Counting microbes in Baltimore Harbor

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IMET-UMCES

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CENTER FOR ENVIRONMENTAL SCIENCE
INSTITUTE OF MARINE & ENVIRONMENTAL TECHNOLOGY

WATERFRONT PARTNERSHIP OF BALTIMORE

Rouse Family Foundation

IMET daily Enterococci testing piloting. Eric Schott, Morgan Shapiro, Khaled Geba

Swimmable Inner Harbor Goal

Goal set in 2010 by the Waterfront Partnership Baltimore under EPA, DOJ sewage consent decree

Associated with sewage and runoff

- **Human pathogens**

Naturally present in brackish water

- *Vibrio vulnificus*
- *Vibrio parahaemolyticus*

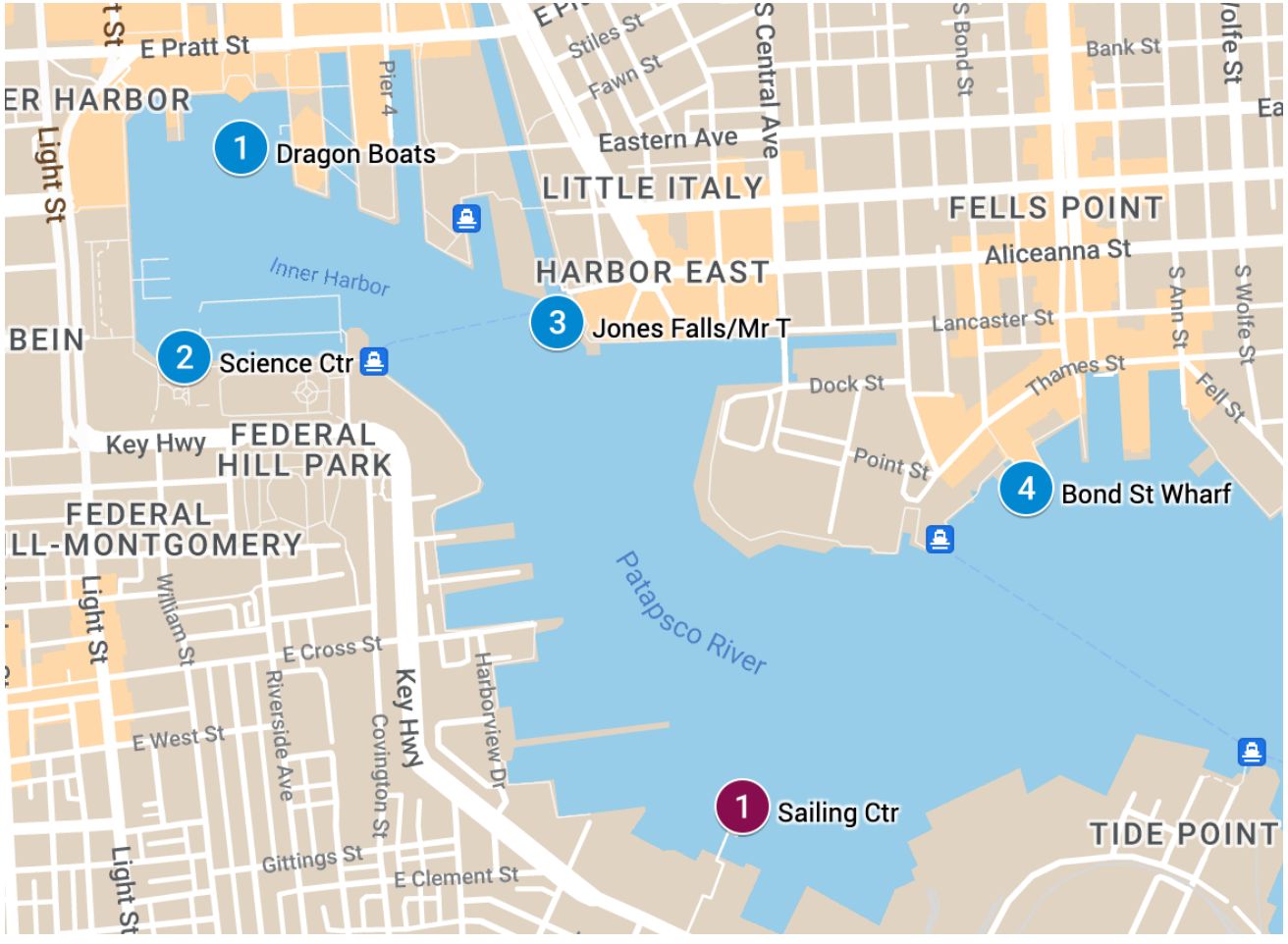
Study sites where water contact is frequent

WPB collections

1.Dragon Boats
2.Science Ctr
3.Jones Falls
4.Bond St Wharf

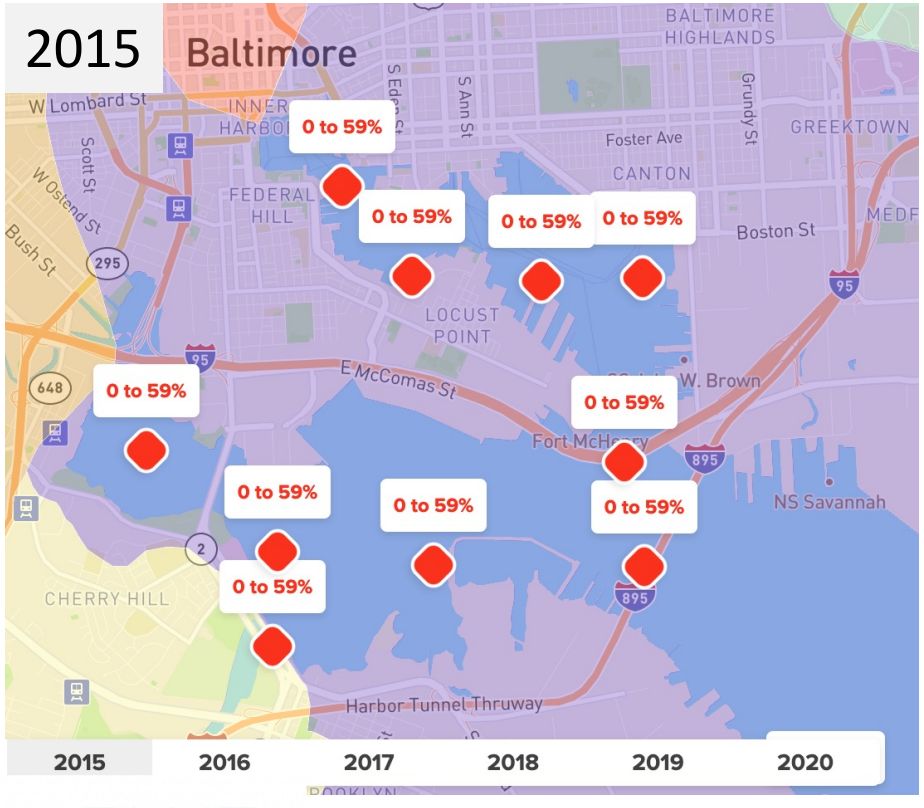
IMET collection

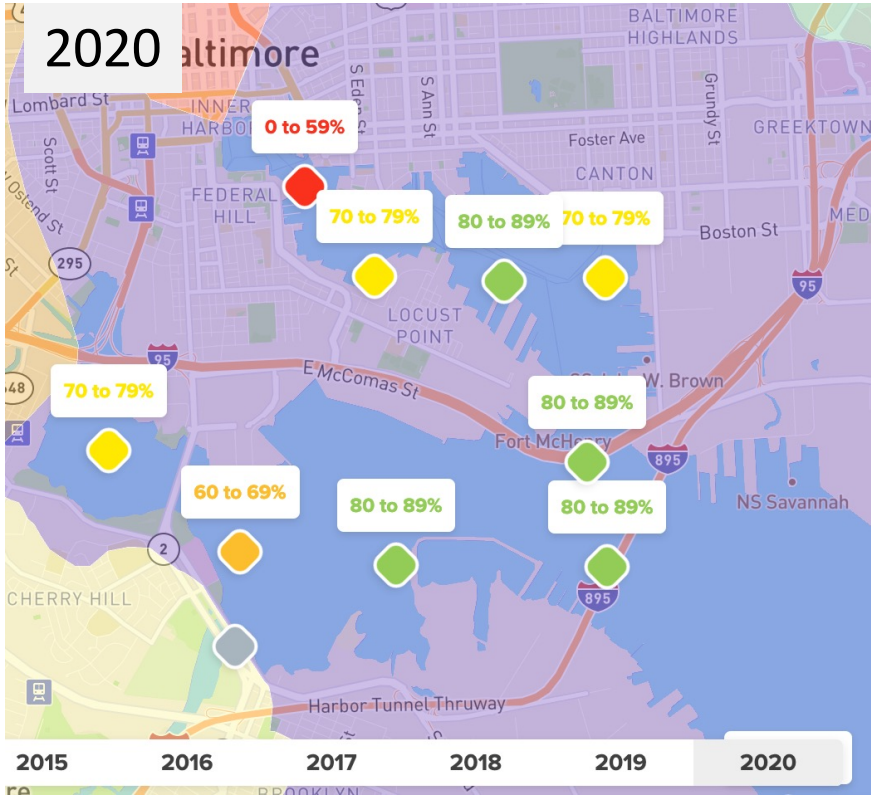
1.Sailing Center



The harbor is clean***er***, but not always

How can we know **when** it's safe to swim?





BLUE WATER BALTIMORE

IDEXX Enterolert methods

Reimagine Middle Branch

master plan approved February 2023

Env justice issue

Neglected communities cannot access clean recreational water

How to measure risk of sewage-related bacteria?

FIB: Enterococcus

colony plating- counting CFU. *18 hours*

IDEXX Enterolert – dilution method and MPN. *18 hours*

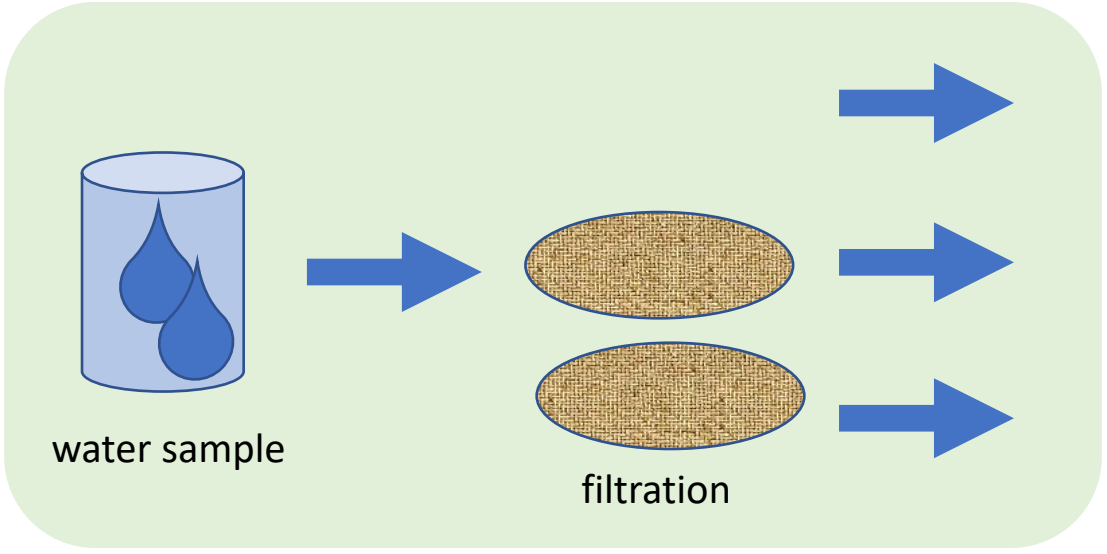
qPCR – EPA method 1611. *5 hours*

More human – specific bacteria, PCR

Bacteroides sp. – “HF183” Site-specific EPA approval.

Lachnospiraceae – Lachno3. Well-tested, no regulatory approval

Enterococcus measured three ways

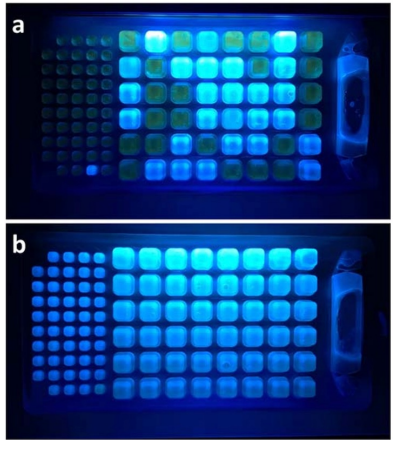


IDEXX liquid cultures → Dilution, MPN

M-entero colony growth → Colonies, large/small

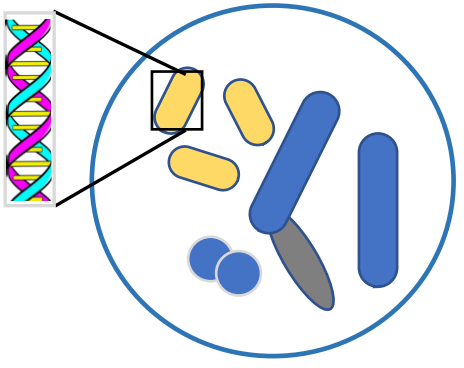
extract DNA → Count bacteria genomes, CCE

IDEXX



M-entero agar

EPA1611 qPCR



Enterococcus: Non-sewage sources

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

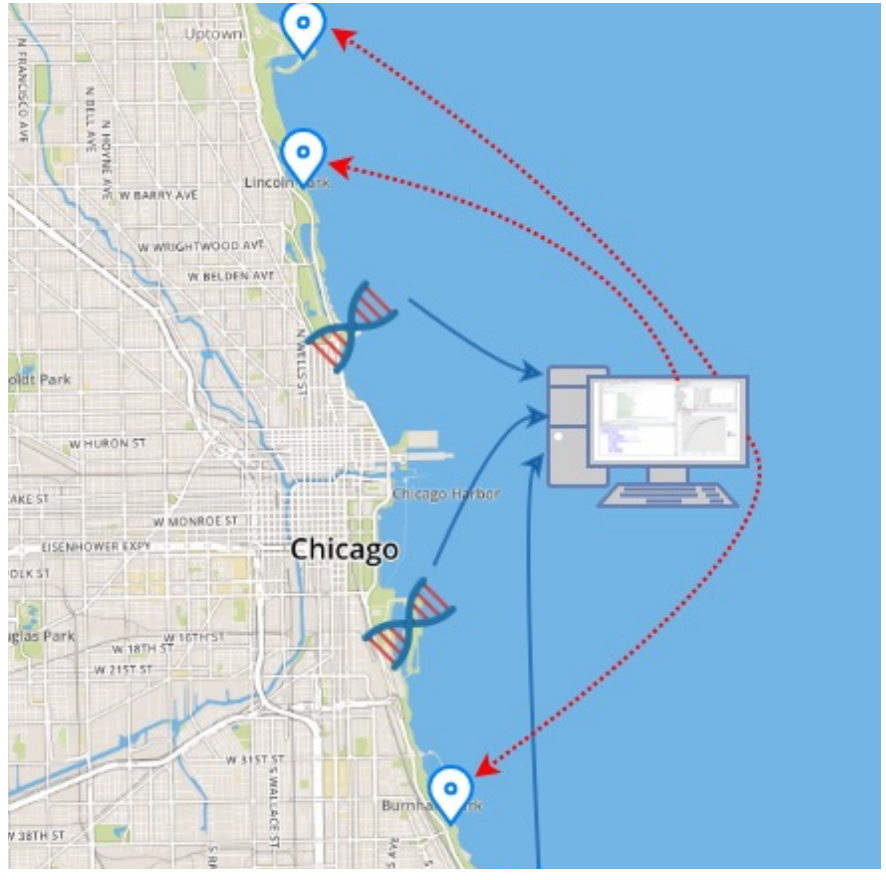
Same day qPCR for *Enterococcus*

EPA United States Environmental Protection Agency

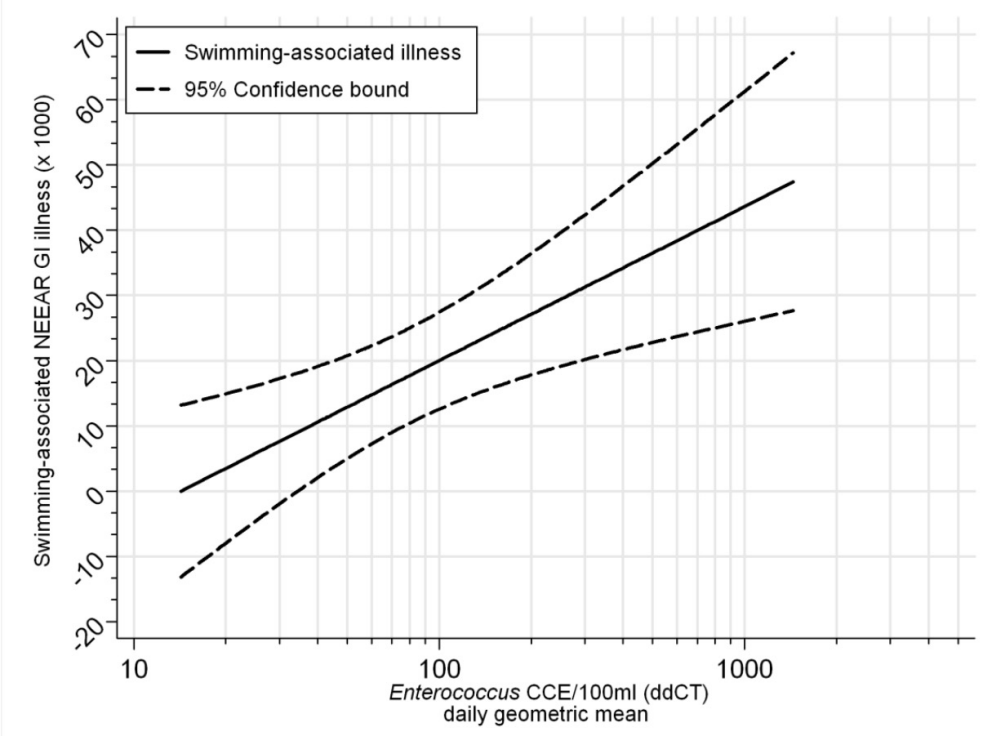
Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay

Indicator	Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators BAV (Units per 100 mL)		Estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators BAV (Units per 100 mL)
Enterococci – culturable (fresh and marine)[a]	70 cfu	OR	60 cfu
E. coli – culturable (fresh)[b]	235 cfu		190 cfu
Enterococcus spp. – qPCR (fresh and marine)[c]	1,000 cce		640 cce

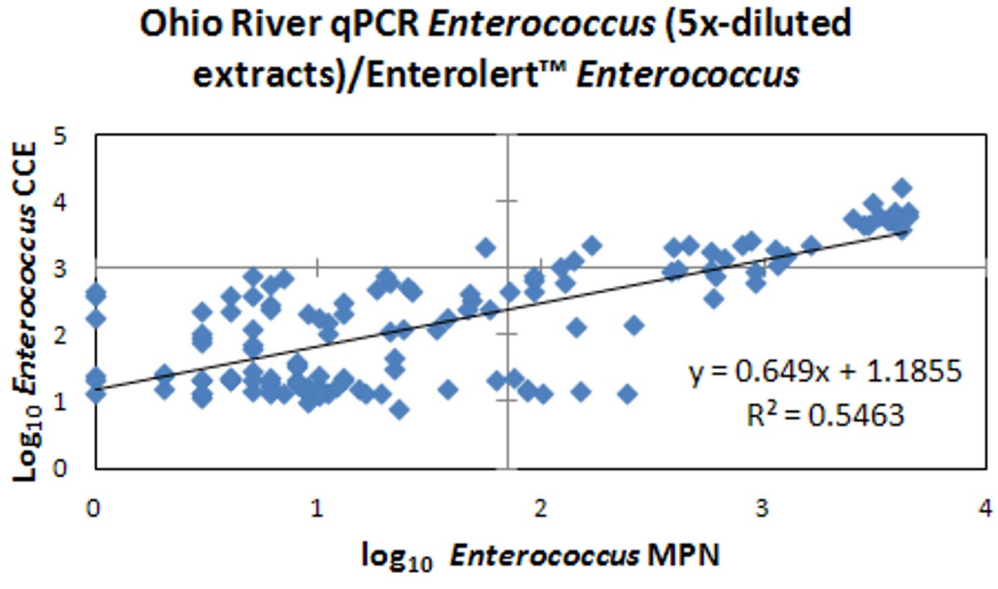
City of Chicago same-day beach reports



Risk vs Enterococcus qPCR signal



Assessment of IDEXX vs EPA1611



Daily water testing

WPB collections

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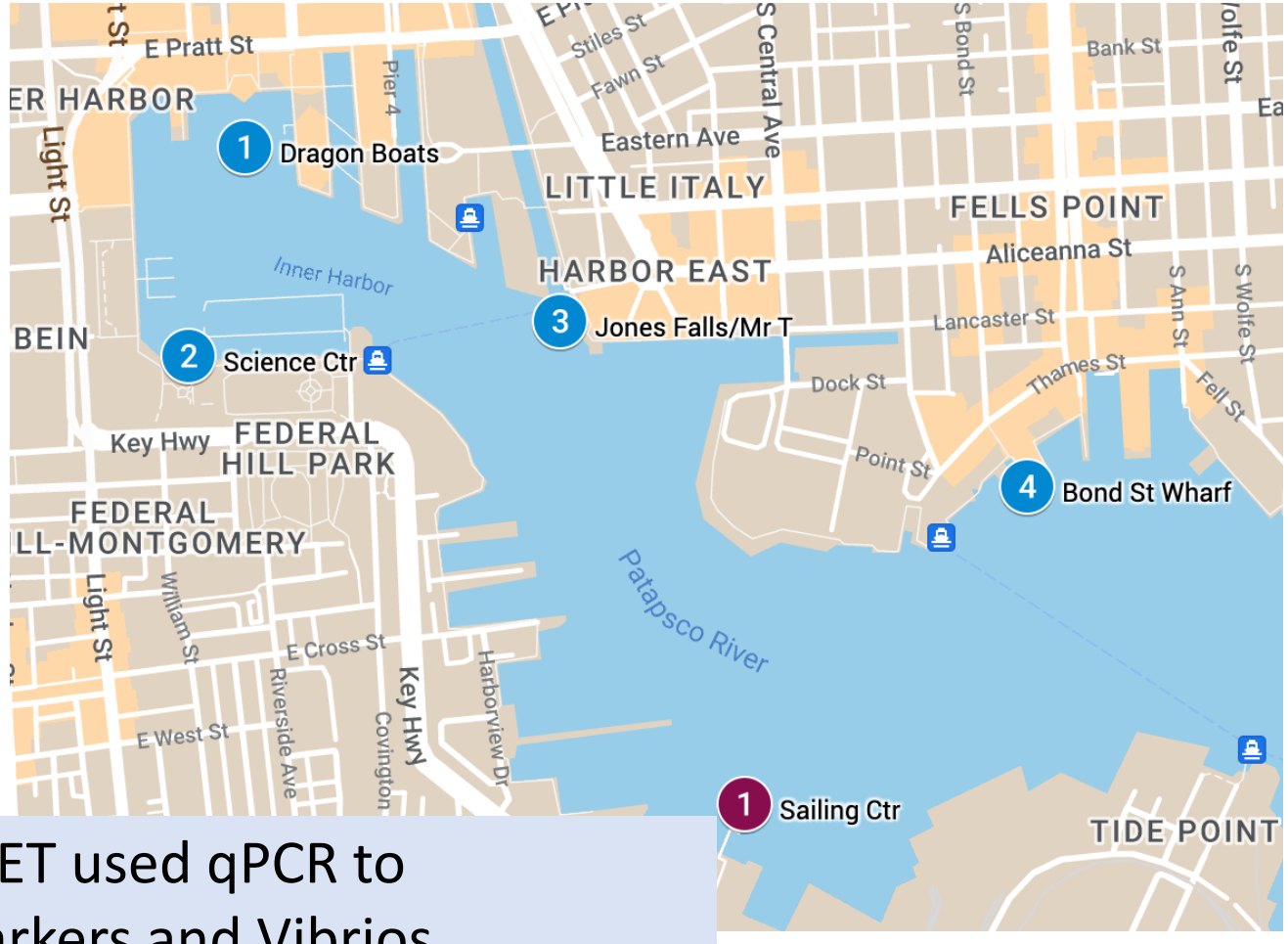
IMET collection

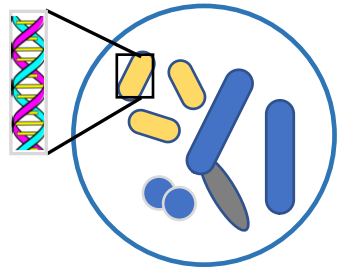
1.Sailing Center



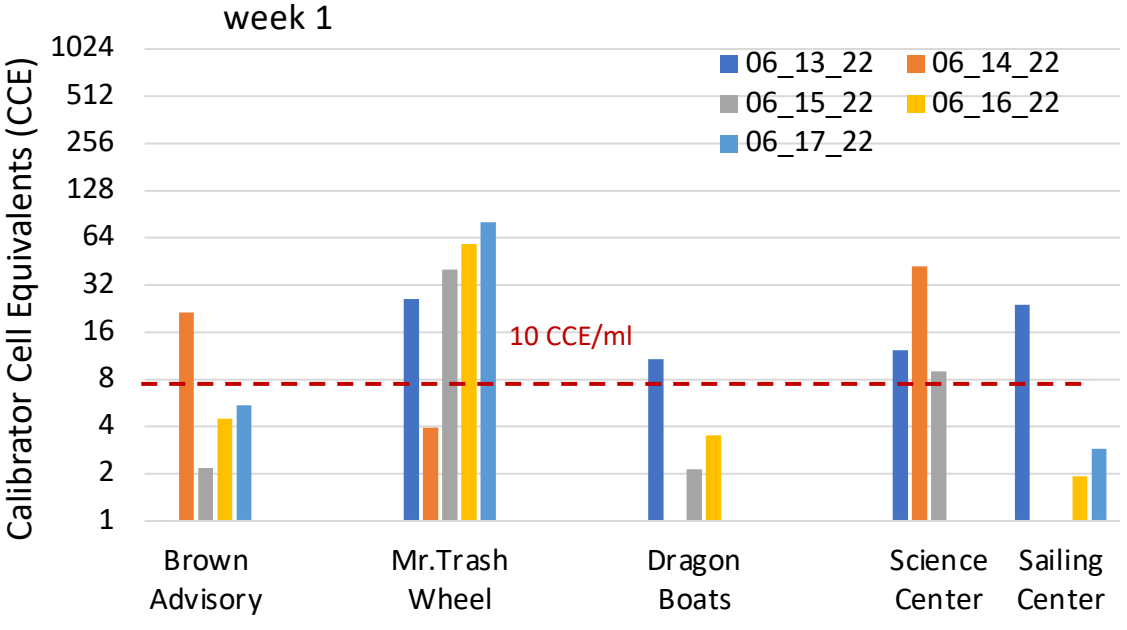
In 2020, 2021, IMET used qPCR to assess sewage markers and Vibrios

2022- trial of daily testing by Enterococcus

EPA1611 qPCR

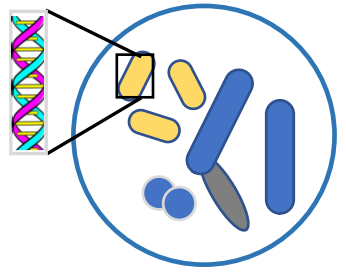


Weekly trends across the Inner Harbor



IMET daily Enterococci testing pilot tests Monday-Thursday. June -July, 2022. Eric Schott, Morgan Shapiro, Khaled Geba

EPA1611 qPCR



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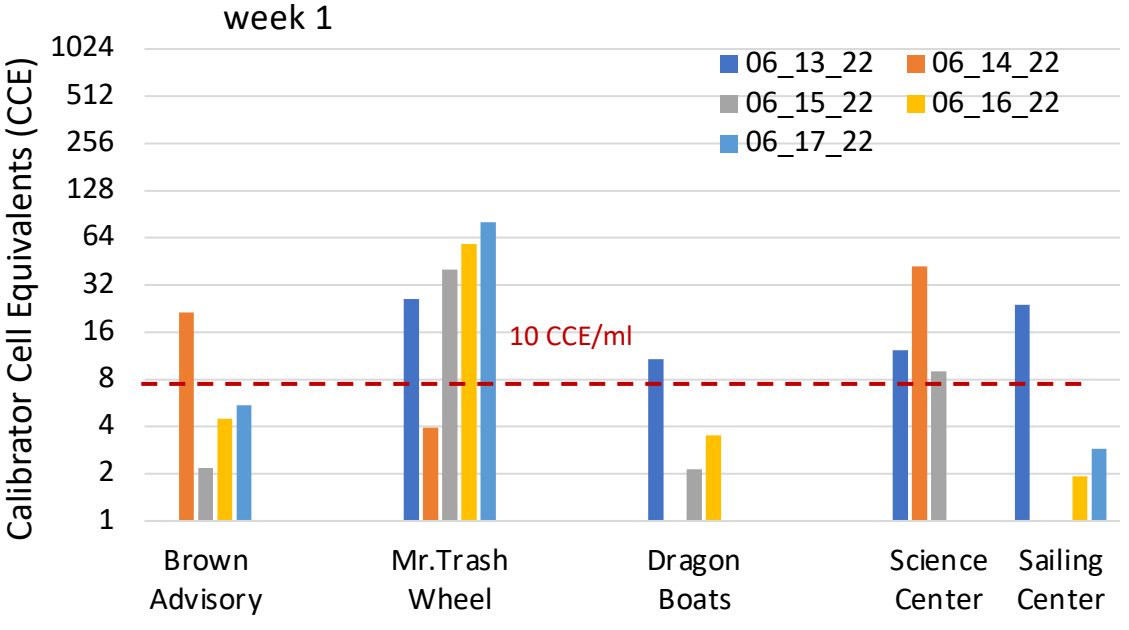
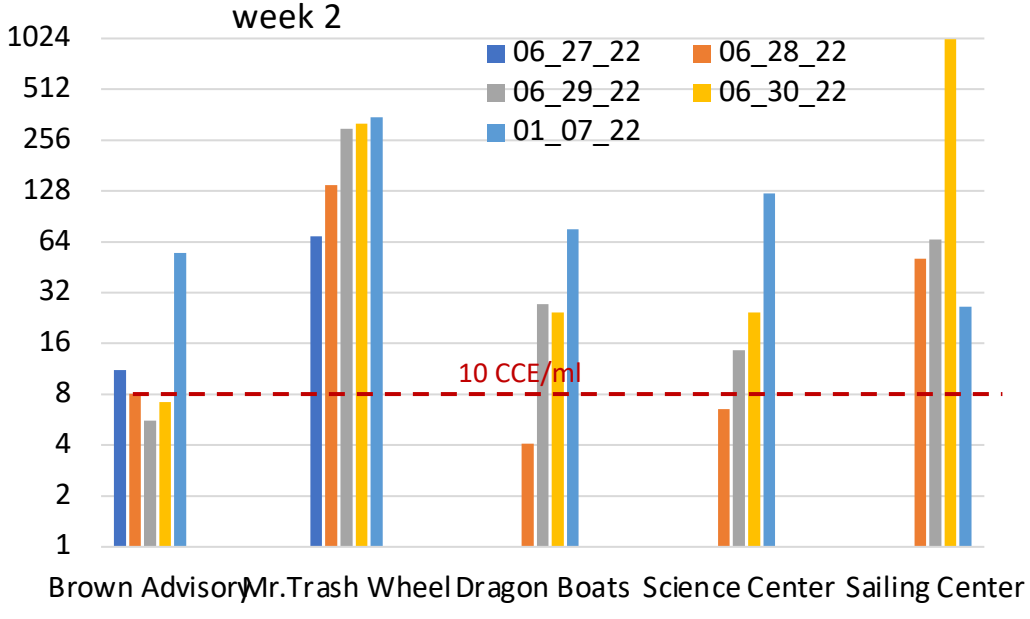
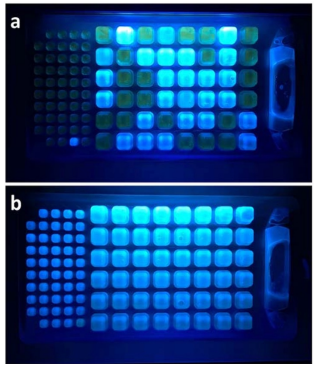




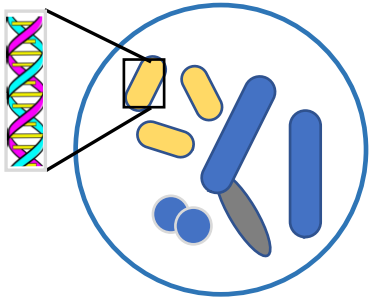
IMET daily Enterococci testing pilot tests Monday-Thursday. June -July, 2022. Eric Schott, Morgan Shapiro, Khaled Geba

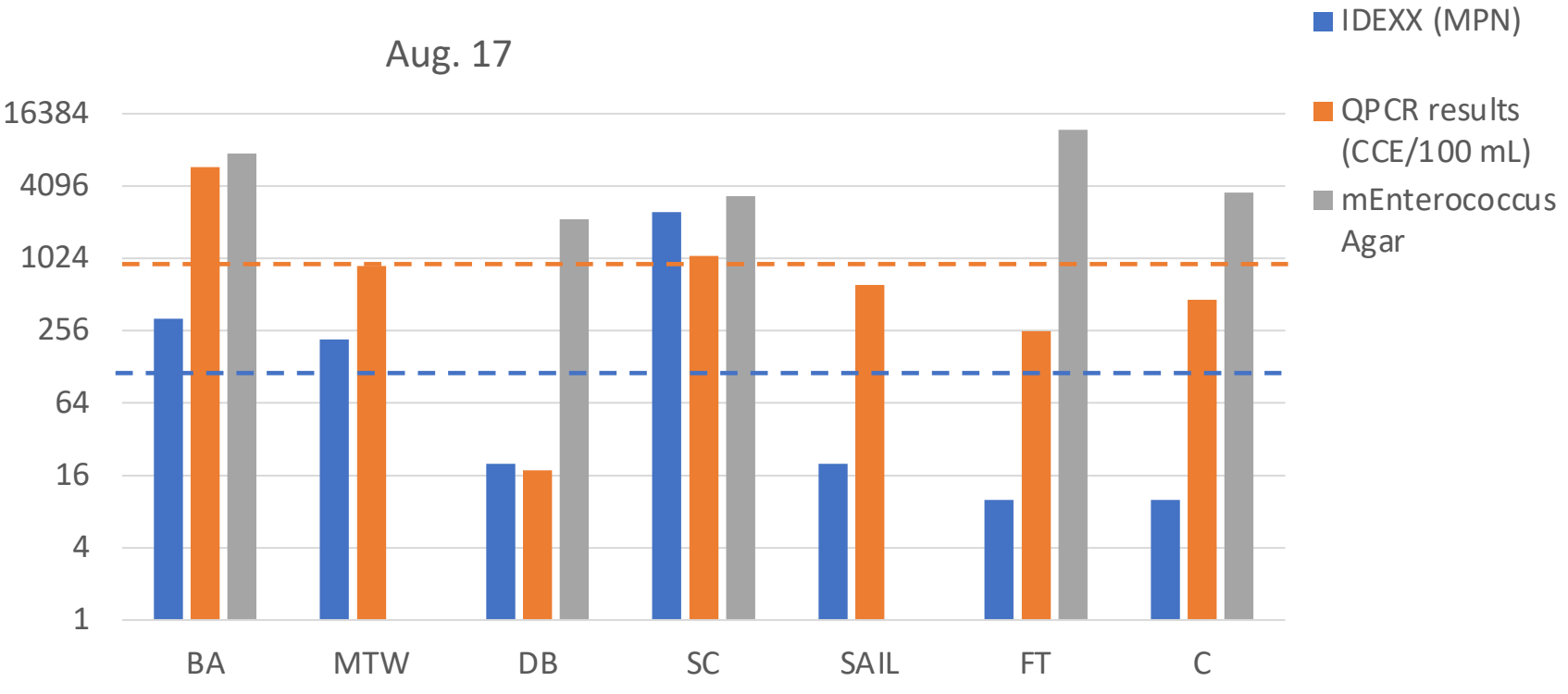
IDEXX



M-entero agar

EPA1611 qPCR

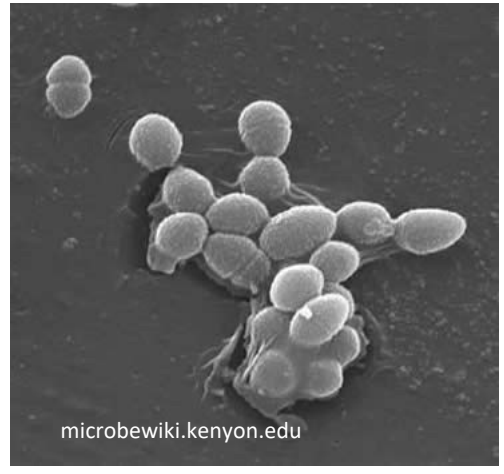




Summary- Enterococcus

We tested same-day qPCR for Enterococcus

- Eventually succeeded in 4-hour turnaround time
- But it raised questions about the correspondence between methodologies
- What about more specific methods?

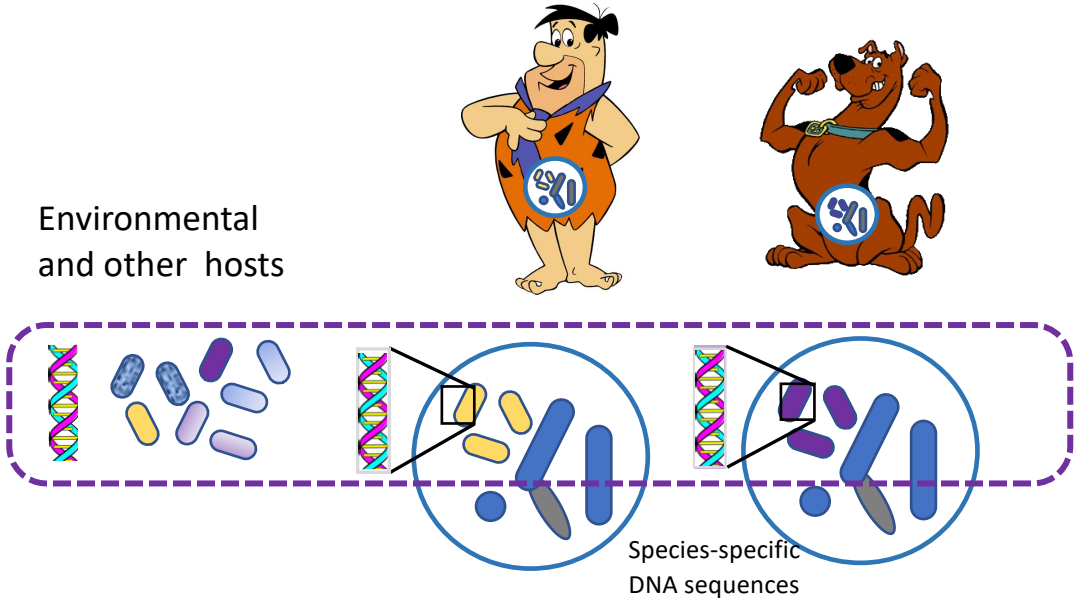


AEM Journals.ASM.org 2015

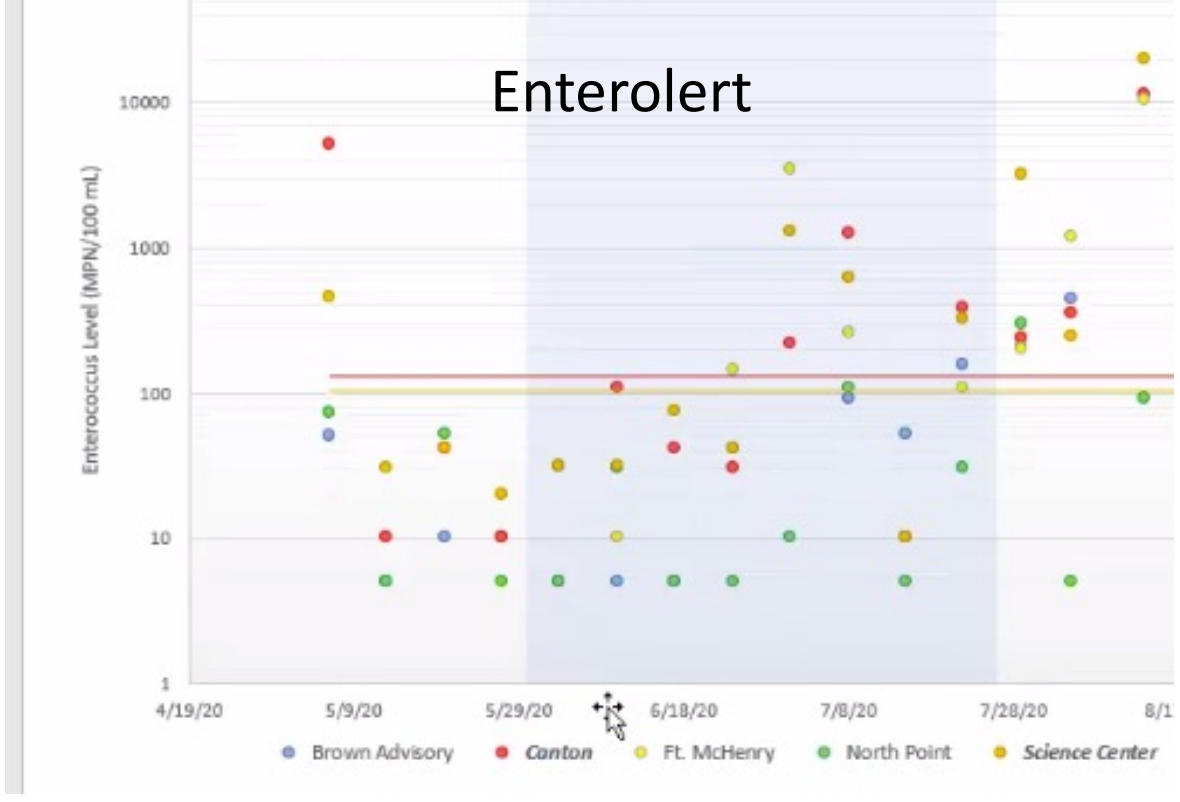
Improved HF183 Quantitative Real-Time PCR Assay for Characterization of Human Fecal Pollution in Ambient Surface Water Samples

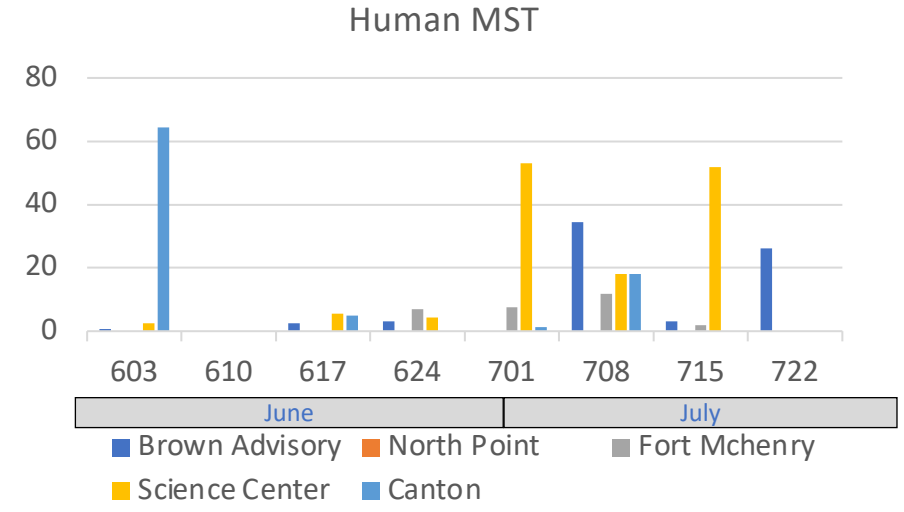
Hyatt C. Green,[a] Richard A. Haugland,[b] Manju Varma,[b] Hana T. Millen,[c] Mark A. Borchardt,[d] Katharine G. Field,[e] William A. Walters,[f] R. Knight,[g,h] Mano Sivaganesan,[a] Catherine A. Kelty,[a] Orin C. Shanks[a]

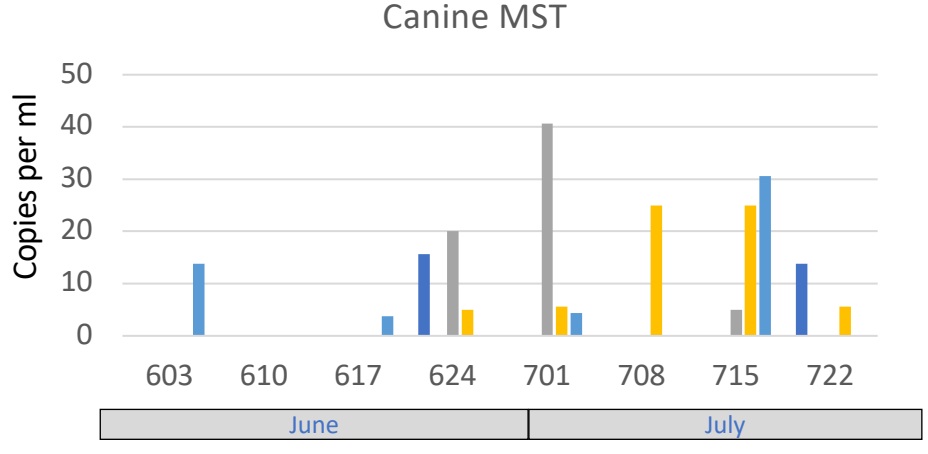
Microbial source tracking using *Bacteroides* spp.



Summer 2020

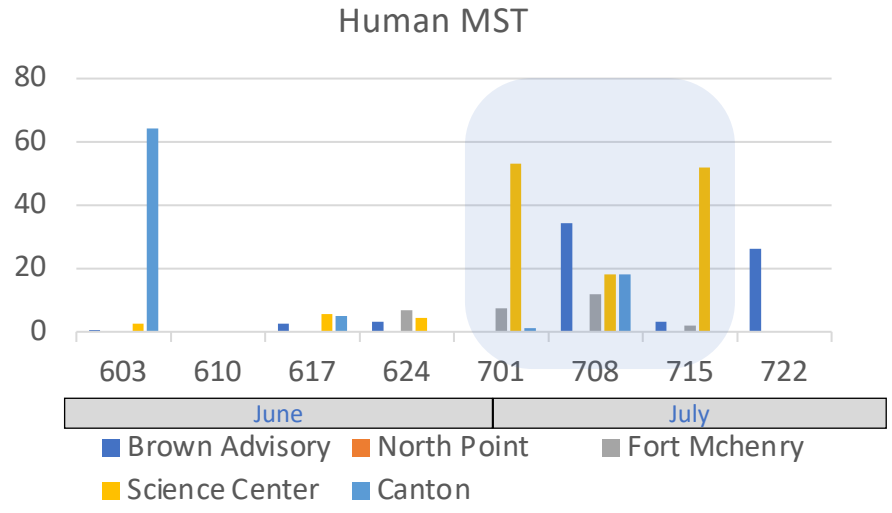




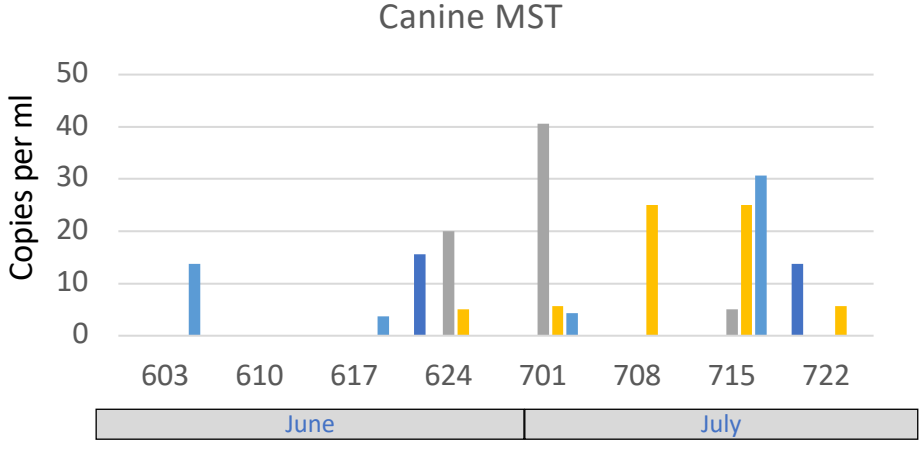


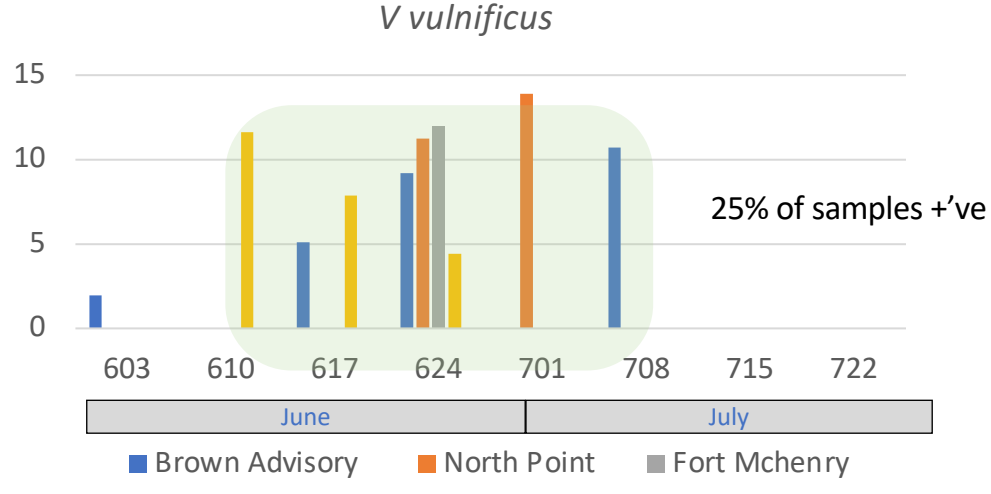
Not all peaks show Enterolert-MST correspondence

Summer 2020

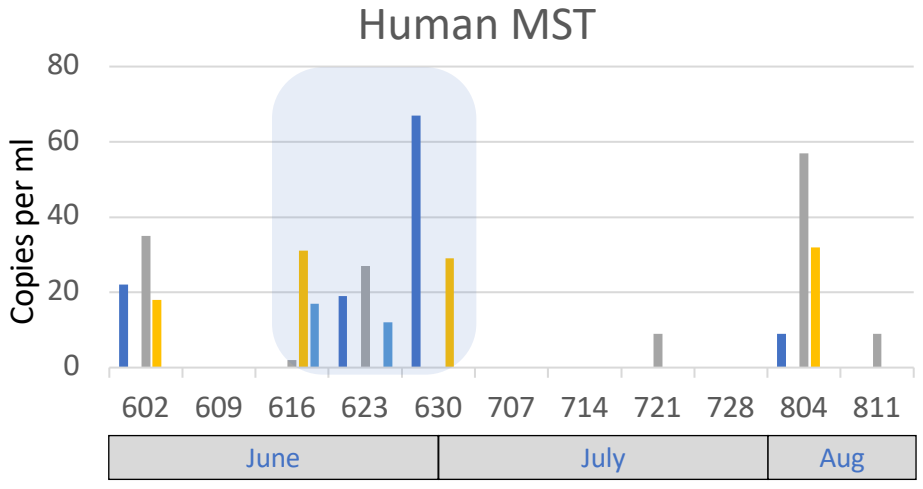

Human MST
80
60
40
20
0
603
610
617
624
701
708
715
722
June
July
Brown Advisory
North Point
Fort Mchenry
Science Center
Canton

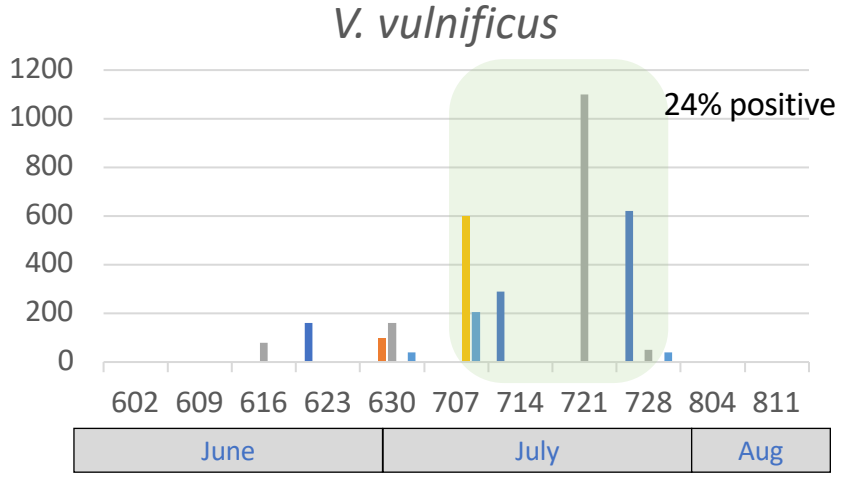
CDC

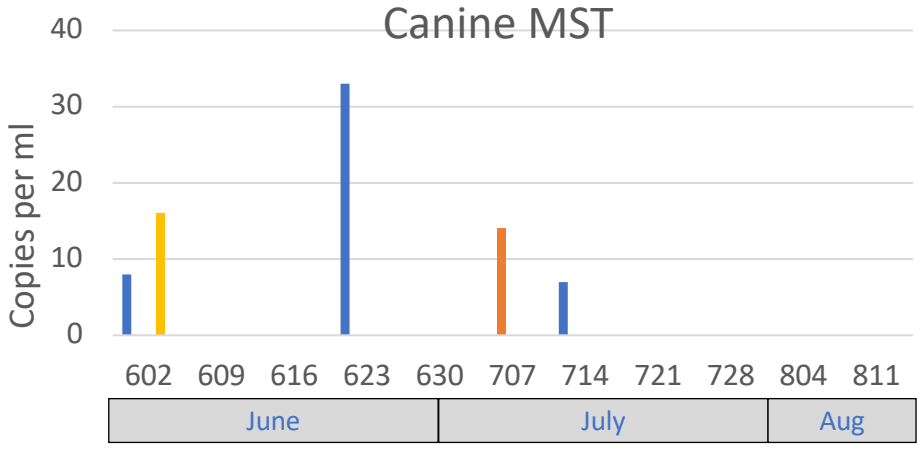

Canine MST
Copies per ml
50
40
30
20
10
0
603
610
617
624
701
708
715
722
June
July

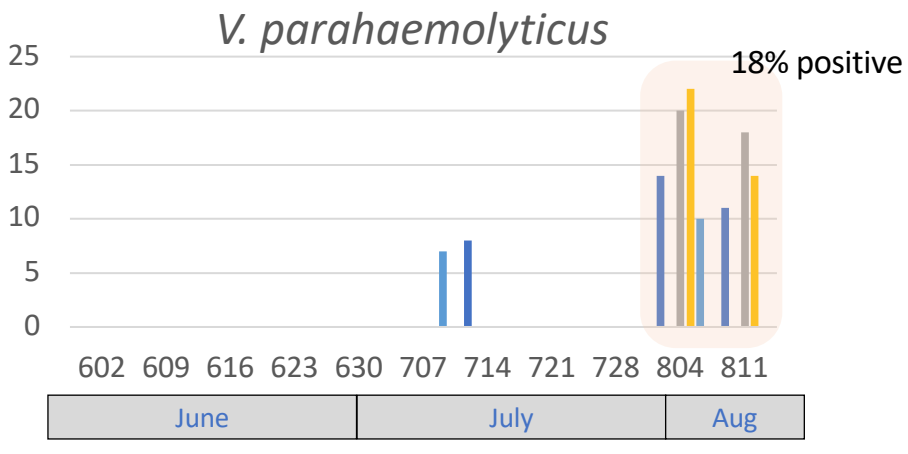

V vulnificus
15
10
5
0
603
610
617
624
701
708
715
722
June
July
Brown Advisory
North Point
Fort Mchenry
25% of samples +'ve

Summer 2021









Br Adv North Point Ft McHenry

Sci Center Canton

Summary and Conclusions:

Each Enterococcus method measures a different feature of the organism

It is increasingly recognized that FIB have limited utility for assessing risk
- Alternative markers are available

HF183 (human MST) has been in use 10 years and can get site specific EPA approval

Other molecular methods on the horizon:
- "Lachno3"
- Microbiome fingerprints

Human pathogenic Vibrios are present episodically

PCR technology with more specific markers can be used to assess recreational waters on the same day, with better certainty than FIB methods.

The reimagined Middle Branch