Addressing our Health with Environmental Surveillance from SARS in Wastewater to *E. coli* on our Beaches:

The Impact of The Michigan Network for Environmental Technology

MICHIGAN STATE UNIVERSITY

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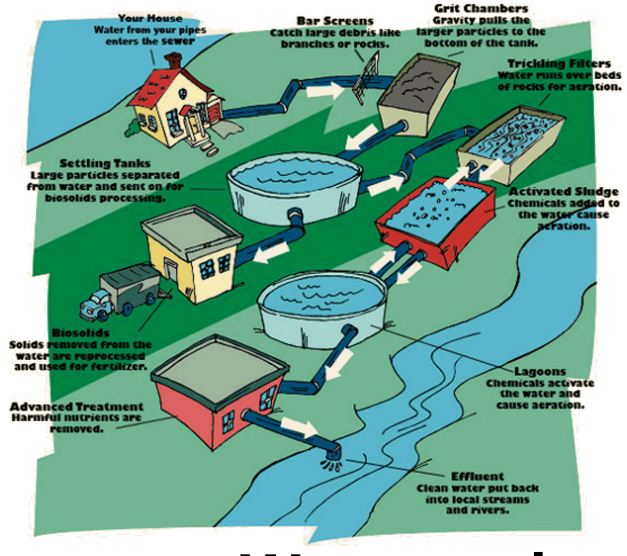
Critical Infrastructure Sectors

https://www.dhs.gov/critical-infrastructure-sectors

Health Care and Public Health

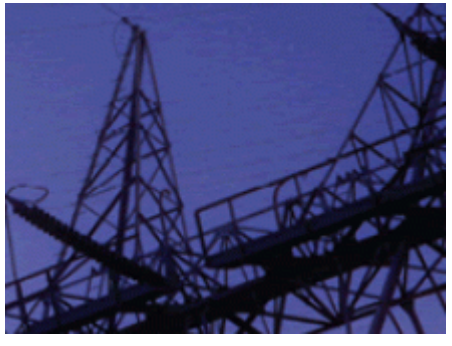
There are 16 critical infrastructure sectors whose assets, systems, and networks, whether physical or virtual, are considered so vital to the United States that their incapacitation or destruction would have a debilitating effect on security, national economic security, national public health or safety, or any combination thereof.



Water and Wastewater

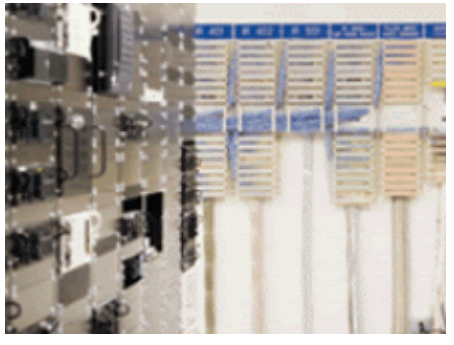
Transportation

Energy



Food and Agriculture

Information Technology



Fecal contamination of water remains one of the largest threats to the biological safety of water today.

FRESH WATER RESOURCES ARE DEGRADING

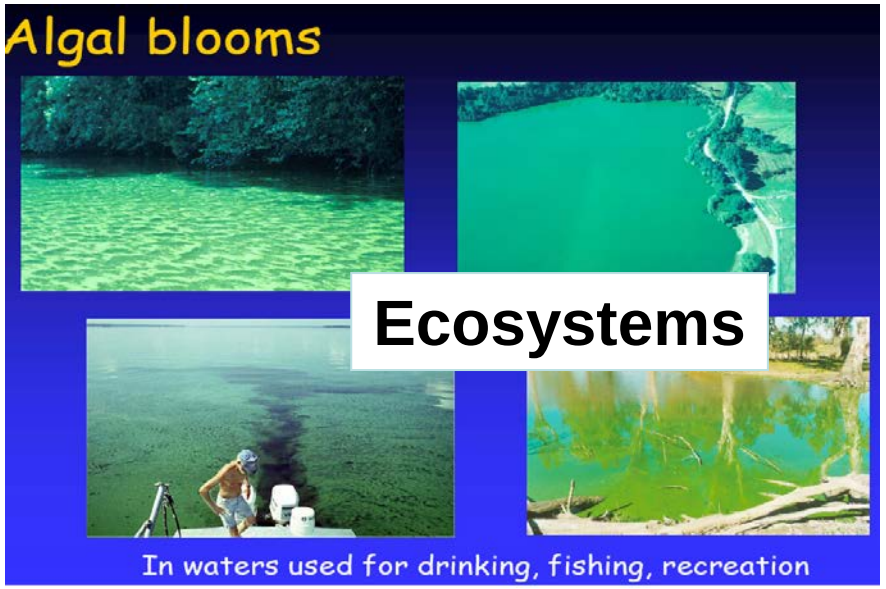
Recreational

Irrigation

By Todd C. Frankel August 11 2014
Follow @tcfrankel The Washington Post

A sample glass of Lake Erie water is photographed near the Toledo water intake crib in Lake Erie. (Haraz N. Ghanbari/Associated Press)

Drinking



Ecosystems

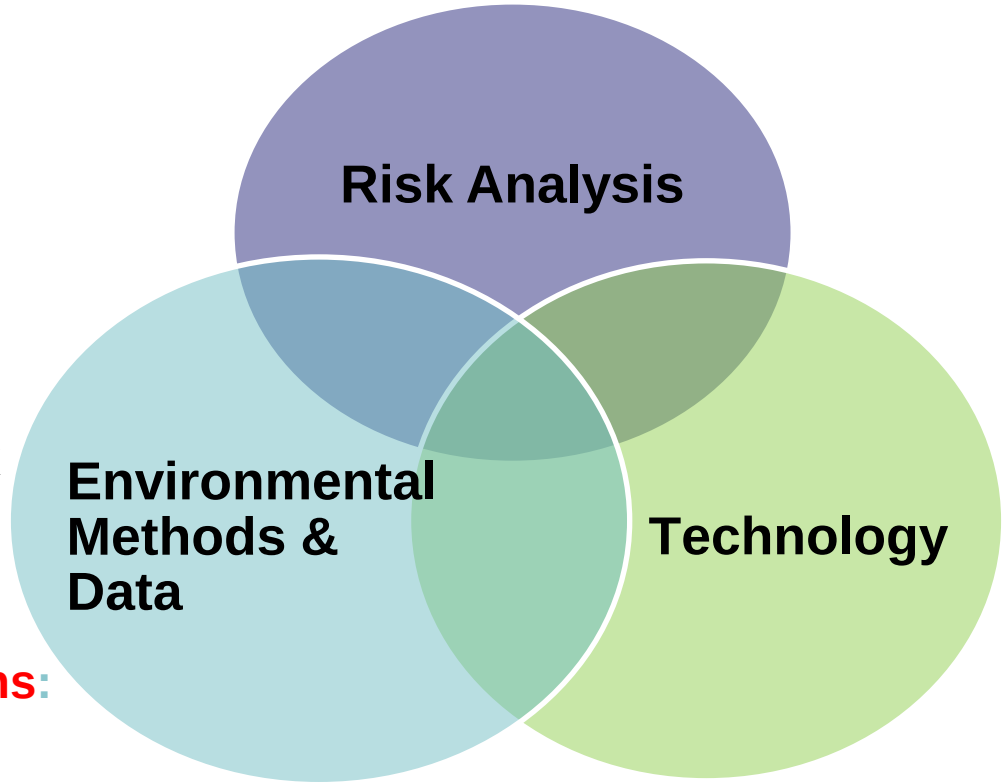
Advances impacting water quality science & health

Science-based & data-driven approaches:

- Support quantitative microbial risk assessment, risk communication and risk management

Emerging methods & pathogens:

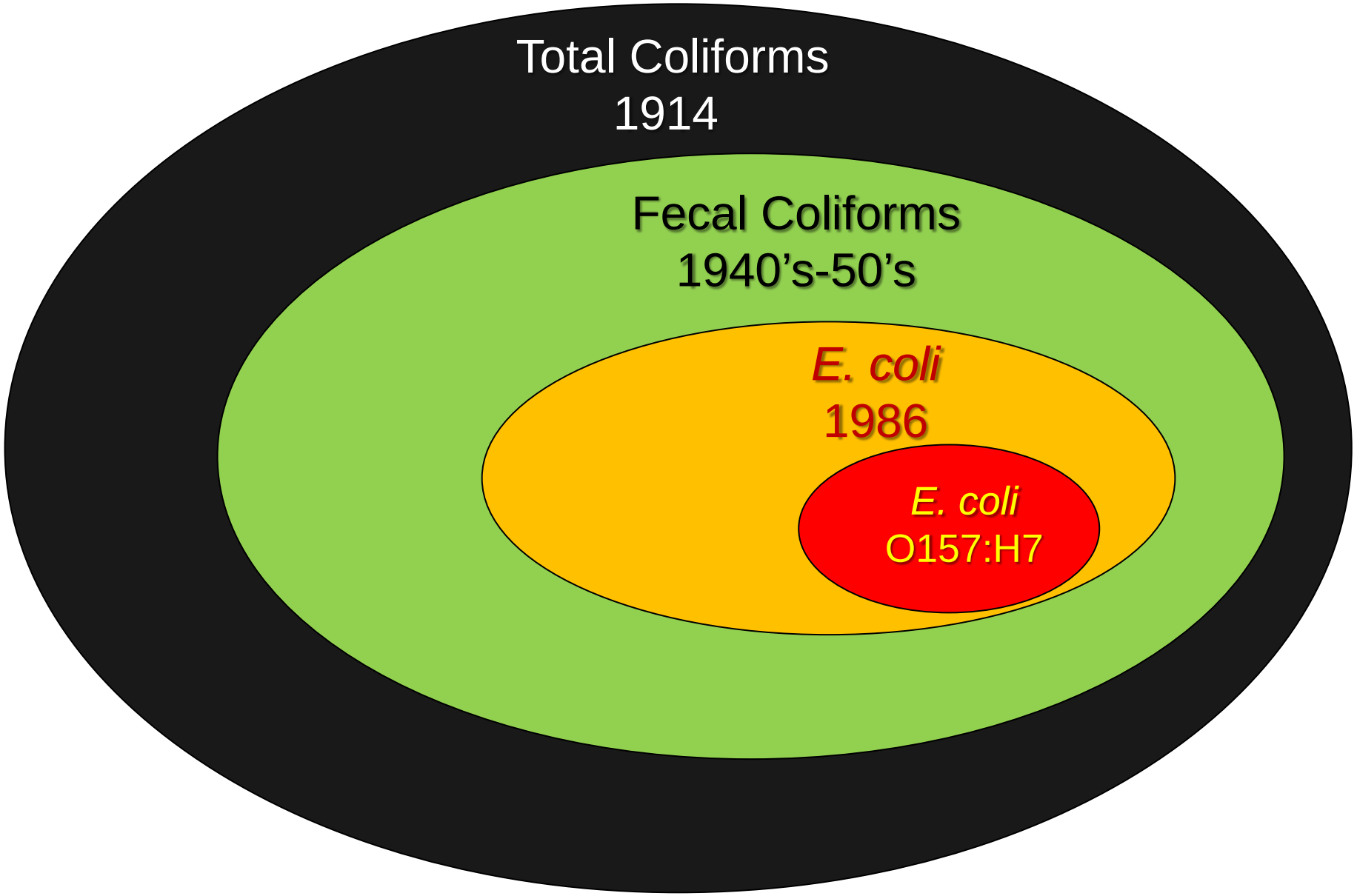
- New molecular methods advance understanding of emerging pathogens
- Now will have data on occurrence in sewage



Green and Blue Economy

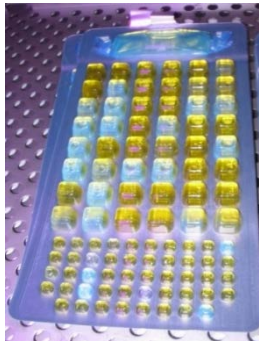
Wastewater Resource Recovery - $37 Billion
Wastewater Treatment - $70.8 Billion
Overall Market (products/services) - $770B

* Pittsburgh's H2Opportunity Report


Total Coliforms
1914
Fecal Coliforms
1940's-50's
E. coli
1986
E. coli
O157:H7

Growth Based Methods: Common Fecal Indicator Organisms for measuring water quality

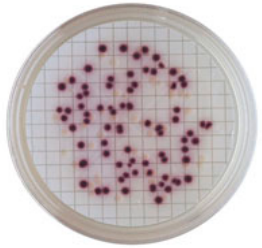
Filtering 100 ml water samples



Total coliforms

E.coli

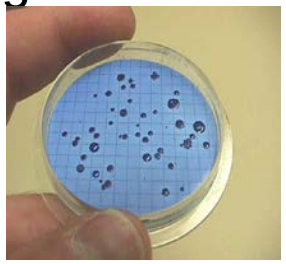
MPN



MPN and colonies

Fecal coliforms

Agar and colonies



Sources of *E.coli* and Pathogens

Septic systems

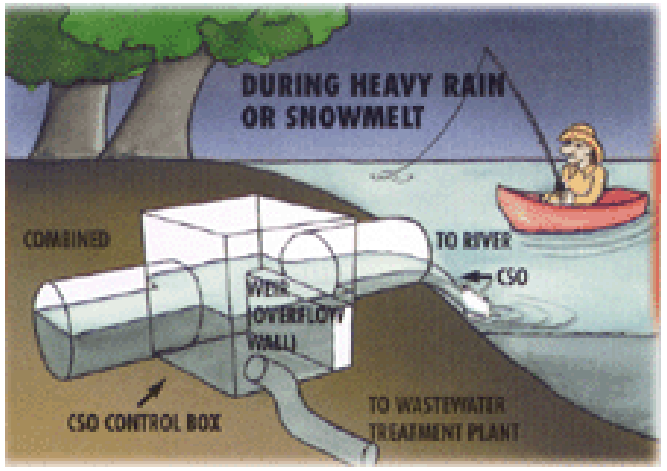
Waste water/Sewage treatment

Animal farming operations

Agricultural run-off

Wildlife

Combined Sewer Overflow



Microbial Source Tracking

- Tools are now available to determine to link specific molecular markers to the source of the fecal pollution

- Health risks
- Remediation
- Prioritization
- Responsibility

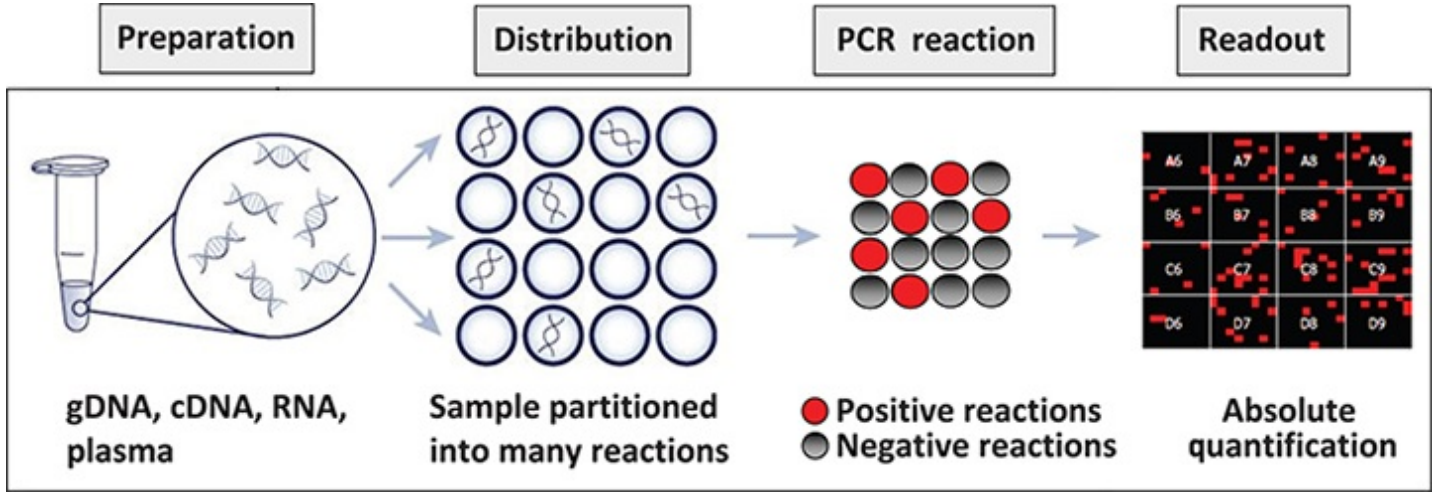
Using molecular methods to determine water quality

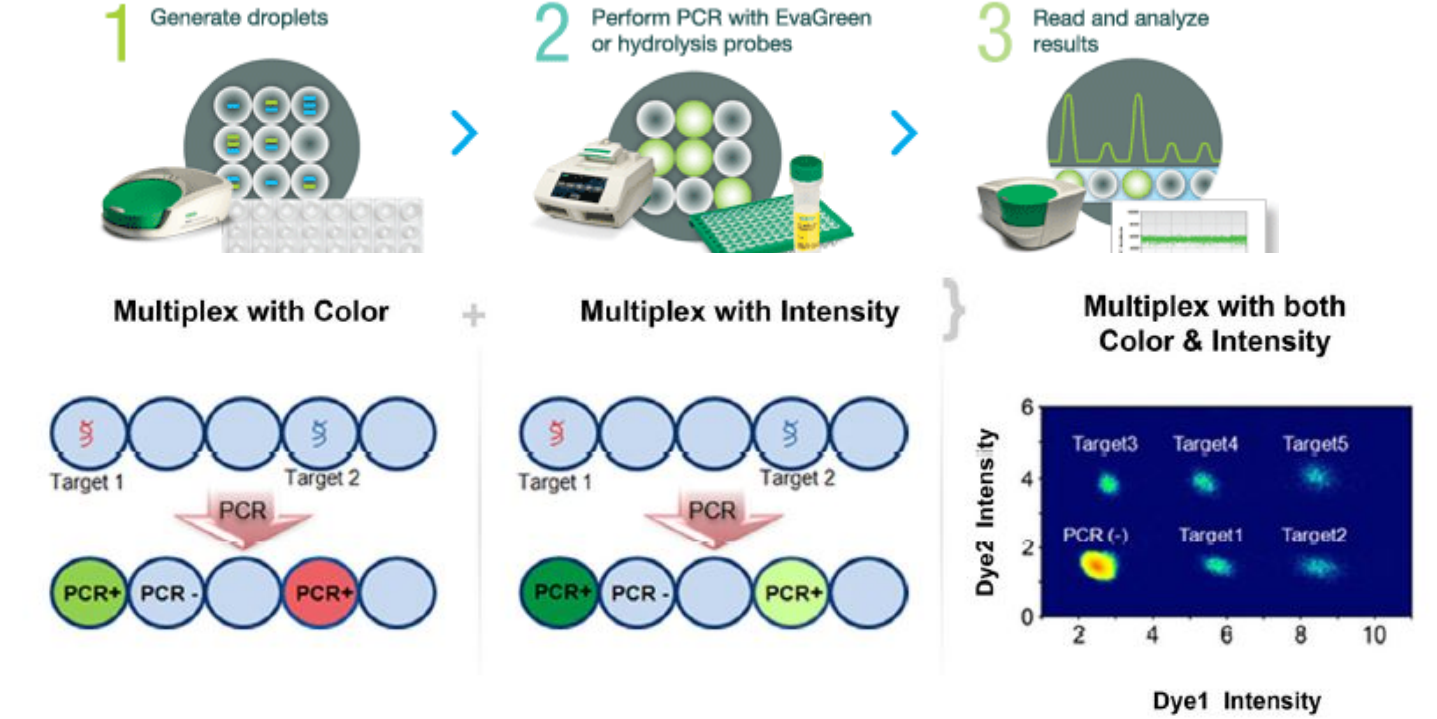
- Advantages:
  - More sensitive
  - More time-efficient
  - Could be more cost-effective?
  - Can target specific pathogens and source tracking markers.
  - Can obtain rapid results during extreme events.
  - Can address quantitative characterization
- Disadvantages:
  - Viability of target organisms needs to be considered
  - Copies/reaction needs to be translated to cells/volume to be able to determine risk

Water Diagnostics using digital droplet polymerase chain reaction

MPN cultivation TC/*E.coli* 24 hrs→ Rapid MPN genetic analyzer

Droplet Digital PCR Platform



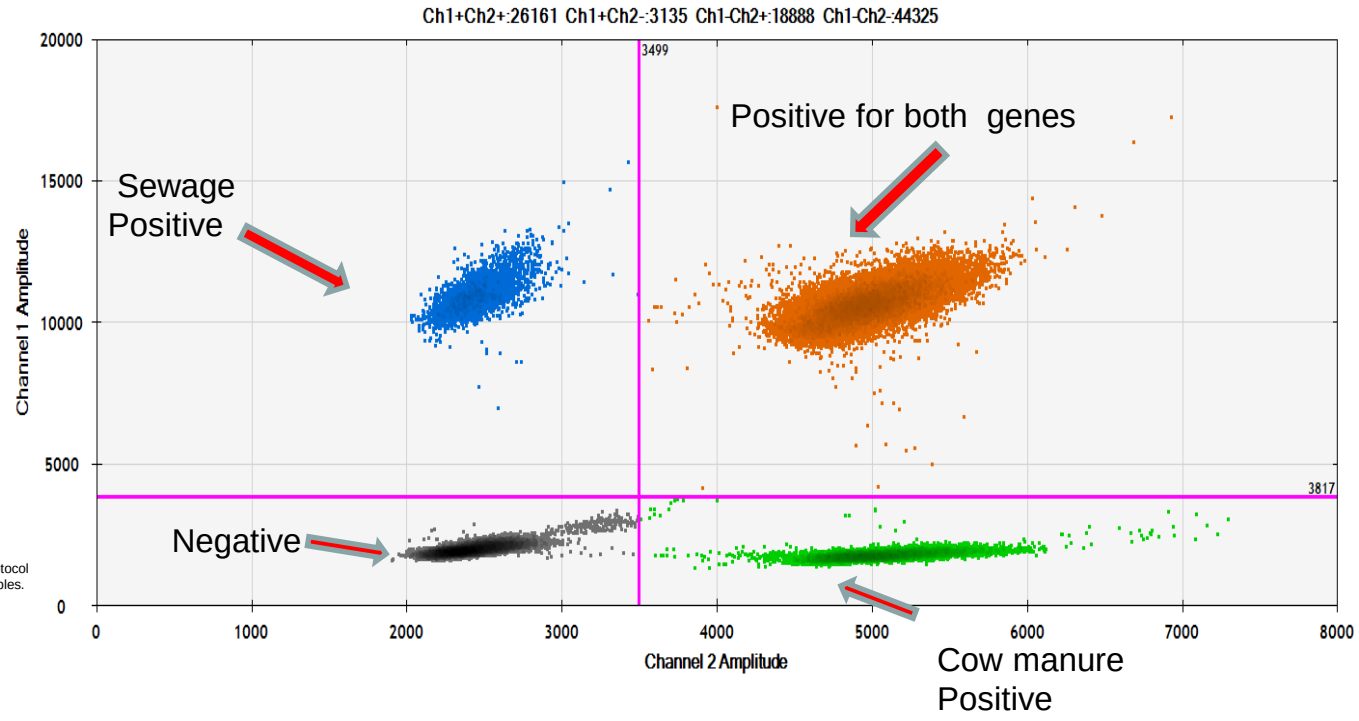


- **The ddPCR enables precise, high sensitivity of nucleic acid quantification.**
- **No need to develop a standard curve.**
- **The target RNA/DNA is partitioned into 20,000 droplets and then amplified.**
- **= > Target sequences are detected by fluorescence – if there is fluorescence then scored as positive and if absent then scored as negative.**
- **Poisson statistical analysis of both + and – droplets yields absolute quantification of the target sequence.**

The Biorad Qx200-droplet-digital-PCR system

ddPCR for Source Tracking

- B.theta for human sewage
- M2 bovine marker
- > 10,000 tests (droplets) per well

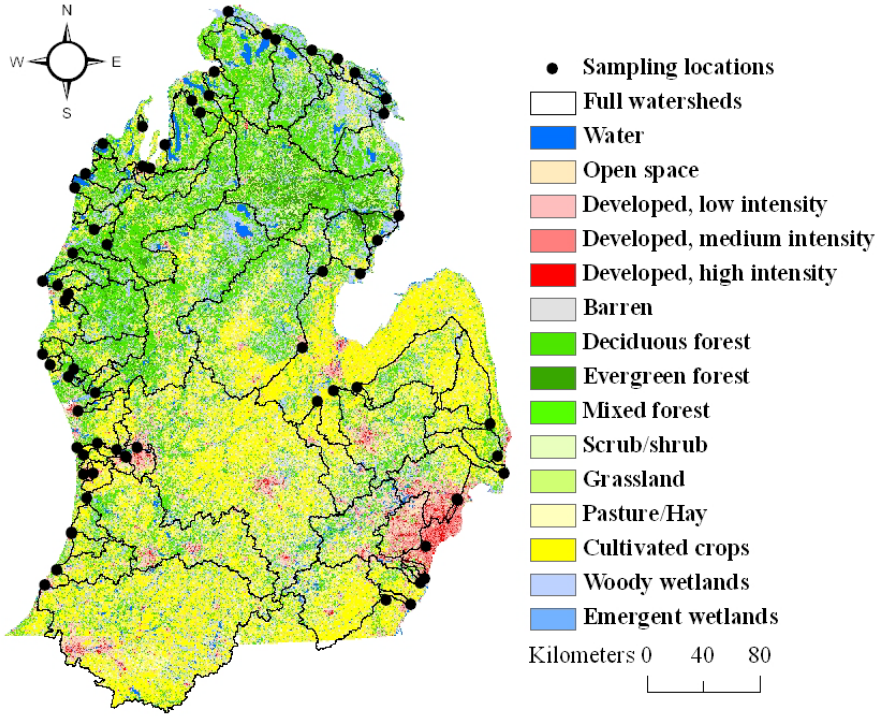


Nazarian, E.J., *et al.* (2008). Design and implementation of a protocol for the detection of *Legionella* in clinical and environmental samples. Diagn. Microbiol. Infect. Dis. 62:125-132.

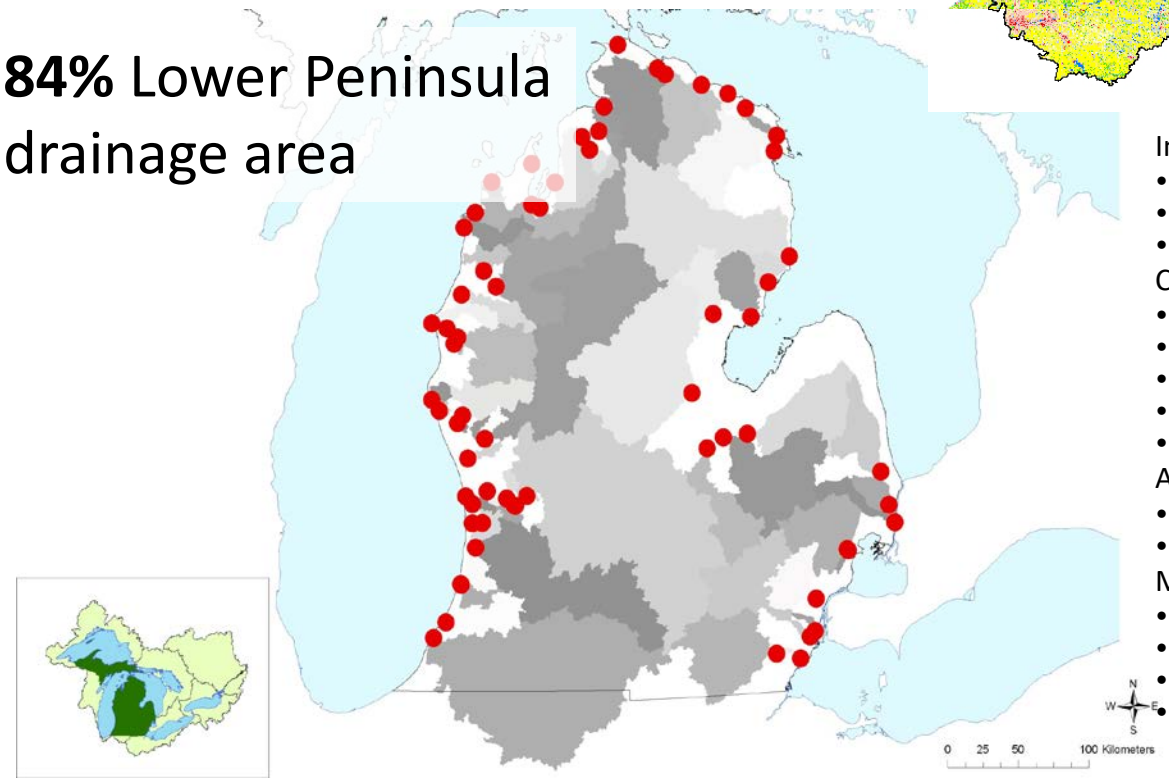
Sampling water quality and the Landscape

Baseflow (October 2010)
Spring thaw (March 2011)
Early summer rain (June 2011)

64 River systems



84% Lower Peninsula drainage area



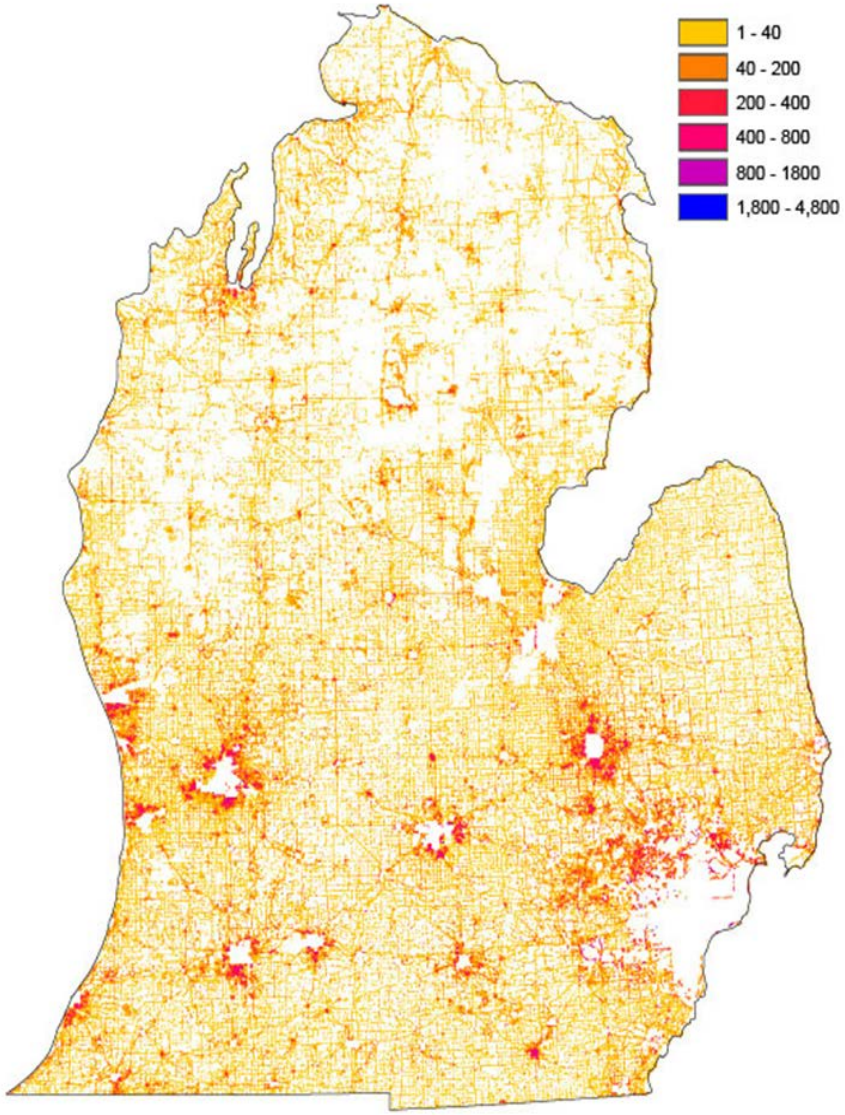
In Stream Conditions:
- River discharge (ADCP and USGS)
- Temperature
- Physical chemistry (pH and specific conductance)

Chemistry and Nutrients:
- Nutrients (N, P, TN, TP, TDN, TDP, SRP)
- Ions (Na, Ca, Mg, Cl, K, NO_3, SO_4, NH_3)
- Dissolved organic carbon
- Alkalinity
- Stable isotopes (δH2 and δO18)

Algae and Chlorophyll:
- Chlorophyll a
- Epiphytic algae (hard and soft substrate)

Microbes and Pathogen Indicators:
- *E.coli*
- *Bacteroides thetaiotaomicron* α-1-6 mannanase (B. theta)
- M2 Bovine marker (*Bacteroides)*
- Pig2bac (*Bacteroides)*

Approach: GIS – Septic Systems



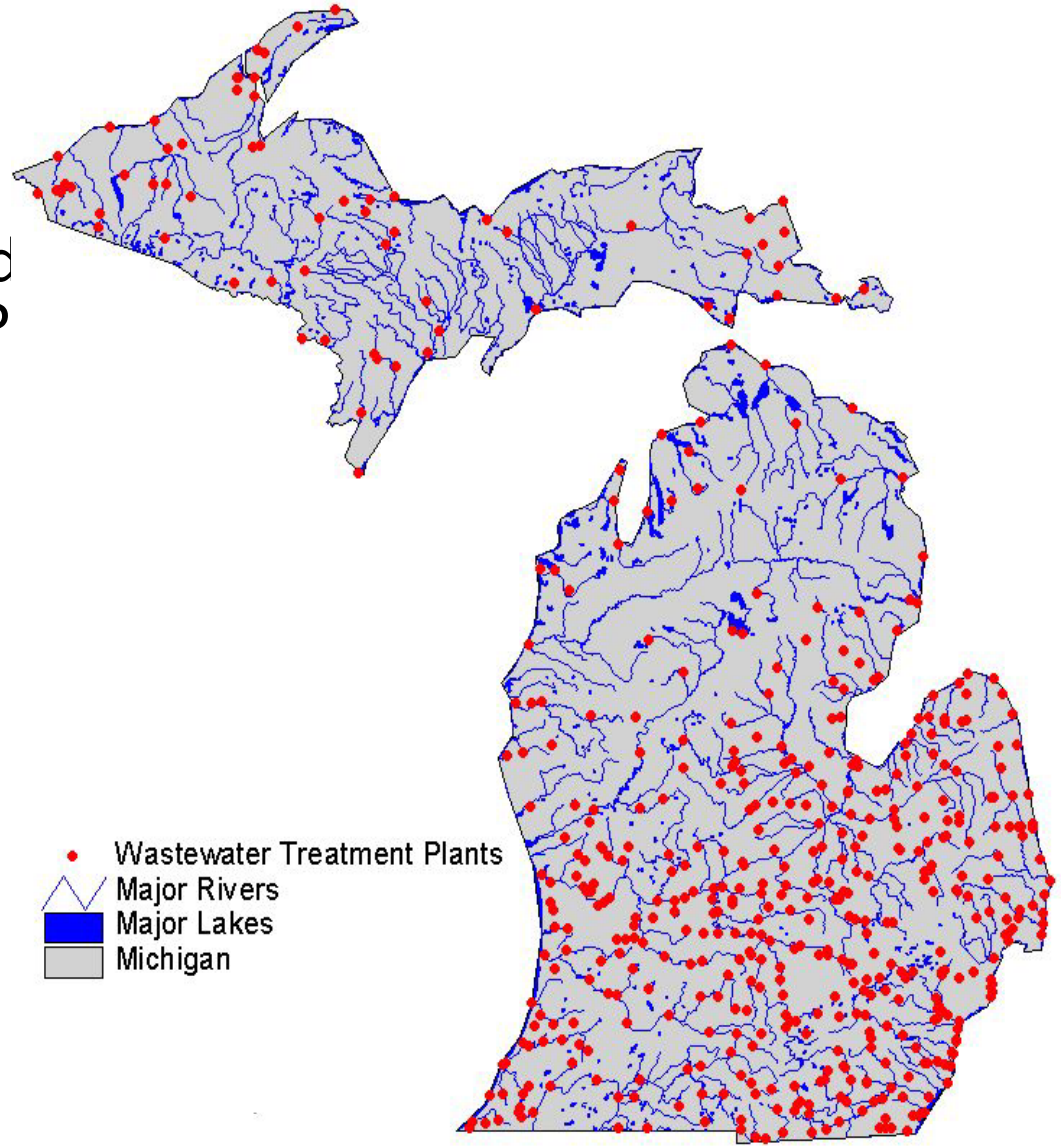
Septic system density

- Identify areas served by wastewater treatment plants
- Estimate number of septic systems in each census block
- Assign system locations:
  - First near known water wells
  - Then, randomly in each block
  - Subject to riparian and road setbacks

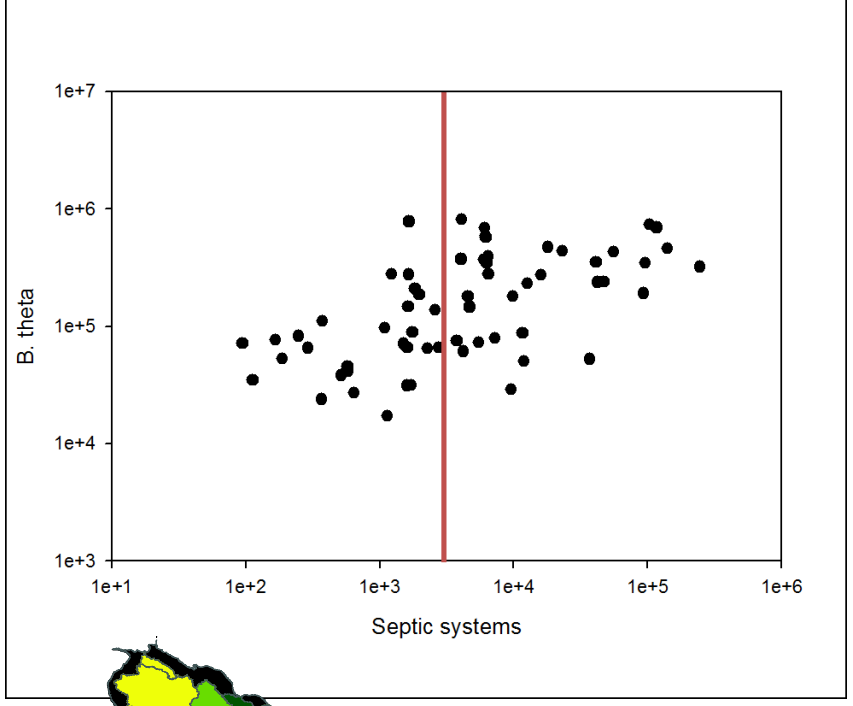
Luscz EC, Kendall AD, Hyndman DW (2015) High resolution spatially explicit nutrient source models for the Lower Peninsula of Michigan. J Great Lakes Res 41(2):618–229.

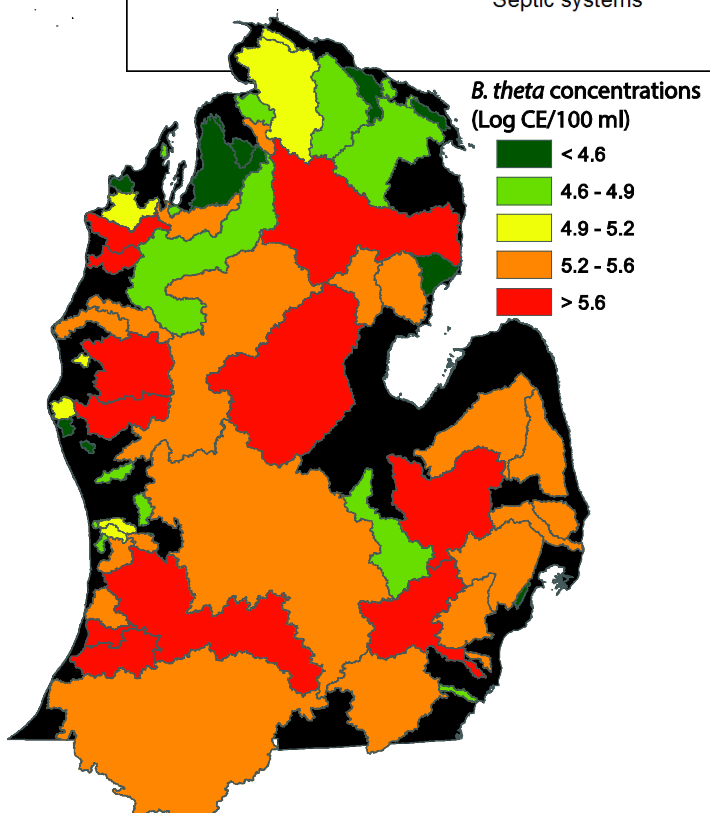
Approach: GIS – National Pollution Discharge Elimination System

- USEPA Discharge Monitoring Report Pollutant Loading Tool used to estimate average WWTP discharge
  - Calculated ratio of WWTP discharge to measured stream discharge
- CSO events not considered for the baseflow
  - No significant precipitation prior to sampling



The distribution of the human sewage marker *Bacteroides*





- Increasing *B. theta* related to more septic tanks
- *More E.coli* related to more total phosphorous and increasing stream temperature

Significant Knowledge Gaps Exist for Septics

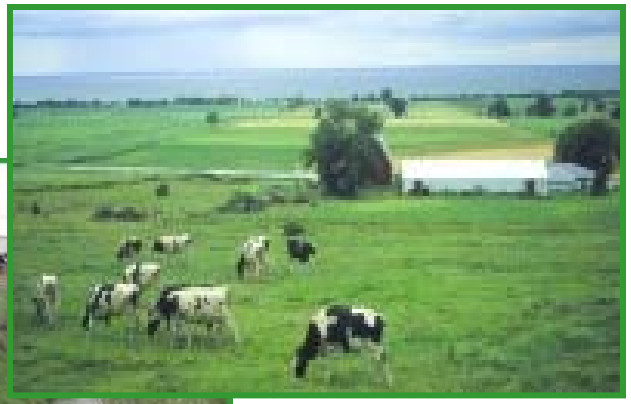




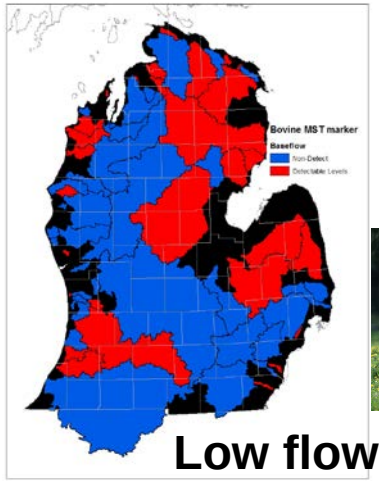
Agricultural Environments

SLIDE PROVIDED BY DR. JEANETTE THURSTON, ARS, NEBRASKA

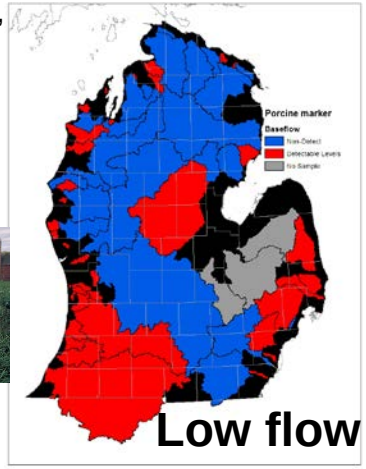


Nshimyimana, J.P., Martin, S.L., Flood, M., Verhougstraete, M.P., Hyndman, D.W., and Rose, J.B. 2018. **Regional Variations of Bovine and Porcine Fecal Pollution as a Function of Landscape, Nutrient, and Hydrological Factors**. *J. Environmental Quality* 47 (5): 1024-1032

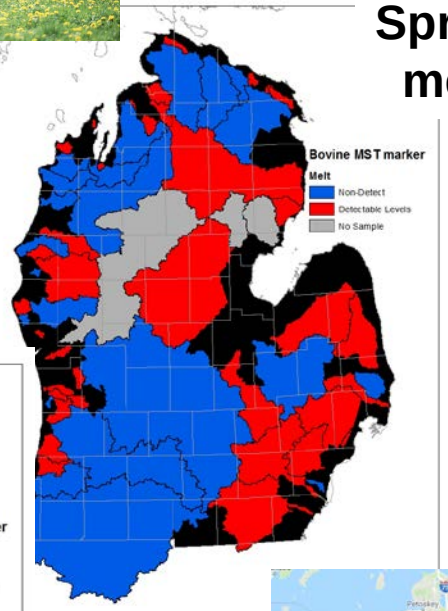


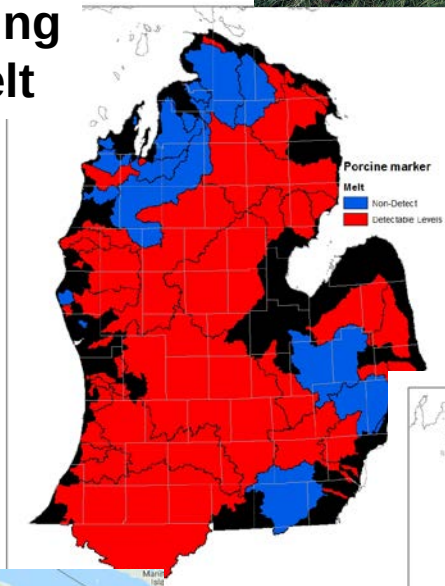
Low flow



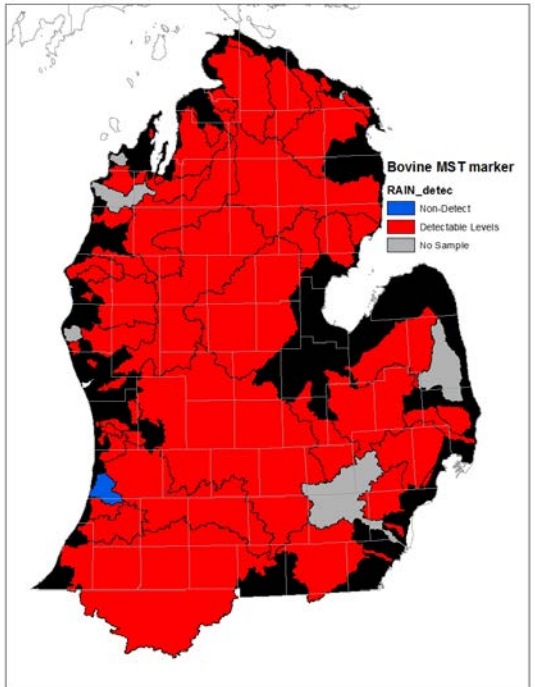
Low flow

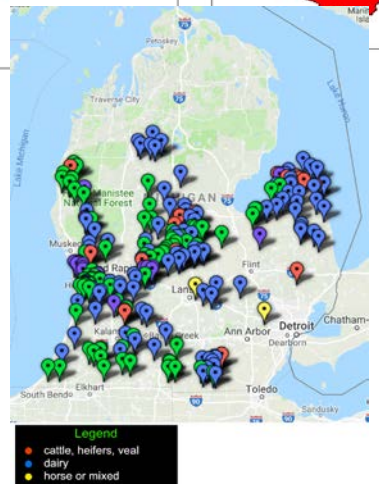
Spring melt



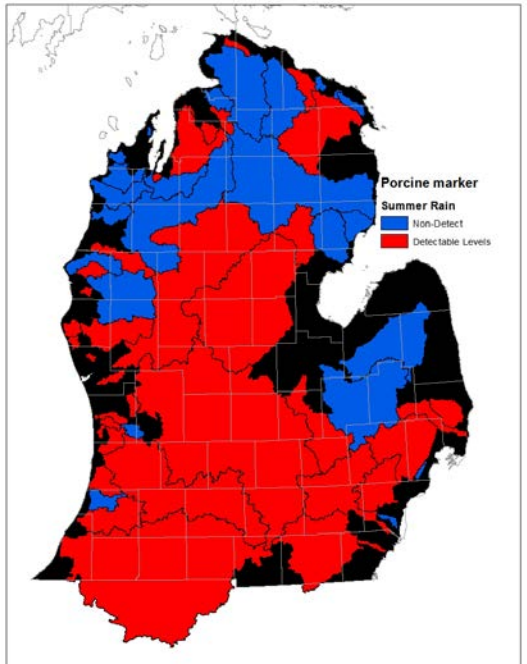


Summer rain





Summer rain



What did we learn?

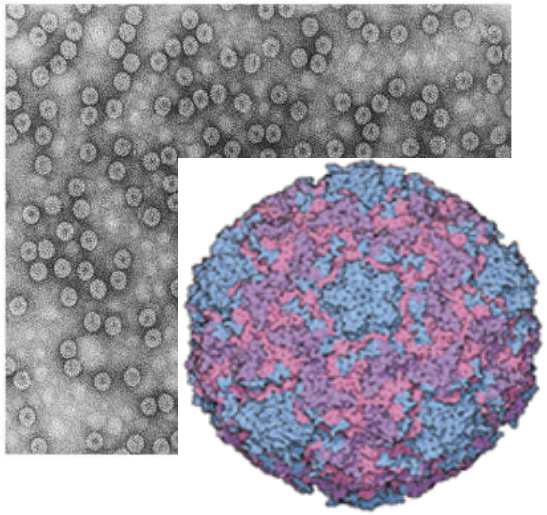
- New microbial source-tracking tools elucidate important nonpoint sources of water quality degradation and potential need for further investigation of human health risks at large scales
- Pollution arising from septic system discharges likely more important than previously realized
- Identifying sources and providing reference levels for water quality provides a basis to assess water quality trends and remediate degraded areas
- Transport was linked to rain

Other Findings

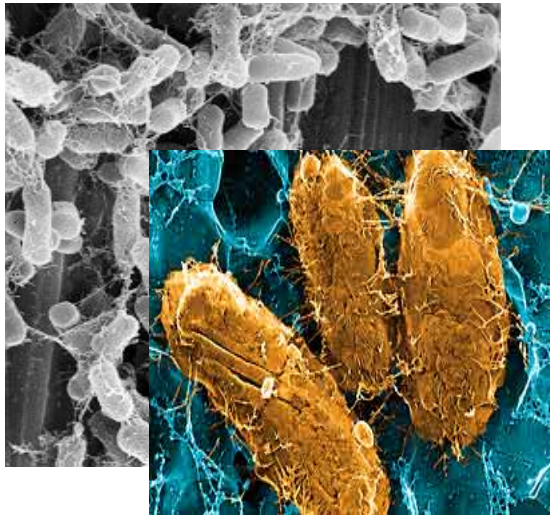
- Streamflow was identified as an important transport factor contributing to the rapid and widespread temporal and spatial increase of manure accumulated markers in rivers draining agricultural and natural fields.
- Streamflow (spring melt) was the strongest predictor of bovine and porcine marker concentrations. Nutrients (TP, TN, and SRP), *E. coli* and temperature were related to the markers during summer rain.
- Watersheds could be identified as hot spots which clustered together based on nutrients, as well as bovine and porcine marker concentrations.
- Wetlands served as a reservoir for markers.

Types of Waterborne Pathogens

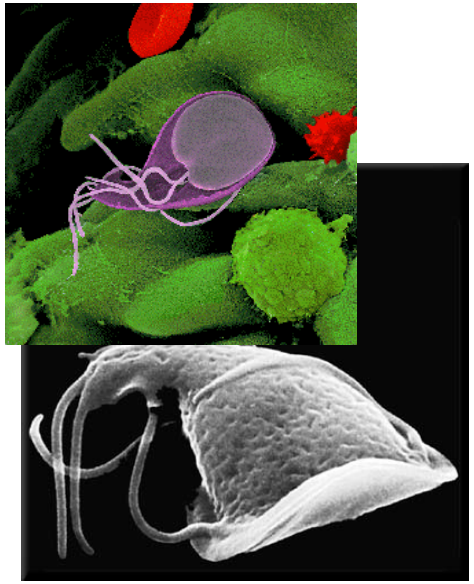
Viruses



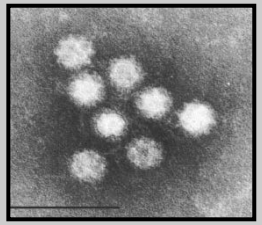
Bacteria



Parasites



THE DISEASES: diarrhea, respiratory illness, liver damage, kidney failure, heart disease, cancer, nervous system disorders, ulcers birth defects, death.



VIRUSES INFECT ALL LIVING ENTITIES

Environmental Health-Related Viruses Infecting Humans

- Adenovirus
- Coxsackievirus
- Echovirus
- Enteroviruses
- **Hepatitis A** and E
- Norovirus
- **Poliovirus**
- Rotavirus

VIRUSES INFECT ALGAE

Algal virus found in humans, slows brain activity

Tweet 293 Share 11k +1 79

By Elizabeth Pennisi | 27 October 2014 3:30 pm | 12 Comments

Liz is a staff writer for *Science*.

Email Elizabeth

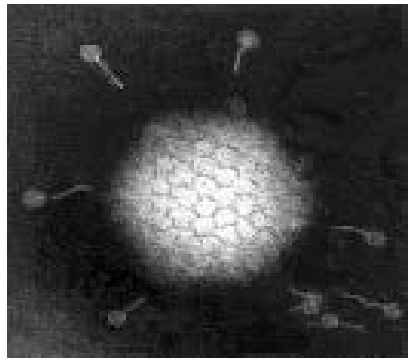
Follow @epennisi

It's not such a stretch to think that humans can catch the Ebola virus from monkeys and the flu virus from pigs. After all, they are all mammals with fundamentally similar physiologies. But now researchers have discovered that even a virus found in the lowly algae can make mammals its home. The invader doesn't make people or mice sick, but it does seem to slow specific brain activities.

The virus, called ATCV-1, showed up in human brain tissue several years ago, but at the time researchers could not be sure whether it had entered the tissue before or after the people died. Then, it showed up again in a survey of microbes and viruses in the throats of people with psychiatric

Bacteriophage are viruses that infect bacteria, associated with moving virulence; antibiotic resistance genes, controlling bacterial populations

VIRUSES INFECT ANIMALS including FISH, BIRDS and MAMMALS



History of a new virus

- December 2019: New outbreak of severe pneumonia began in Wuhan city, the capital of Hubei province in China,

- January 2020: Chinese scientists had isolated a novel coronavirus, severe acute designated as respiratory syndrome coronavirus 2 (SARS-CoV-2)

- February 2020: Disease named COVID-19 by WHO

- March 11, 2020, WHO declared the COVID-19 a global pandemic

Coronaviridae

A family of viruses with positive-sense, single-stranded RNA.

Contains an enveloped layer with glycoproteins

Found in humans and animals and infects over 200 different hosts

The first CoV was identified in 1932 includes the Common Cold, SARS (Feb 2003 outbreak lasted 6 months) and MERS (2012 the largest outbreak lasted ~4 months)

SARS-CoV-2 (COVID-19)

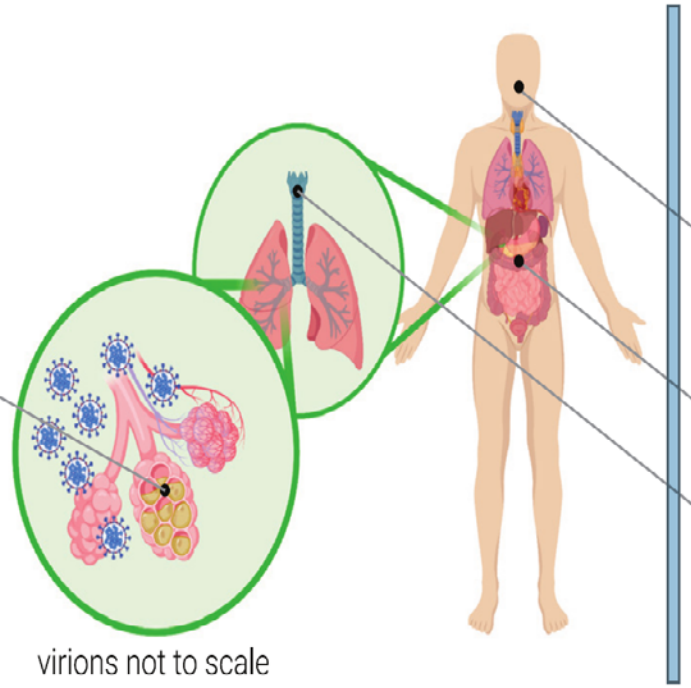
Diarrhea in 2-10% of patients
Detection in feces

https://bit.ly//2WOeN64

Host Cells

(tentative list; number of cells per person)

Type I & II pneumocytes (~10^{11} cells)
Alveolar macrophage (~10^{10} cells)
Mucous cell in nasal cavity (~10^{9} cells)
Host cell volume: ~10^{3} μm^3 = 10^{3} fL



Concentration

maximal observed values following diagnosis
(Woelfel et al. 2020; Kim et al. 2020; Pan et al. 2020)

Nasopharynx: 10^{6}-10^{9} RNAs/swab
Throat: 10^{4}-10^{8} RNAs/swab
Stool: 10^{4}-10^{8} RNAs/g
Sputum: 10^{6}-10^{11} RNAs/mL

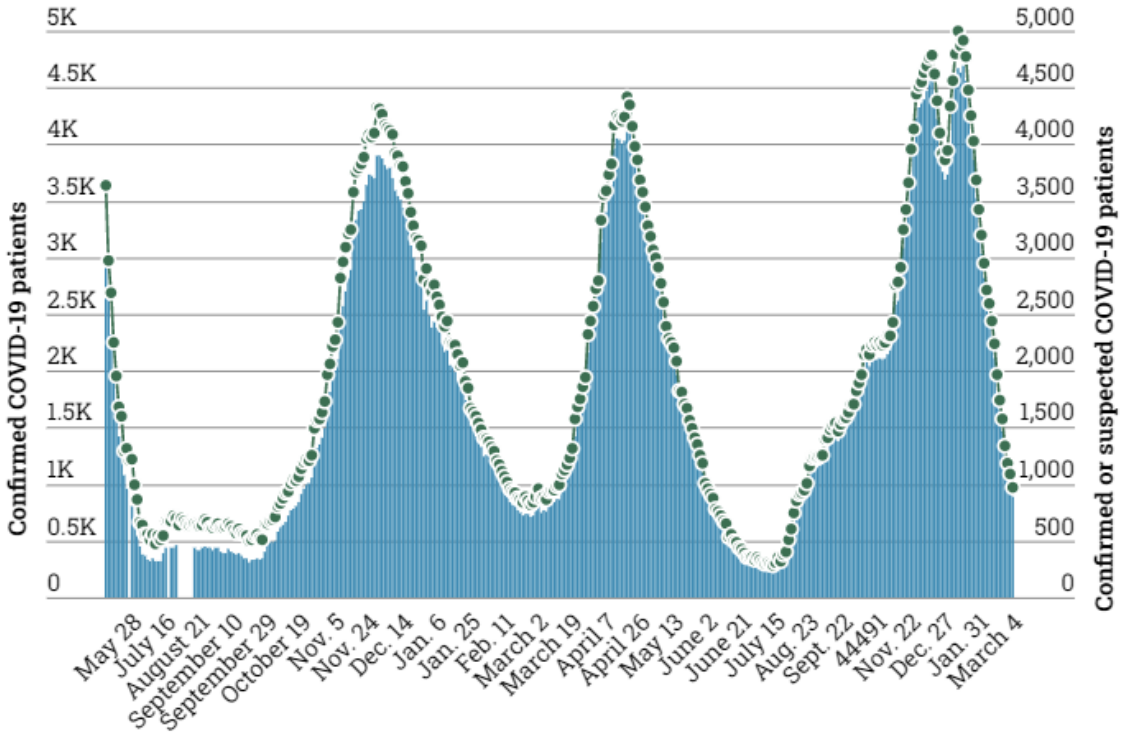
RNA counts can markedly overestimate infectious virions

✓ **Prolonged viral excretion may have important public health implications if responsible for spread of the virus to other persons or to the environment**

The Four Waves of COVID in Michigan

Hospitalizations

This is hospitalizations as reported several times a week by Michigan public health officials.



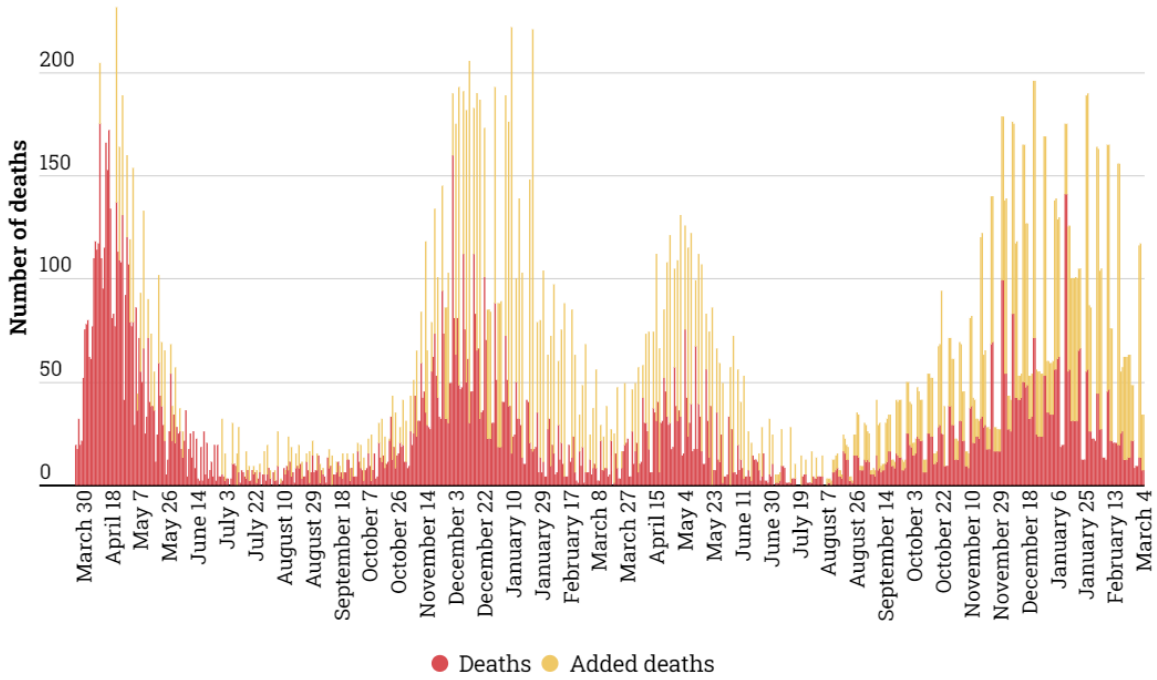
Source: Michigan Department of Health and Human Services and Western Michigan University Libraries.

Bridge MICHIGAN

— *Mike Wilkinson*

Daily deaths

Deaths by day reported, since March 25



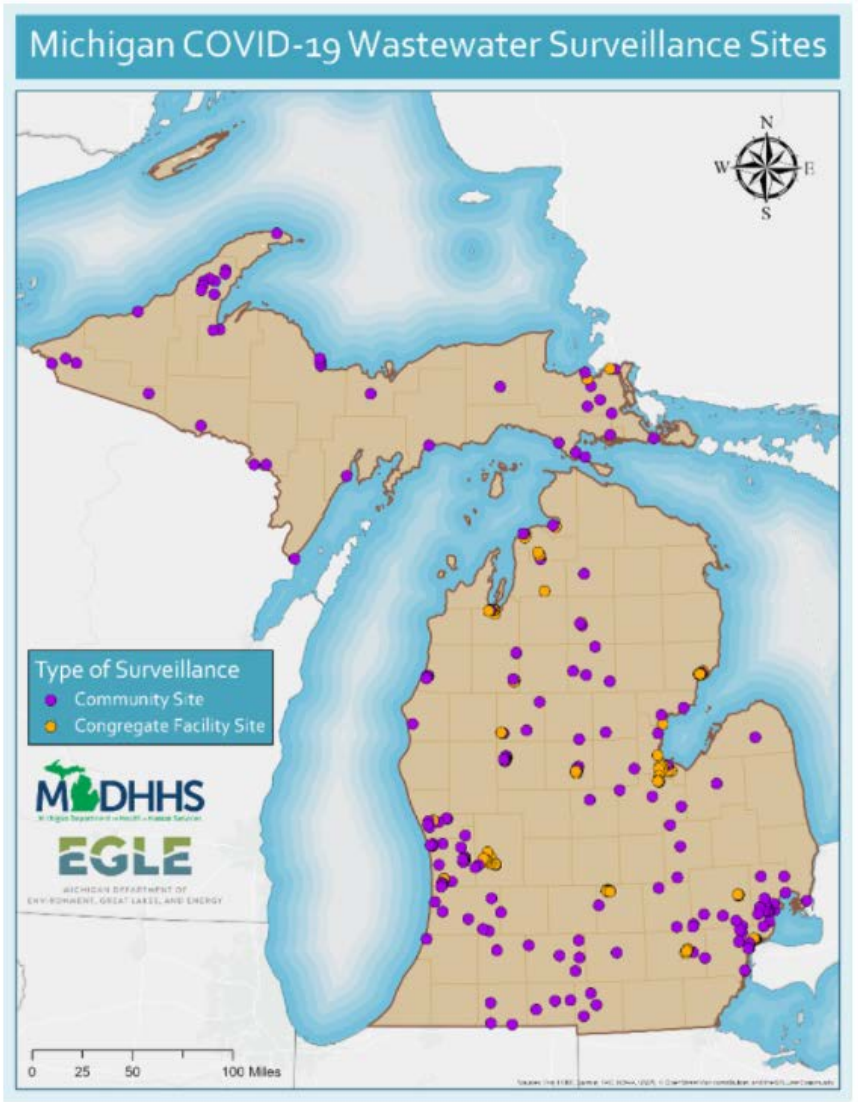
Added deaths are those added after a review of earlier death records.

Michigan Network for Environmental Health and Technology

SARS-CoV-2 Epidemiology - Wastewater Evaluation and Reporting (SEWERS) Network Project (2021-2023)

19 Labs

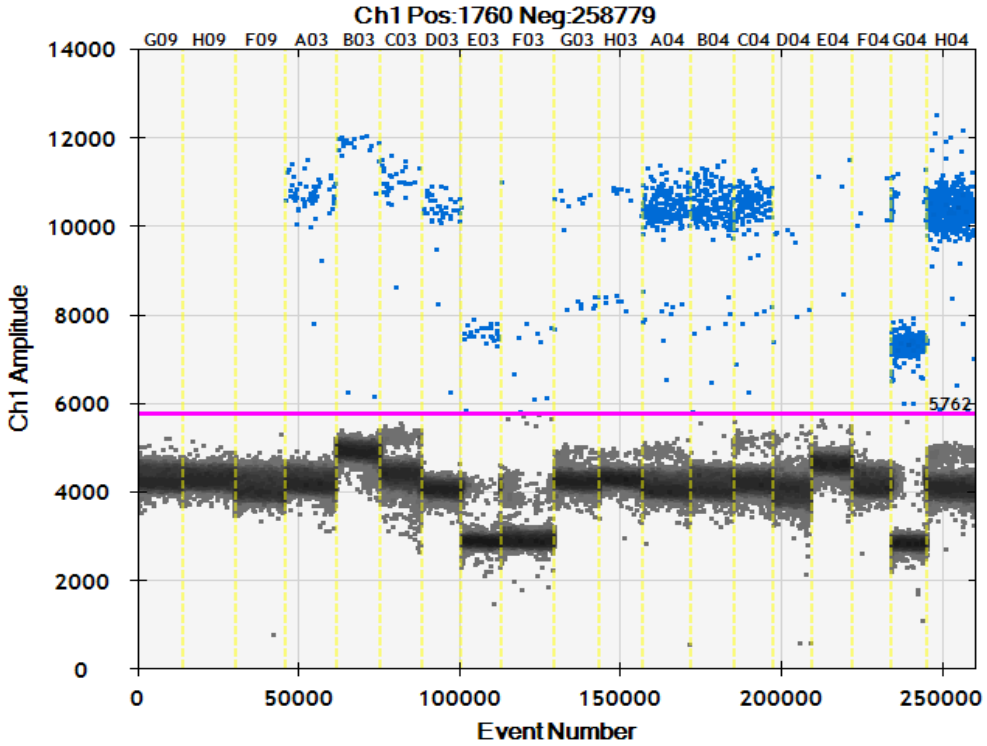
~460 sites being monitored



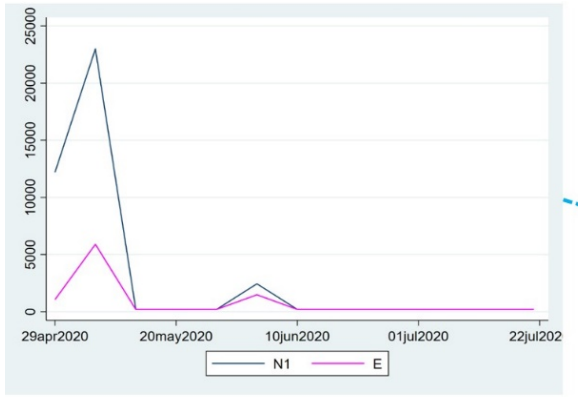
https://www.michigan.gov/coronavirus/0,9753,7-406-98163_98173-545439--,00.html

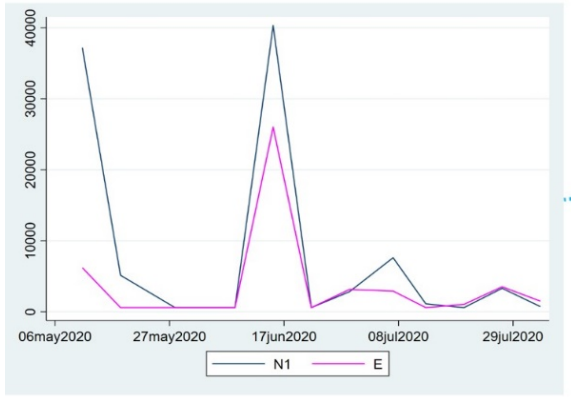
https://storymaps.arcgis.com/stories/f2996168197c4bbfa05e76b893fd9a8e

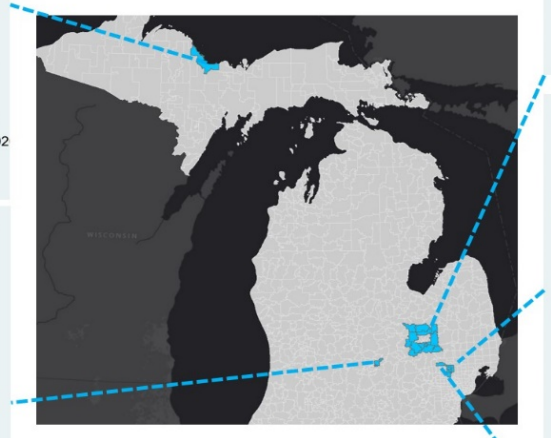
Workflow sample collection to ddPCR

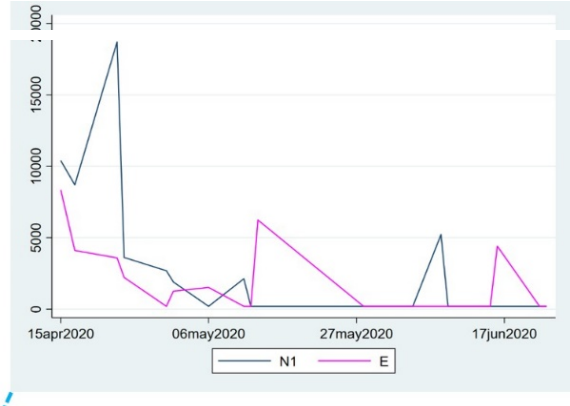


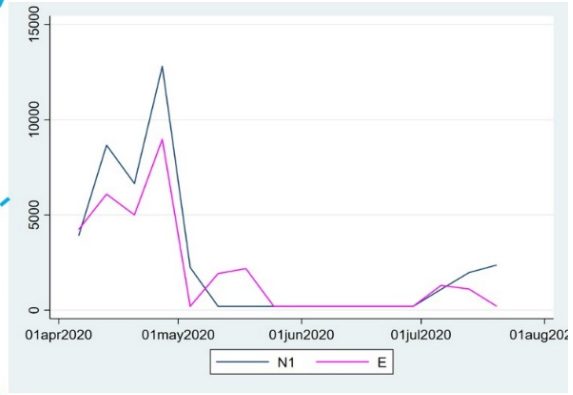
Wastewater assessment Michigan’s First Wave

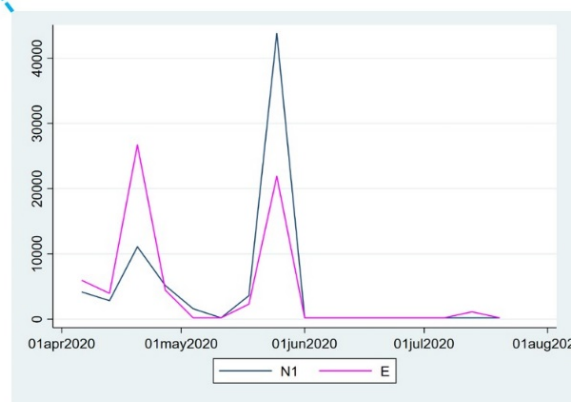




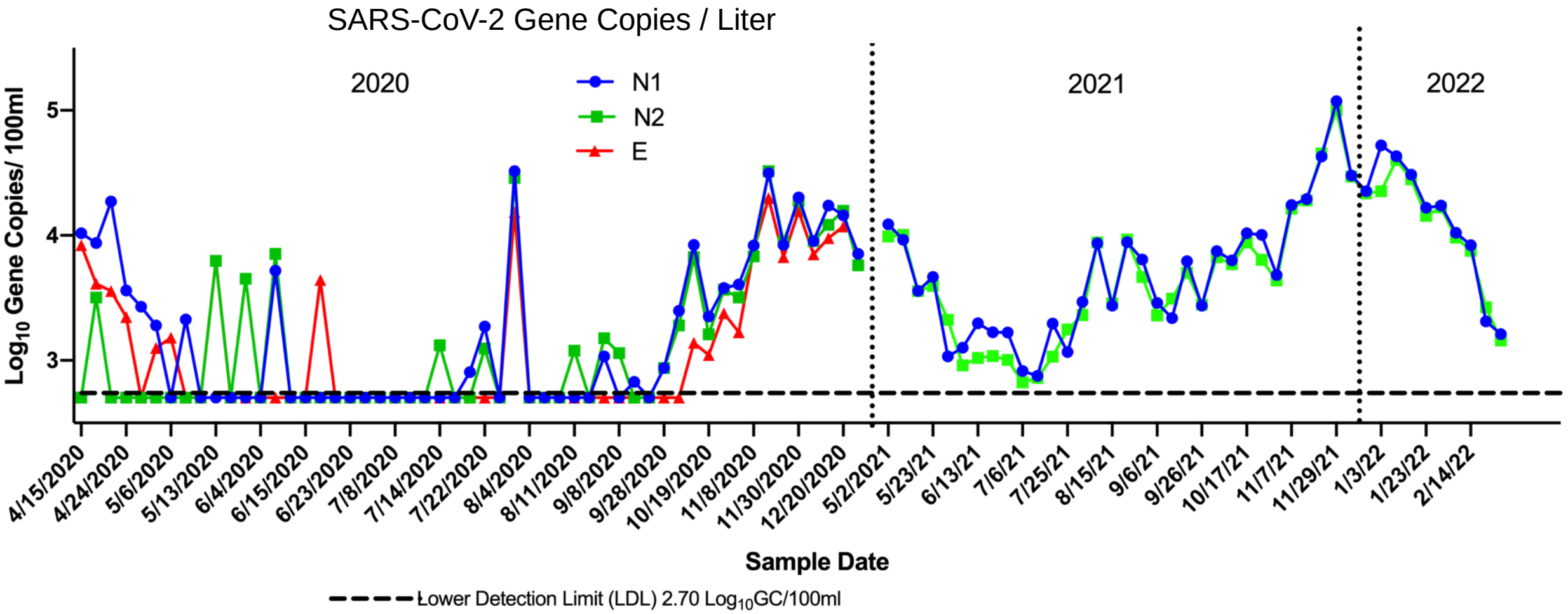




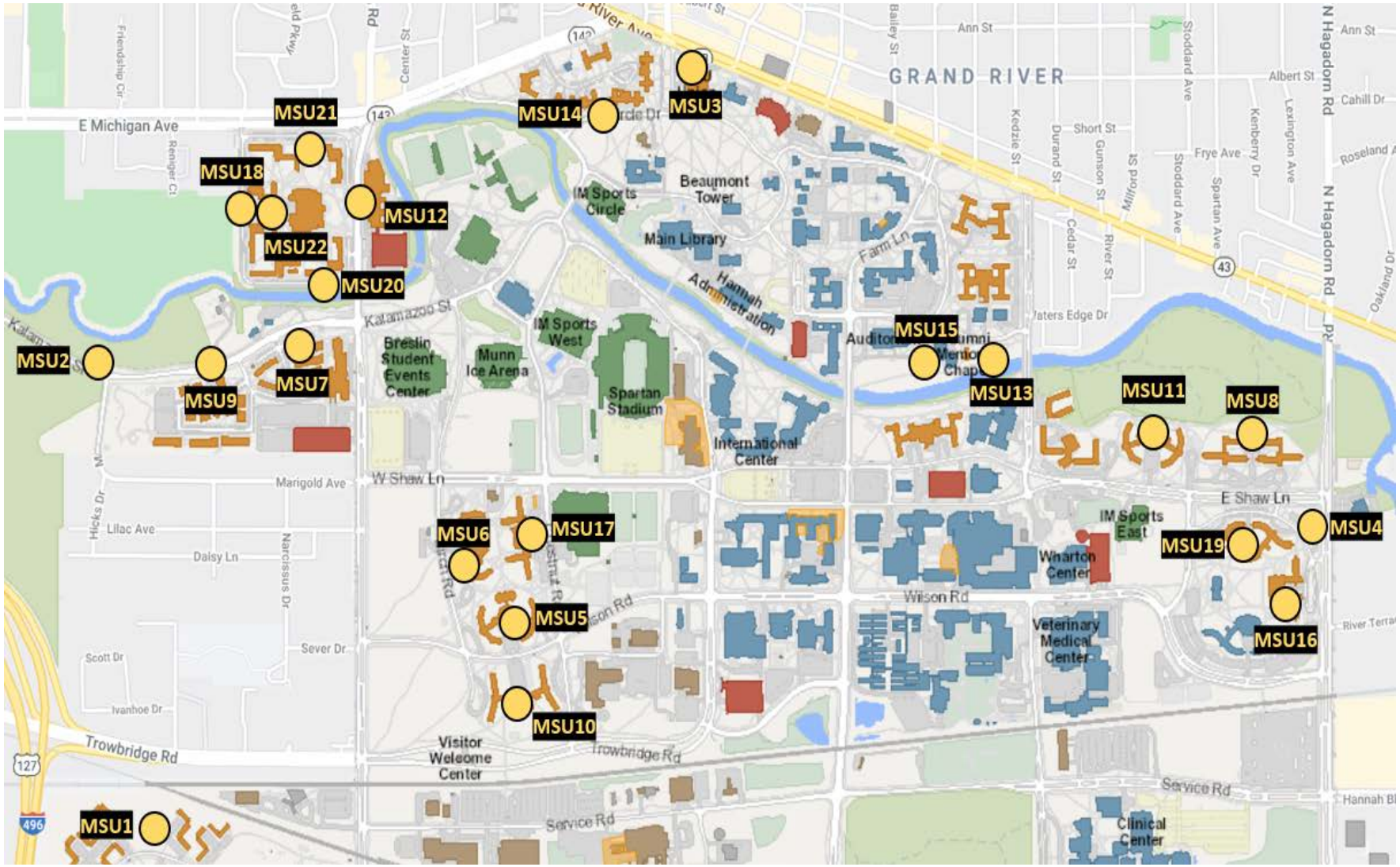




Michigan's Waves

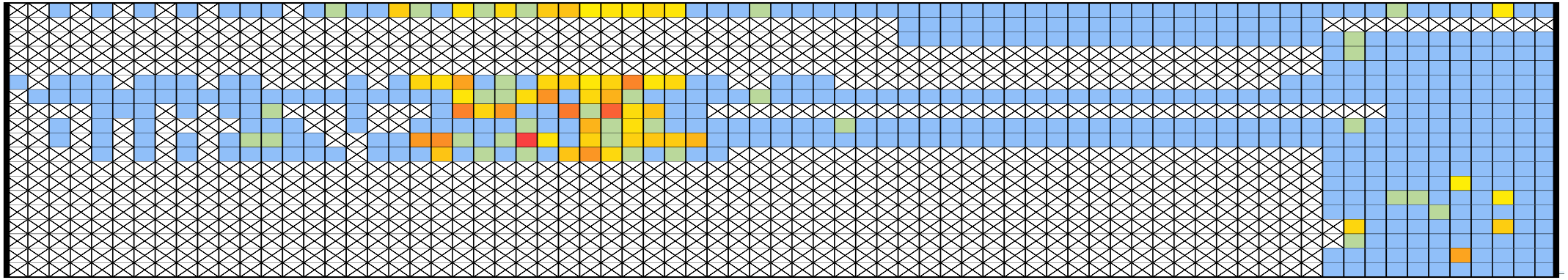


MSU sanitary sewer wastewater surveillance sites

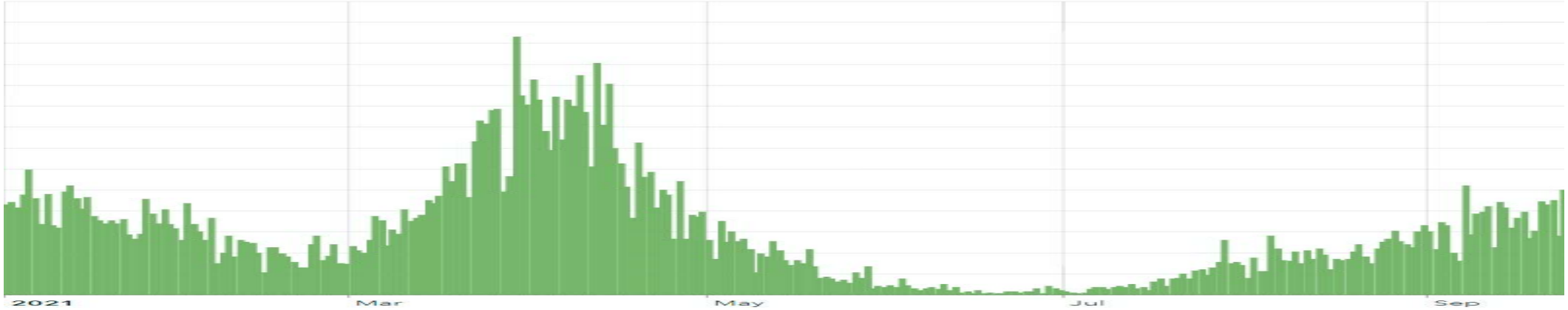


INGHAM COUNTY- Alpha & Delta variant detects Vs SARS CoV-2 Cases

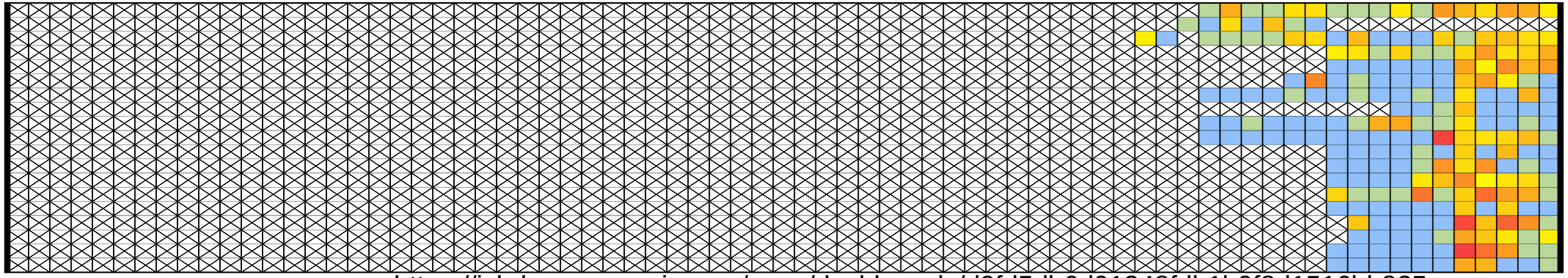
ALPHA



CASES

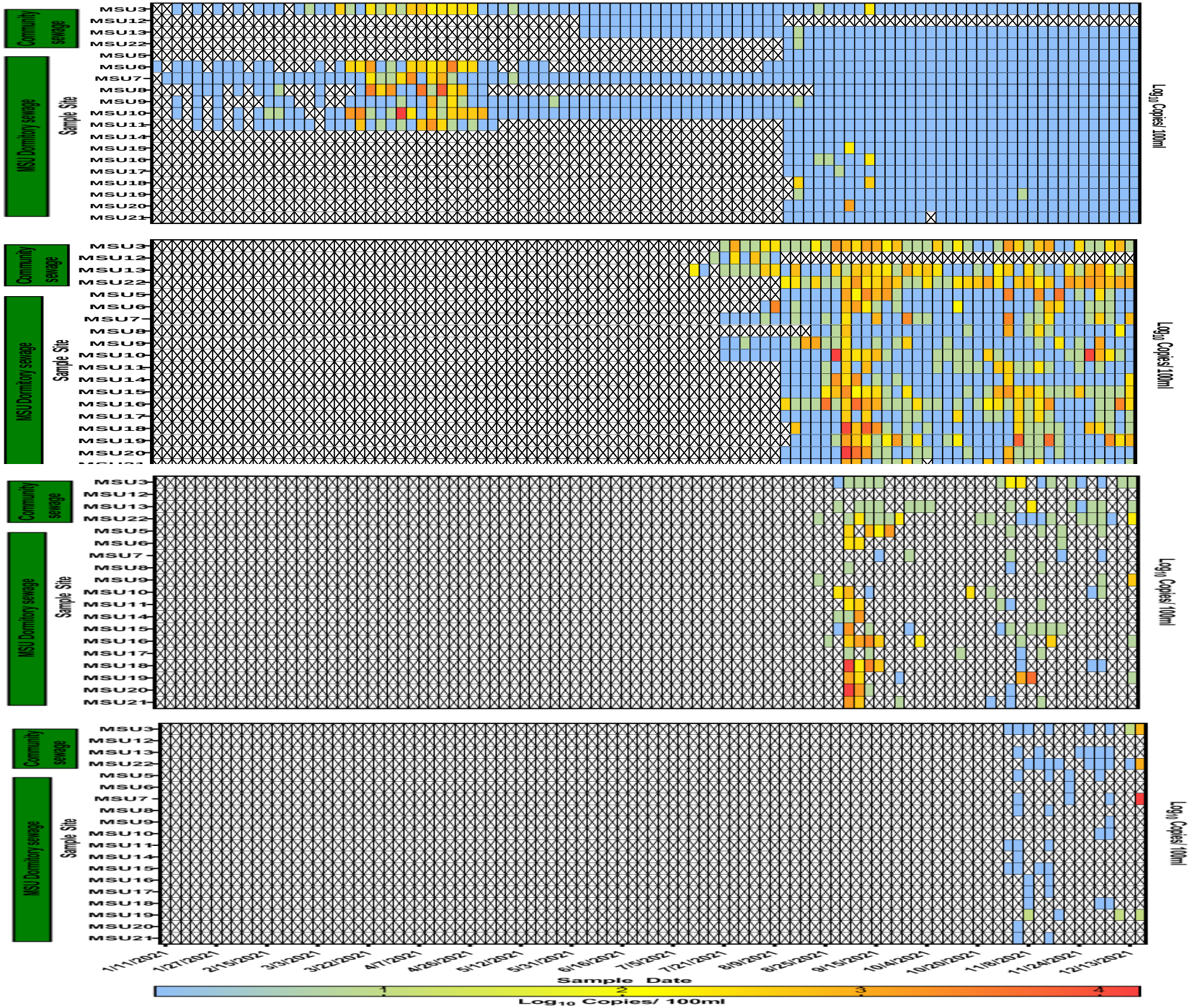


DELTA



https://ichd.maps.arcgis.com/apps/dashboards/d9fd5db6d01348fdb1b9f8d1516bb825

Variant Trends



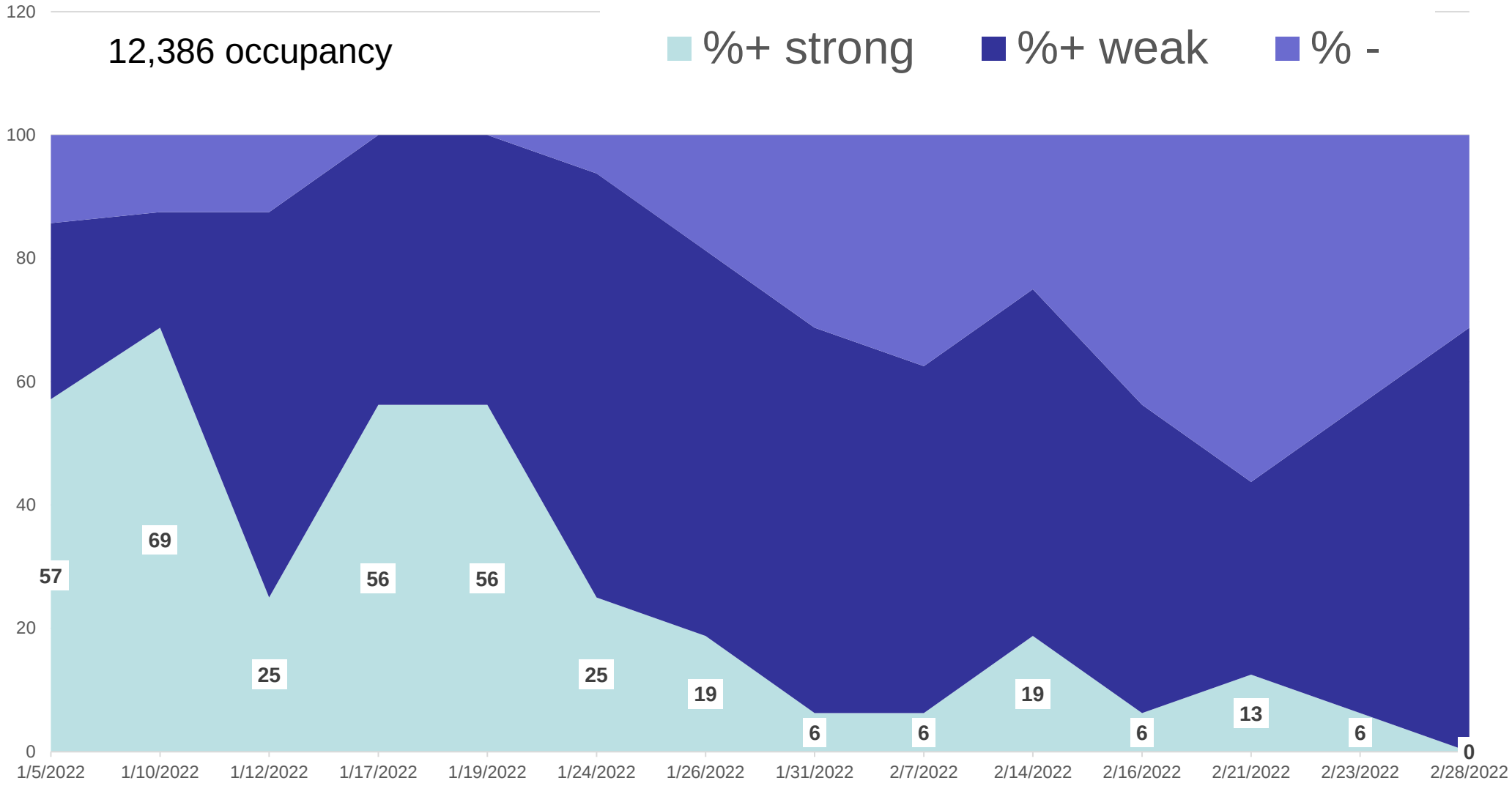
ALPHA

DELTA

DELTA PLUS

OMICRON

Change in SARS-CoV-2 concentrations in Dorm Wastewater Spring 2022 with Omicron



Why Monitor Wastewater and pollution sources?

Can use the information as a leading indicator and early warning of disease.

Will support testing for downstream evaluation of sewage treatment needs.

Can demonstrate relationships between existing diagnostic testing methods and wastewater surveillance data.

Will support identification of sewage, fecal waste impacts on watersheds.

Can produce risk maps

Will support sequencing/ source tracking and appearance of new variants

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