Advance Research Methods Training
Bacterial Water Quality Monitoring at The River Project
September 2019 Workshop Summary

- the date, location, instructors, and number of participants
  - Date: 9/19/19 and 9/19/19
  - Instructors: Melissa Rex, Director of Education, The River Project
    Toland Kister, Education Coordinator, The River Project
  - Location: The River Project Wetlab (Pier 40, 353 West St, New York, NY 10014)
  - Number of participants: 1

- a brief summary of aspects of the protocols and curriculum plan that appeared to be particularly effective

  The workshop provided instruction in a bacteria-testing method commonly used at local laboratories (the IDEXX method) and a second less sensitive method (the Hyserve method) that is better suited to classroom use based on the equipment needed for the procedure. The participant noted that partaking in these testing methods made her "feel like a scientist" and that using the two methods helped her understand what kind of data each method can produce.

  On the second day of the workshop, the participant used provided resources, including data banks and interactive maps, to investigate bacteria data for the Hudson River site where she teaches. She was able to graphically compare rainfall and bacteria data over several weeks to learn how long bacteria persisted at her site after rain. She appreciated gaining the tools to predict bacterial counts, an important safety metric, at her site through data analysis.

- a brief summary of any areas for improvement in protocols or curriculum

  In this workshop, participants began by collecting water samples and later received background information on the environmental issue of combined sewer overflows and the testing methods used to detect them. In the future, I would provide more background information before sampling, so participants can choose sample sites based on information they learned about sewage outfalls, and therefore have more independence in directing their own research.

- a brief summary of any notable feedback from participants.

In a follow-up email, the participant wrote, "I wanted to thank you again for an amazing training last week- I feel more confident discussing CSO's and water quality data with students now, and it's thanks to you!" She explained during the training that she was looking for ways to incorporate more quantitative data into her curriculum. Additionally, she said that understanding the connection between rainfall and sewage overflows would help her make better informed decisions

about whether it was safe for students to interact with the river at her site after it rained.

Water Quality Testing for Enterococcus 2-Day PD

Day 1

10:00-10:15 (15 min) Intro - teachers' names, what they teach, why they are attending PD
10:15-11:15 (60 min) Sampling - 5 samples along Pier 40; General water quality
11:15-11:35 (20 min) Wetlab tour
11:35-12:15 (40 min) Presentation
12:15-12:25 (10 min) Idexx pipetting practice
12:25-12:55 (30 min) Idexx
12:55-1:30 (35 min) Lunch
1:30-1:40 (10 min) Hyserve pipetting practice
1:40-2:10 (30 min) Hyserve
2:10-2:40 (30 min) Analyze prepared samples
2:40-3:00 (20 min) Questions, review

Day 2

11:00-11:40 (40 min) Intro to data sources; relationships between CSOs and oxygen, nutrients, etc
11:40-12:40 (60 min) Graphing/data analysis

1. MPN vs Rainfall, 2018: Pier 101, Prospect Park Lake, Gowanus Caroll
2. MPN vs Rainfall, 2017: Coney Island Creek-21st St, Canarsie Pier
3. MPN vs DO, 2017: Dutch Kills Head, Dutch Kills Mouth, English Kills
4. MPN vs. Rainfall, 2018: Peter Sharp Boathouse, W 172nd St, Pier 40, Stuyvesant Cove
5. MPN 2012-2019: Gowanus 2nd, Gowanus Carroll

12:40-1:15 (35 min) Lunch
1:15-1:35 (40 min) Analyze Idexx and Hyserve, discuss results
1:35-2:35 (60 min) Finish graphing, present
2:35-3:00 (25 min) Surveys, wrap-up

Water Quality Resource List

Data Banks

1. **New York City Water Tail Association - Citizens' Water Quality Testing Program**
   Enterococcus data for ~70 NYC sites, 2011-2019
   Recorded daily rainfall at Central Park (NOAA)
   https://www.nycwatertrail.org/water_quality.html

2. **Newtown Creek Alliance**
   Enterococcus, dissolved oxygen, etc at several sites in Newtown Creek, 2016-2018
   Recorded daily rainfall at LGA (NOAA) and Newtown Creek (Weather Underground)
   http://www.newtowncreekalliance.org/water-quality-sampling/

Current and Historical Environmental Conditions

1. **Weather Underground**
   Site-specific precipitation and other weather data
   https://www.wunderground.com/

2. **Hudson River Environmental Conditions Observing System**
   Real time precipitation, dissolved oxygen, etc. at Pier 84 and upriver sites
   https://www.hrecos.org/ >> https://ny.water.usgs.gov/maps/hrecos/

Maps

1. **Open Sewer Atlas**
   Water quality, CSO-shed, and sewershed maps for NYC (2016)
   https://openseweratlas.tumblr.com/maps

2. **HabitatMap - Where does my toilet flush to?**
   Detailed sewershed map (also houses NYS watershed map, NYC garbage map, etc)
   http://habitatmap.org/markers?colors=0_3_1&lat=40.705705&lng=-73.978195&maps=212_213_192&nogrp=1&t=terrain&z=10

3. **OASIS NYC**
   CSO sites, land use, land cover, etc, in NYC
   http://www.oasisnyc.net/map.aspx

4. **Newtown Creek Aerators**
   Sites where NYCDEP have installed mechanical aerators in Newtown Creek
   http://www.newtowncreekalliance.org/wp-content/uploads/2015/01/Screen-shot-2015-02-11-at-11.02.06-AM.png

Background Reading & Water Quality Reports

1. **SWIM (Stormwater Infrastructure Matters) Coalition**
   Factsheets, reports, and advocacy information on NYC stormwater infrastructure
   https://www.swimmablenyc.org/

2. **NYCDEP Harbor Water Quality Report, 2017**
   NYC water quality
   https://www1.nyc.gov/assets/dep/downloads/pdf/water/nyc-waterways/harbor-water-quality-report/2017-new-york-harbor-water-quality-report.pdf

3. **Riverkeeper - How's the Water?, 2017**
   Hudson River Estuary water quality report
   https://www.riverkeeper.org/wp-content/uploads/2017/11/Riverkeeper_WQReport_2017_final-1.pdf

Related News Articles and Other Sources

1. *City's Pumping Air Into Polluted Newtown Creek has Critics Worried*. City Limits, 2018
   https://citylimits.org/2018/08/08/citys-pumping-air-into-polluted-newtown-creek-has-critics-worried/

2. *A Park to Sop Up Pollutants Before They Flow Into the Gowanus Canal*. NYTimes, 2015
   https://www.nytimes.com/2015/12/16/nyregion/sponge-park-in-brooklyn-to-treatpolluted-waters-of-gowanus-canal.html

3. *Beach Haven Apartments Fined $400,000 for Dumping Raw Sewage Into Coney Island Creek*. Bklyner, 2017.
   https://bklyner.com/beach-haven-apartments-fined-400000-for-dumping-raw-sewage-into-coney-island-creek/

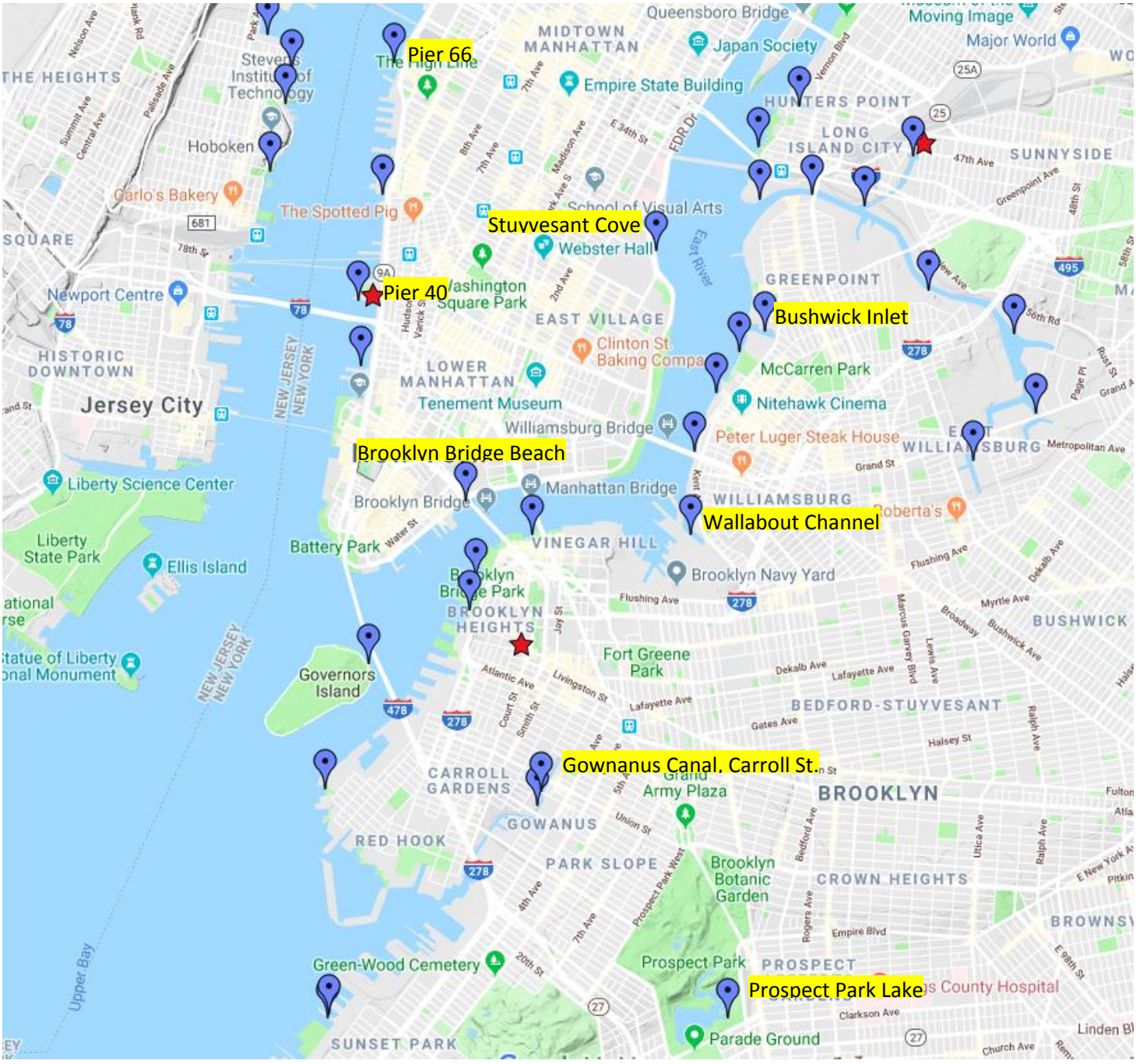
Further Reading

1. **Enterococci as Indicators of Environmental Fecal Contamination**
   Boehm AB, Sassoubre LM. *Enterococci as Indicators of Environmental Fecal Contamination.* 2014 Feb 5. In: Gilmore MS, Clewell DB, Ike Y, et al., editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Inrmary; 2014.
   https://www.ncbi.nlm.nih.gov/pubmed/24649503

2. **Volunteer Water Quality Monitoring - Presenting Bacteria Data Effectively**
   http://blog.uvm.edu/kstepenu/files/2016/11/Bact-Present_XV.pdf

THE RIVER PROJECT

2019 Citizens' Water Quality Testing Program
Partial Site Map



Credit: New York City Water Trail Association

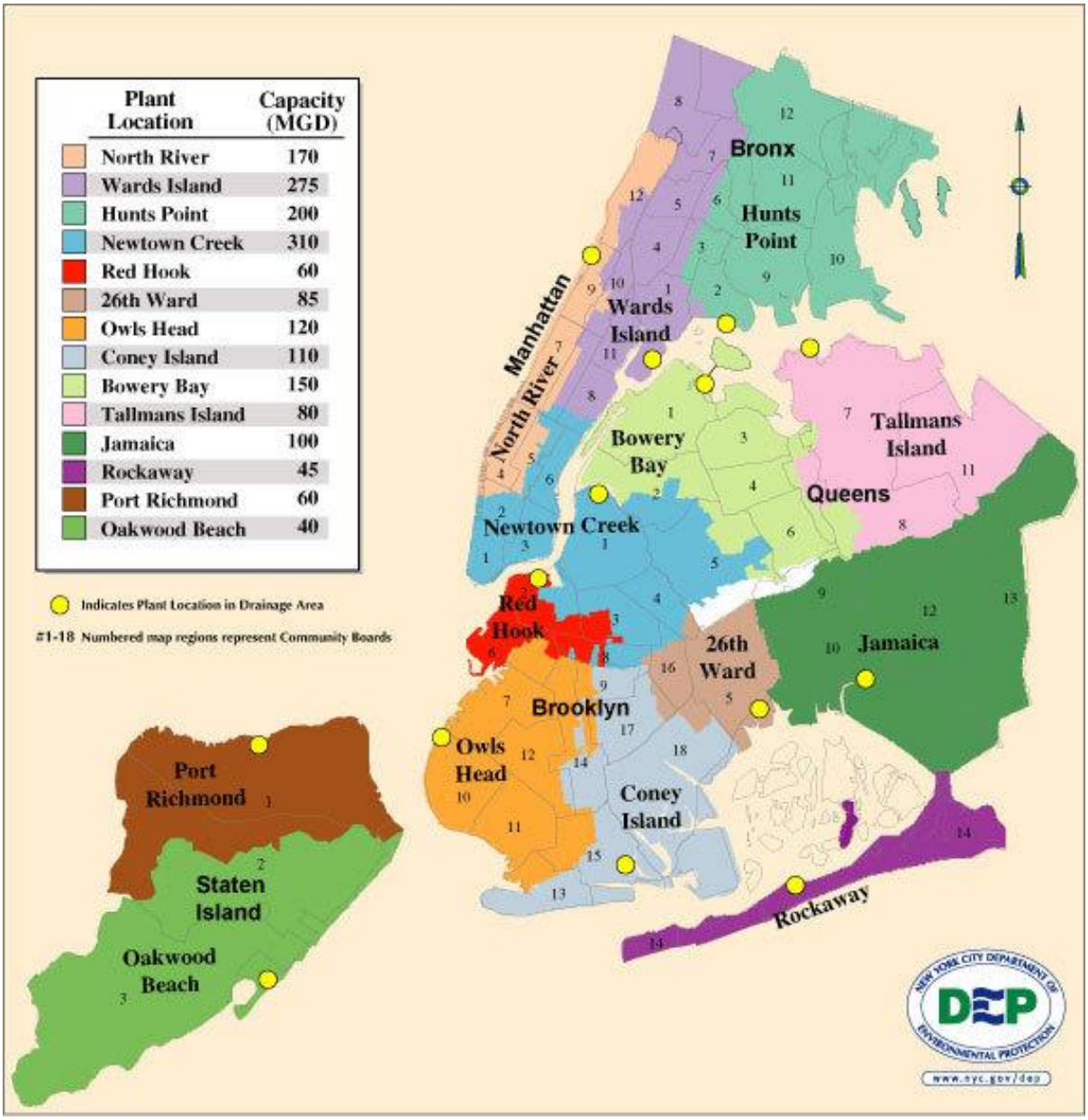
THE RIVER PROJECT

Map of NYC Combined Sewer Outfalls

Credit: Open Sewer Atlas

THE RIVER PROJECT

NYC “Sewershed” Map



Credit: Habitat Maps

THE RIVER PROJECT

Name_____ Date_____

Central Park Rainfall (inches), Source: NOAA

9/6/2019	0.32
9/7/2019	0.02
9/8/2019	0
9/9/2019	Trace
9/10/2019	0.01
9/11/2019	0
9/12/2019	0.17

NYC DOH Enterococcus Standards (for swimming)
Green: <35 MPN--acceptable
Yellow: 35-104 MPN--unacceptable if levels persist
Red: >104 MPN--unacceptable

Sample Date: 9/12/2019

Site	Large Wells	Small Wells	MPN
Pier 66, Hudson River			
Pier 40, Hudson River			
Bushwick Inlet, East River			
Stuyvesant Cove, East River			
Wallabout Channel (BNY), East River			
Brooklyn Bridge Beach, East River			
Gowanus Canal at Carroll St.			
Prospect Park Late			

MPN = most probable number of Enterococci/100mL water

Sources

2019 Citizens' Water Quality Testing, New York City Water Trail Associate
https://www.nycwatertrail.org/water_quality.html

Wet Weather Map (Combined Sewer Outfalls), Open Sewer Atlas
https://openseweratlas.tumblr.com/wetweathermap

Where does my toilet flush to? (Sewershed Map), Habitat Maps
http://habitatmap.org/markers?marker_id=144-newtown-creek-sewage-treatment-facility

What is a CSO?, Akron Waterways Renewed!
http://www.akronwaterwaysrenewed.com/about-us/combined-sewer-overflow-cso-explained.aspx

HyServe Compact Dry ETC Enterococcus Testing Protocol

Precautions and Notes:

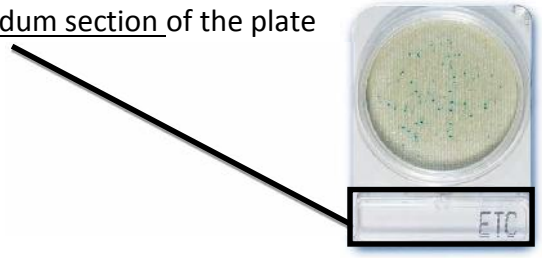
The HyServe Compact Dry ETC count plate contains *Enterococcus* growth media that is light yellow in color. This media is food for *enterococci* to grow into colonies that can be visibly seen so that they can be counted and quantified. While the media itself does not pose a threat to the user, it is **imperative** that one does not touch the media at any time otherwise the test will be compromised.

The units used for bacteria monitoring is MPN/100mL or CFU/100mL and are somewhat interchangeable. MPN stands for "Most Probable Number" and CFU stands for "Colony Forming Units". CFU is literally the number of colonies you see (say on a plate), while MPN is the probability of seeing that number of colonies **if** the same water sample was tested on a plate. In the case of the HyServe Compact Dry ETC Plates, the units are CFU/100mL since plates are used. Idexx does not involve plates and so they use the units MPN/100mL. In general, units should always be noted in any test so that the reader knows what he/she is dealing with.

Since only 1 mL of water sample will be used for the HyServe Compact Dry ETC plates, the number of colonies will need to be multiplied by 100 in order to standardize the units to other types of bacteria tests that use a full 100mL.

Directions:

1. Write the Date, Time and Site Name in the memorandum section of the plate



2. When you are ready to sample, open the disposable plastic pipette and depress the top bulb portion to expel some of the air.

3. Insert the disposable plastic pipette into the sample of river water and very slowly release until the water line reaches the 1 milliliter (mL) mark.

While holding the pipette still slightly depressed with the correct volume (1 mL) at hand, take the pipette completely out of the water and then release it fully. All the water will then accumulate to the top bulb of the pipette.

4. Remove the plastic lid covering the growth media on the HyServe Dry ETC count plate and depress the bulb of the pipette to release the 1mL of water in the center of the plate. The water sample will diffuse automatically. Keep the pipette as you will use the same one later to sterilize the plate before disposal.

5. Put the plastic lid back on top of the plate and wait a minute for the medium to gel up. Then turn the capped plate upside down and incubate the plate at 35 ± 2 °C for 20-24 hours.

6. After 20-24 hours have elapsed, count the number of blue/blue green colored colonies that have formed on the plate:

    a. You should count all colonies formed, regardless of variation in size and shape or even if they are not quite blue/blue green in color. Bacteria other than Enterococcus are inhibited to grow and they do not form any colonies.
    b. Placing a white piece of paper under the plate may help you see the colonies more clearly for counting purposes.
    c. Also, the growth area is 20 cm^2. The back of the plate has a grid carved of 1 cm x 1 cm to make the colony counting easier. In case of any difficulty counting the colonies due to the large number of colonies grown, total viable count can be obtained by multiplying 20 by the number of colonies per 1cm^2 square counted from several squares.
    d. In cases where there are hundreds (100-300) of colonies, you could count the number of colonies using the method “c” above, or the old fashioned way. For values above 300 colonies (i.e. 30,000 CFU/100mL), it is beyond the enumeration capabilities of the plate. We then count that as “TNTC” which stands for “Too Numerous To Count.”

7. Take a close-up picture of a plate (the flower setting on your camera) so that we have a record of the plate and can refer back to it and re-count as needed.

8. When the plate have been counted, take the used pipette and drop 1mL of bleach on the plate. It can now be disposed of properly.

IDEXX Protocol for Enterococcus Testing

1. Incubators are preheated to 41°C and the IDEXX sealer is turned on.
2. Samples are transported to the lab in an opaque bag and stored in refrigerator.
3. Lab technicians should wash hands past wrists and avoid touching anything until inside lab. Wear gloves once inside lab.
4. The supplies for sample preparation are set out:
    a. Sterile pipettes
    b. Sterile mixing vessels
    c. Sterile water
    d. Quanti-trays
    e. Reagent
    f. Data sheet
5. When lab bench is prepared, samples are removed from refrigerator and organized by time from earliest to latest.
6. Mix one reagent packet into 90mL of sterile water by swirling the vessel. Make sure that the powder dissolves completely.
7. Pipette 10mL of the sample into the same mixing vessel, cap, and invert.
8. Open the Quanti-tray carefully and pour the sample into it. Make sure that there are no air bubbles in the small wells.
9. Place the Quanti-tray into the rubber mold and run them through the IDEXX sealer.
10. Label each Quanti-tray with the sample location and time of incubation and then put into the incubator.
11. On data sheet, record date, sample location, time of collection, time of incubation, time of analysis (day 2), number of large and small fluorescing cells (day 2), and Most Probable Number [MPN] (day 2).
12. On day two, 24-26 hours of incubation, at least two technicians count all large and small fluorescing cells, and calculate MPN using the IDEXX Quanti-tray/2000 MPN table and multiplying by ten.

Quality control protocol:

1. Mix one packet reagent into 90mL of sterile water by swirling the vessel. Make sure that the powder dissolves completely.
2. Pipette 10mL of the sterile water into the same mixing vessel, cap, and invert.
3. Follow steps 8 and 9 above.
4. Label the Quanti-tray with “Control”, time of incubation, and then put into incubator.
5. Follow steps 11 and 12 above.

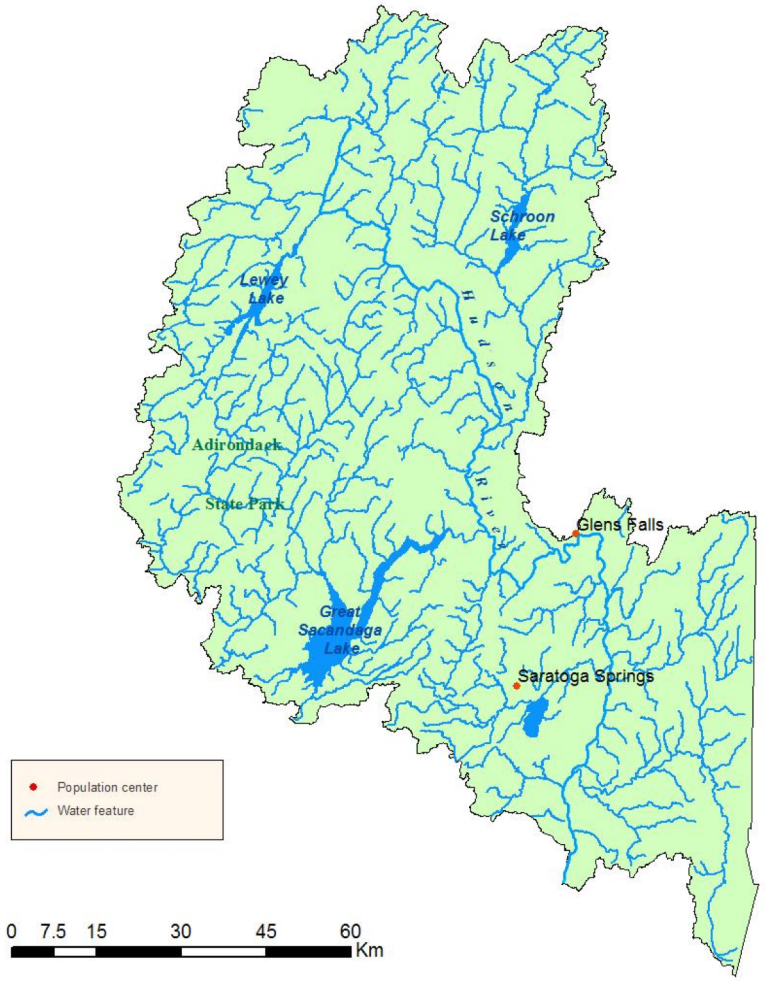
IDEXX Enterolert Data Sheet

Date						
	Time sample		Time	Enterococci Results		
Site	taken	Incubated	Analyzed	Small	Large	MPN

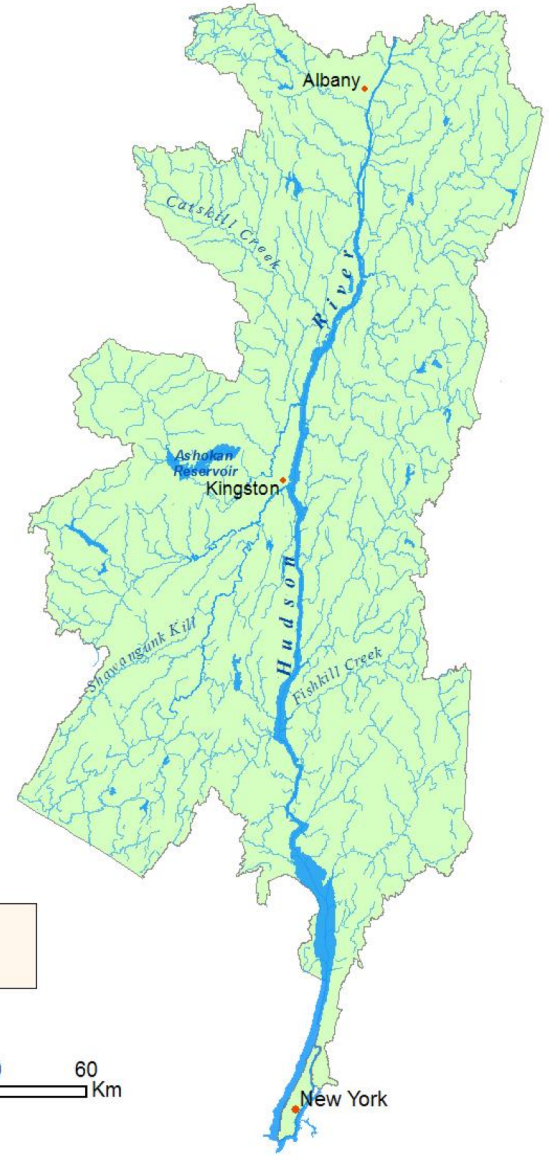
Hyserve Compact Dry Data Sheet

Date				
Site	Time sample taken	Incubated	Time Analyzed	Entero CFU

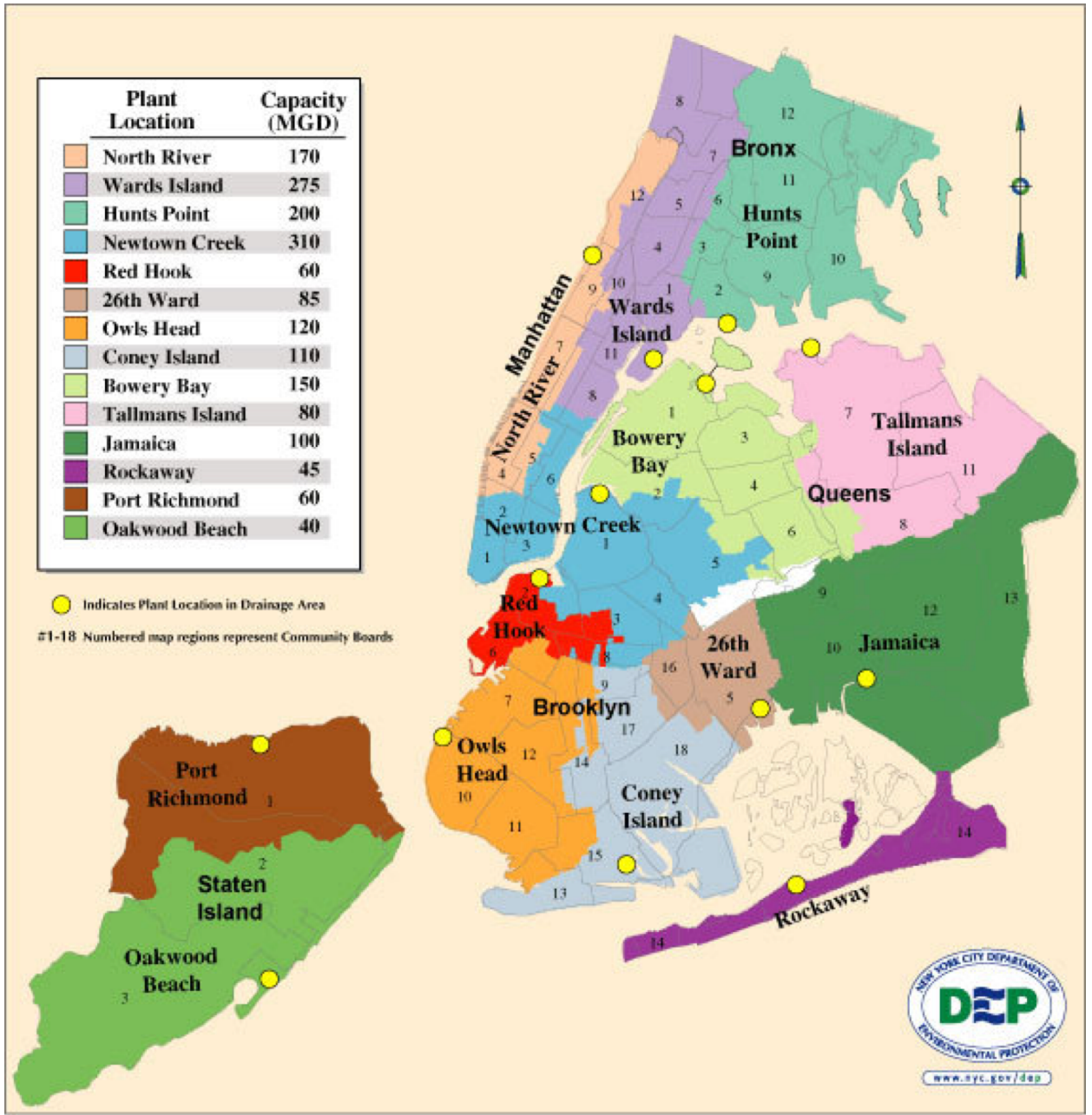
Upper Hudson



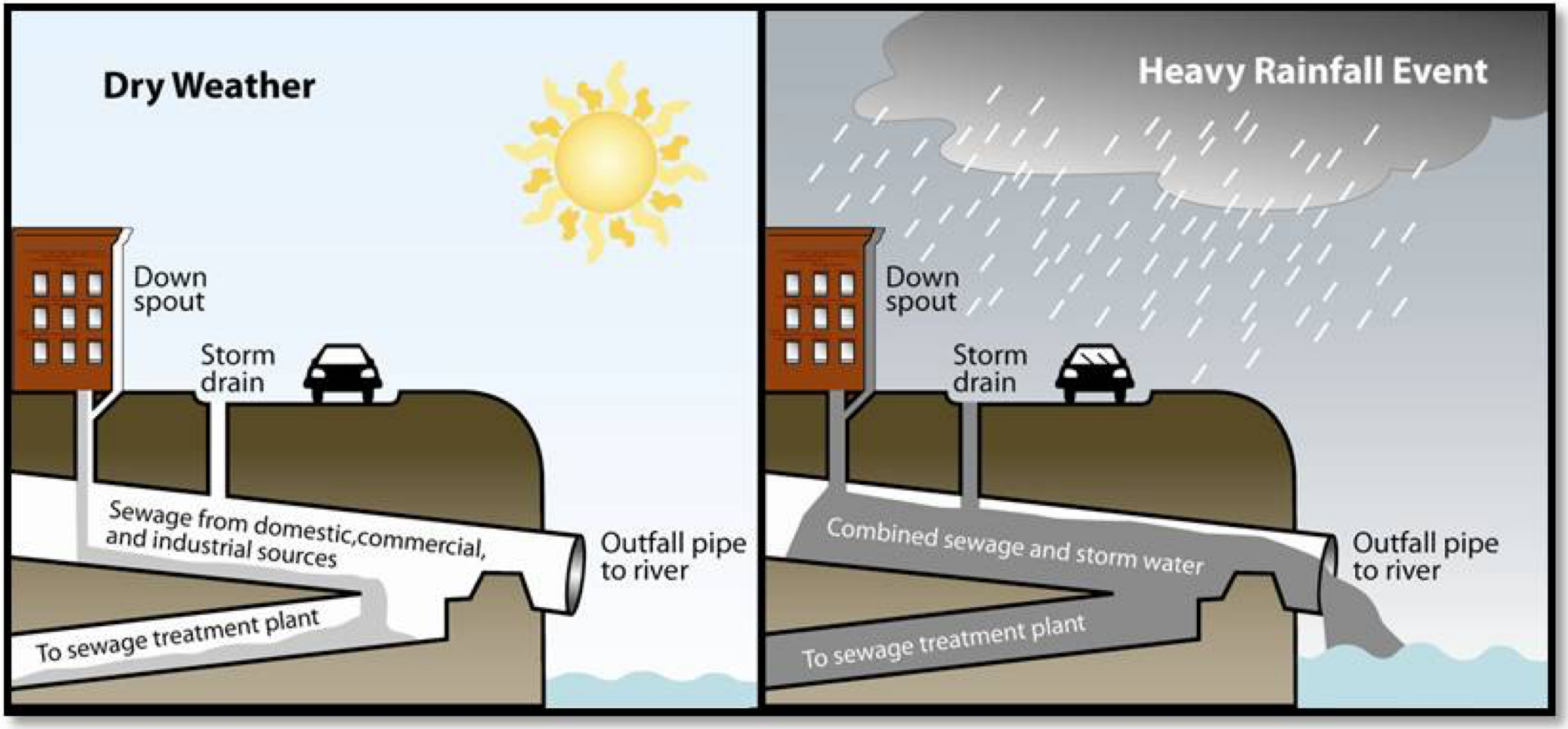
Lower Hudson



NYC “Sewershed”



Combined Sewer System & Combined Sewer Overflows (CSO)



CSO Outfall Map

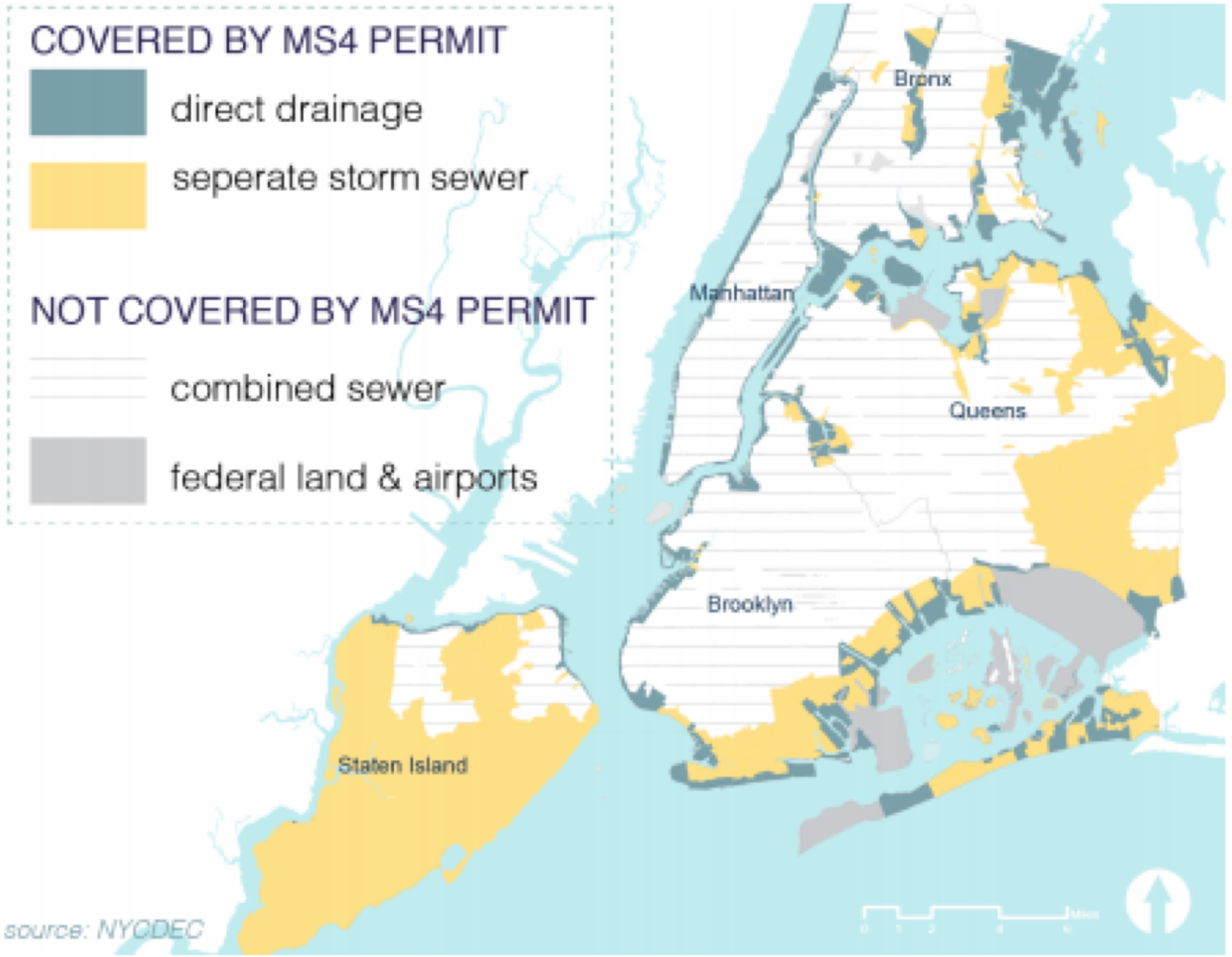
New York City has 450+ CSO outfalls from the Harlem River to the Gowanus Canal

CSOs discharge over 20 billion gallons of polluted water into NYC waterways each year

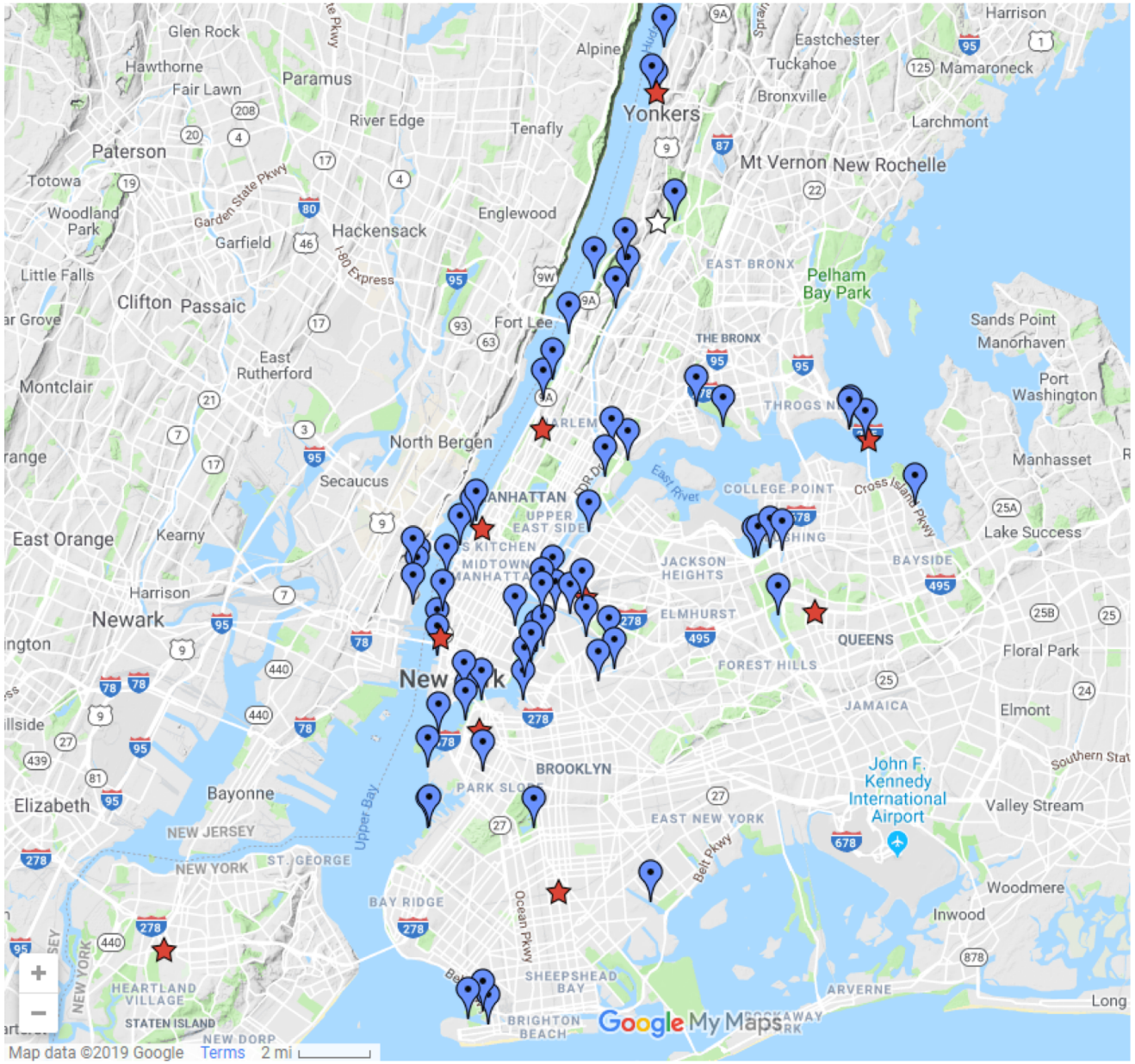
Combined sewer systems make up ~60% of New York City's sewershed

Credit: SWIM Coalition / Open Sewer Atlas

Municipal Separate Storm Sewer System (MS4)



Citizens' Water Quality Testing Program



= Sampling site; includes boat launches and other water access points

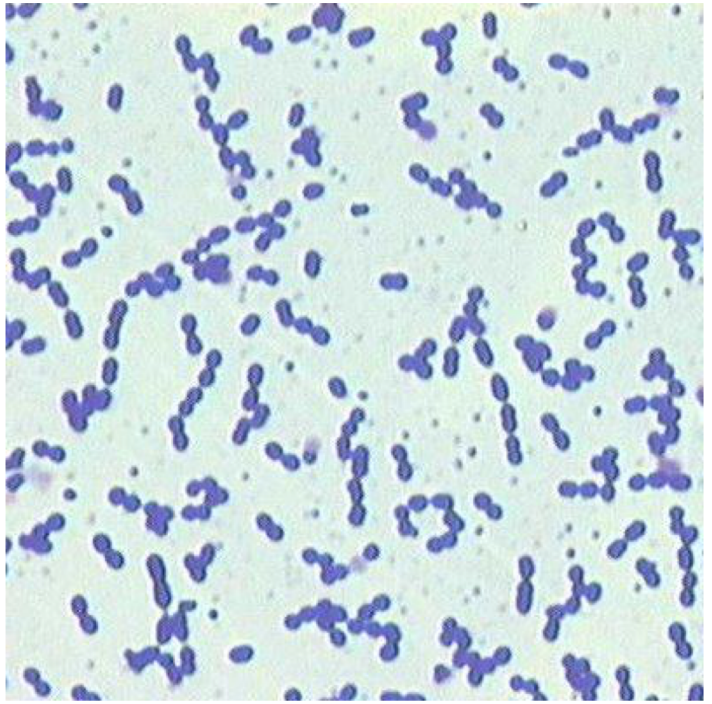
= CWQTP laboratory

Enterococci as fecal indicator bacteria

- Found in fecal matter of warm-blooded animals
- Strong correlation between swimmer illness and enterococci levels (although entero probably don't cause illness)
- Survive in salt water
- Generally not found in environment

Considerations

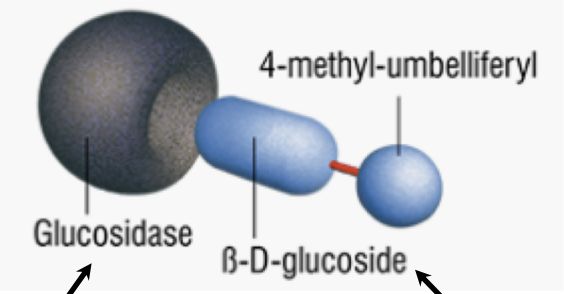
- Killed by UV
- Somewhat associated with particulate matter



Boehm AB, Sassoubre LM. *Enterococci as Indicators of Environmental Fecal Contamination.* 2014

IDEXX Enterolert method for detecting Enterococcus

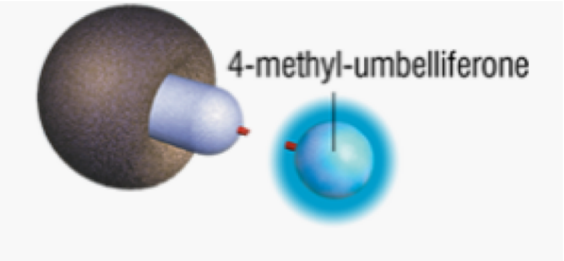
- Results in 24 hours
- Enumerates <10 – 24,190 enterococci per 100 mL brackish water
- EPA-approved method



Enzyme found in enterococci

Reagent: modified carbohydrate

Enzyme cleaves reagent, producing fluorescent molecule

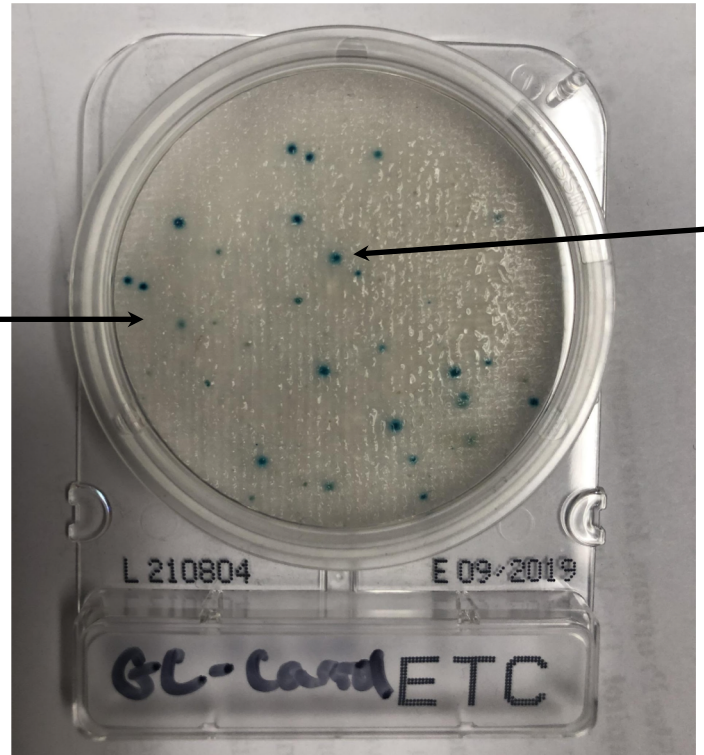


Wells containing enterococci fluoresce

HyServe Compact Dry ETC method

- Results in 24 hours
- Lower equipment cost
- Enumerates 100-30,000 enterococci per 100 ml
- Not EPA-approved

Media contains antibiotics that kill bacteria other than *Enterococcus*



Enterococcus colony.
X-glucoside in media is cleaved by *Enterococcus* enzyme that produces blue product

Aseptic Technique

Sterile work surface

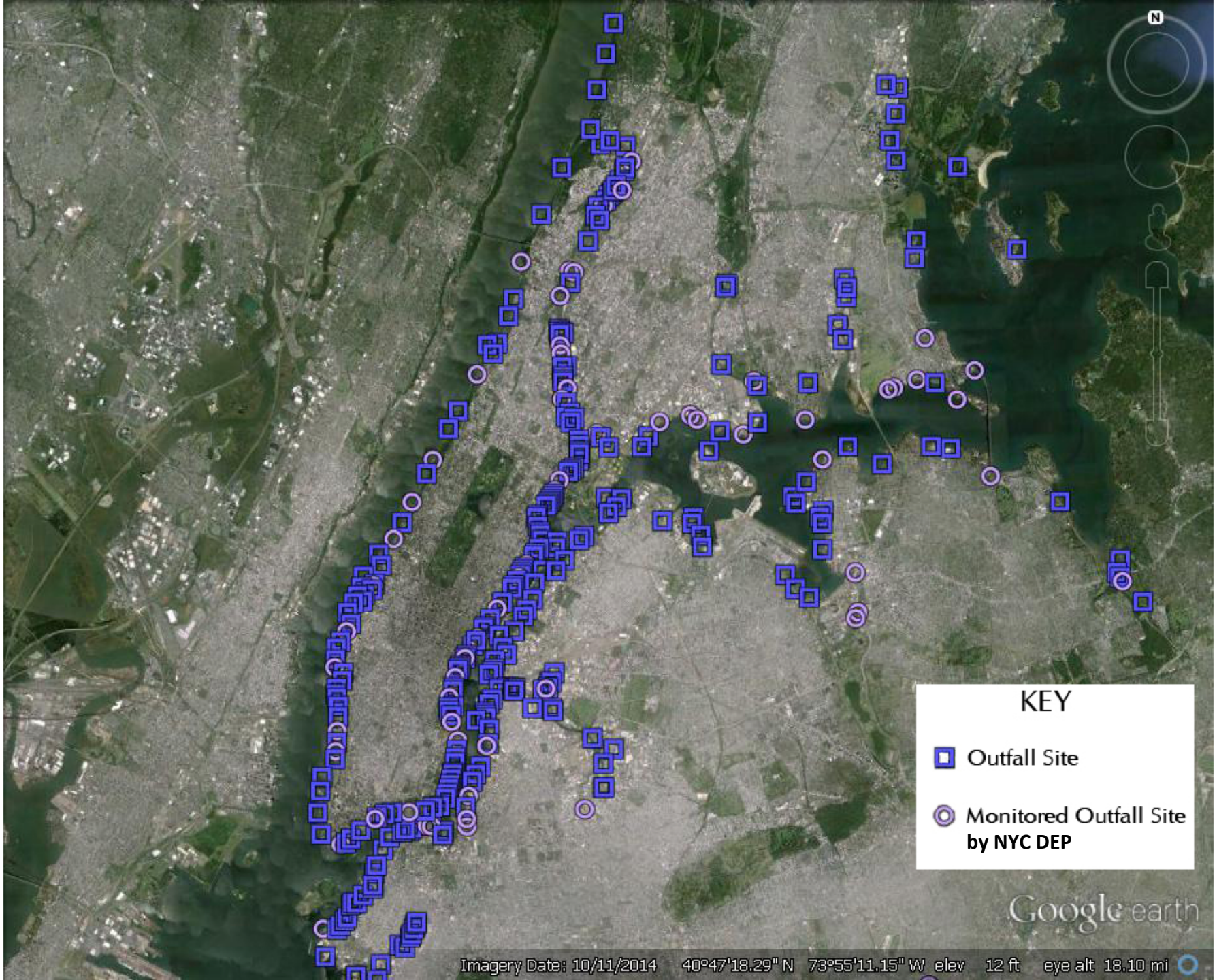
- uncluttered work area
- wipe down work surface with bleach or 70% ethanol

Personal hygiene & protective gear

- wash hands past wrists before/after lab work
- always wear lab gloves while working in lab
- do not touch face, body, unsterile surfaces/items with gloves

Sterile Handling

- only use pipettes once to avoid cross-contamination
- pipette should transfer from one vessel to the next without touching other surfaces
- only uncover a vessel when you are ready to use it and replace cap as soon as possible


KEY
Outfall Site
Monitored Outfall Site
by NYC DEP
Google earth
Imagery Date: 10/11/2014 40°47'18.29" N 73°55'11.15" W elev 12 ft eye alt 18.10 mi

Street	ID	Volume of CSO (gal/year)	Number of CSO events	Rainfall that triggered a CSO event in 2016 (in.)
Vestry	NC-074	4 million	11	0.8
Watts	NC-075	38 million	20	0.57
Charlton	NC-080	6 million	23	0.52
Pier 40	NC-076	130 million	39	0.34
Charles	NC-081	11 million	25	0.51
Bank	NR-019	3 million	22	0.53
Pier 51	NR-020	11 million	22	0.53
Gansevoort South	NR-021	3 million	19	0.57
Gansevoort North	NR-050	0 million	4	1.61
W. 14th	NR-049	7 million	29	0.48
W. 17th	NR-022	6 million	13	0.78
Pier 59	NR-023	18 million	10	0.96
W 22nd	NR-024	8 million	16	0.7
Pier 63	NR-025	8 million	14	0.76
Pier 66	NR-026	13 million	22	0.53
W. 30th	NR-027	82 million	84	0.05
W. 33rd	NR-052	1 million	7	1.42
Pier 76 (NYPD Tow Pound)	NR-028	3 million	7	1.42
W. 40th	NR-029	3 million	13	0.78
W. 42nd	NR-048	4 million	18	0.67
Pier 83 South	NR-053	no data available		
Pier 83 North	NR-030	1 million	6	1.53
Pier 84	NR-031	2 million	10	0.96

WATER QUALITY STANDARDS FOR CITY, STATE AND FEDERAL AGENCIES

AGENCY	Indicator bacteria standard used	Significance of bacteria levels*		
		Safe for swimming	Unsafe if levels persist	Unsafe for swimming
DEC/DEP	Fecal Coliform	<200	200-1,000	>1,000
EPA	Enterococcus	<35	35-104	>104

*Unit: bacteria cells per 100mL of marine water

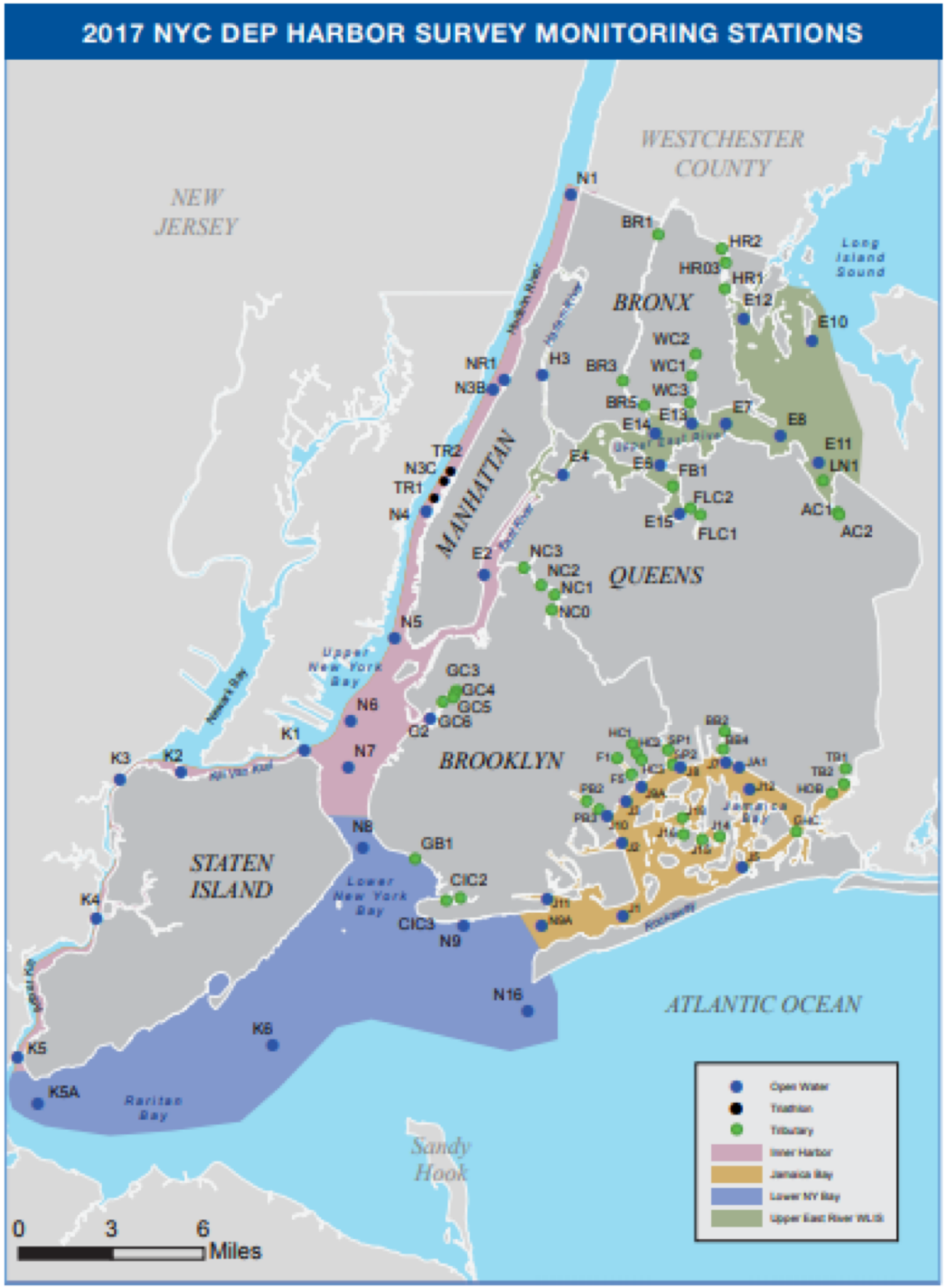
NYC DOH Enterococcus Standards (for swimming)
Green: <35 MPN--acceptable
Yellow: 35-104 MPN--unacceptable if levels persist
Red: >104 MPN--unacceptable


2019 Citizens Water Quality Testing Program
Central Park Rainfall in inches (source:
Sampling Date
Battery High Tide
Sampling Sites (roughly arranged north to south)

COMMON WATER USE AND NYSDEC STANDARDS FOR SALINE WATERS

Class	Best Usage of Waters	Fecal Coliform	Dissolved Oxygen (never-less-than)	Enterococcus
SA	Shellfishing and all other recreational use	No standard	5.0 mg/L	N/A
SB	Bathing and other recreational use	Monthly geometric mean* less than or equal to 200 cells/100 mL from 5 or more samples	5.0 mg/L	(monthly geometric mean) - < 35 Cells / 100mL (single sample) - Max 104 Cells / 100mL
I	Fishing and Boating	Monthly geometric mean less than or equal to 2,000 cells/100 mL from 5 or more samples	4.0 mg/L	N/A
SD	Fish survival	No standard	3.0 mg/L	N/A

*A geometric mean is a weighted average that is less sensitive to outliers than an arithmetic mean



Hudson River & Upper NY Bay

N1	Mt. St. Vincent
NR1	North River WWTP
N3B	West 125th Street
N4	West 42nd Street
N5	Pier A - The Battery
N6	Bell Buoy No. 31
N7	Robbins Reef Buoy No. 28
G2	Gowanus Channel

East River

E2	East 23rd Street

Staten Island

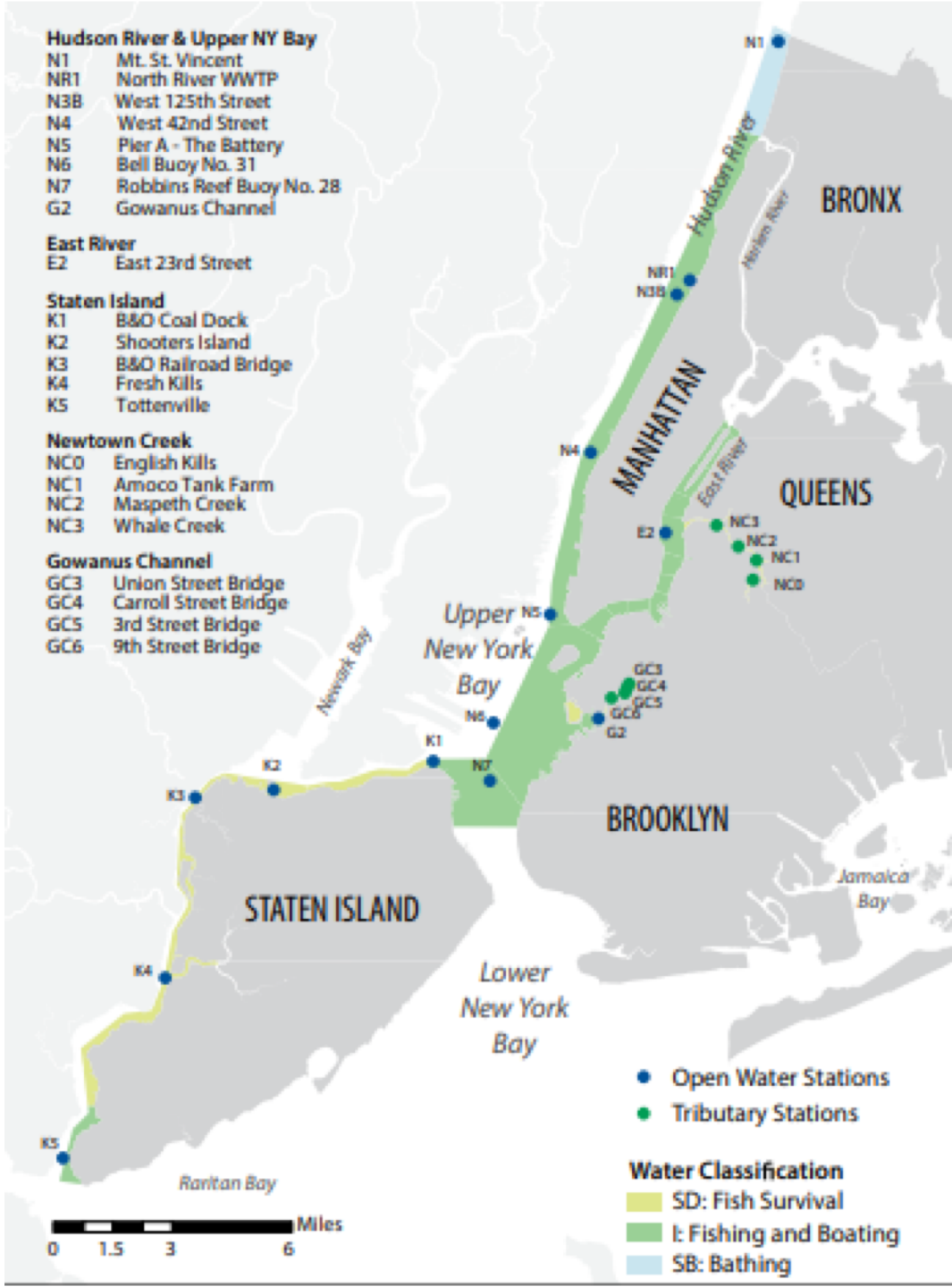
K1	B&O Coal Dock
K2	Shooters Island
K3	B&O Railroad Bridge
K4	Fresh Kills
K5	Tottenville

Newtown Creek

NC0	English Kills
NC1	Amoco Tank Farm
NC2	Maspeth Creek
NC3	Whale Creek

Gowanus Channel

GC3	Union Street Bridge
GC4	Carroll Street Bridge
GC5	3rd Street Bridge
GC6	9th Street Bridge



SUMMER GEOMETRIC MEANS FOR FECAL COLIFORM IN SURFACE WATERS

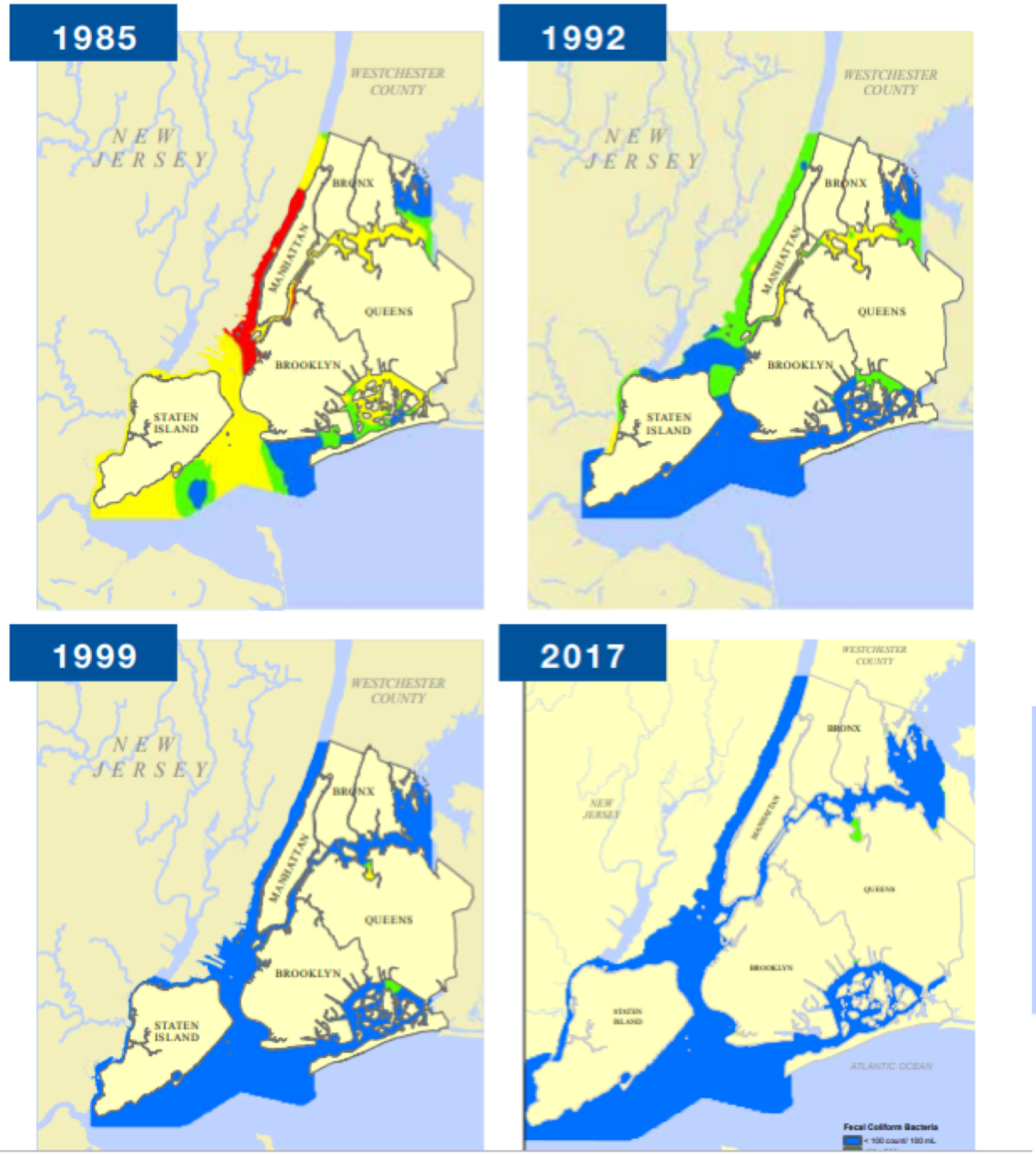
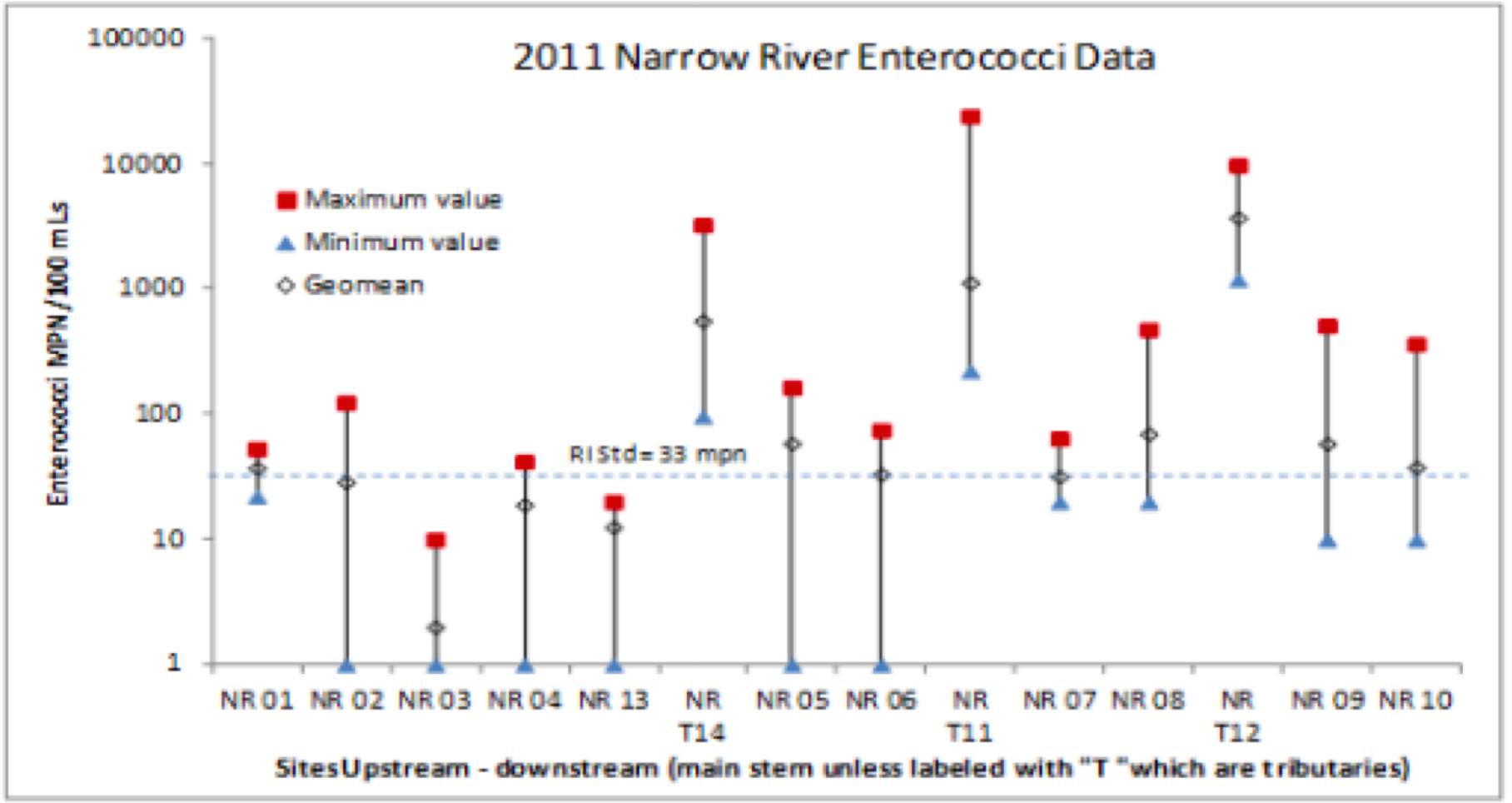
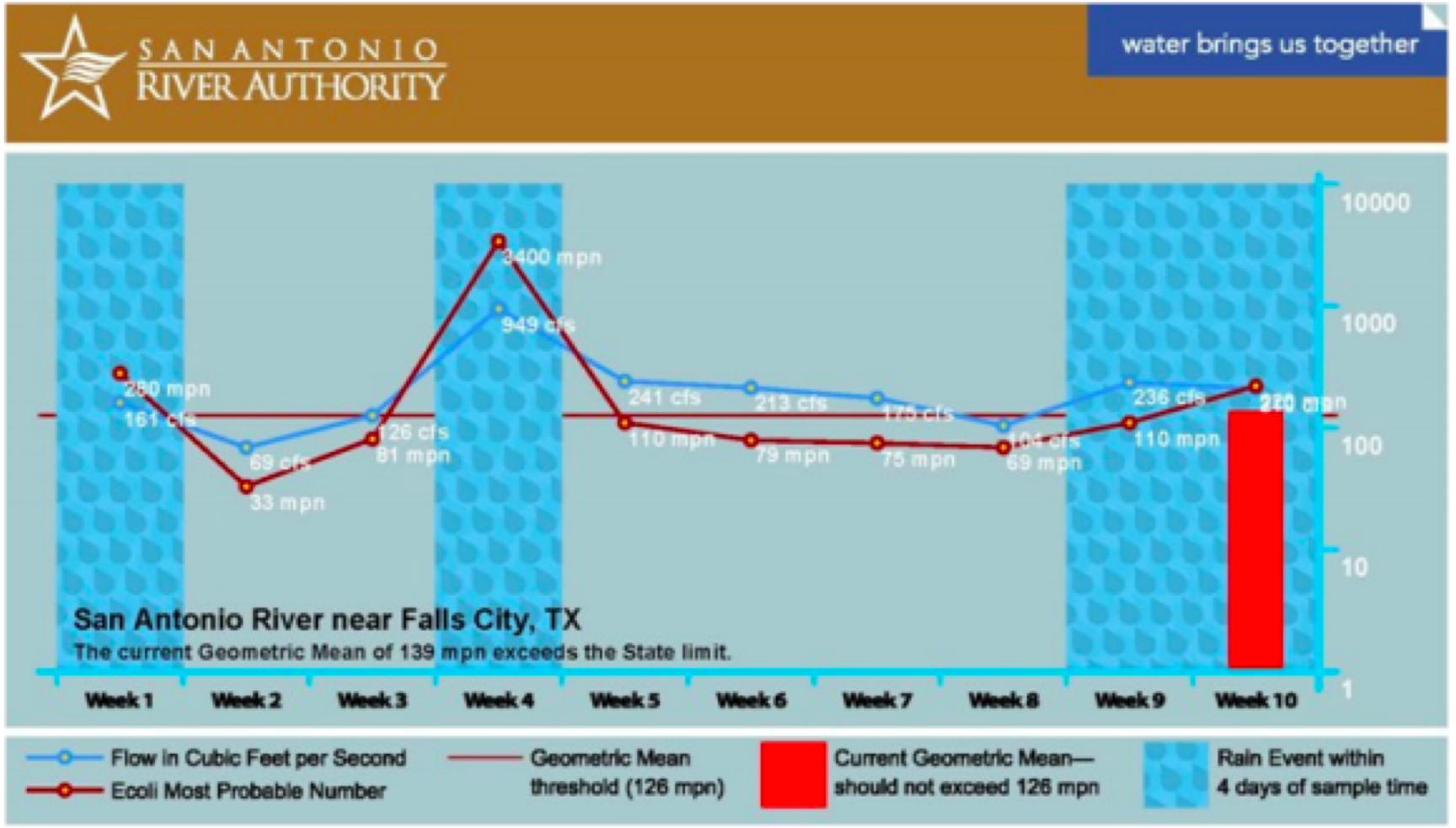


Figure 1. Example of a Stock Chart Modified for Use with Bacteria Data



http://norwalkriver.org/wpcontent/uploads/2014/11/NR-winter-report-Oct-2010-through-April-2011.pdf

http://blog.uvm.edu/kstepenu/files/2016/11/Bact-Present_XV.pdf



San Antonio River Authority: https://www.sara-tx.org/apps/bacteria_charts/ecoli_chart_logrithm.php

http://blog.uvm.edu/kstepenu/files/2016/11/Bact-Present_XV.pdf

Newtown Creek- Metropolitan Ave. Bridge

Enterococcus counts

On 8/6/2019: **2700**

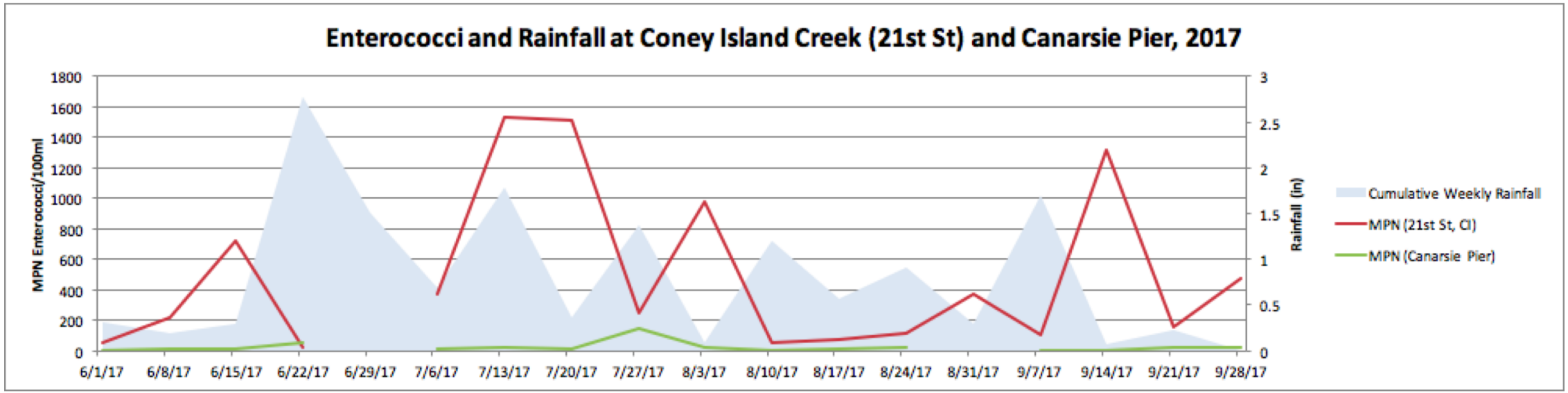
Prior 4 Days Rain: **0.3"**

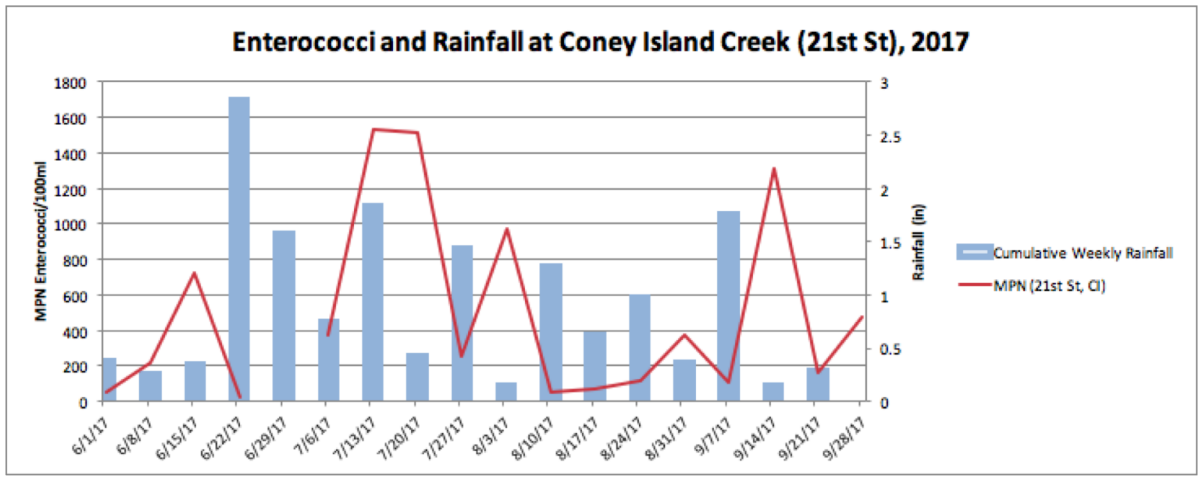
Last 6 Measurements

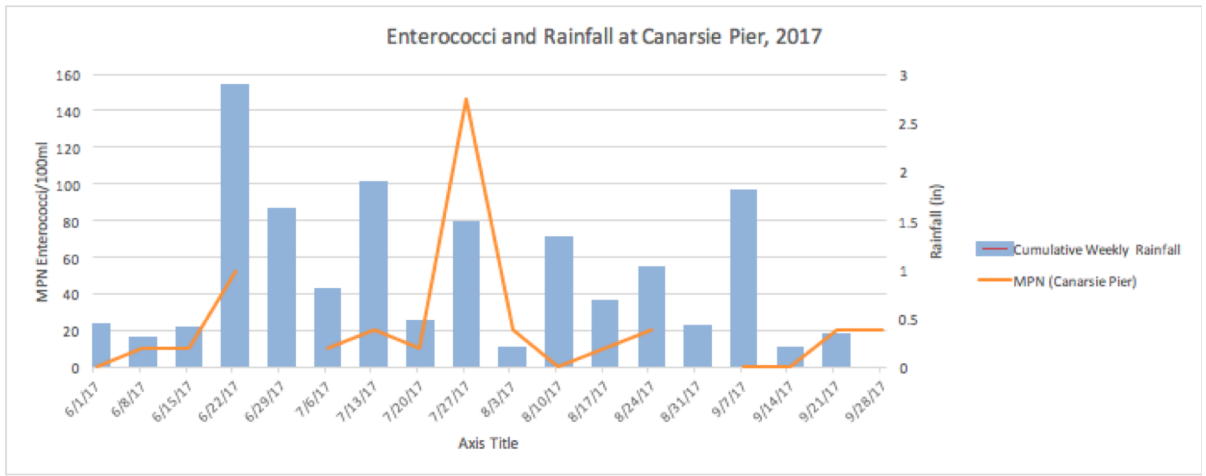
Entero Count:

Rainfall:

https://www.riverkeeper.org/water-quality/hudson-river/nyc-hudson-bergen/newtown-creek-metropolitan-ave-bridge/


Enterococci and Rainfall at Coney Island Creek (21st St) and Canarsie Pier, 2017
MPN Enterococci/100ml
Rainfall (in)
Cumulative Weekly Rainfall
MPN (21st St, CI)
MPN (Canarsie Pier)
6/1/17
6/8/17
6/15/17
6/22/17
6/29/17
7/6/17
7/13/17
7/20/17
7/27/17
8/3/17
8/10/17
8/17/17
8/24/17
8/31/17
9/7/17
9/14/17
9/21/17
9/28/17


Enterococci and Rainfall at Coney Island Creek (21st St), 2017
MPN Enterococci/100ml
Rainfall (in)
Cumulative Weekly Rainfall
MPN (21st St, CI)


Enterococci and Rainfall at Canarsie Pier, 2017
MPN Enterococci/100ml
Rainfall (in)
Cumulative Weekly Rainfall
MPN (Canarsie Pier)
Axis Title