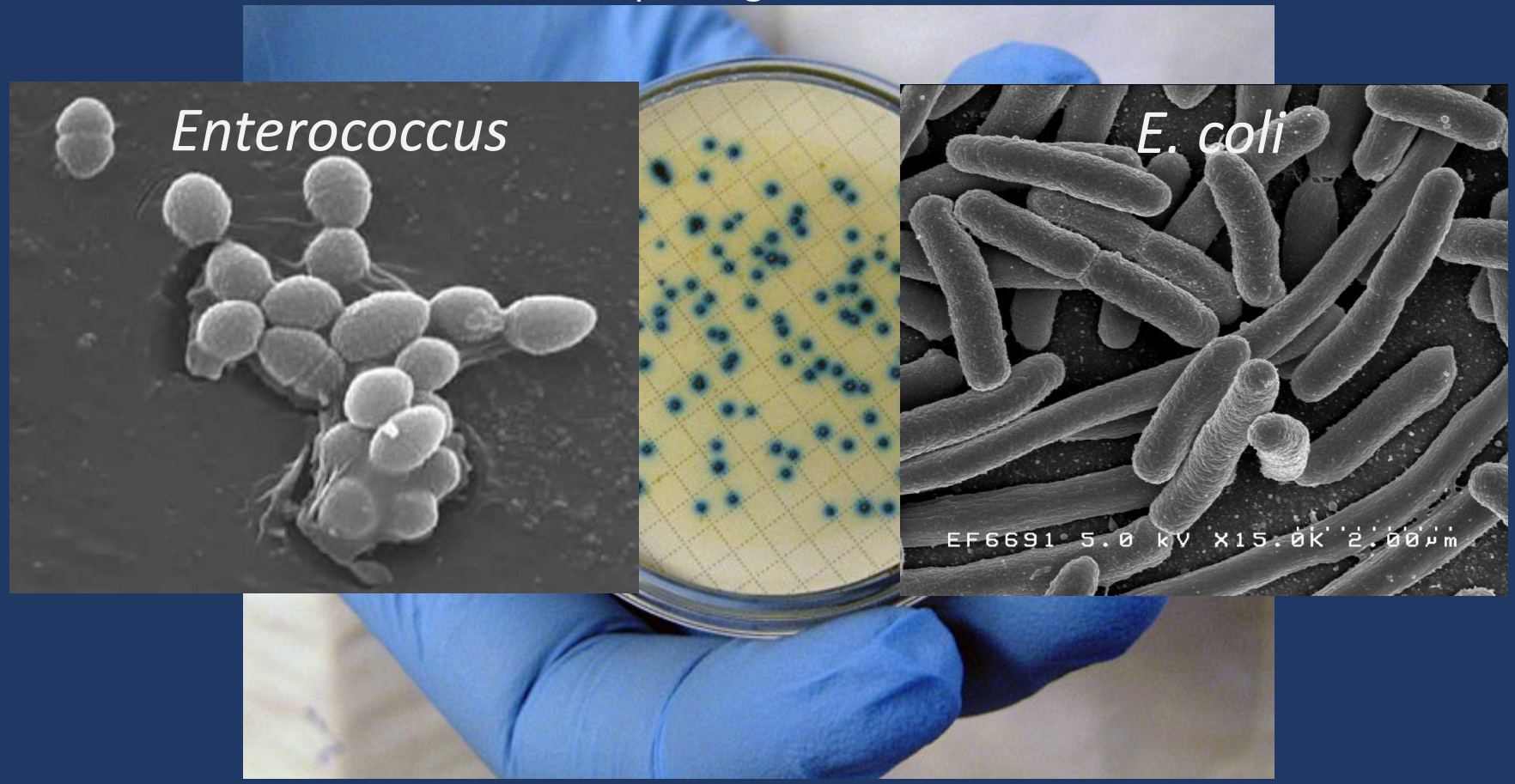
Cryptosporidium



Enteropathogenic *E. coli*

Markers for sewage presence

- FIB = fecal indicator bacteria
- Semi-selective culture methods
- Facultative anaerobes
- Not pathogenic



Basic conclusions from Enterolert data

1. We should have diluted 1/10 from the start (July)
2. High entero correlates with rain
3. Upstream usually higher than downstream

    This needs statistical tests

Basic conclusions from Human MST data

1. Upstream usually higher than downstream
2. High Human MST correlates with rain
3. Sci Ctr on Aug 19?
4. Large % of not detected is unexpected. More QC needed.

Basic conclusions from Canine MST data

1. Upstream higher than downstream
2. High Canine MST correlates with rain
3. Mr Trash and Harris Creek often positive