"Microbial pollution in urban stormwater: reasons for expanded management and potential application of an antimicrobial porous treatment media"

Gregory O'Mullan & William Blanford:

stormwater microbial pollutant data with student collaborators including:
Angel Montero and Azlan Maqbool

QUEENS COLLEGE

CITY UNIVERSITY OF NEW YORK THE GRADUATE CENTER

Overarching motivation: to better manage urban stormwater pollution and improve the quality of receiving waterways

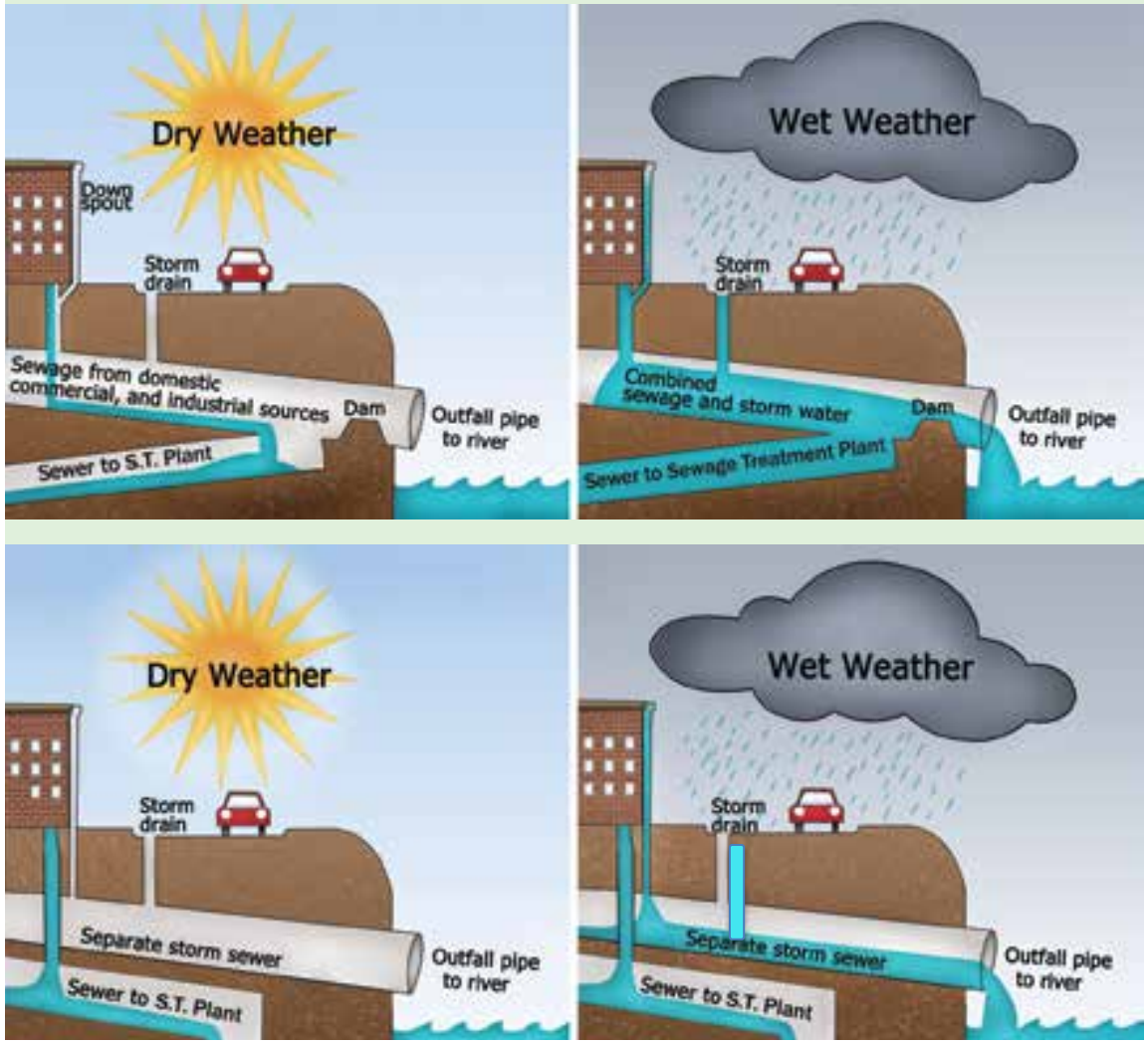
Progress can come from improved monitoring, expanded partnerships, wider management implementation of controls and innovation of treatment techniques.

Outline

i. Brief background
ii. Abundance and diversity of microbial pollutants of concern in NYC stormwater
    - Fecal bacteria/indicators
    - Antibiotic resistant bacteria
    - *Legionella pneumophila*
iii. Description of our water treatment media and bacterial removal performance examples
iv. Conclusions

Combined and separated sewers

What are they and what are some of the things we can do to manage them in NYC?



Modified from EPA

<u>Combined sewer systems and combined sewer overflow (CSO)</u> control plan options include:

- retention tank & tunnels
- sewer optimization
- ***sewer separation***
- reduced water use
- disinfection of overflow
- ***stormwater infiltration***
- nature based treatment solutions after discharge

<u>Municipal separated stormwater system (MS4)</u> control plan options include:

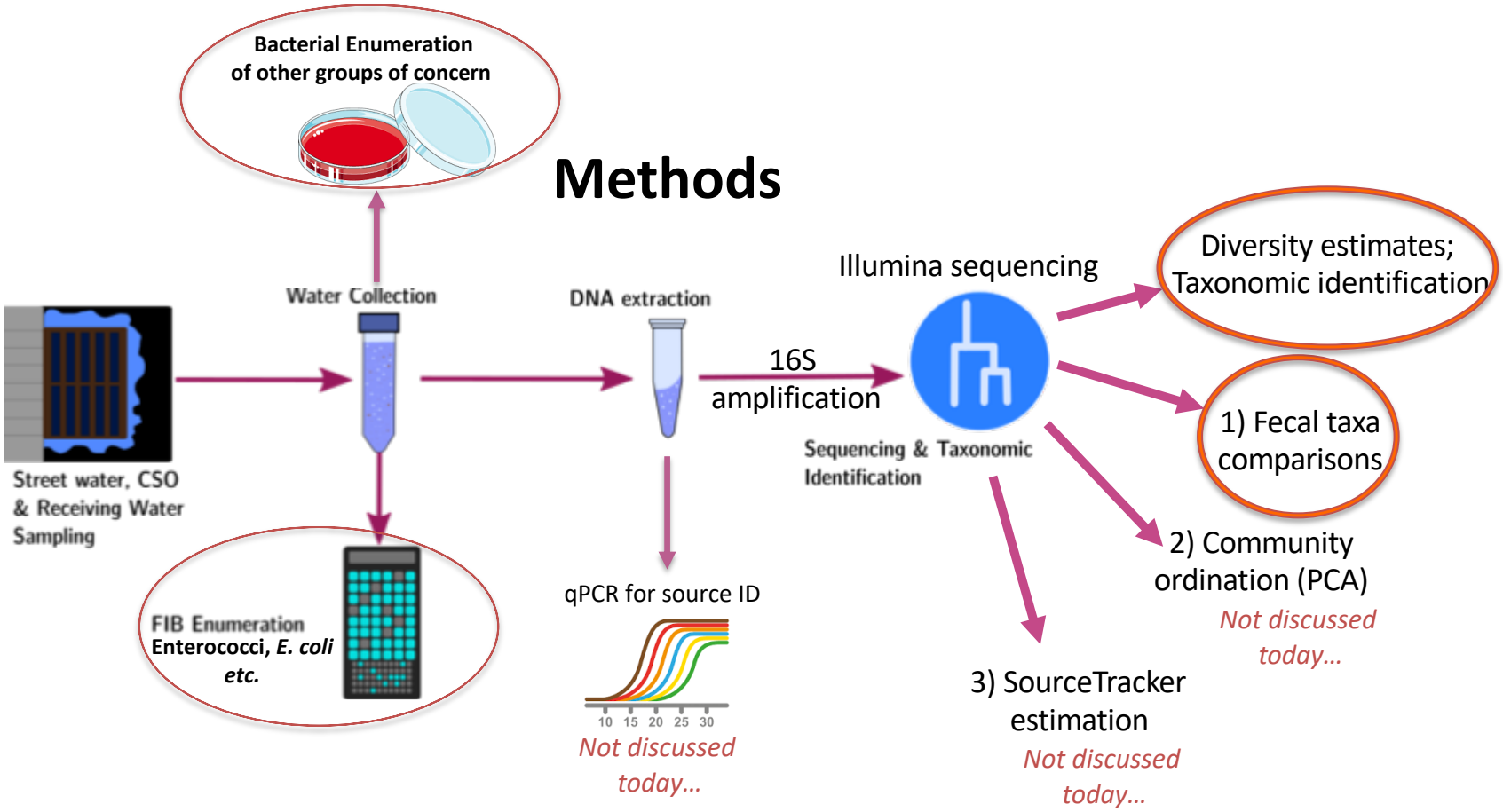
- watershed/street surface pollutant reduction
- ***stormwater infiltration***
- ***stormwater treatment***
- nature based treatment solutions after discharge

Addressing CSO pollution is a major management focus in NYC, and it should be.

However, not all waterways in need of management attention have CSOs and untreated sanitary sewage is not the only source of microbial contaminants that we need to consider.

In this talk we will focus on stormwater sampled primarily from **streets** and on **microbial** contamination concerns

Methods used to explore microbial pollutants in stormwater



It is fairly well known that stormwater can deliver considerable microbial pollution

Available at www.sciencedirect.com
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ELSEVIER

Confirmation of putative stormwater impact on water quality at a Florida beach by microbial source tracking methods and structure of indicator organism populations

M.J. Brownell[a], V.J. Harwood[a,*], R.C. Kurz[b], S.M. McQuaig[a], J. Lukasik[c], T.M. Scott[c]

Available at www.sciencedirect.com
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journal homepage: www.elsevier.com/locate/watres
ELSEVIER

Characterizing fecal contamination in stormwater runoff in coastal North Carolina, USA

J.K. Parker[a], D. McIntyre[b], R.T. Noble[c,*]

Contents lists available at ScienceDirect
Water Research
journal homepage: www.elsevier.com/locate/watres
ELSEVIER

Quantification of pathogens and markers of fecal contamination during storm events along popular surfing beaches in San Diego, California

Joshua A. Steele [a,*], A. Denene Blackwood [b], John F. Griffith [a], Rachel T. Noble [b], Kenneth C. Schiff [a]

Available online at www.sciencedirect.com
SciVerse ScienceDirect
journal homepage: www.elsevier.com/locate/watres
ELSEVIER

Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia

J.P.S. Sidhu[a,b,*], L. Hodgers[a], W. Ahmed[a,b], M.N. Chong[a], S. Toze[a,c]

FIB have been found to have a complicated association with illness in non-sewage impacted waters

But this doesn't mean that we can ignore it..

The influence of stormwater must be understood in our wider monitoring data, and our receiving water regulations are not source specific..

ORIGINAL ARTICLE

Water Quality Indicators and the Risk of Illness at Beaches With Nonpoint Sources of Fecal Contamination

John M. Colford, Jr., Timothy J. Wade,† Kenneth C. Schiff,‡ Catherine C. Wright,* John F. Griffith,‡ Sukhminder K. Sandhu,* Susan Burns,§ Mark Sobsey,¶ Greg Lovelace,¶ and Stephen B. Weisberg‡*

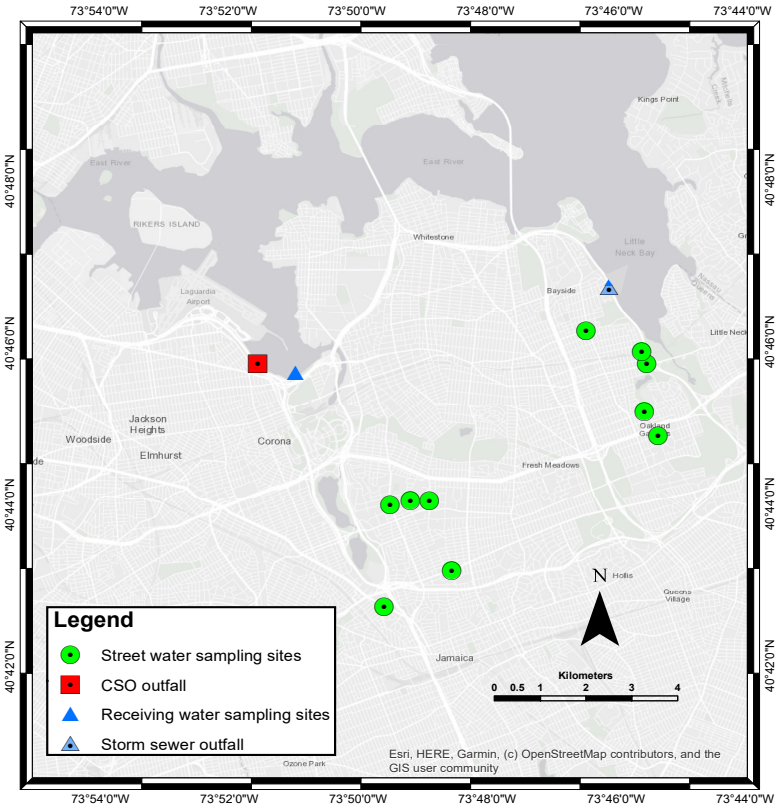
Assessing Pathogen Risk to Swimmers at Non-Sewage Impacted Recreational Beaches

MARY E. SCHOEN* AND
NICHOLAS J. ASHBOLT

Sampling Locations

CSO outfalls BB06, BB08

Inner Flushing Bay



Street Water

Stormwater outfalls

Research by former O'Mullan lab student Angel Montero

Research Question 1

Does NYC “street” water act as a major source of **Fecal Indicator Bacteria (FIB),** and “other microbes of concern”, to waterways?

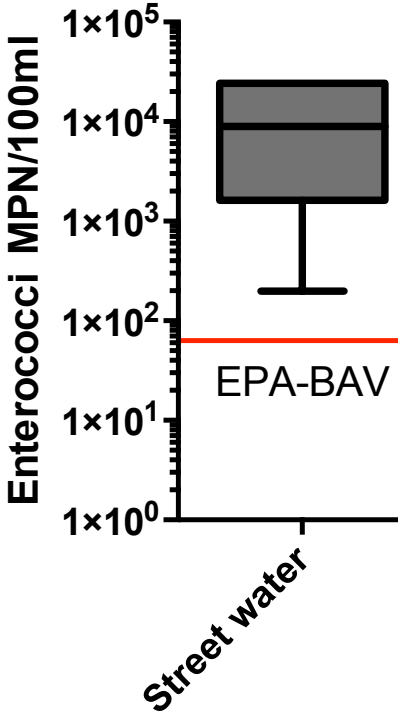
- **Approach:** Use EPA approved cultivation methods to enumerate enterococci in street water (Enterolert, mEI)

- **Hypothesis:** street water will contain high levels of culturable FIB with potential to act as a source for adjacent waterways

Montero & O’Mullan in prep

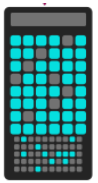
Does “street” water act as a major source of FIB to waterways?

Approach: cultivation (Enterolert) to enumerate FIB



- FIB were abundant in all 69 urban streetwater samples, collected across 10 locations.

- Median FIB concentration was more than 2 orders of magnitude above EPA-Beach Action Value (BAV)



Montero & O’Mullan in prep

Does “street” water act as a major source of FIB to waterways?

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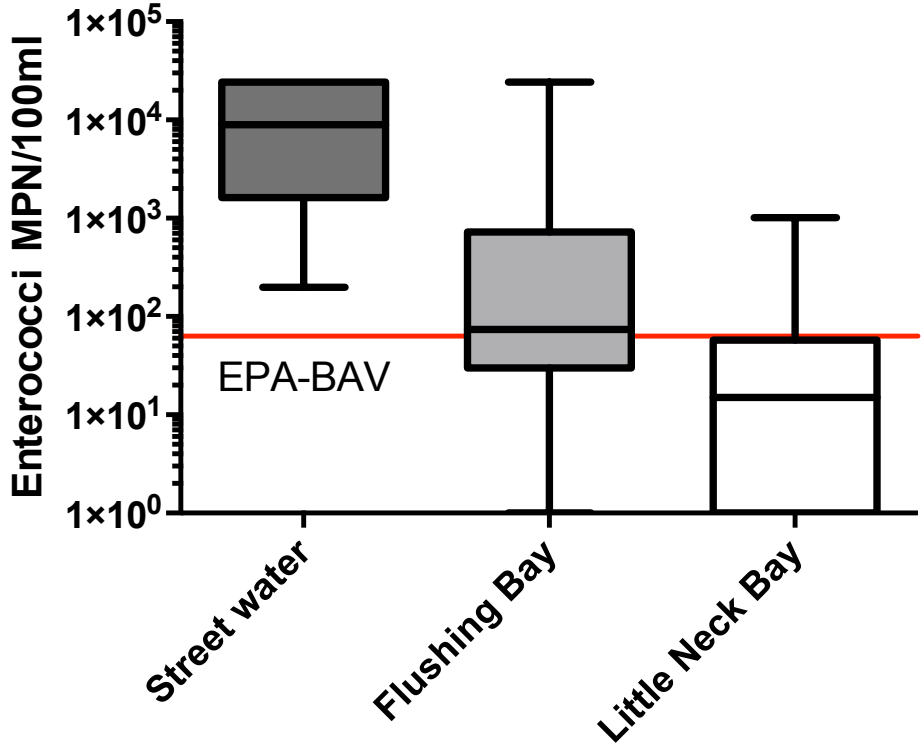
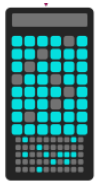


Street water enterococci concentrations were **significantly higher** than in the two embayments sampled in parallel data sets (Kruskal-Wallis, $p < 0.01$).



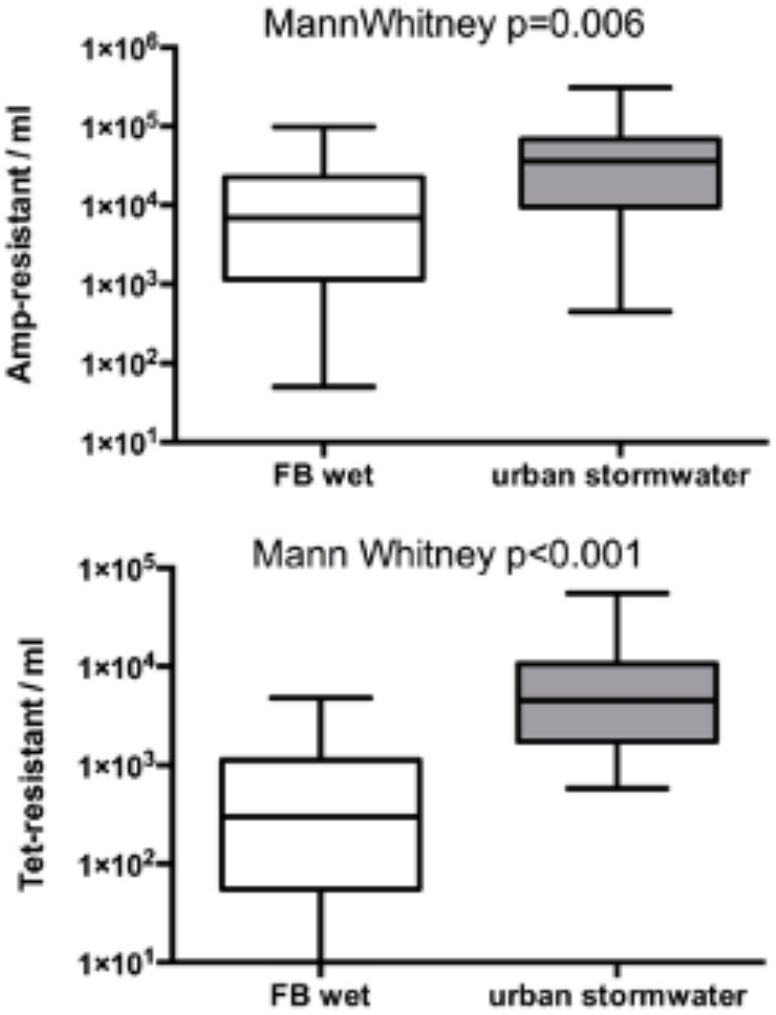
Urban stormwater, not just CSOs, can be a significant source of FIB to our waterways..

Montero & O’Mullan in prep

Is street water a major source of non-FIB microbes of concern?

Approach: Enumeration of Antibiotic Resistant Bacteria (ARB)

The concentration of ARB was higher in street water than either of the waterways studied at the same time, despite Flushing Bay being a major target of an LTCP.



Montero & O'Mullan in prep

Research Question 2

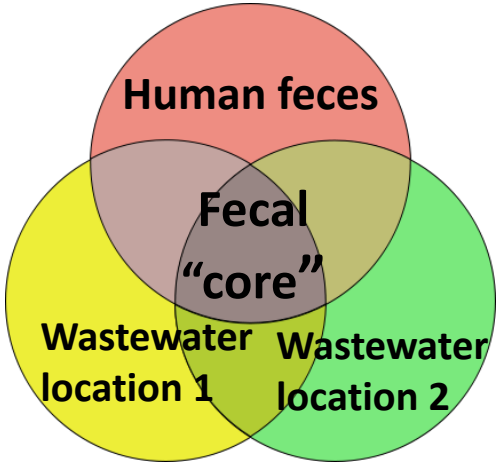
Do fecal bacteria ("fecal core") in street water differ from those in CSO and receiving waterways?

- **Approach:** Use 16S rRNA gene sequencing of DNA samples to explore the microbial community of street water, CSO, and receiving water

- **Hypothesis: "**Fecal core" microbes in street water will differ significantly from CSO and receiving water

Prior literature has used high throughput DNA sequencing to identify taxonomic groups from a "fecal core" commonly shared by human feces and sanitary wastewater

7 Fecal "core" families:
Bacteroidaceae, Porphyromonadaceae, Clostridiaceae, Lachnospiraceae, Ruminococcaceae, Rikenellaceae, Prevotellaceae (VandeWalle et al 2012; Shanks et al 2013; Newton et al. 2013; Newton et al. 2015)



Comparison of the Microbial Community Structures of Untreated Wastewaters from Different Geographic Locales

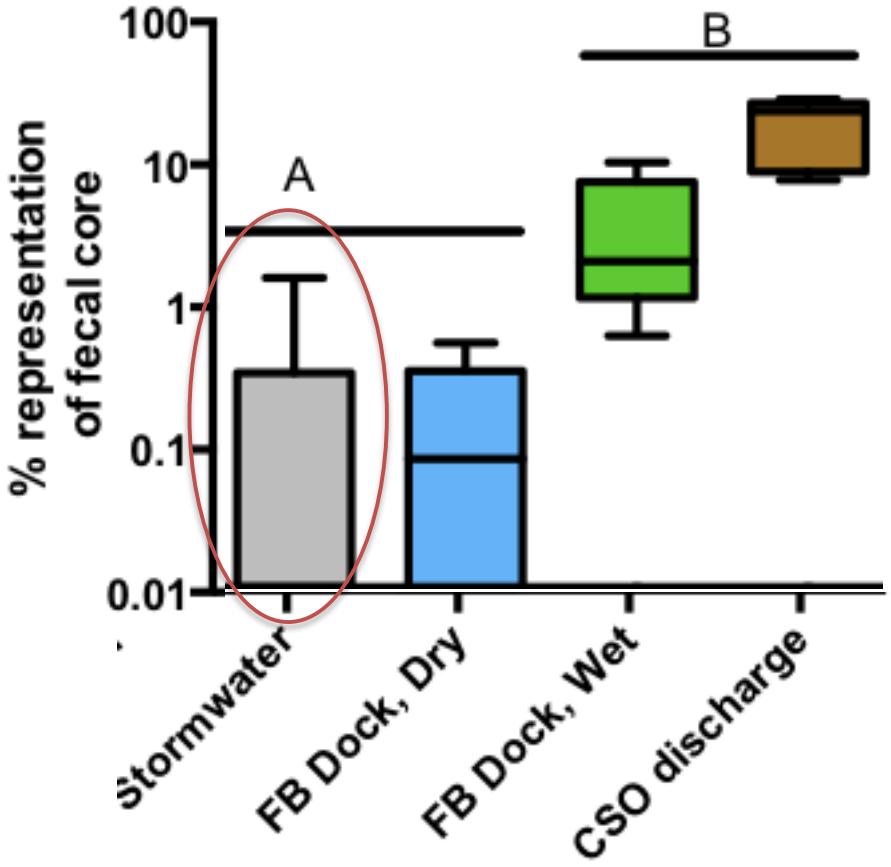
2013 Applied & Environmental Microbiology 79(9):2906-2913

Orin C. Shanks,[a] Ryan J. Newton,[b] Catherine A. Kelty,[a] Susan M. Huse,[c,d] Mitchell L. Sogin,[c] Sandra L. McLellan[b]

Newton et al 2013. Microb Ecol 65:1011-1023
Newton et al. 2015. Mbio 6(2):e02574-14

Representation of "Fecal core" sequences increase following rain in Flushing Bay (FB)

Kruskal Wallice p<0.01



fecal core max:
CSO 27%
Wet 10%
Dry 0.5%

Despite high levels of culturable FIB, "fecal core" sequences were rare in urban stormwater

Angel Montero and O'Mullan, in prep

Research Question 3

Is *Legionella pneumophila* widespread in NYC urban polluted waterways?

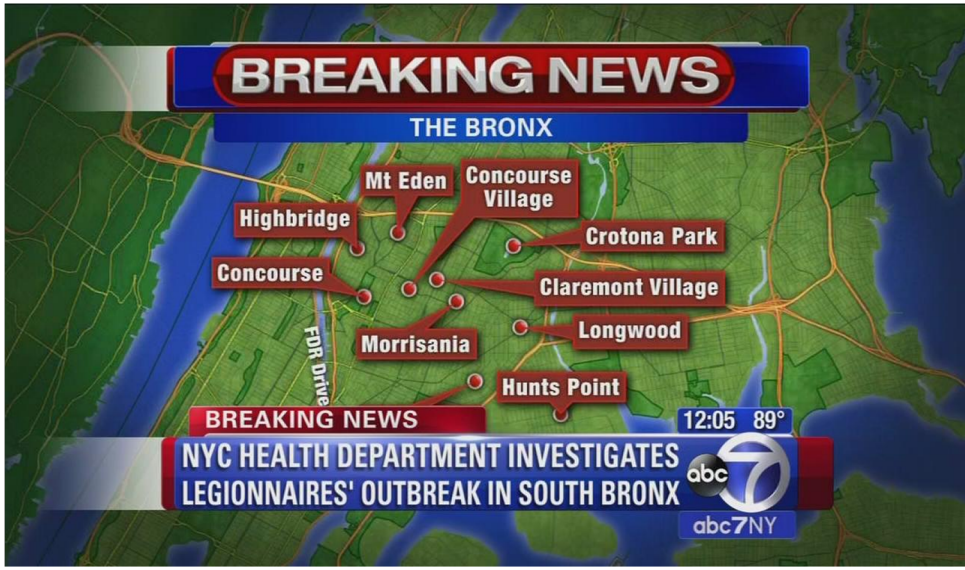
- **Approach:** Use 16S rRNA gene sequencing of DNA samples from the Hudson River Estuary in archived samples, and cultivation based enumeration of Legionella

- **Hypothesis:** *Legionella pneumophila* will be commonly detected in sewage impacted waterways and in CSO, but have lower concentrations in stormwater.

Research by former O'Mullan lab MS student **Azlan Maqbool**

Legionella and Legionnaires Disease

- *Legionella pneumophila* is the bacterium causing Legionnaires Disease, transmitted via inhalation and widespread in water and soil (Chamberlain et al 2017), but of most concern in **cooling tower aerosols**.

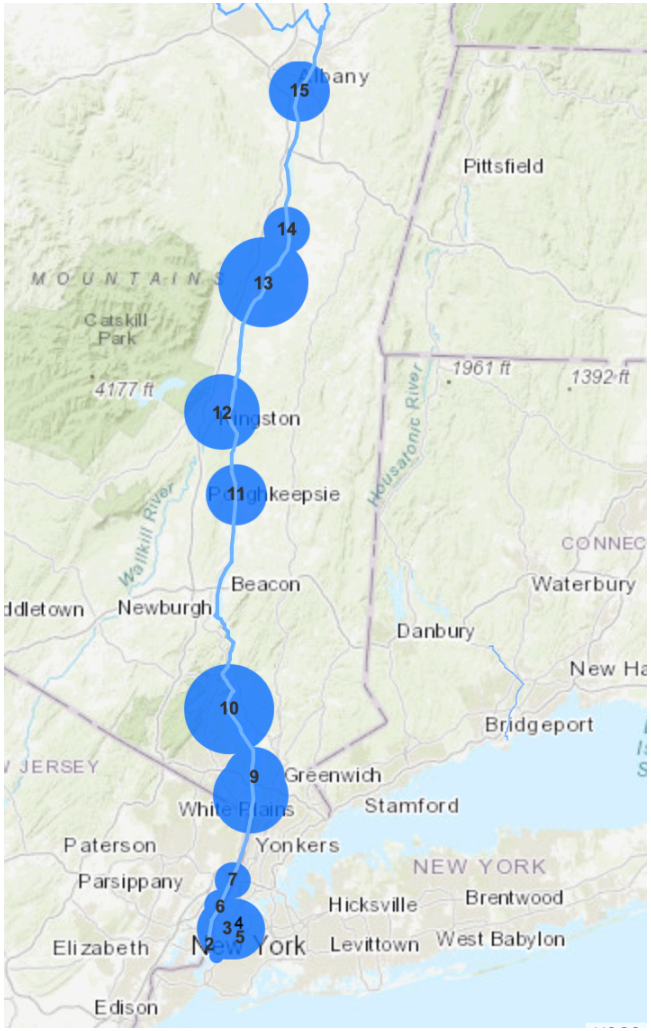


- In 2015, New York City had its worst Legionnaires outbreak in history. One outcome = Local Law 77

* *Legionella* has also been reported in municipal WWTPs (Caicedo et al., 2018)

Legionella was frequently detected in the Hudson River Estuary in our DNA based surveys

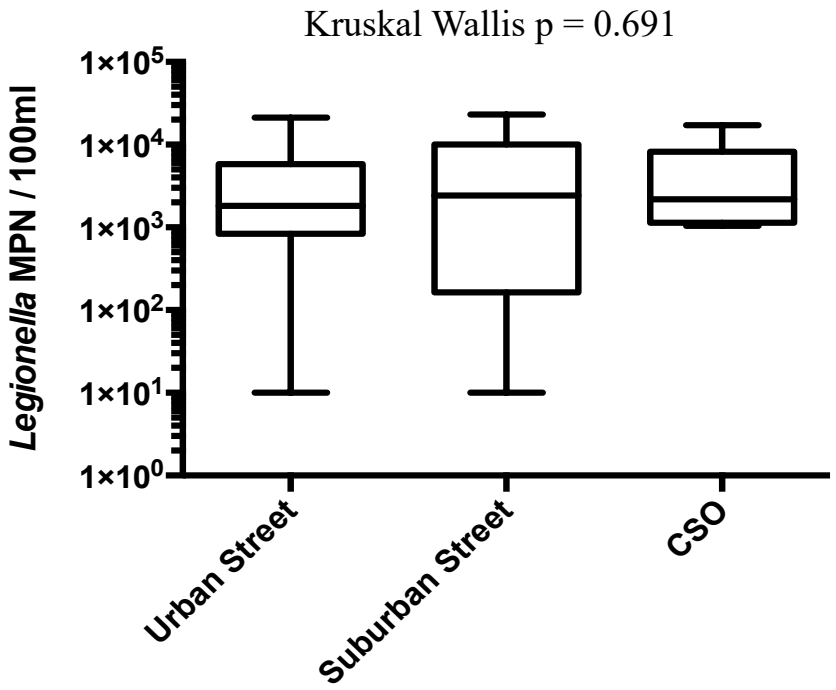
Site #:	# of Samples:	% of samples w/ Detection:
1	12	50%
2	13	54%
3	13	85%
4	13	85%
5	13	54%
6	13	69%
7	14	71%
8	13	92%
9	13	85%
10	13	100%
11	13	85%
12	13	92%
13	12	100%
14	13	77%
15	13	85%
Total	194	79%



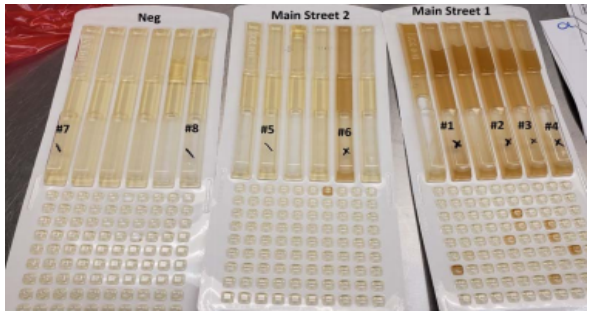
- 16S rRNA gene sequences associated with the genus Legionella were detected at all 15 HRE sites examined.
- The % of samples with detection at each site ranged from 50% to 100%

Azlan Maqbool, in prep

Legionella detection in NYC waterways increased after wet weather (data not shown), and stormwater appears to be a common source

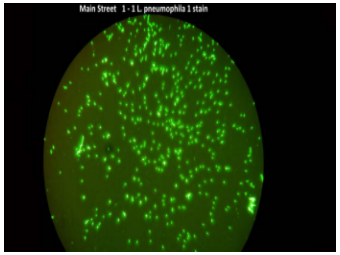


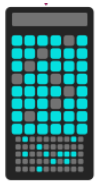
Enumerated with "Legiolert"



sub-cultured by contract lab

confirmed as *L. pneumophila* w/ immunofluorescence

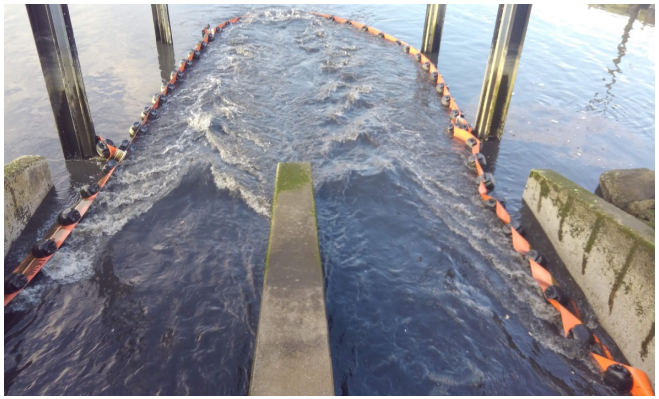




Azlan Maqbool, in prep

Conclusions about stormwater microbial pollution

- FIB, ARB and *Legionella* were commonly found in urban stormwater
- Stormwater microbial pollutant levels often exceeded those in local waterways considered heavily polluted
- In contrast, genera associated with human fecal contamination had much lower representation in stormwater compared to polluted waterways
- It is reasonable to consider the need to treat stormwater before release into waterways, and maybe even before infiltration in bioswales..
- The public also needs reasonable expectations for waterways when CSOs are controlled…we should not expect FIB to be absent, and may not be acceptable with NYS WQ standards, unless stormwater is also managed…



Goal: Development of antimicrobial water treatment approach, using quats fixed in stable coatings to the surface of objects or porous media (e.g. pea gravels, recycled glass, etc) to allow high flow rates with microbes killed as the water passes through the treated material.

Water Science & Technology

IWA PUBLISHING

Water Science & Technology Vol 87 No 10, 2457 doi: 10.2166/wst.2023.076

Evaluation of a novel porous antimicrobial media for industrial and HVAC water biocontrol

William James Blanford* and Gregory D. O'Mullan

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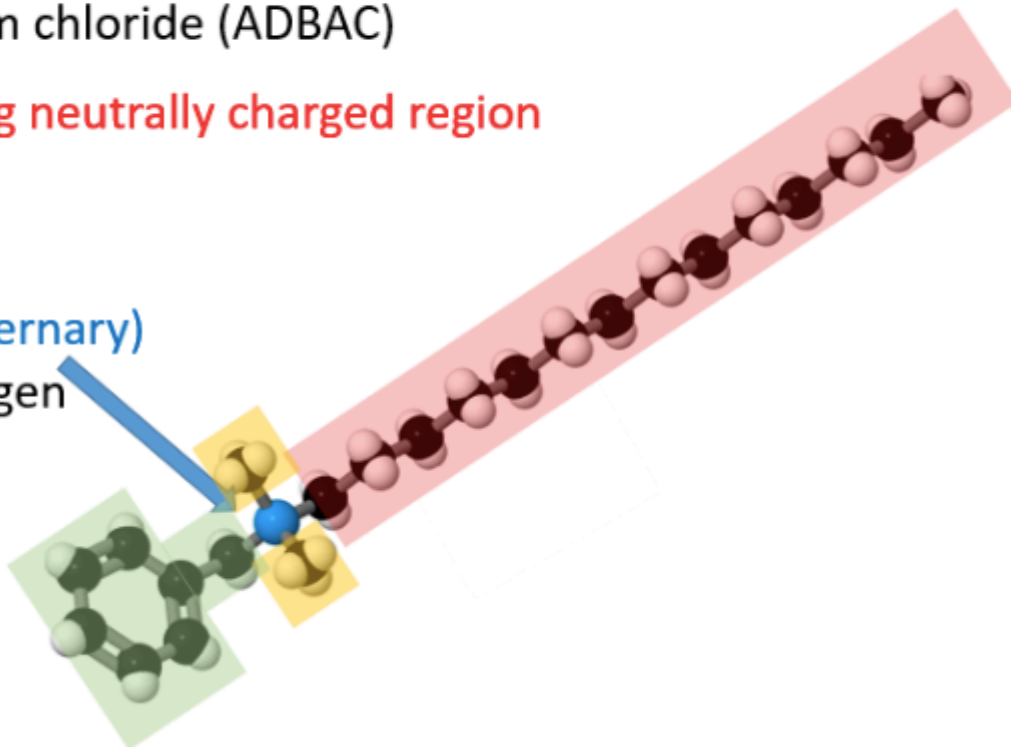
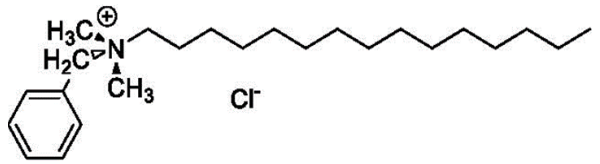
- low cost
- flexible application of coating to different materials/different treatment systems
- can be gravity fed, high permeability, low/no energy use
- quats are not consumed, reduces chemical use compared to treatments added in solution that

Quats - Quaternary ammonium compounds, a common class of sanitizers/disinfectants

- One of the most common: Benzalkonium chloride
  Alkyldimethylbenzylammonium chloride (ADBAC)
- Alkyl chain (8-18 carbons): long neutrally charged region
- Two methyl groups (CH_3)
- Benzyl group
- Four bonds off Nitrogen (Quaternary)
- Result Positively charged nitrogen

And a cationic surfactant

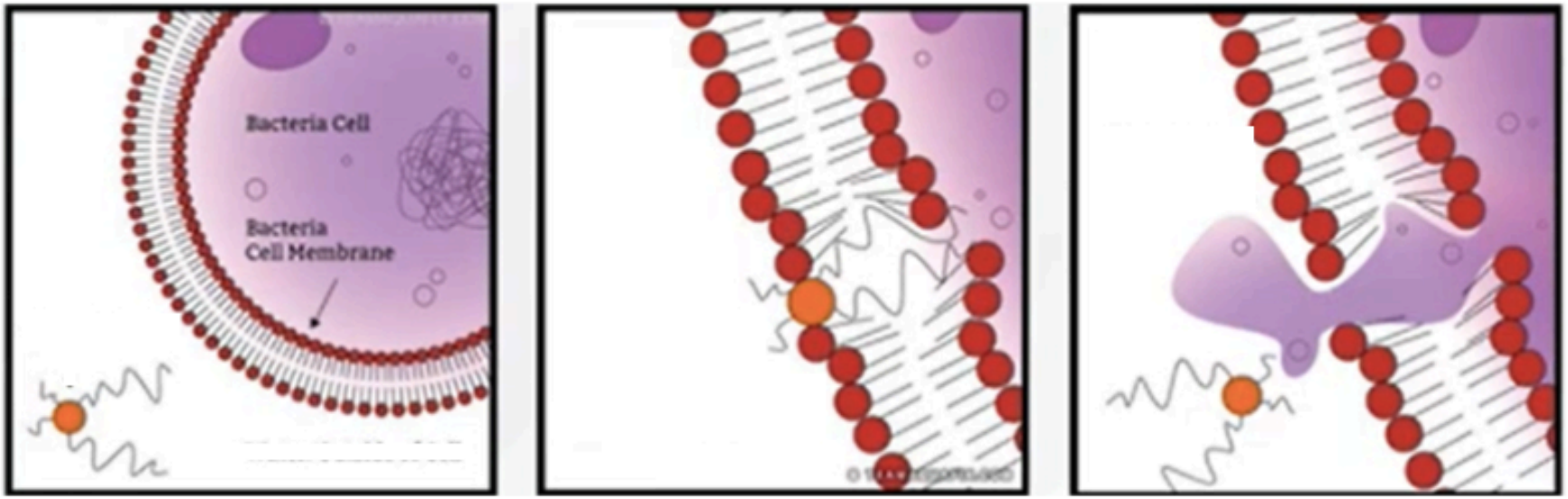




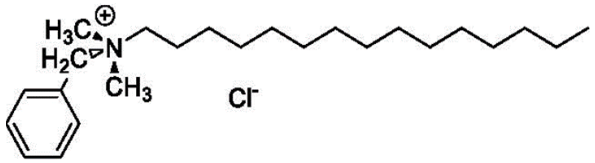
(Benzalkonium chloride) ADBAC is an active ingredient in many hand soaps and Lysol, the quat used in solution in those cases

Quats

- Known effective against bacterial groups (Legionella, Enterococcus, Pseudomonas)
- Some evidence for denaturation proteins (viruses) and inactivation of enzymes
- Bactericidal action primarily attributed to disruption of cell membrane
- Cationic surfactant attracted in environment to negatively charged membrane

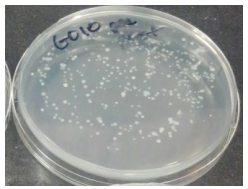


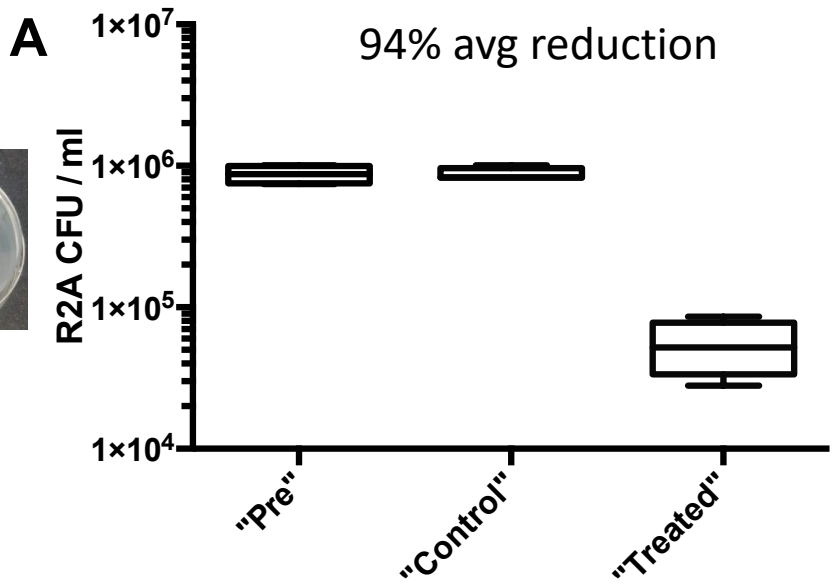
https://www.youtube.com/watch?v=kzuAspWfEyk

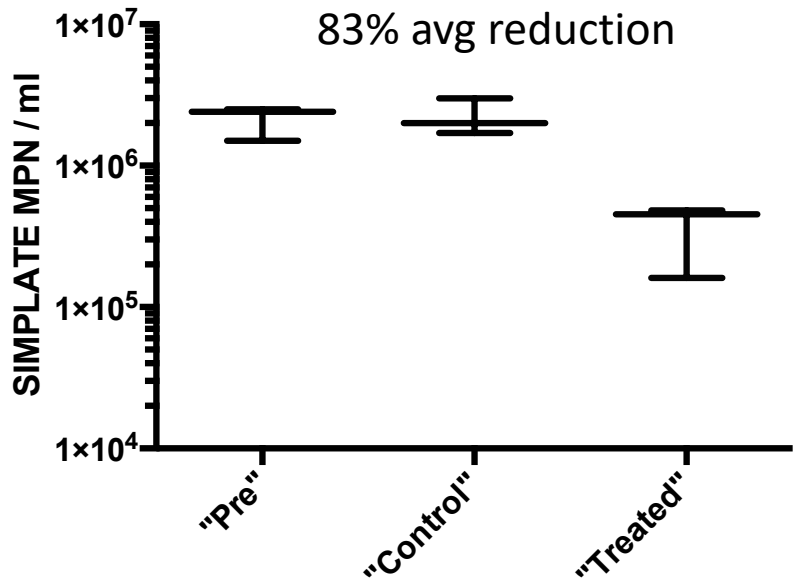


HVAC Cooling tower water (complex microbial community), gravity fed through (4 column reps per treatment), 5-foot long, 2 inch diameter PVC column filled with untreated versus treated (quat-coated) pea-gravel (<0.5 inch diameter), with flow rate of approx. 4L/min and <15 sec water contact time in this single pass column exposure experiment.

Treated columns had >1000L DI pre-flush (to reduce any leaching from coating). 2 different heterotrophic plate counts (R2A, in-house; IDEXX SIM plate, external lab)





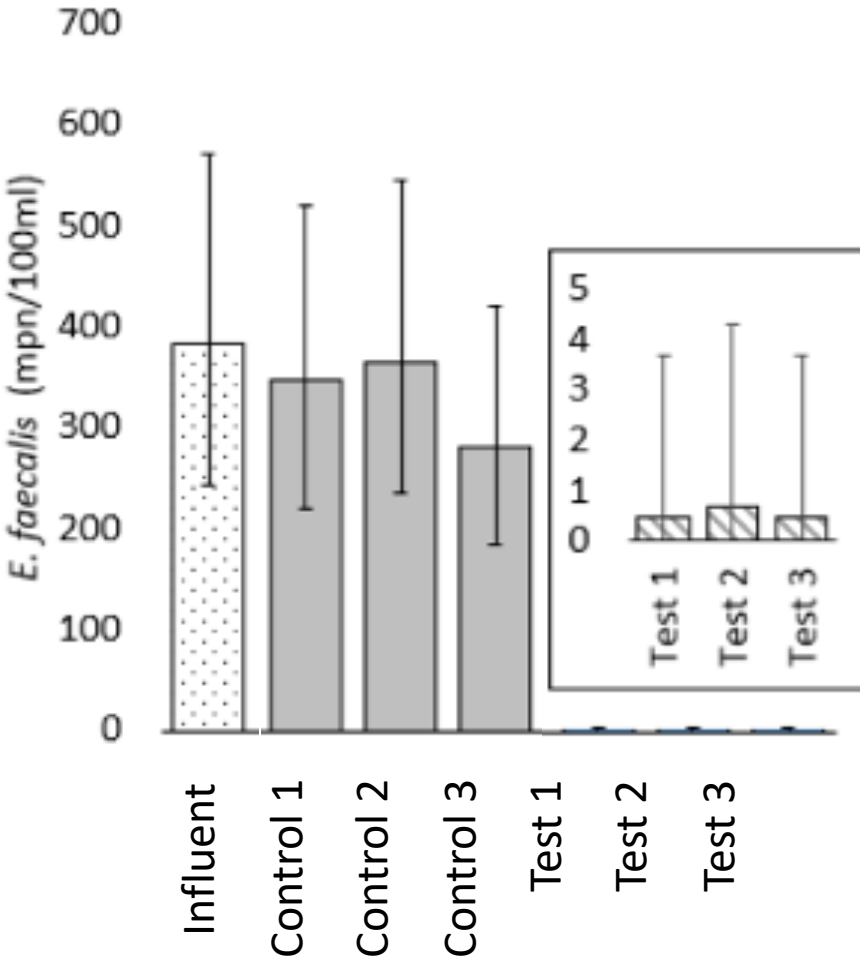


Enterococcus faecalis *(ATCC #19433)* pure culture tests, with updated low-VOC antimicrobial coating on small diameter pea gravel (33” bed depth; 300 L pre-flush & 19L culture exposure).

External (UnivAZ WEST Center) lab tests, 3 rep control and test columns, w/ 3 reps per column.

- No significant difference between influent and controls

- >99% reduction of FIB in columns with treatment media



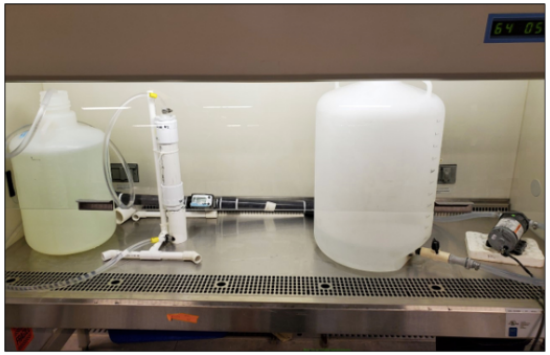
Simplified from Blanford and O’Mullan 2023

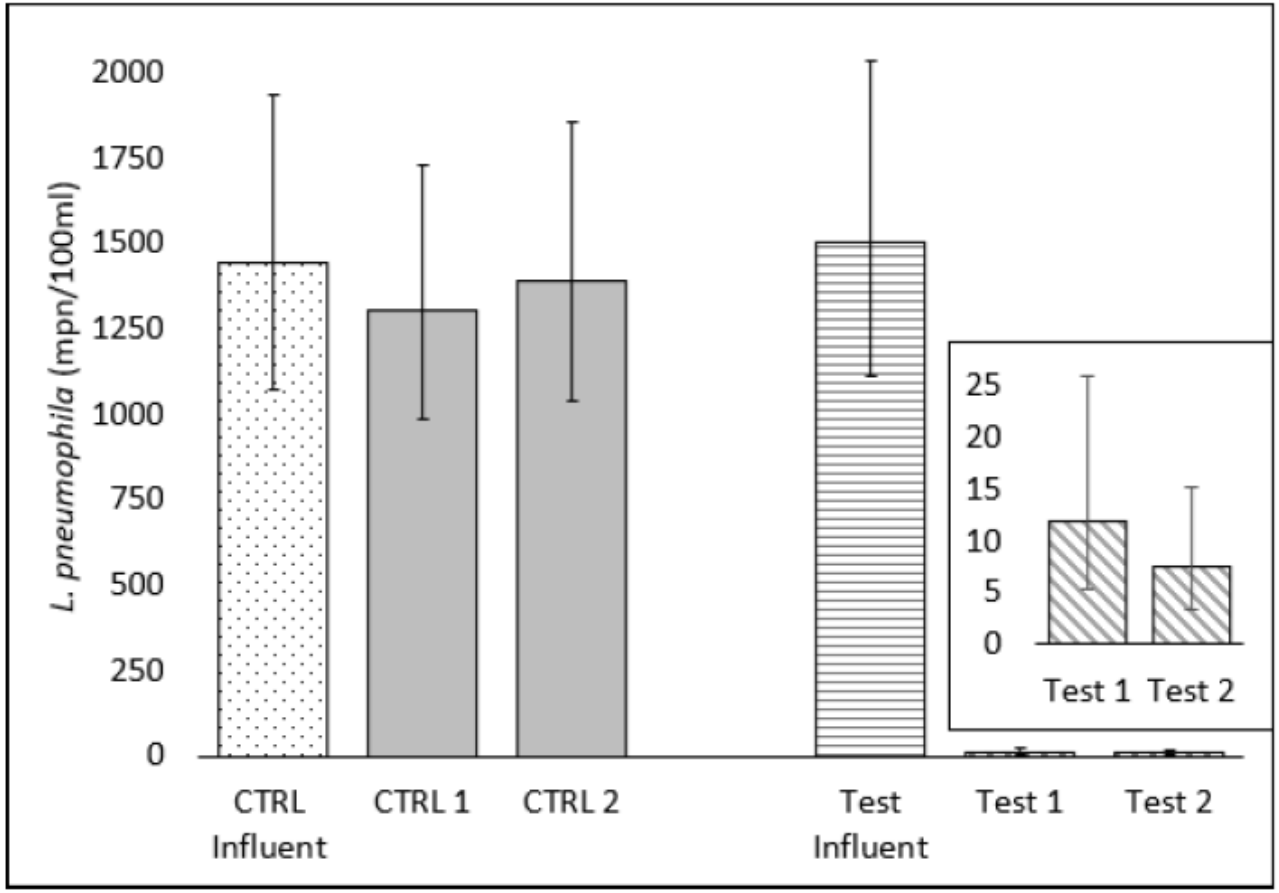
Legionella pneumophia *(ATCC #33152)* pure culture tests, with updated low-VOC antimicrobial coating on small diameter pea gravel (9” bed depth due to biosafety cabinet size, 300 L pre-flush).

External (Univ AZ WEST Center) lab tests, 2 rep control and test column experiments, with 3 reps per test. The control and test had to be run separately due to biosafety cabinet space, so have 2 slightly different influent concentrations.

- No significant difference btwn Influent and controls (p=0.13)

- >99% reduction with 3 of 6 tests were below minimum detection





Blanford & O’Mullan 2023

Conclusions for porous media treatment

- Proof of concept on FIB and Legionella treatment
- We expect that the porous media has potential for use in cooling tower and stormwater treat applications
- Stormwater (and cooling tower water) is in need of expanded risk assessment, treatment approaches, and broader management.
- Management discussion- We should be considering expanded microbial treatment of stormwater in MS4 discharges and bioswale infiltration.

Acknowledgements:

Funding from:
Riverkeeper, NY Powerbridge, CUNY I-corps, NSF I-corps

Student collaborators on stormwater microbial pollutant description:
Angel Montero and Azlan Maqbool

Town & Gown:
We excited to expand collaborations with agencies, including DEP and DOHMH, in an effort to expand water monitoring and treatment

Extra slides

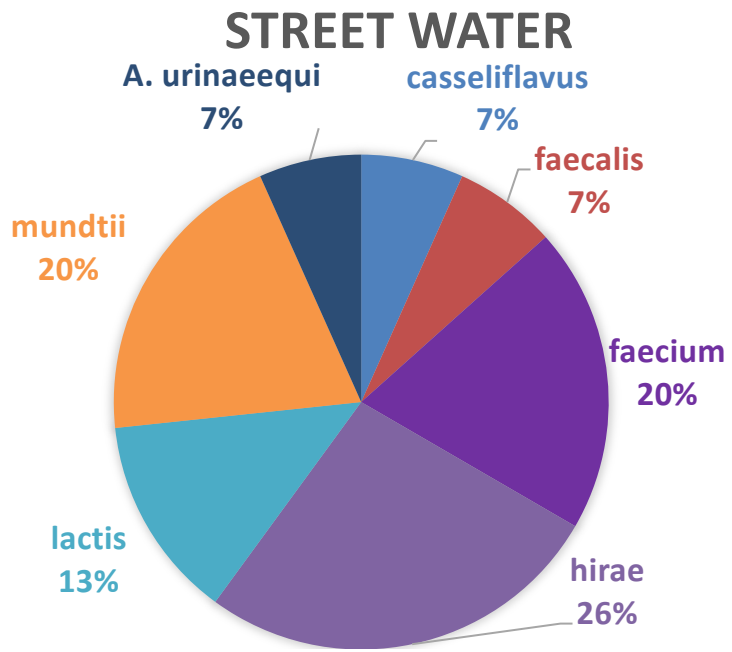
Research Question 4

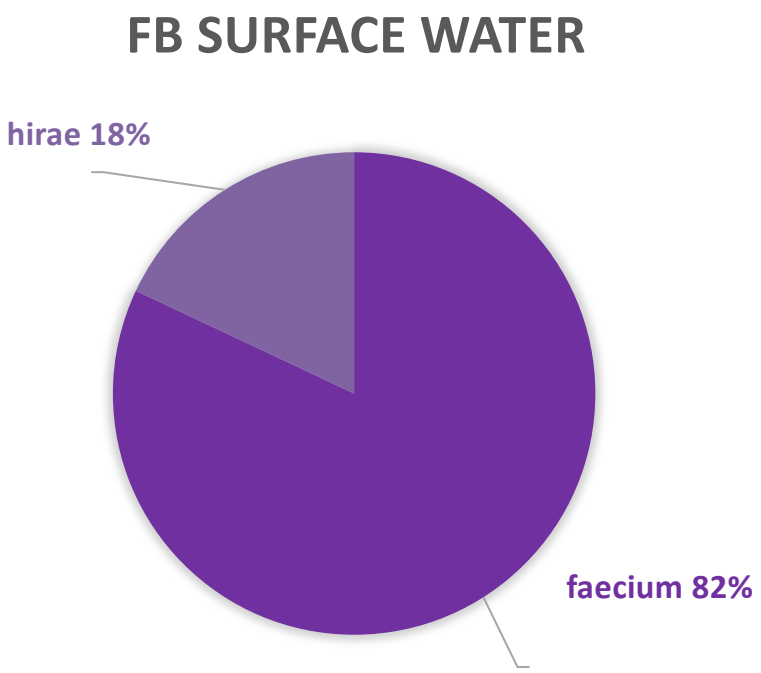
Do *Enterococcus* species in street water and receiving waterways differ?

- **Approach:** cultivation and 16S rRNA gene based identification of FIB in street water and receiving waterways
- **Hypothesis:** Street water enterococci isolates will differ from receiving water

Prior literature shows that the dominant human Enterococcus species = E. *faecium*, E. *faecalis*

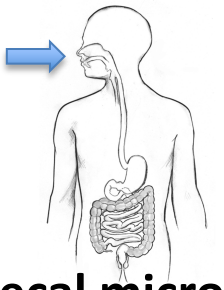
Do the species of FIB in street water differ from those in receiving waterways?



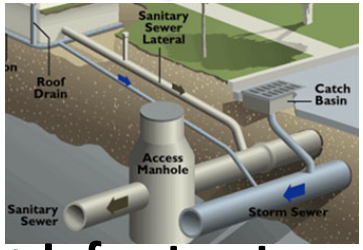


Sequencing of 16S rRNA confirmed 14/15 street water isolates tested were from the genus enterococci, but the strain diversity was much broader and less likely human associated

Enterococci and % "fecal core" representation from DNA are correlated in Flushing Bay water samples

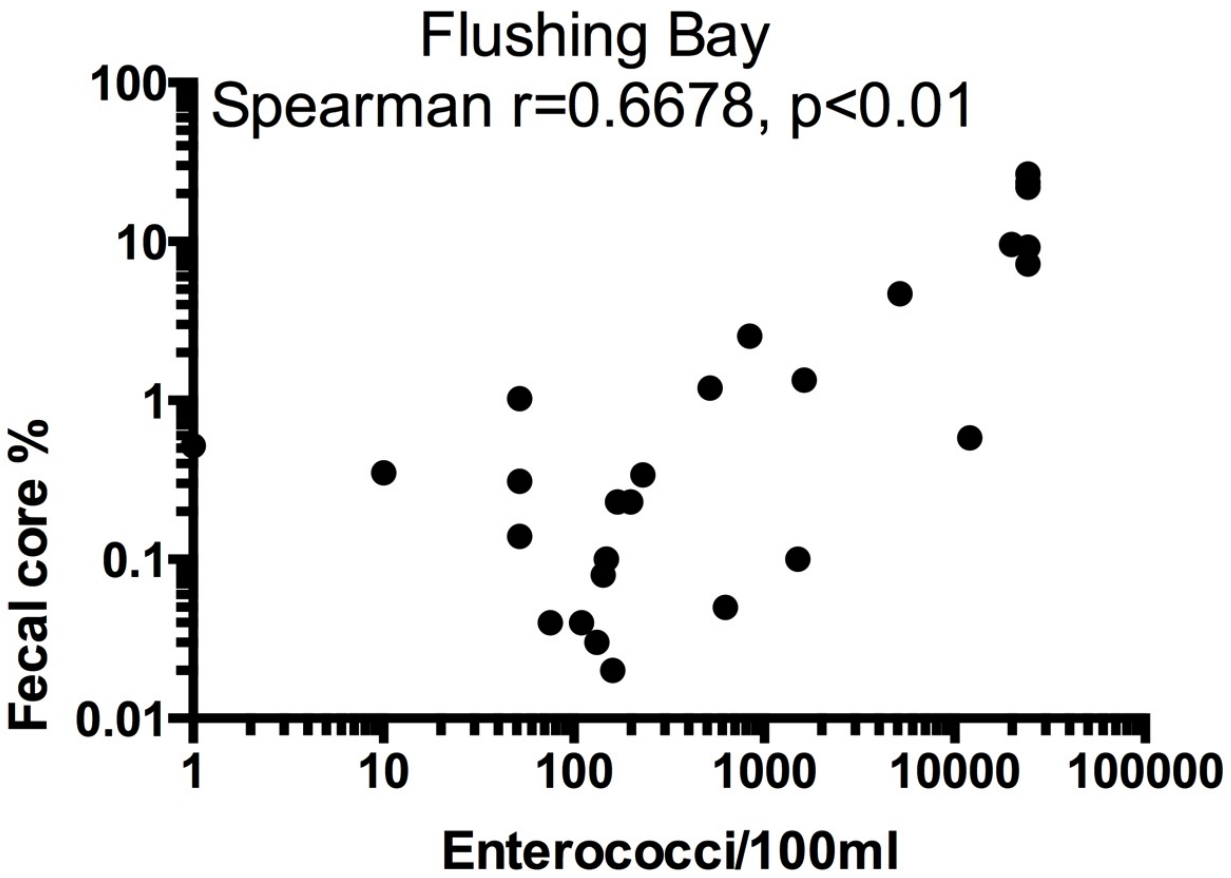


Fecal microbes



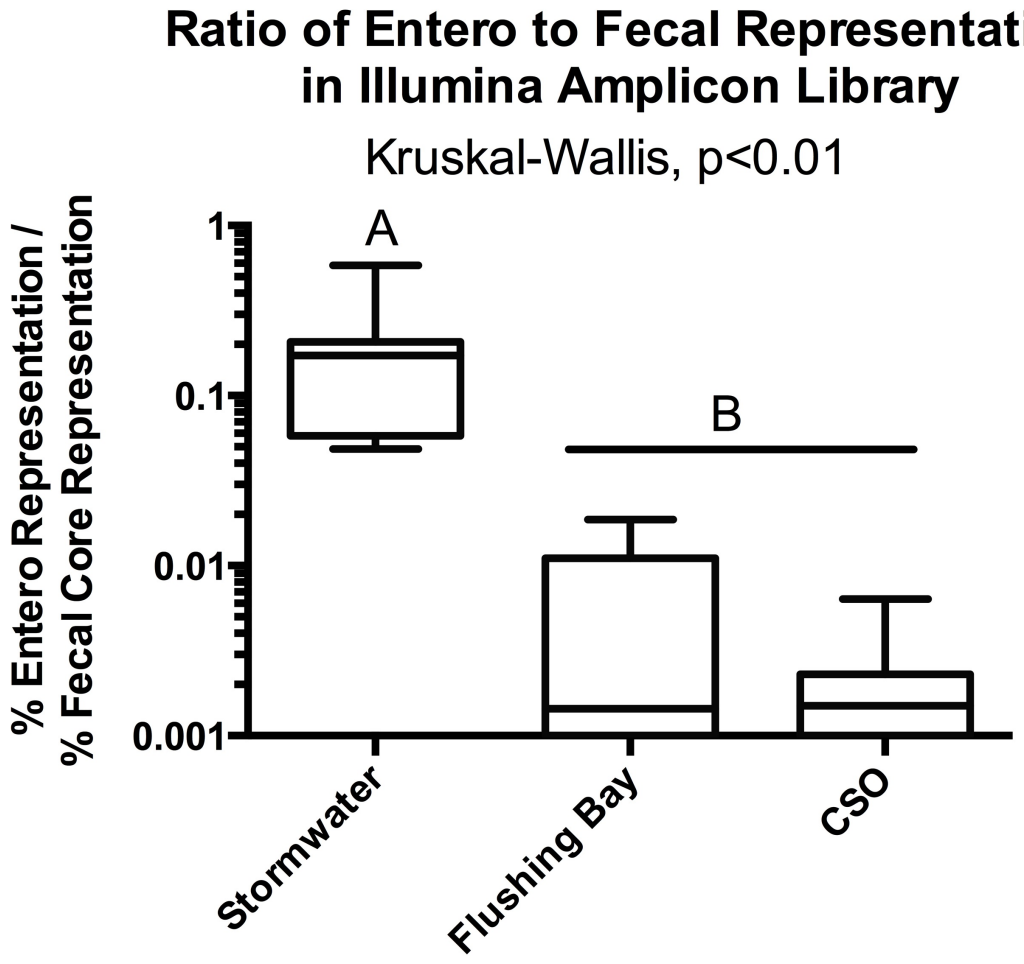
+ Infrastructure microbes

CSO input



Angel Montero and Roman Reichert

Ratio of FIB to fecal core sequences is significantly greater in stormwater…



- Significantly higher ratio of FIB to Fecal sequences in stormwater
- **(Not shown) Unlike the receiving waterway, FIB and fecal sequences were not correlated…**
- **Consistent w/ epidemiology suggesting lower risk from stormwater FIB (e.g. Soller et al 2010)**

This may complicate management/monitoring…

Montero & O'Mullan in prep