Microbial populations in the Macatawa watershed

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Hope COLLEGE

NSF

What's in our water? And is it safe?

The water research group at Hope College:

- Dr. Aaron Best
- Dr. Brent Krueger
- Dr. Jon Peterson
- Dr. Michael Pikaart
- Sarah Brokus
- Randy Wade
- Adam Slater

…and lots of Hope students including Day 1 Watershed first-year seminar/laboratory since 2014

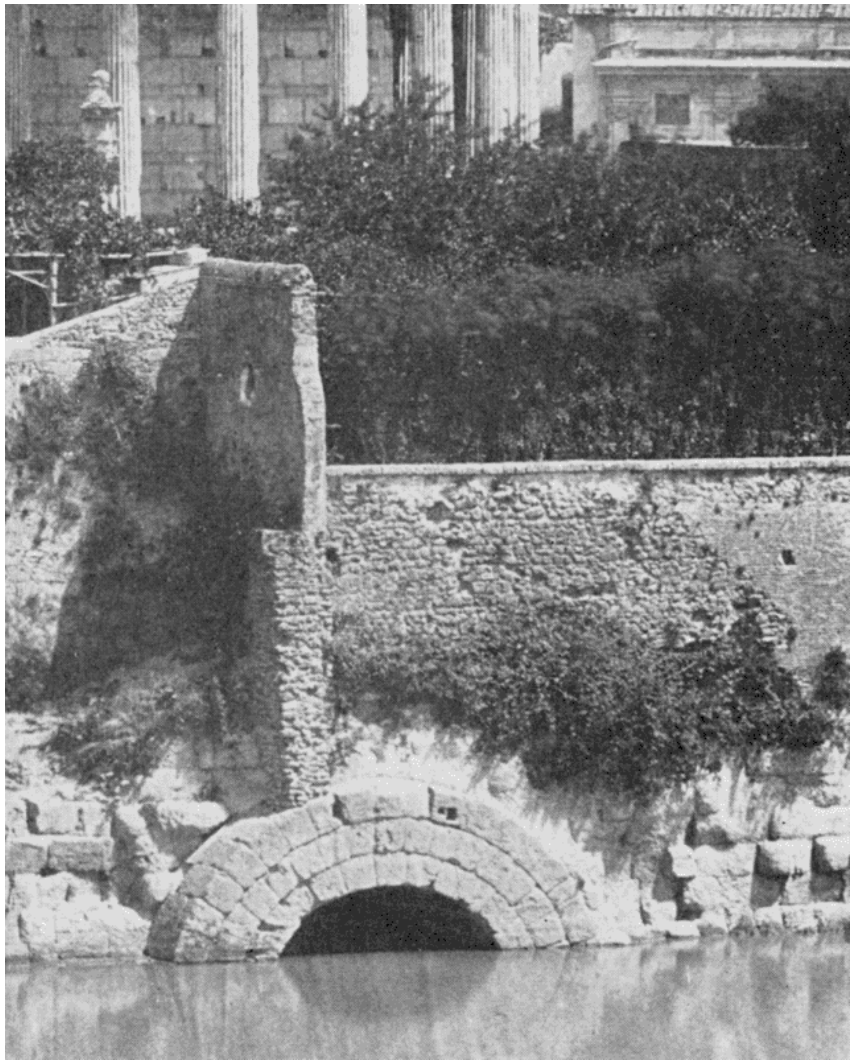
Support from

- National Science Foundation
- Dow Foundation

In in cooperation with

- Outdoor Discovery Center/Project Clarity
- Macatawa Area Coordinating Council

Best public health invention ever: separation of sewage from drinking water.



Dr. John Snow – first modern epidemiology investigation; tracked London's 1854 cholera outbreak to a contaminated pump.

Modern sanitation removes risk of waterborne pathogens:

- Sterilization of water supply
- Hygiene
- Sanitary sewers
- Sewage treatment prior to discharge
- Testing and monitoring

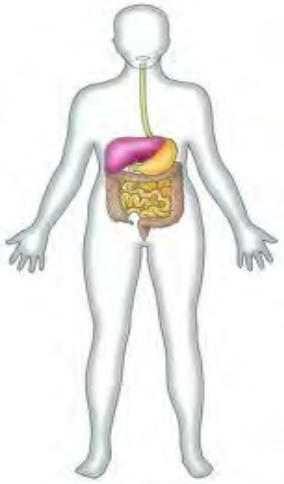
Microbiological culturing techniques going back to 1920's led to "Total coliform" concept.

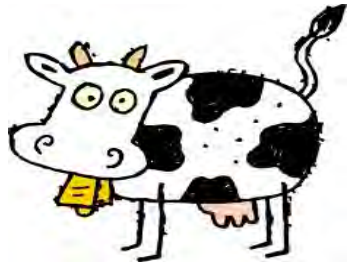
Established in law with the Safe Drinking Water Act of 1974

Some microbiology terms:

Total coliform: Rod-shaped, Gram-negative, lactose-fermenting, acid-producing.

Fecal coliform: Rod-shaped, Gram-negative, lactose-fermenting, acid-producing AND grow at 44°C.





E. coli: A particular genus/species found in normal gut microorganisms.

EC O157H7, O104H4

Enterococci: A class of related organisms found in normal gut.

The trouble is, coliforms (except the few E. coli strains that are pathogenic) do not actually make you sick!

Some of the real bad actors include…

Protozoans like:

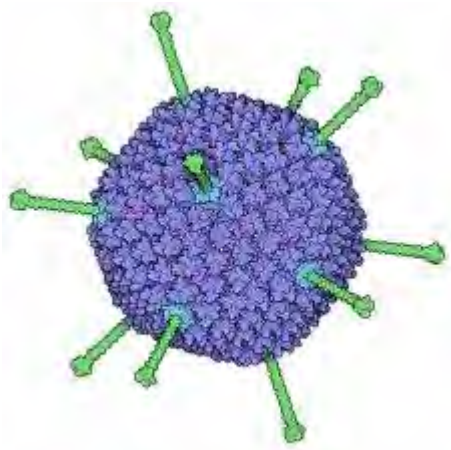
- Entamoeba
- Cryptosporidium
- Giardia

Bad bacteria like:

- C. botulinum
- Campylobacter
- V. cholerae
- Shigella
- Salmonella

Viruses like:

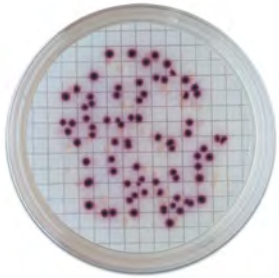
- Adeno, parvo, corona
- Hepatitis A
- Polio



So why do we bother with coliform (or *E. coli*, entero) “counts”?

- Because we can, using classical microbiology culture.

Plate assays give us colony-forming units (cfu) per 100mL of water sample.



“Colisure” tray cultures give us essentially the same thing (technically a “most-probable number” of cells per 100mL)

These are “Fecal Indicator Bacteria.” These numbers are tracked at Lake Michigan beaches (once a week, in summers); Dunton Park on Lake Macatawa until 2014

Lake Macatawa - Dunton Park

There is no monitoring information for Dunton Park this year.

Beach Detail | Closures and Advisories | Sample Results | Monitoring | Surveys

All historical sampling results are shown below.
Results for the current year are available here.

Sample Year	Sample Date	Sample Type	Analysis Method	Result Value
2014				
	08/14/2014 12:00 PM	Daily Mean	Colilert-18 hour	320 *
	07/30/2014 10:00 AM	Daily Mean	Colilert-18 hour	15.5
	07/24/2014 11:30 AM	Daily Mean	Colilert-18 hour	125
	07/16/2014 10:30 AM	Daily Mean	Colilert-18 hour	7.8
	07/09/2014 8:40 AM	Daily Mean	Colilert-18 hour	4.8
	07/02/2014 10:20 AM	Daily Mean	Colilert-18 hour	13
	06/26/2014 11:15 AM	Daily Mean	Colilert-18 hour	16.2
	06/19/2014 1:15 PM	Daily Mean	Colilert-18 hour	165
	06/10/2014 11:00 AM	Daily Mean	Colilert-18 hour	9.2
	06/05/2014 9:00 AM	Daily Mean	Colilert-18 hour	8

Lake Michigan - Holland State Park

Holland State Park is open.

Holland State Park is monitored by Ottawa County Health Department this year.

Sample Date	Sample Type	Analysis Method	Result Value
8/8/2019	Daily Mean	Colilert-18 hour	334.267
8/1/2019	Daily Mean	Colilert-18 hour	3.7075
7/25/2019	Daily Mean	Colilert-18 hour	13.5272
7/18/2019	Daily Mean	Colilert-18 hour	19.6516
7/11/2019	Daily Mean	Colilert-18 hour	243.724
7/2/2019	Daily Mean	Colilert-18 hour	9.8854
6/27/2019	Daily Mean	Colilert-18 hour	18.5189

Why not close beaches for >300 cfu/100mL any more?

These counts tell you the E. coli load **yesterday**

Current public health practices suggest beach closings based on E. coli testing have little effect on health risk

→ These are natural waters; we don't expect them to be sterile (in contrast – drinking water; public swimming pools)

Possible sources of historically high FIB counts at Dunton Park, Lake Macatawa:

Point source –

- Ineffective municipal treatment
- Illicit discharge
- Bad septic tank nearby
- Sewer overflow

Non-point source –

- Upstream septic tank or sewerage leakage
- Agricultural (either animal facility or manure spread on fields)
- Wildlife
- Persistent indigenous growth in environment

Figuring out the origin of microbes in the environment = **source tracking**

Our previous work on source tracking was based on single-marker PCR detection. This proved not sensitive enough to be reliable,

The Macatawa Watershed

Began longitudinal sampling protocol Sept 2016 at 12 sampling sites.

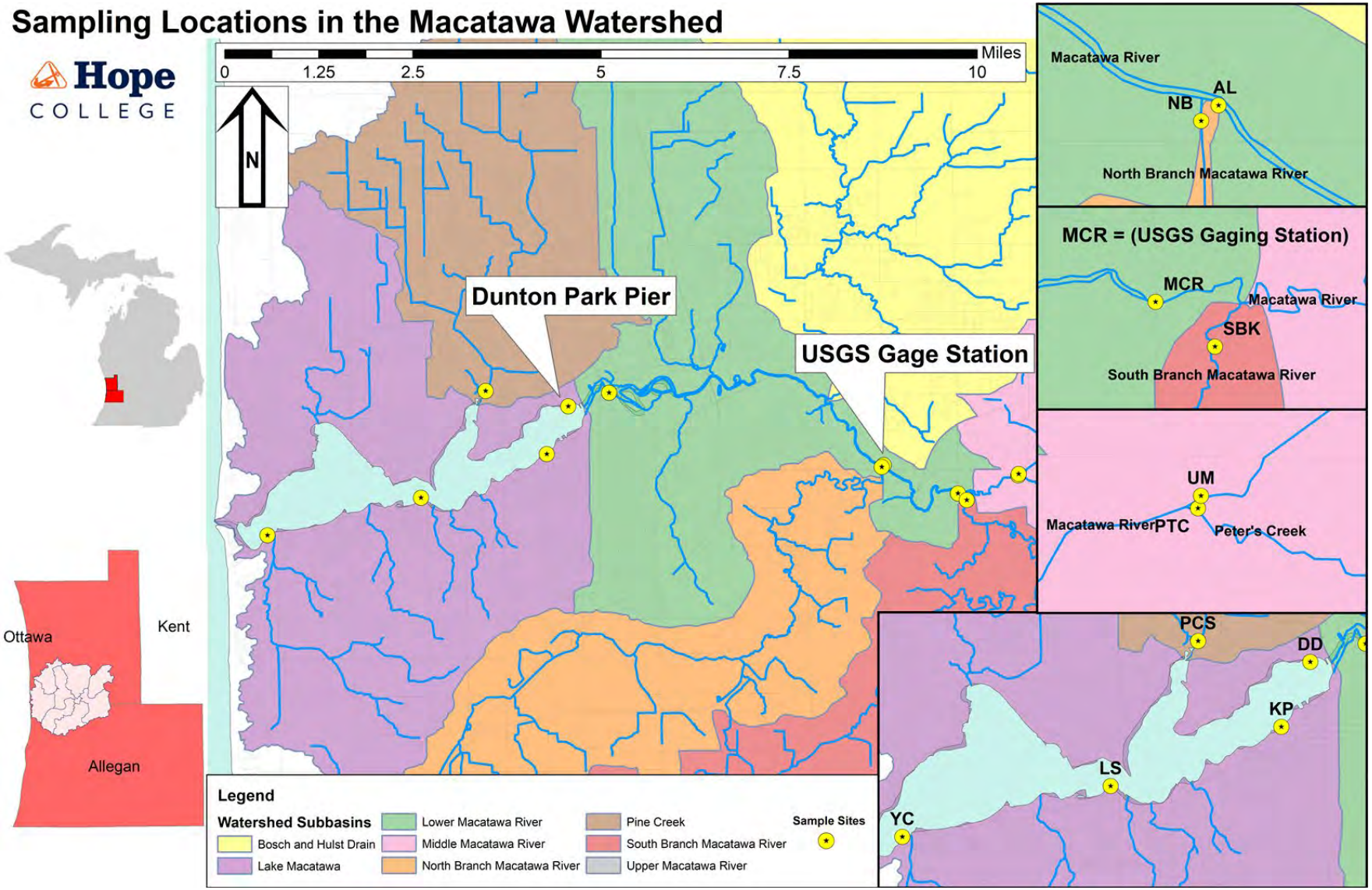
4 lake – MacBay yacht club, Lake ave (old firedock), Kollen Park, Dunton Park.

6 stream – Main branch at Adam's Landing; North branch; South branch; Peter's creek; Upper Mac; Pine creek.

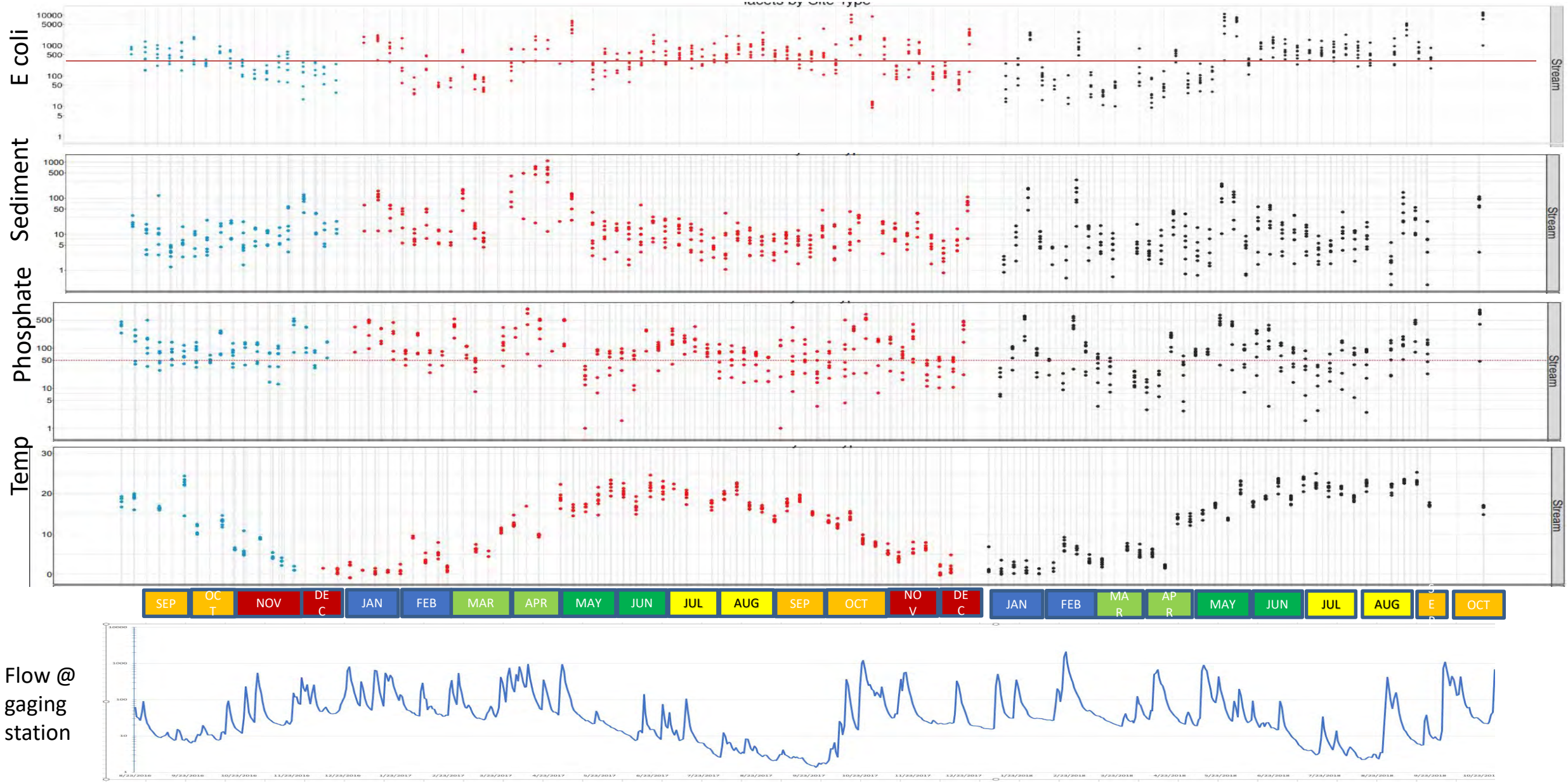
Window on the Waterfront was initially considered a stream site but it is ambiguous.

Measurements taken:

- E coli mTEC (cfu/100mL)
- Sediment (TSS; mg/L)
- Phosphate (ppb)
- Nitrate (ppm)
- Water temperature (°C)
- pH; dissolved O_2
- Flow at USGS station

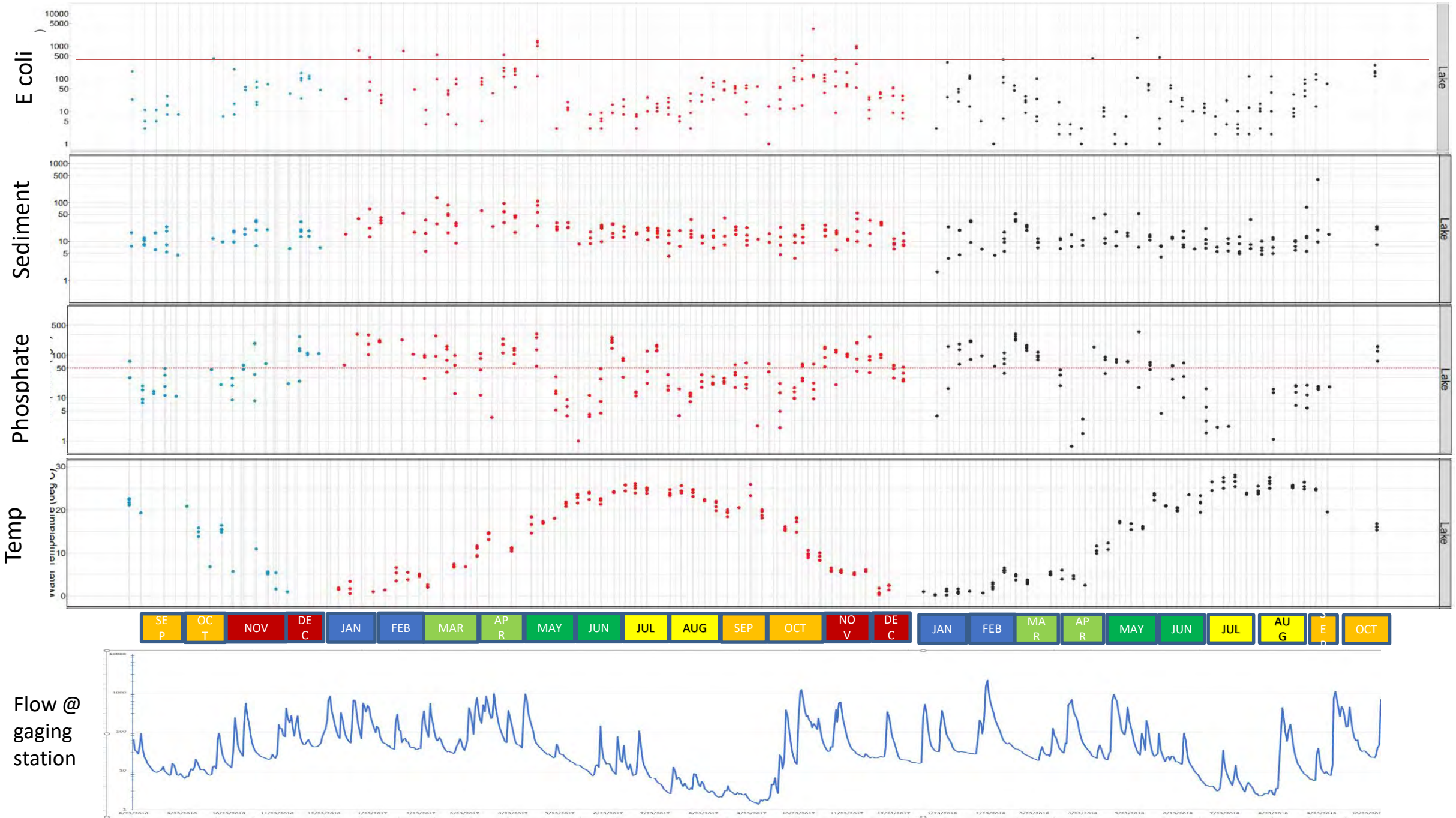


Snapshot of combined results – Stream sites



Flow @ gaging station

Snapshot of combined results – Lake sites



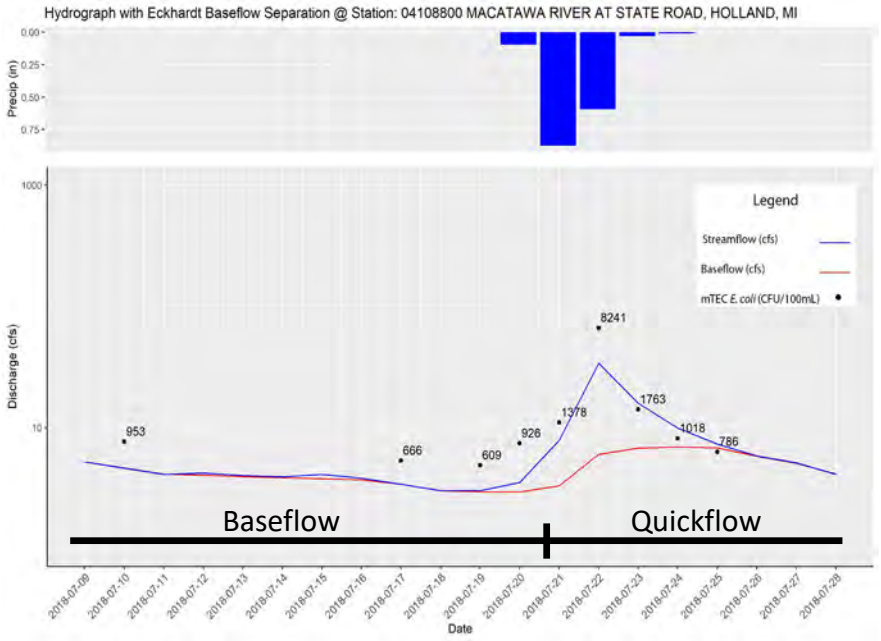
Current focus:

- Experimentally distinguish base versus quick flow
- Model populations as endogenous (represented by base flow population) versus transient (quick flow)
- Once these are defined, compare to populations found at representative source sites and/or types
    - Human, nonhuman mammal, bird; agricultural, residential, industrial; environmental, standard cultures

Evidence of Environmental Adaptation?
Different Populations between Baseflow and Quickflow Conditions?

Mixed transient and endemic populations exist

Populations isolated during long running baseflow conditions represent predominantly endemic Escherichia, whereas isolates from quickflow conditions represent populations with increased levels of transient Escherichia.



Sample Collection and Data Processing

- Sampling as part of course-based undergraduate research experience (CURE)
- Standard EPA Method 1603 – water filtration for isolating *E. coli* on mTEC and obtaining individual *E. coli* strains
- Base-Quick samples - Daily sampling at two sites (stream and lake) for a seven day period; Triplicate sampling at each site, mTEC counts; Obtain 30 isolates from each site (total of 60 isolates per day)
- NexteraXT library preparation, Illumina MiSeq v2 2x250 paired end sequencing
- Comparative Genomics - Assembly via aRAST Spades pipeline, Annotation via RASTtk and PATRIC