

Macatawa Watershed *Escherichia coli* Levels and Population Genomics

Aaron A. Best, Ph.D.
Harrison C. and Mary L. Visscher Professor of Genetics

November 30, 2017

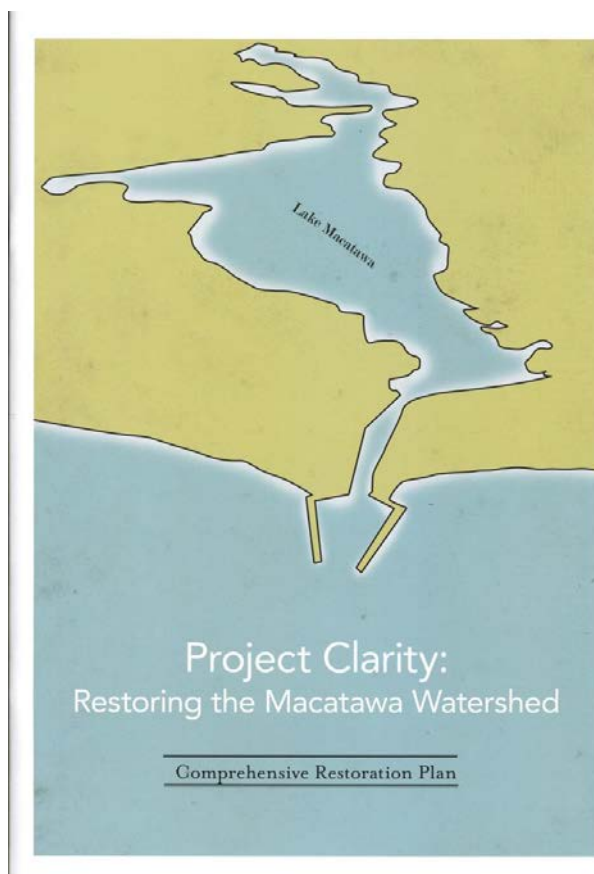
best@hope.edu | @aaron_best



Macatawa Watershed Restoration

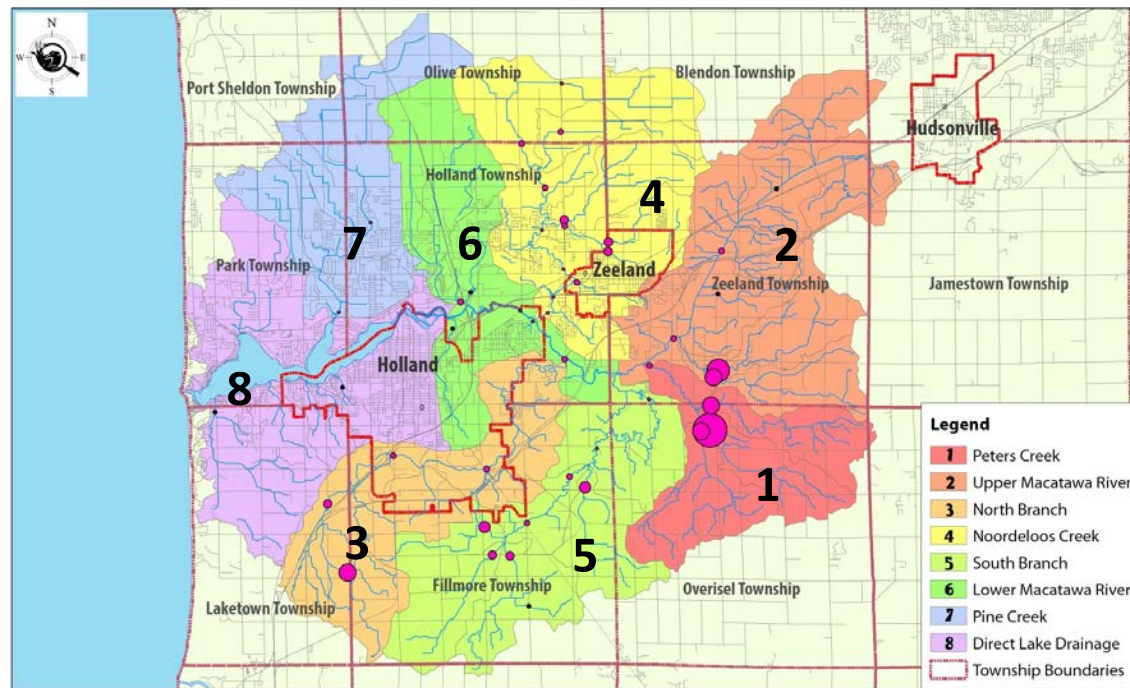


Project Clarity Restoration Plan



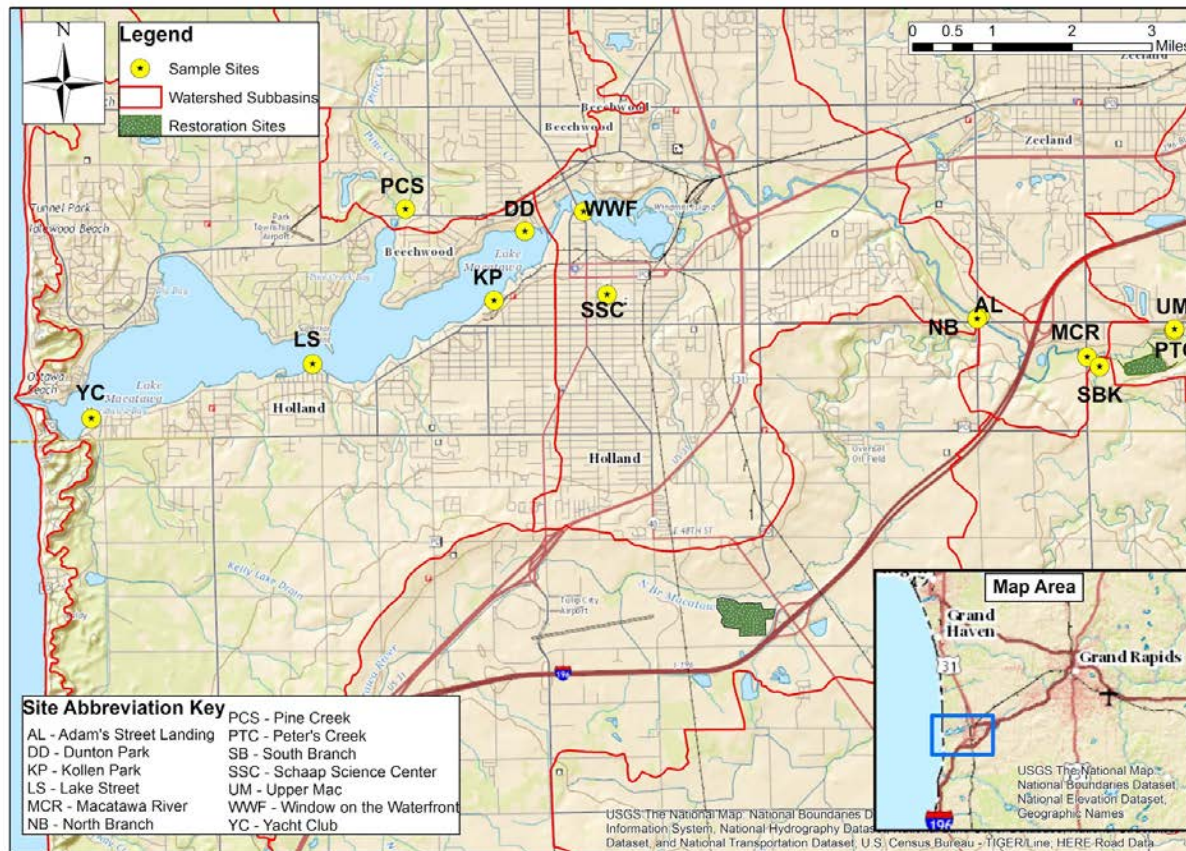
- ✓ Phase One: Research Results
- ✓ Phase Two: Implementation
- ✓ Restoration Team
- ✓ Investment of \$11,976,000
- ✓ Multi-faceted approach
 - ID & Secure Land
 - Restoration
 - Best Management Practices (BMP)
 - Education & Information
 - Maintenance

Macatawa Watershed



Macatawa Watershed Priority Areas

Day1: Watershed Sampling sites



Day1: Watershed

Residential Research Community

Improving retention in the STEM fields for broad set of students:

Research experience
Residential component
Pre-college component
Peer mentors in courses
First Year Seminar
Intro Chem Lab
Intro Bio Lab



Day1
Watershed

Hope College's innovative Day1 programs offer first-year students early and real research opportunities in science, engineering, and math. Gain professional skills while tackling real-world problems through research that begins on day one of your college experience. Explore your interests as you choose the Day1 program that's right for you!

 **Hope** COLLEGE

Let us know you are interested in Day1.  hope.edu/day1

The graphic features a dark green background with white text. It includes two small inset photos: one showing students in a field and another showing students in a lab setting. A larger photo on the right shows a student in a lab coat and gloves holding a test tube.

www.hope.edu/Day1

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Primary Projects

- Establishing Baseline Data for Chemical & Microbial Loads
 - Weekly sampling
 - Year long (August 2016 – August 2017)
 - Chemical – Total Suspended Solids, Phosphate, Nitrate, Dissolved Oxygen, Biochemical Oxygen Demand
 - Physical – Flow rates, Temperature, pH
 - Microbial – *Escherichia coli* counts; Total bacterial community census
 - How do microbial communities respond to large perturbation (rain events)?
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 - Ultimately – “who” are they? Are there better ways to monitor?

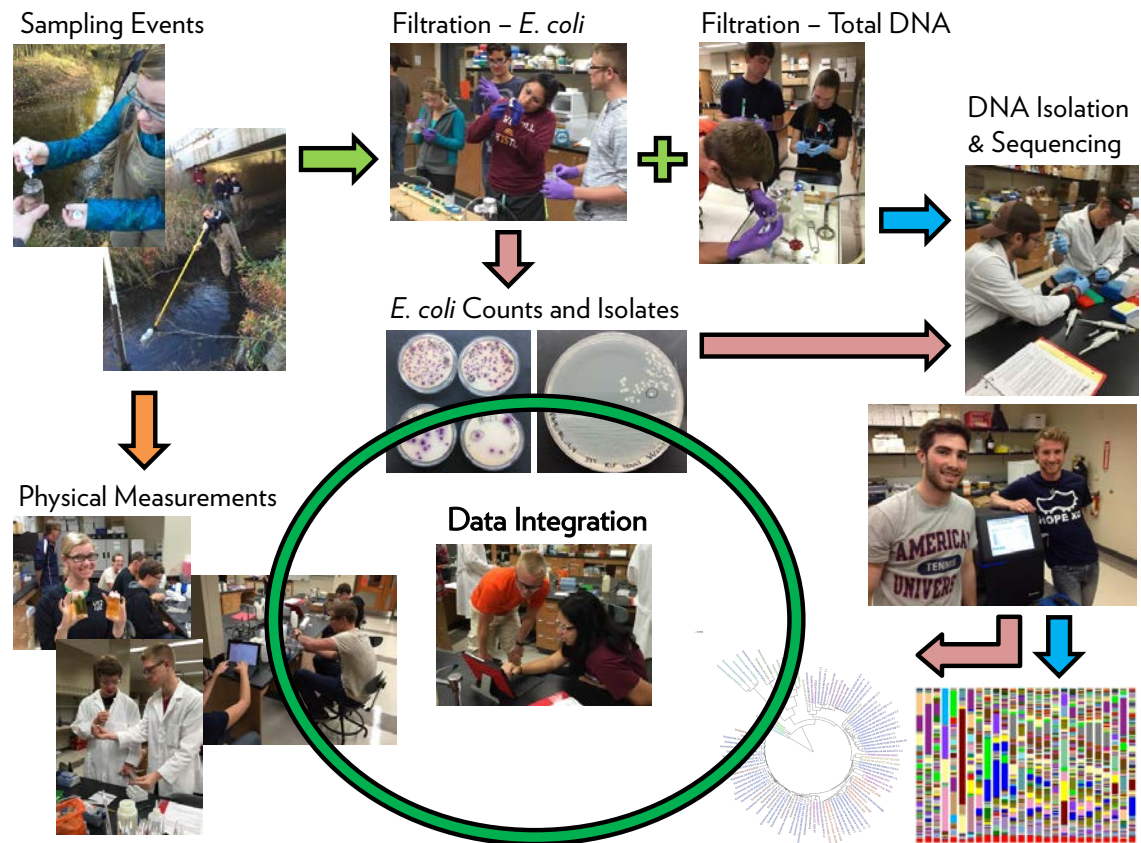


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Day1 Watershed Course Research Experience

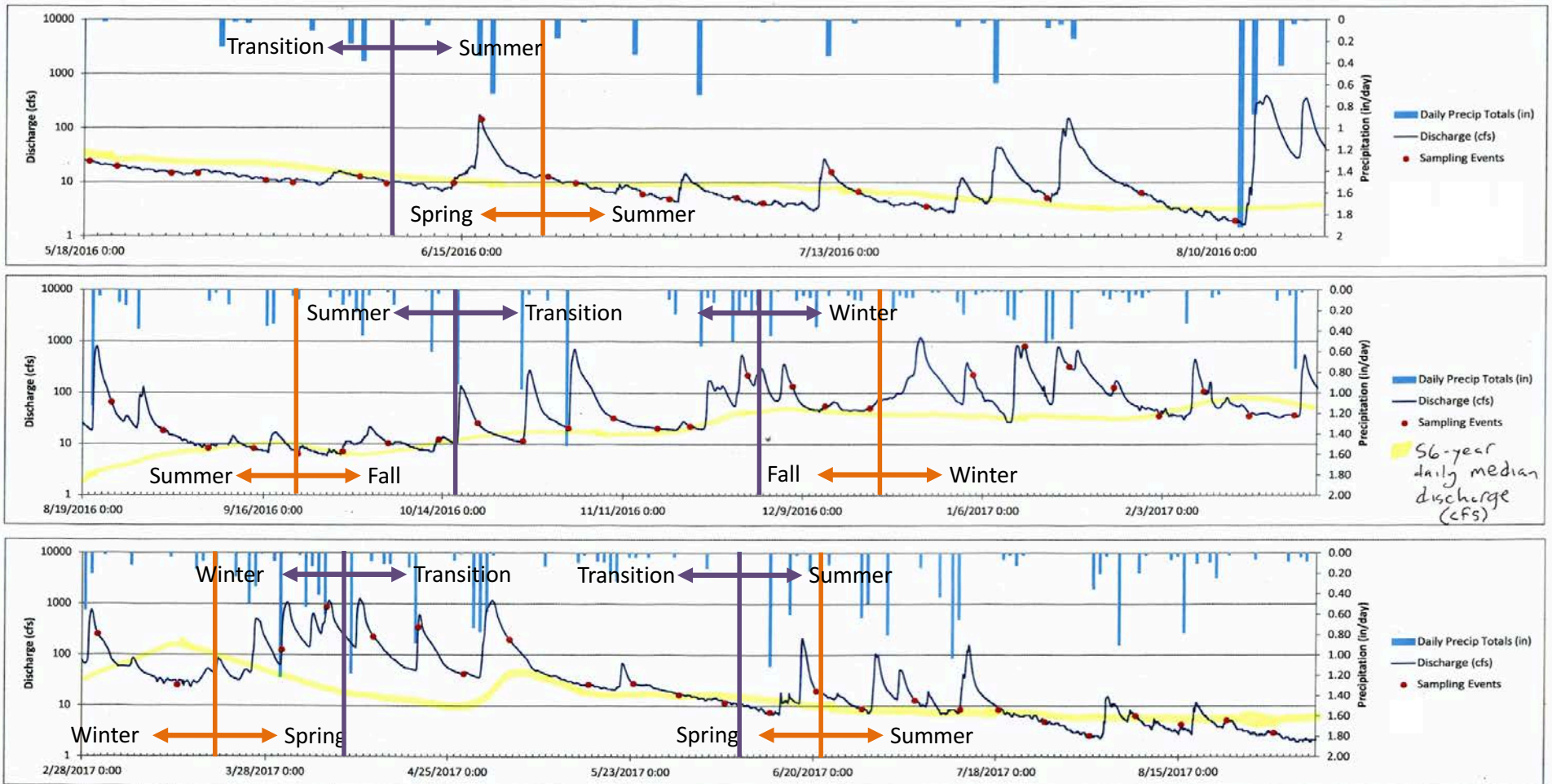


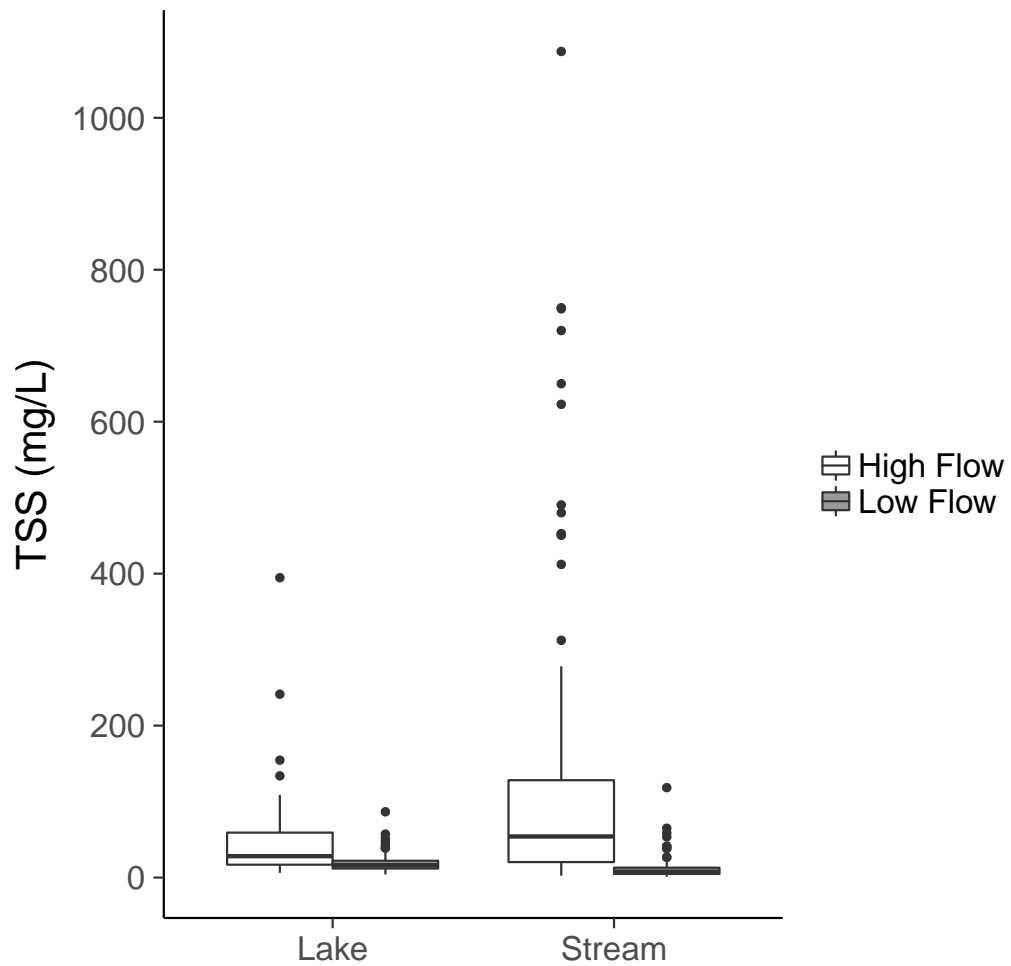
Weekly Sampling – Full Year



Weekly Sampling - Full Year

■ Calendar Season
■ Water Temperature

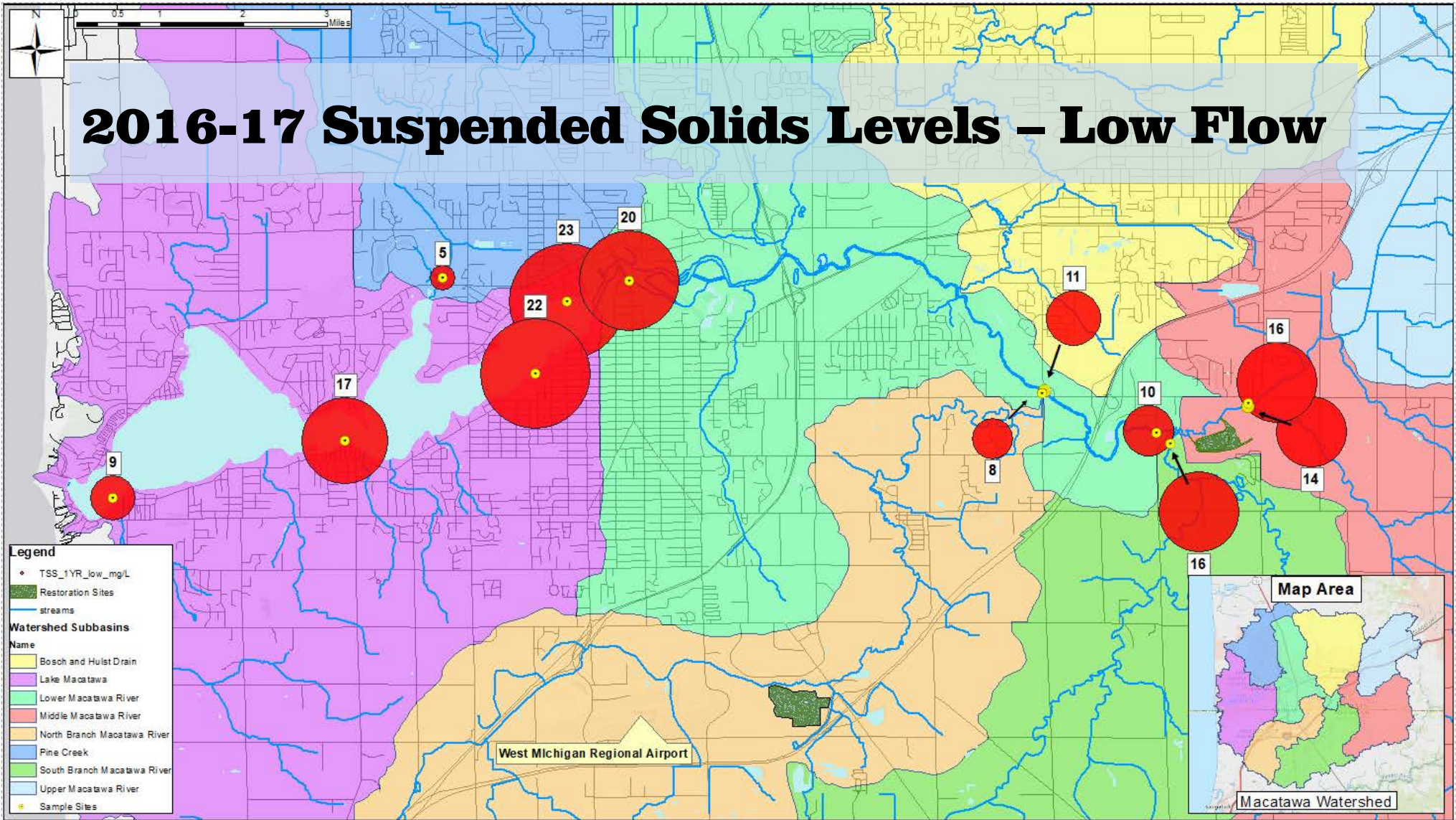




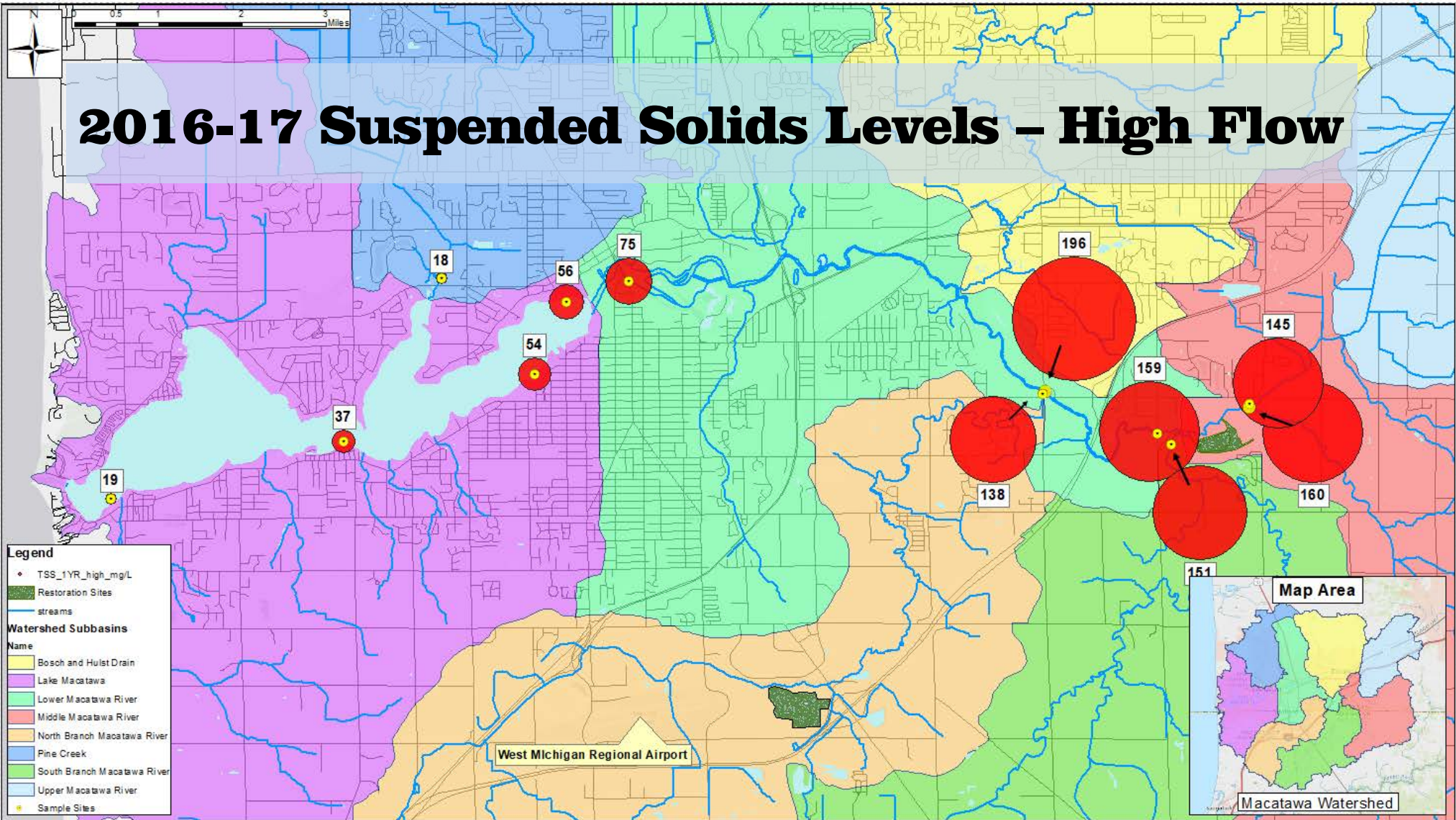
Total Suspended Solids

- Total Suspended Solids (TSS)
- High flow conditions
 - Stream sites on average **higher** than Lake sites
 - Extreme outliers observed in Stream sites
 - Lake site average levels vary by 2-fold range compared to low flow conditions
- Dilution effect from east to west in Lake sites
- Correlated with PO_4^{3-}

2016-17 Suspended Solids Levels - Low Flow



2016-17 Suspended Solids Levels - High Flow



19

37

54

56

75

138

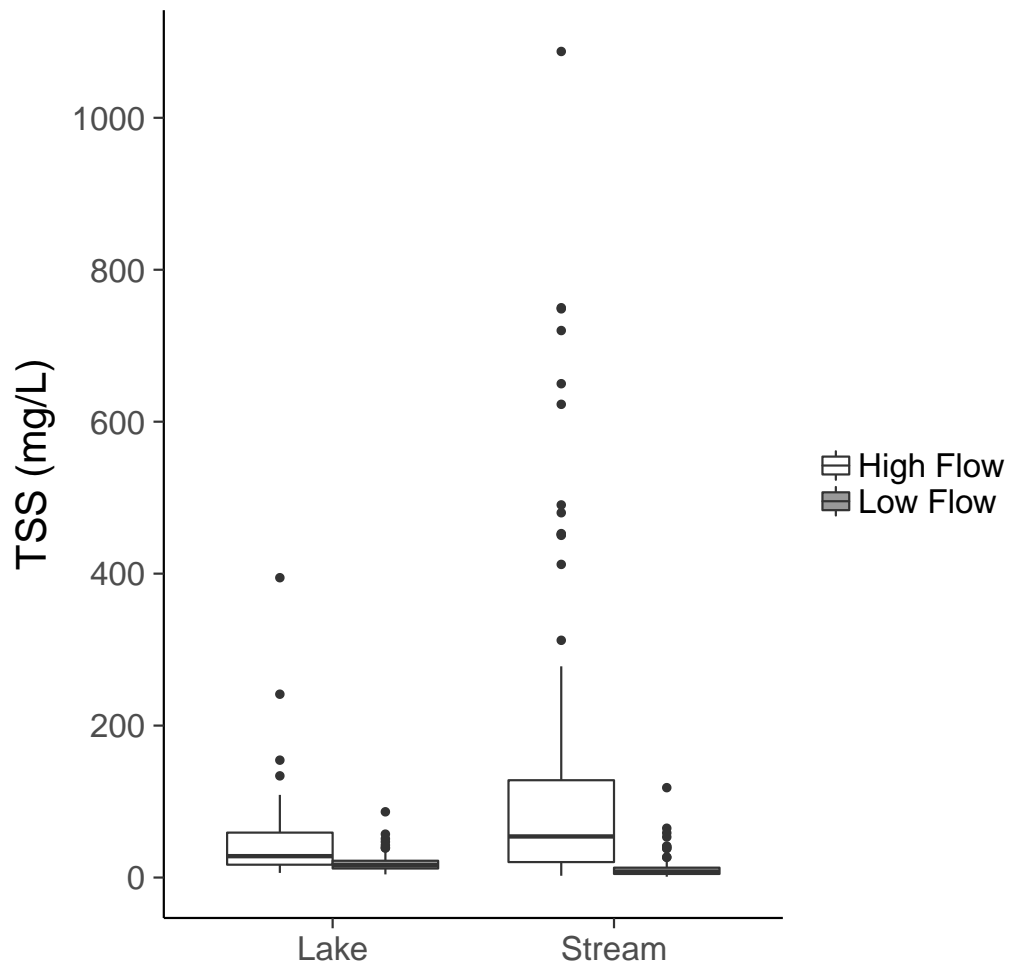
196

159

151

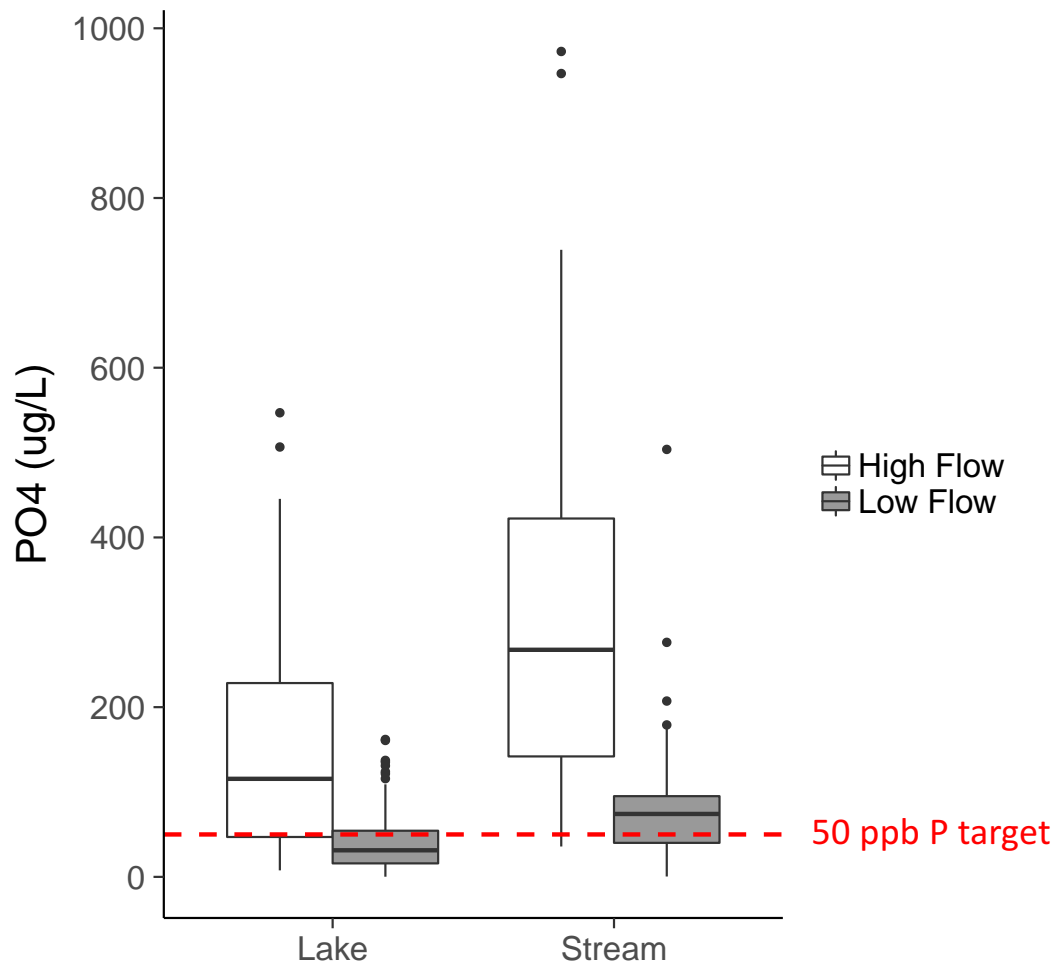
145

160



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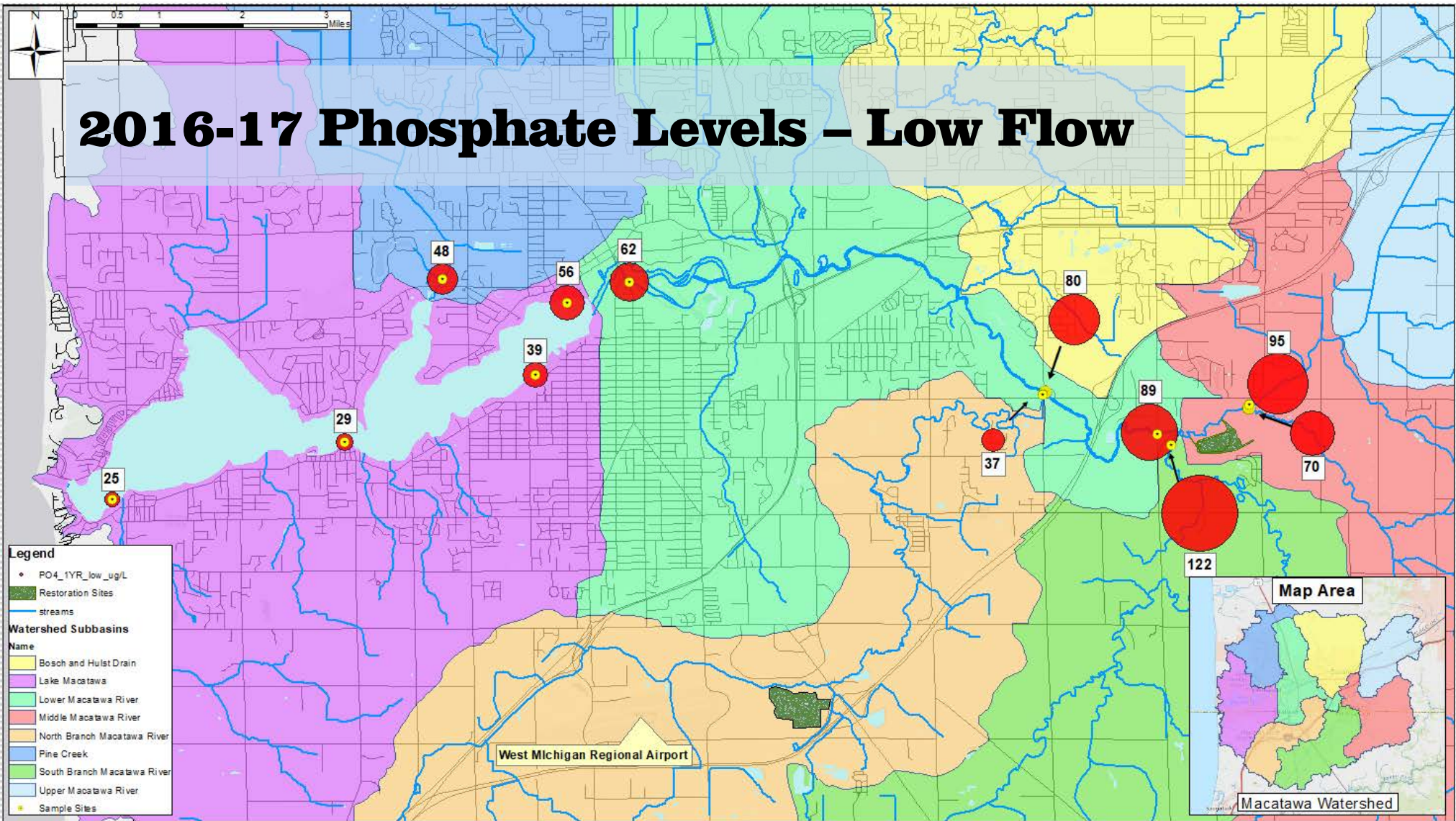


Phosphate Levels

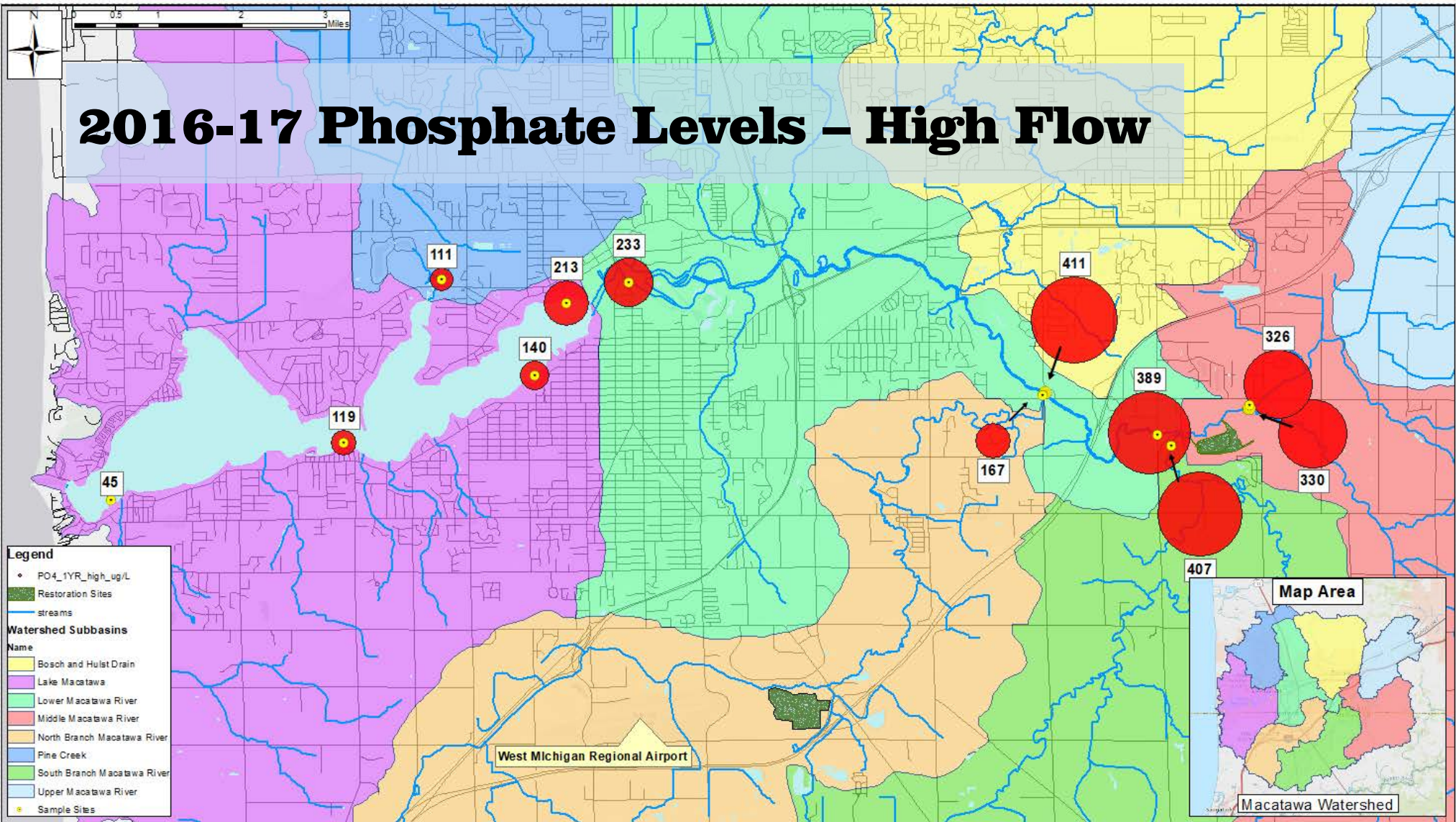
- Goal is under 50 ppb total P (70% reduction)
- Measuring PO₄²⁻ -- underestimates total P
- Low Flow Conditions
 - Lake Sites – generally **below** target
 - Stream Sites – generally **above** target for lake
- High Flow Conditions
 - **All** sites **above** target for lake
 - Increases by 2 to 4-fold at all sites compared to low flow conditions

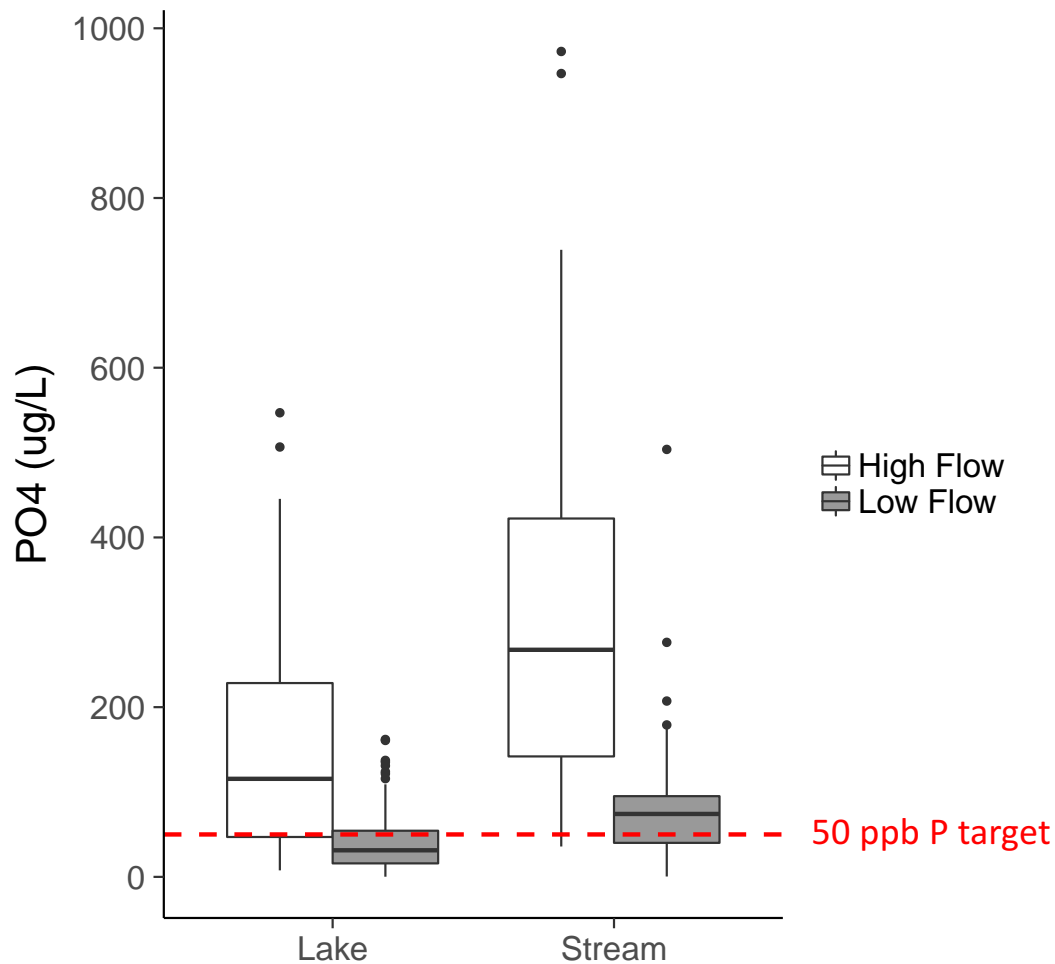


2016-17 Phosphate Levels – Low Flow



2016-17 Phosphate Levels - High Flow

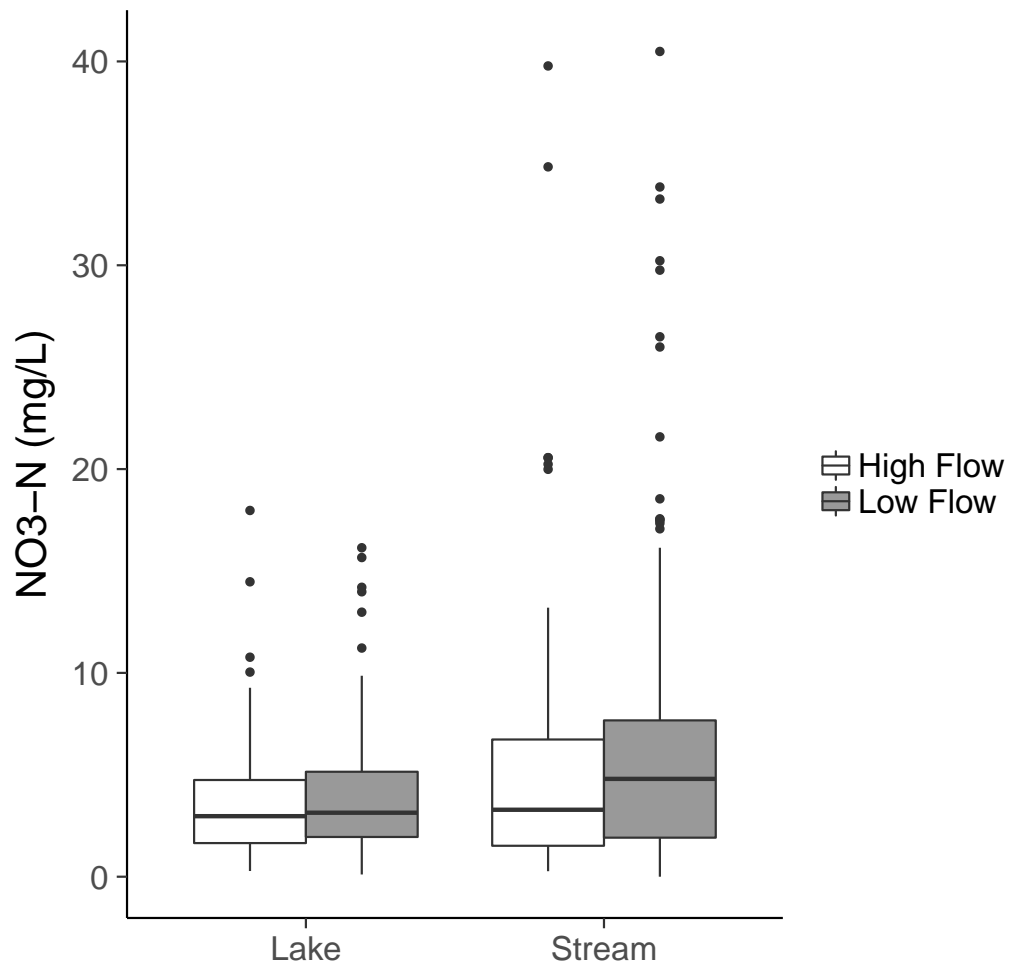




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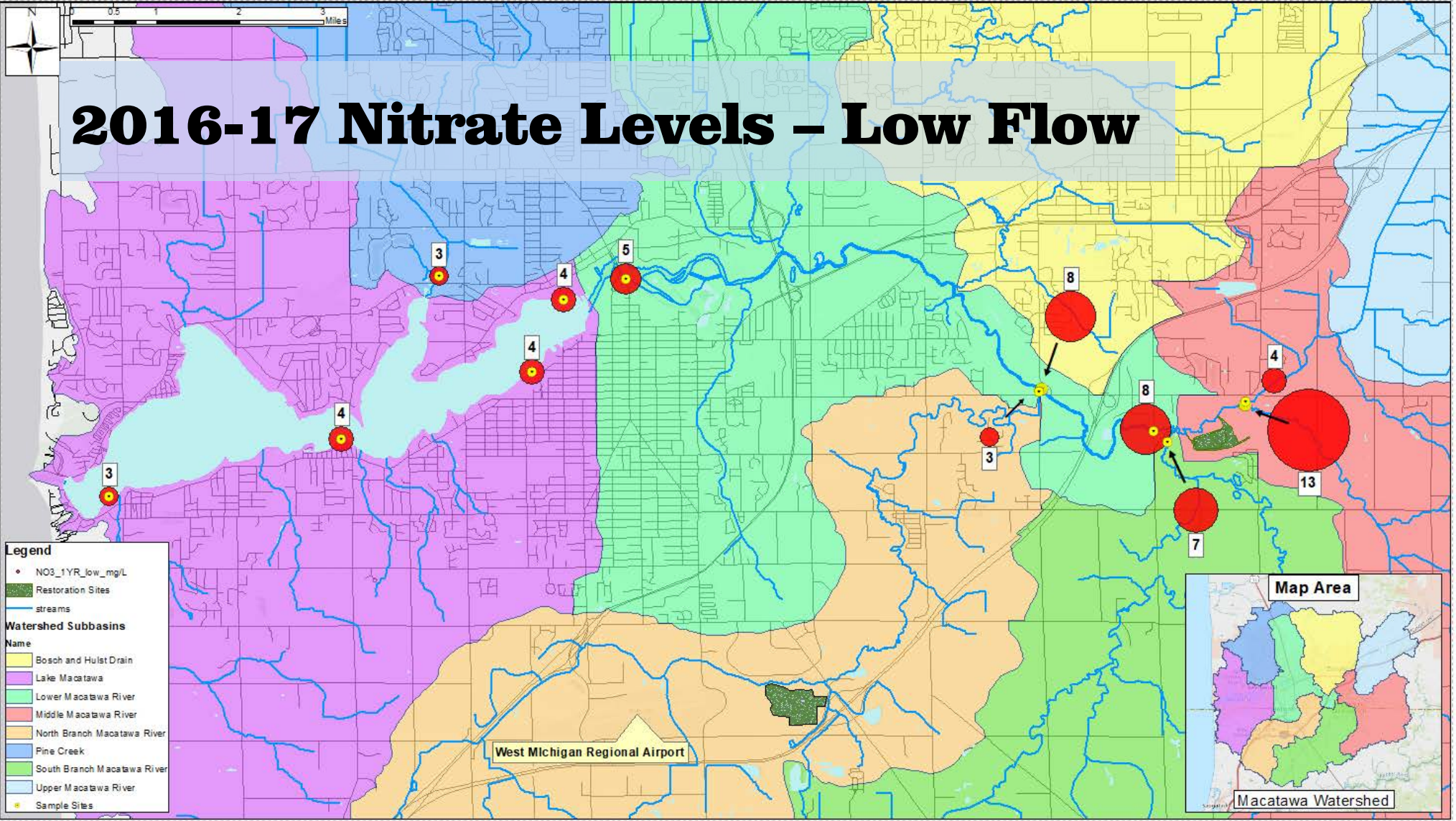


Nitrate Levels

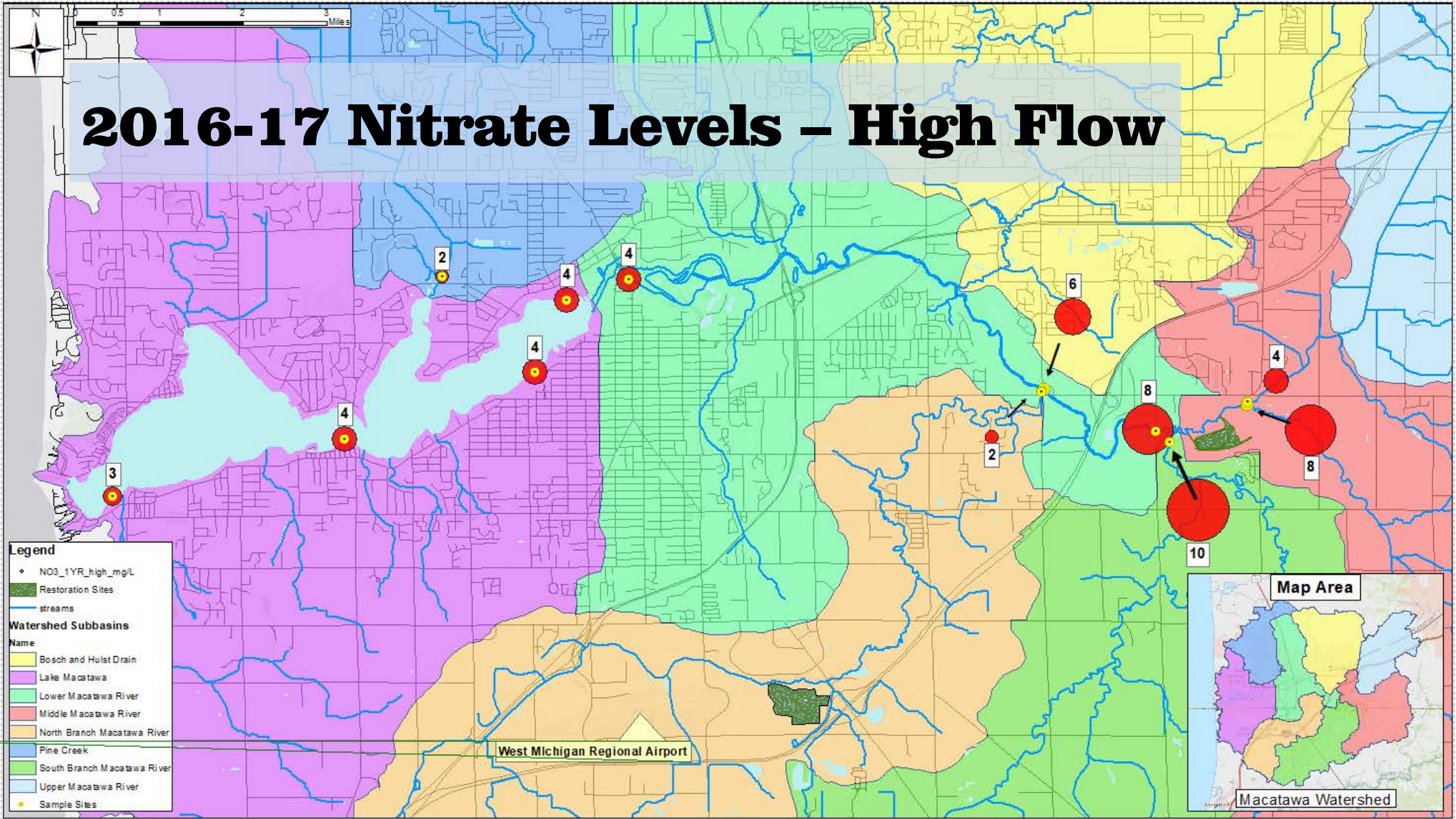
- Drinking water regulated – below 10 ppm
- Not regulated for streams and lakes
- Low Flow Conditions **higher** in both lake and stream sites
- Outlier sampling events with high measured levels
- Can be limiting nutrient in cyanobacterial (algal) bloom formation



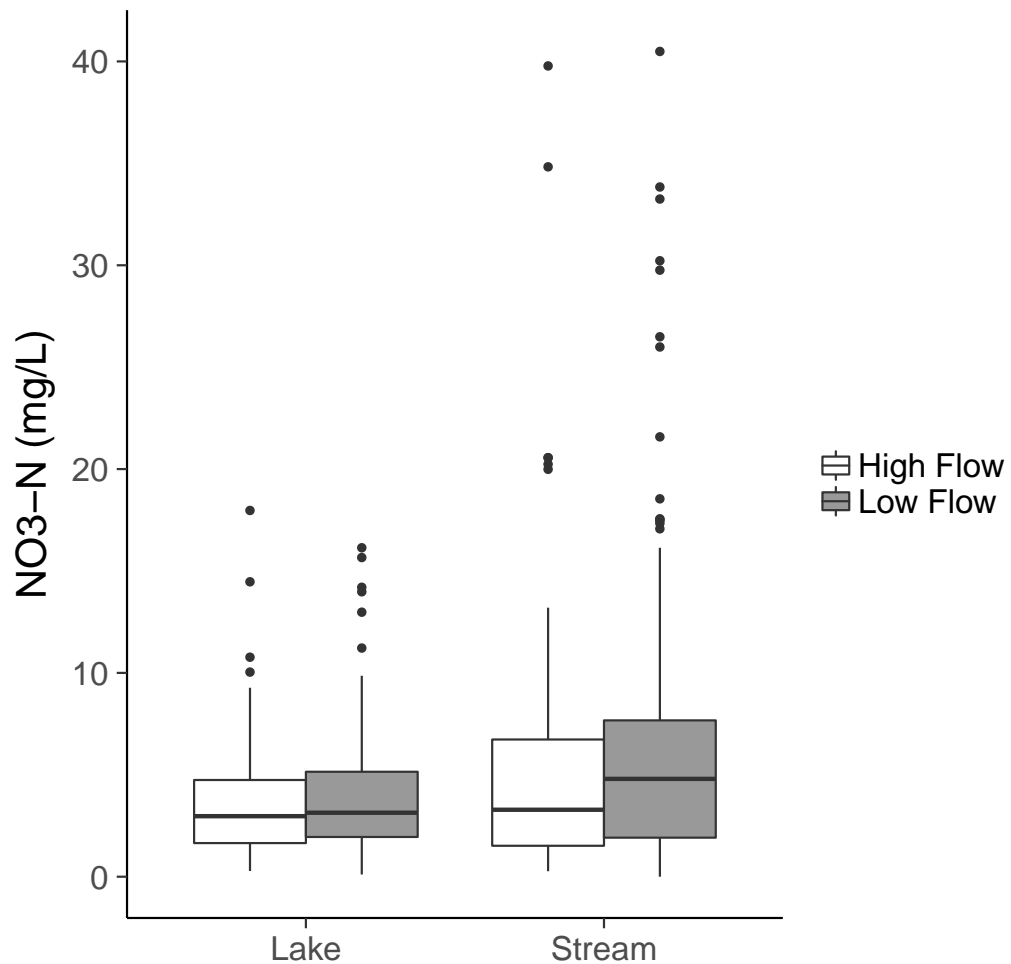
2016-17 Nitrate Levels - Low Flow



2016-17 Nitrate Levels - High Flow



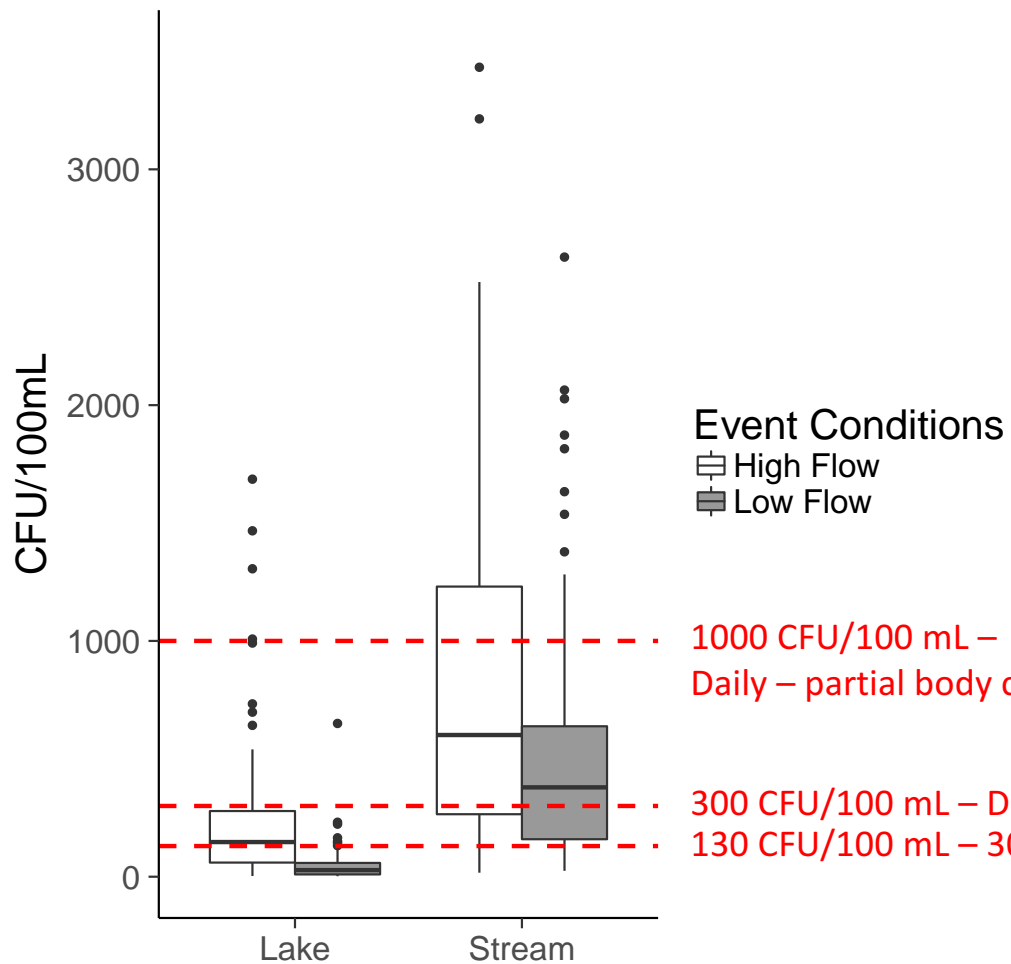
Sample Site Number	Approximate Nitrate Level (mg/L)	Subbasin
2	2	Upper Macatawa River
3	3	Lake Macatawa
4	4	Lake Macatawa
4	4	Lake Macatawa
4	4	Upper Macatawa River
4	4	Upper Macatawa River
6	6	Upper Macatawa River
8	8	Upper Macatawa River
8	8	Upper Macatawa River
10	10	Upper Macatawa River



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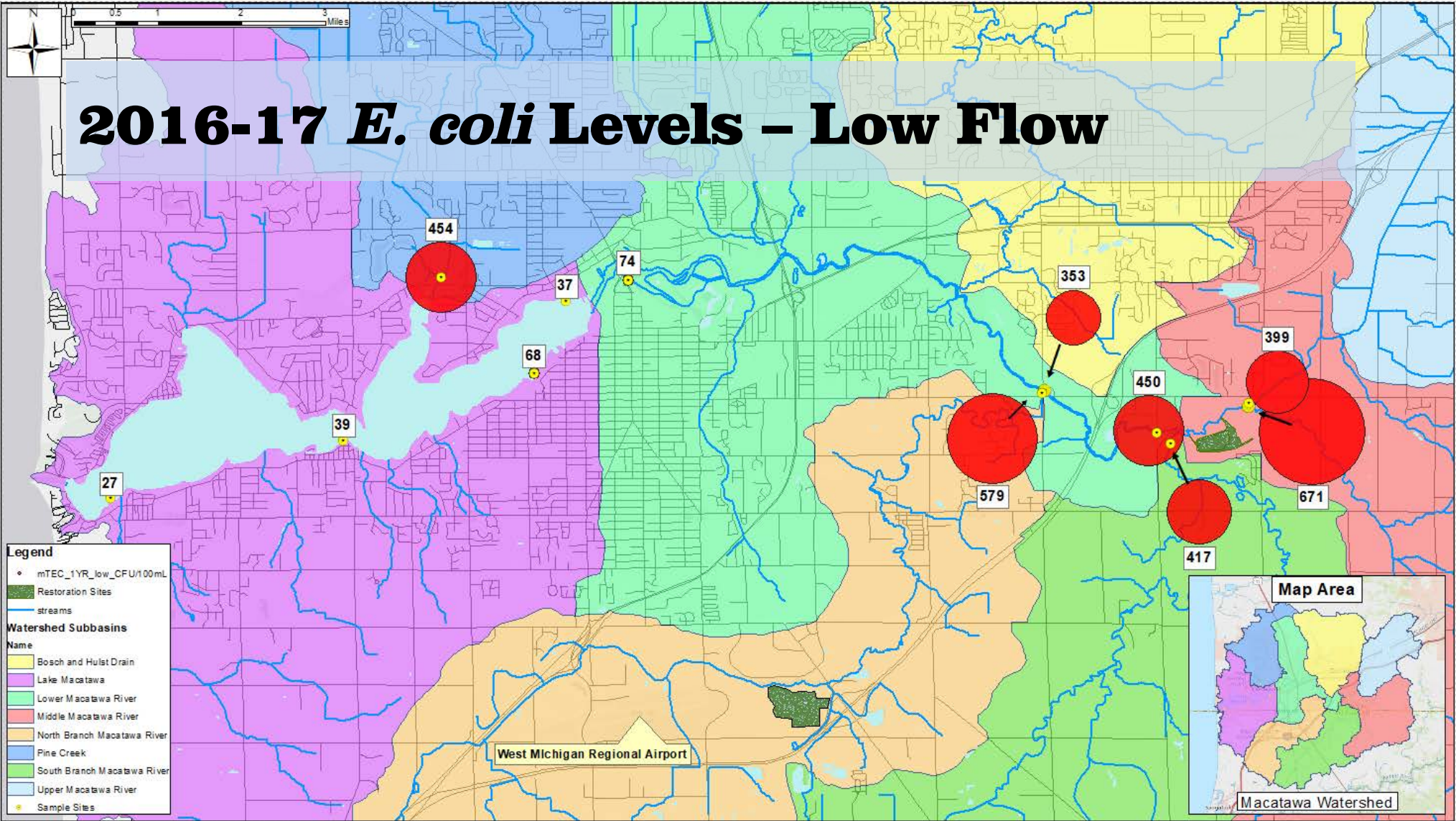


E. coli Counts

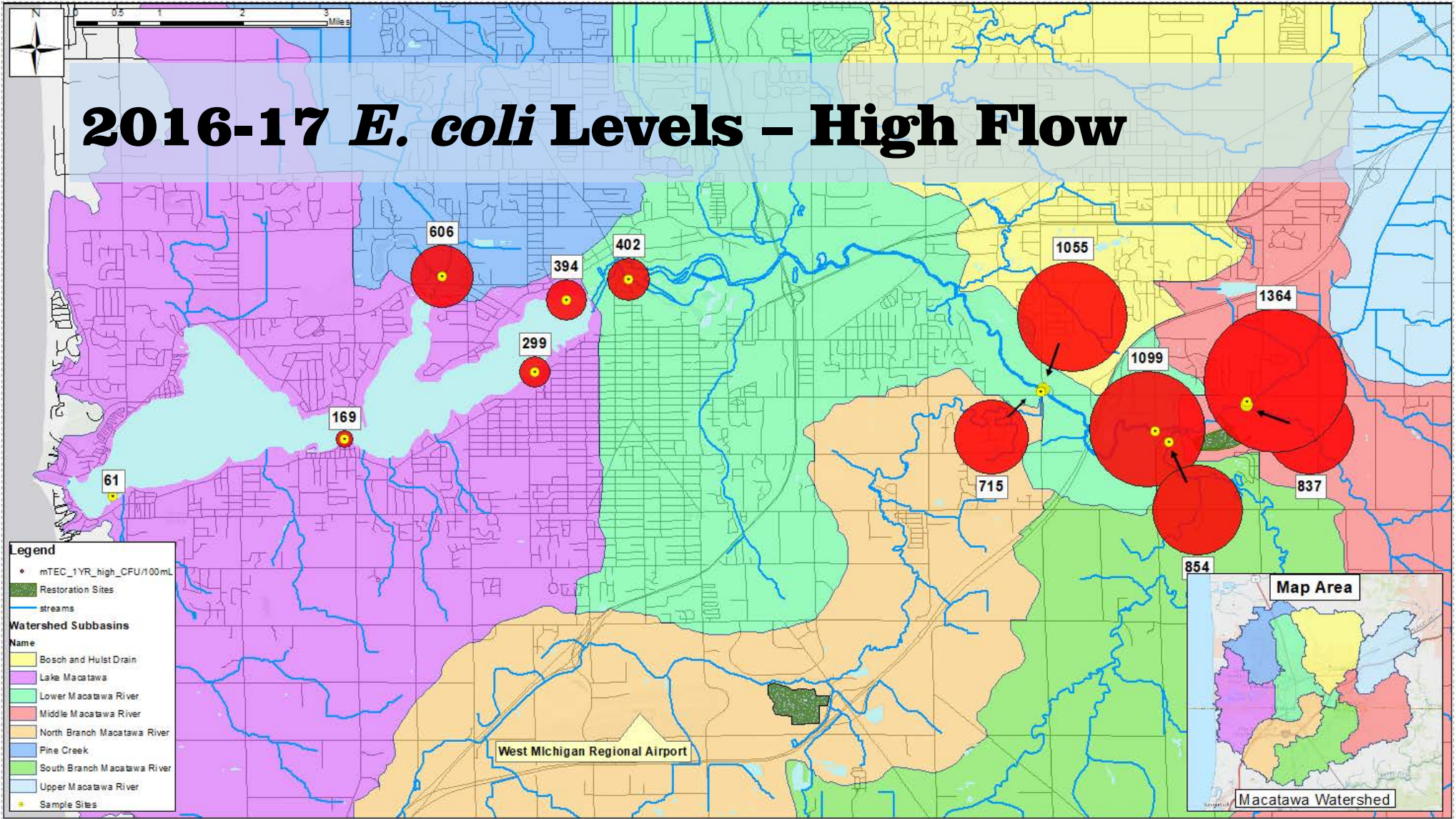
- Colony Forming Units (CFU)
 - EPA Method 1603 (mTEC plate counts)
 - Lake Sites generally **lower** than total body contact limit in high and low flow conditions – with exceptions
 - Stream Sites generally **above** total body contact limit in high and low flow conditions – sometimes well above partial contact limit in high flow conditions
- 1000 CFU/100 mL – Daily – partial body contact limit (All Year)
- 300 CFU/100 mL – Daily – total body contact limit (May to October)
- 130 CFU/100 mL – 30 Day geometric mean – total body contact limit

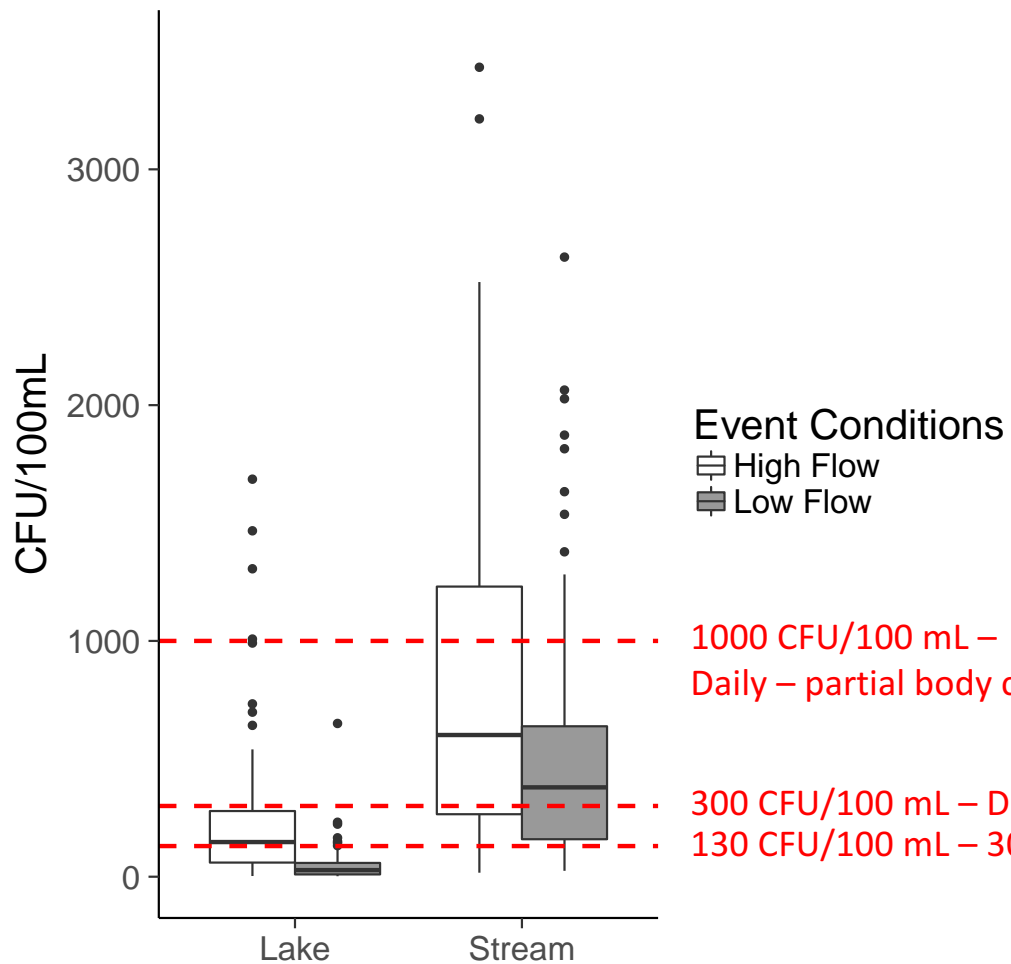


2016-17 *E. coli* Levels - Low Flow



2016-17 *E. coli* Levels - High Flow





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WATER

- Great Lakes
- Drinking Water
- Lakes & Streams
- Aquatic Nuisance Control
- Water Quality Monitoring
- Lakes & Streams Protection
- Marinas
- Related Links
- Wetlands
- MiWaters
- Permits
- Wastewater
- Water Management

DEQ / WATER / LAKES & STREAMS / WATER QUALITY MONITORING

Michigan's Statewide *E. coli* Total Maximum Daily Load

Contact: [Molly Rippke](mailto:molly.rippke@deq.state.mi.us) 517-284-5547

 [Get the latest updates - subscribe to receive TMDLs and Integrated Reporting \(Clean Water Act Sections: 303d, 305b, and 314\) emails.](#)

When a water quality standard is exceeded, the Federal Clean Water Act requires Michigan to address pollution issues with either a [Total Maximum Daily Load \(TMDL\)](#) or by fixing the problem through other means. For some issues, a remedy is already in progress to reduce pollution. This is not the case for *E. coli* across the state. This TMDL will provide a framework for restoration of water quality.

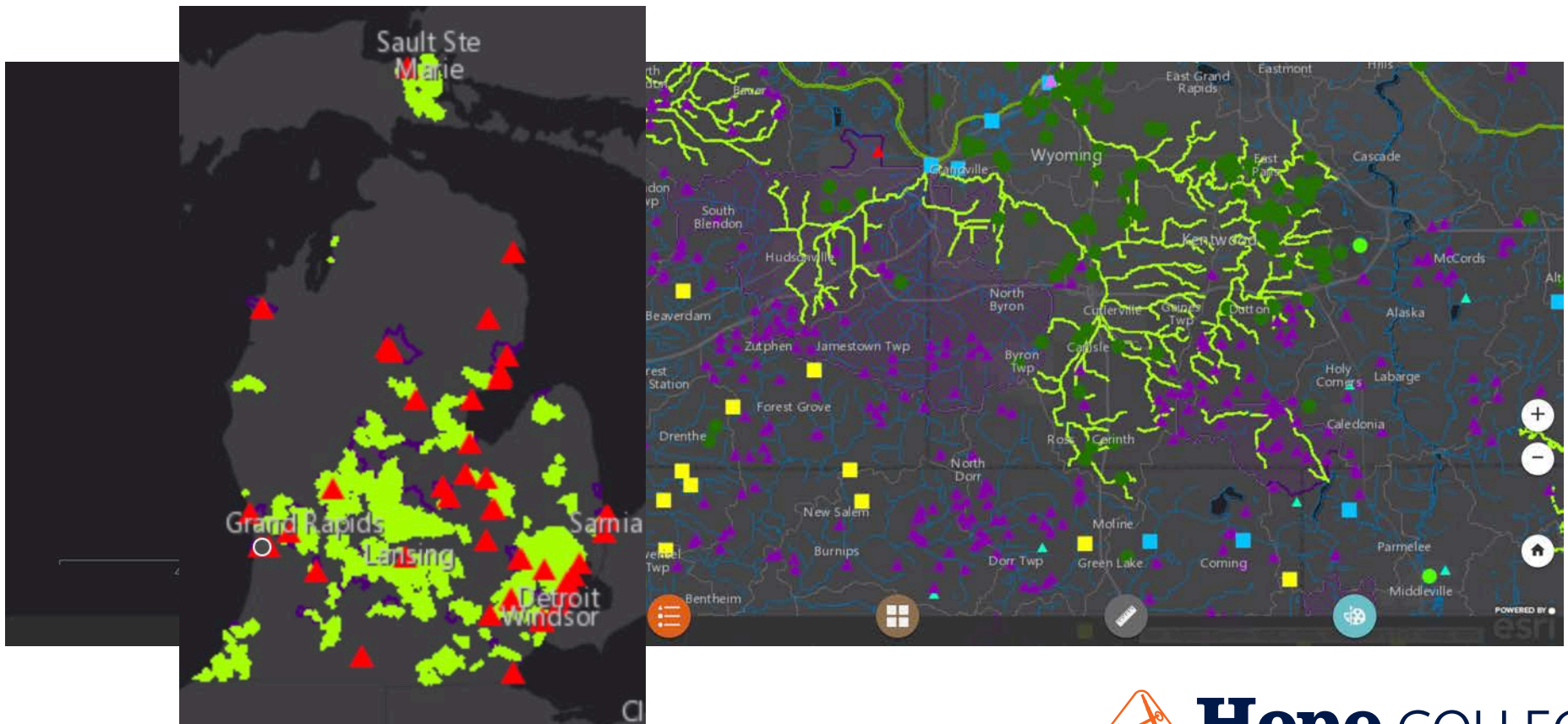
Routine testing has shown *E. coli* levels in many areas are above the standard. These levels increase the risk of illness upon contact or incidental ingestion of the water. Given the extent of this problem, and the multitude of potential sources, a statewide approach will be more effective and more efficient at addressing this issue. To learn more, please visit the [E. coli in Surface Waters website](#).

Long term solutions to bacterial problems can only be accomplished through a collaborative approach. In addition to its work on effective National Pollutant Discharge Elimination System (NPDES) permit requirements and corrective actions on illegal sources, the MDEQ is looking for assistance from landowners, local health departments, conservation districts, other state and local agencies, and environmental groups to focus voluntary improvements in areas where nonpoint sources are a problem. Please see our [Guide for Homeowners](#) to find out how you can help reduce *E. coli* contamination of our rivers, lakes and beaches.

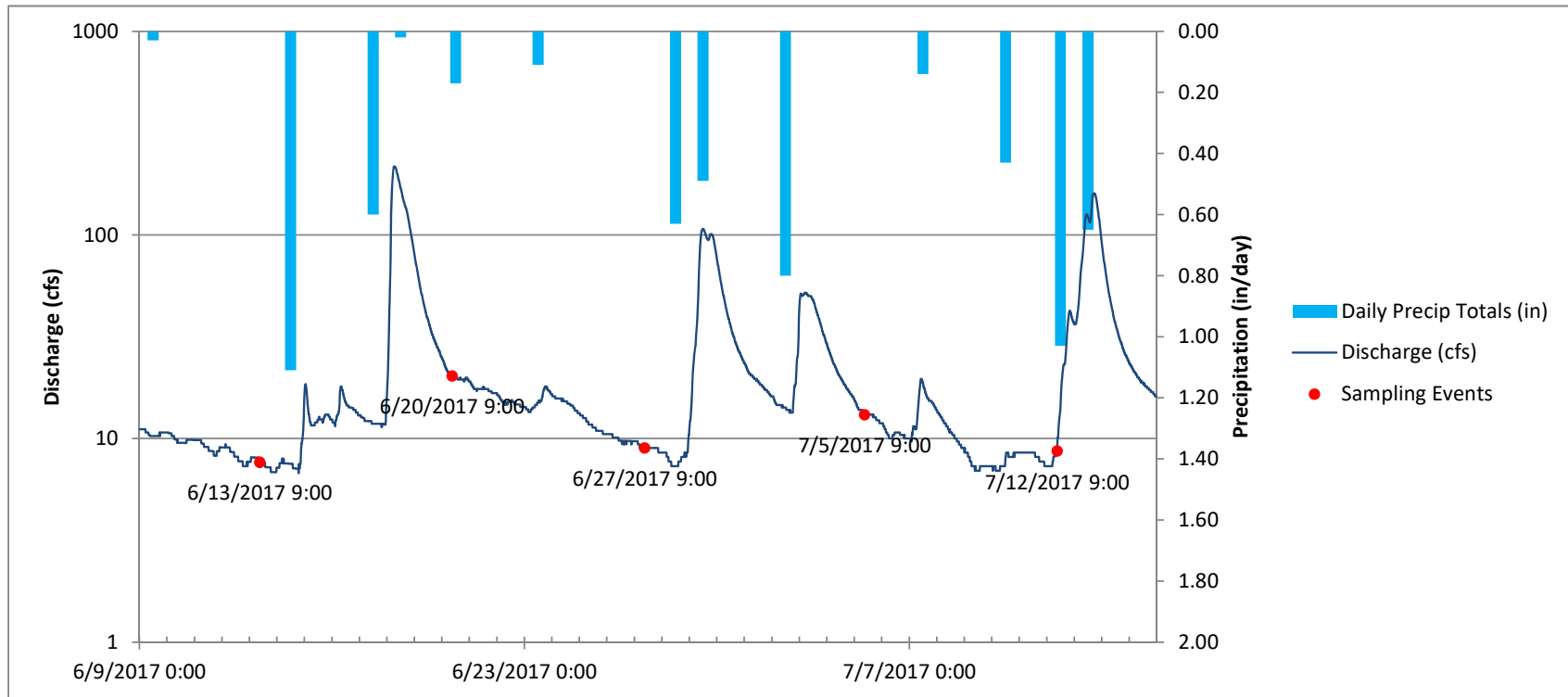


Google Search Term: michigan deq e coli
Also see "E. coli in Surface Waters" web page

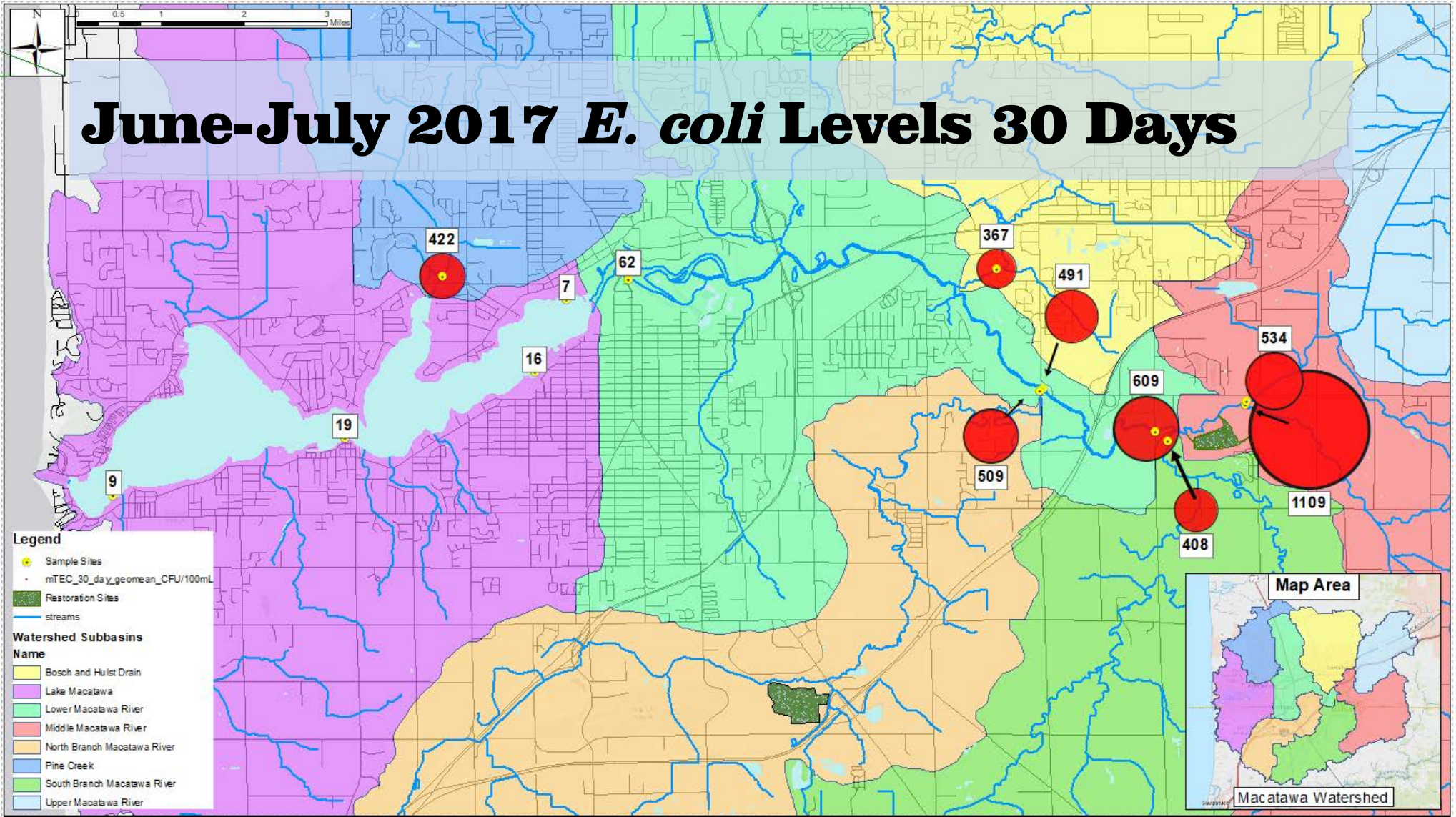
MDEQ Interactive Map – *E. coli* TMDL



Weekly Sampling - June/July 2017



June-July 2017 *E. coli* Levels 30 Days



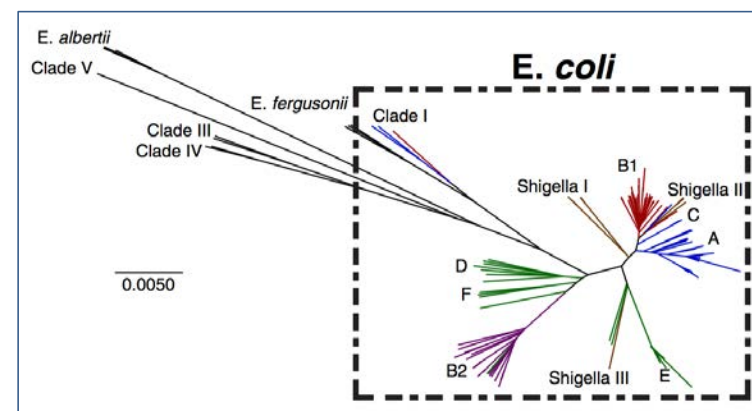
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Not All *E. coli* Are Created Equal

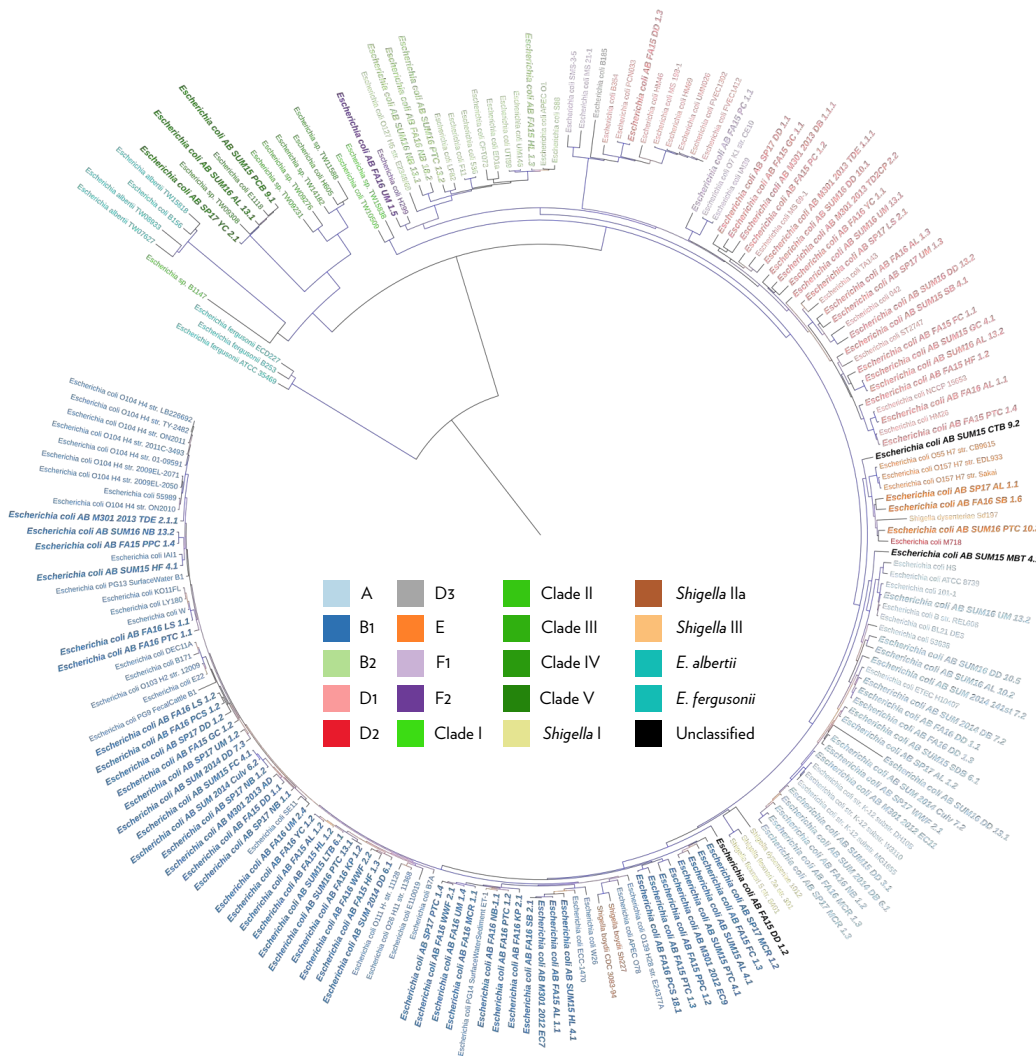
- Model organism for microbiology, genetics and molecular biology
- Commensal organism in animal guts
- Pathogenic Strains – gastrointestinal, UTIs, food poisoning, many classifications (e.g., EPEC, ExPEC, EHEC, ETEC, EAEC, EIEC, UPEC, APEC)
- Used in routine water quality monitoring – EPA and State Guidelines
- Thousands of publically sequenced genomes
 - Vast majority are from clinical sources
 - Highly diverse genus with respect to genome content
 - Evidence for adaptation to secondary environments (e.g., soil, water)
 - Is *E. coli* a good organism to use as proxy for sewage contamination of water resources?



Kaas RS, et al. 2012. BMC Genomics 13:577.

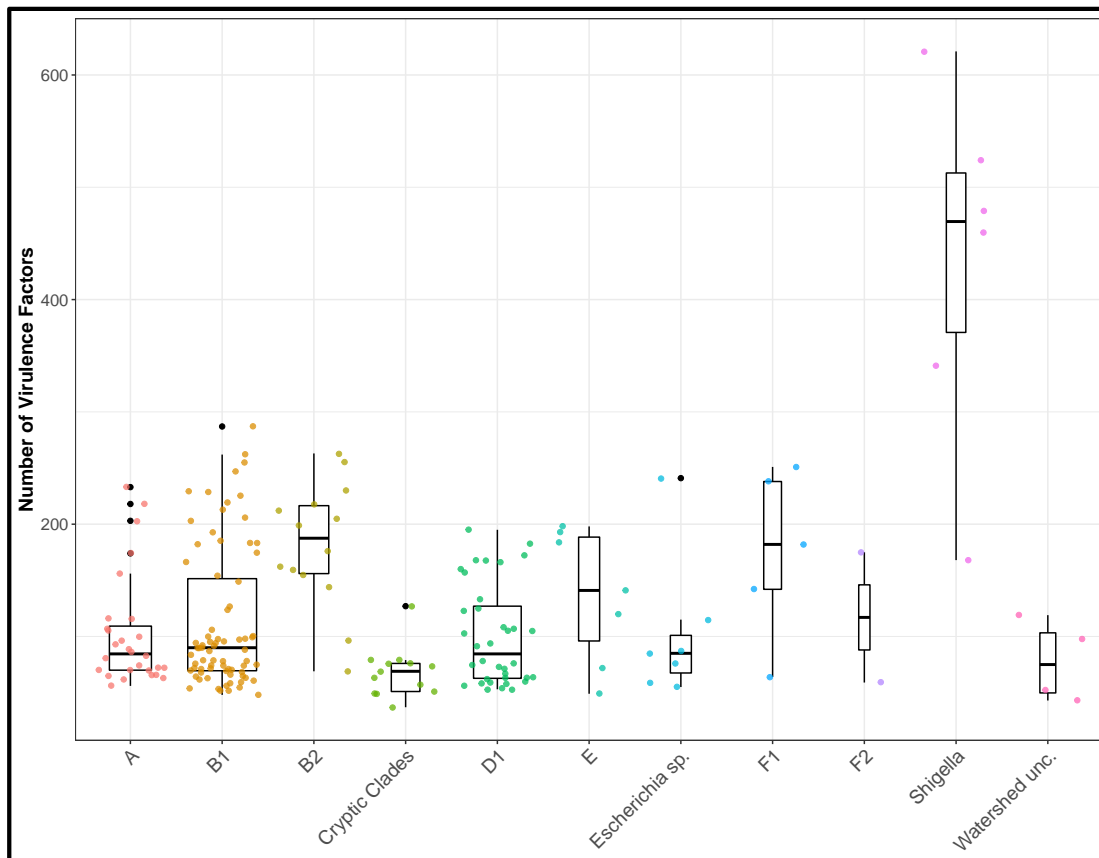


Tree scale: 0.01



- Family Tree of *E. coli*
- 104 watershed strains
- 97 reference strains
 - Environmental
 - Commensals
 - Laboratory
 - Pathogens
- Watershed
 - All subgroups found
 - 46% group B1 – found often in water
 - Present year round

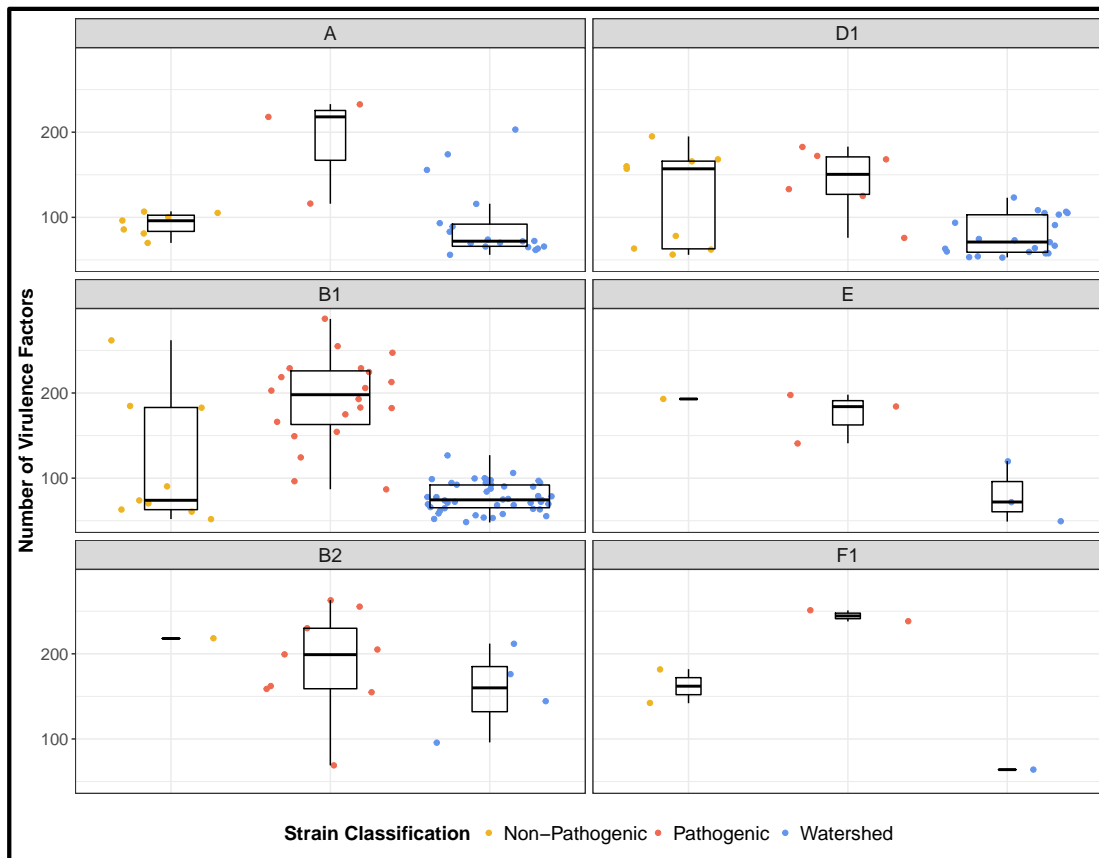
Virulence Factors Summary by Phylogroup



- Virulence Factor Database (VFDB)
- PATRIC (www.patricbrc.org) specialty gene interface
- Groupings include reference and watershed strains
- Average number of VFs for major groups of *Escherichia* and *Shigella* vary significantly (ANOVA)
 - *Shigella* significantly higher than all other groups ($p < 0.01$)
 - Phylogroup B2 significantly higher than phylogroups A, B1, D1 and cryptic clades ($p < 0.01$)
 - Phylogroup F1 significantly higher than cryptic clades ($p < 0.05$)

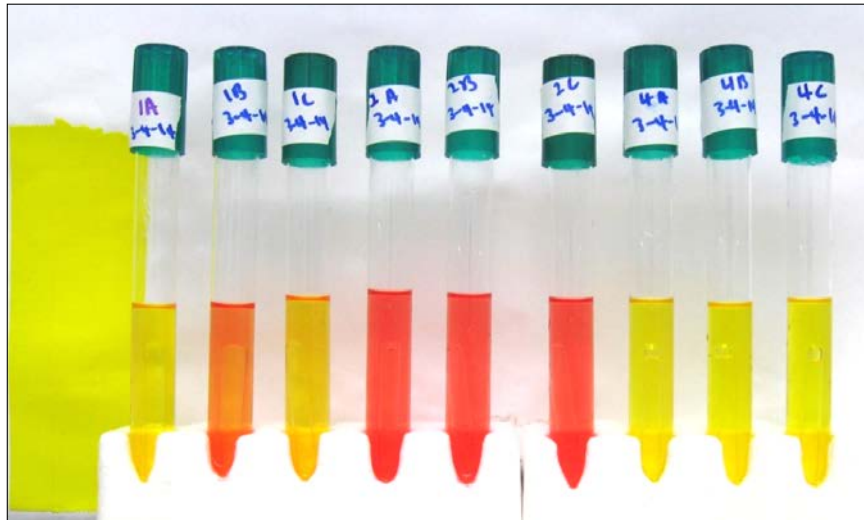


Virulence Factors Summary by Strain Classification

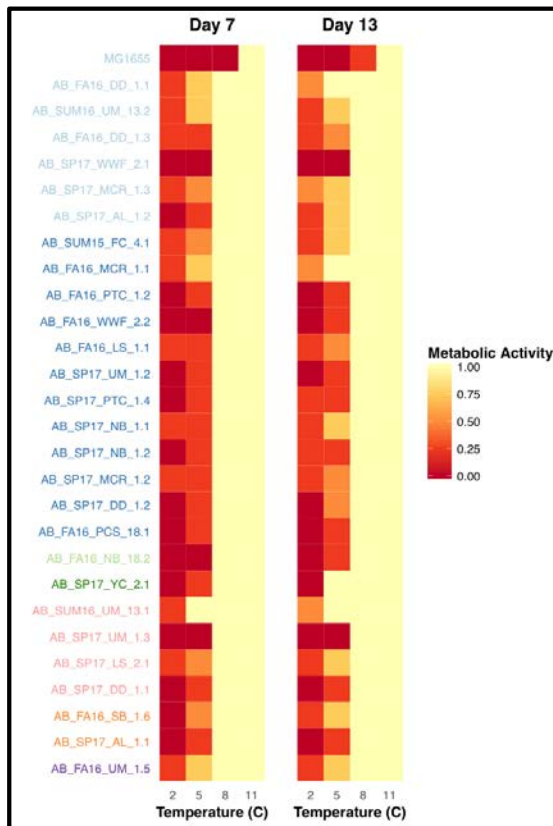


- Virulence Factor Database (VFDB)
- PATRIC (www.patricbrc.org) specialty gene interface
- Subgrouping of All Strains
 - Reference Pathogenic Strains
 - Reference Non-Pathogenic Strains
 - Watershed Strains
- Significant differences in average number of VFs for groups (ANOVA)
 - All phylogroups combined ($p < 0.01$)
 - Phylogroup B1 pathogenic higher than both non-pathogenic and watershed ($p \ll 0.001$)
- Phylogroup A Watershed Strain Outliers – functions of VFs enriched in same categories as for known pathogen reference strains





Physiological Adaptation? Cold Metabolism



Fermentation Day 13	2°C (# of strains)	5°C (# of strains)	8°C (# of strains)	11°C (# of strains)
No Change	12	3	0	0
Weak	12	9	1	0
Weak Acid	4	4	0	0
Acid	0	8	0	0
Strong Acid	0	4	27	28





Hope College students since Fall 2015 – Over 120

Hamilton High School Stream School – 30 students

Holland Christian High School Winterim Internships – 3 students



Acknowledgements

Project Clarity

Travis Williams; Dan Callam; Lynn Kotecki

Naber Family; Koostra Family

Sarah Brokus; Frank Moen; Randy Wade, Chelsea Payne;
Matt Hughes

Dr. Brent Krueger; Dr. Michael Pikaart; Dr. Graham
Peaslee

Dr. Catherine Mader; Sarah Harvin

Herbert H. and Grace. A. Dow Foundation

National Science Foundation

Hope College Departments of Biology and Chemistry

Hope College Day1 Program

Hope College Day1 Watershed Students and TAs 2015-17

Hope College Microbiology Students and TAs 2014-17

Marian Schmidt; Michelle Berry

