

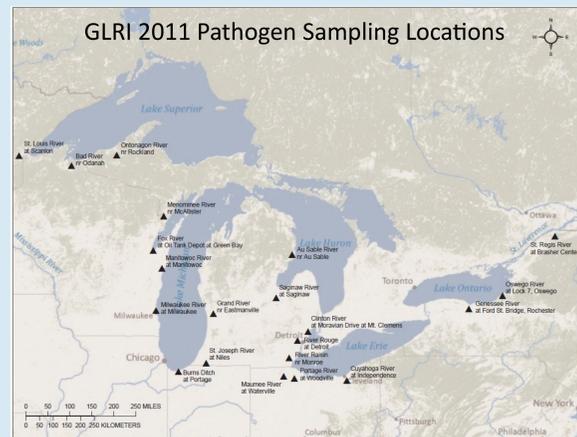
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Introduction

From March through October 2011, the U.S. Geological Survey (USGS) Michigan Water Science Center (MI-WSC), in conjunction with USGS Water Science Centers in Indiana, Minnesota, Ohio, New York, and Wisconsin, conducted a study to determine the frequency of occurrence of genetic markers of bacterial pathogens and densities of fecal indicator bacteria (FIB) in tributaries to the Great Lakes. This project was funded as part of the Great Lakes Restoration Initiative, and included analysis of 21 sampling locations throughout six states that border the Great Lakes.



Methods & Approach

A total of 134 environmental samples were collected at USGS stream gaging locations during high flow, as well as during routine normal/low flow conditions, and analyzed by the Michigan Bacteriological Research Laboratory (MI-BaRL), located at the MI-WSC. The majority of samples were collected according to USGS protocol⁴ using the equal width increment method (EWI) in order to obtain the most representative sample at each site.

Water samples were analyzed for the presence of FIB concentrations (fecal coliform bacteria¹, *Escherichia coli* (*E. coli*)², and enterococci³) according to EPA Standard Methods using standard membrane filtration and serial dilution methods. The results were quantified and calculated as Colony Forming Units (CFU) per 100 mL.

Samples were also analyzed using polymerase chain reaction (PCR) to determine the occurrence of pathogen gene markers for *Shigella* spp., *Campylobacter*, *Salmonella*, and pathogenic *E. coli* including Shiga toxin-producing *E. coli* (STEC).

Differences in log median FIB concentrations were compared using the Kruskal-Wallis test. Differences in pathogen gene frequencies were compared using the Fisher Exact test. A test was considered significant if the p-value < 0.05.

Gene Targets and Associated Organisms

Gene target	Virulence Trait	Method	Organism
<i>eaeA</i>	Attachment	Enumeration/Enrichment	<i>E. coli</i>
<i>stx2</i>	Severe toxin	Enumeration/Enrichment	<i>E. coli</i>
<i>stx1</i>	Moderate toxin	Enumeration/Enrichment	<i>E. coli</i>
<i>rfb0157</i>	Pathogenic strain marker	Enumeration/Enrichment	<i>E. coli</i>
<i>ipaH</i>	Invasion	Enumeration/Enrichment	<i>Shigella</i>
<i>invA</i>	Invasion	Enrichment only	<i>Salmonella</i>
<i>spvC</i>	Plasmid	Enrichment only	<i>Salmonella</i>
16s rDNA (Campy)	Pathogenic strain marker	Enrichment only	<i>Campylobacter jejuni</i> and <i>coli</i>

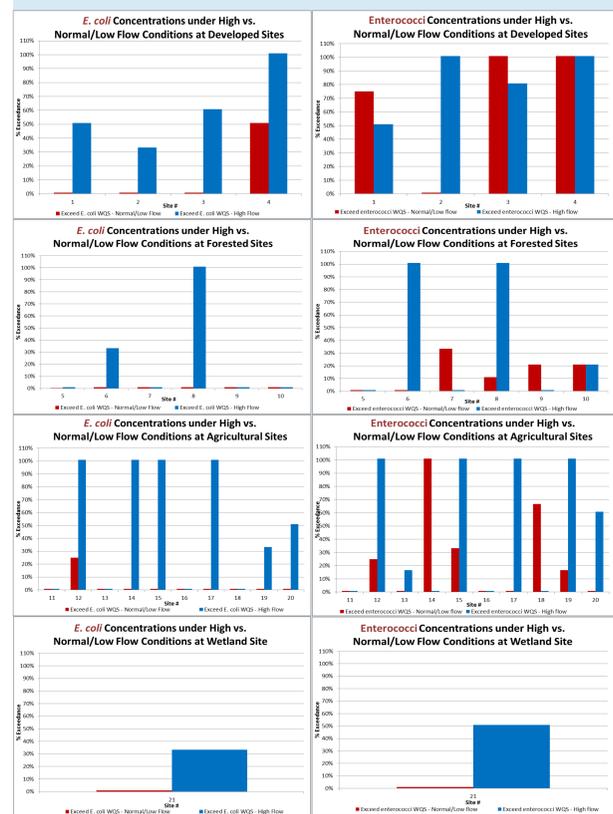
STEC, including *E. coli* O157:H7, can cause illness ranging from mild intestinal disease to severe kidney complications and death in animals and humans. *Shigella* acts similarly to STEC, however mainly affects humans. *Campylobacter* is one of the most common causes of diarrheal illness in the United States, with symptoms including cramping, abdominal pain, and fever. *Salmonella* infection can cause diarrhea, fever, and abdominal cramps, and can even lead to death⁵.

Data Analysis & Results - Fecal Indicator Bacteria Concentrations

Enterococci and *E. coli* were evaluated based on whether or not the data met or exceeded the EPA criteria for Moderate Full Body Contact Recreation for freshwater for a single sample⁶. The 21 sampling locations were divided into predominant land cover categories based on highest land cover percentage, according to the 2006 National Land Cover Database (NLCD)⁷, and include Developed, Forested, Herbaceous Planted/Cultivated, and Wetlands land cover.

Moderate Full Body Contact Recreation Criteria (CFU/100 mL) (EPA 1986) ⁶	
Enterococci	<i>E. coli</i>
78	298

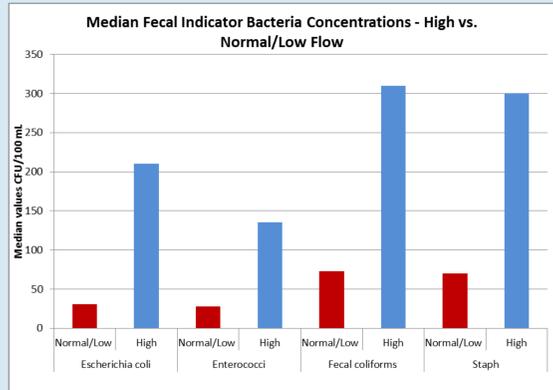
The graphs below represent the relationship between land cover and recreational water quality criteria (RWQC) for *E. coli* and enterococci under different flow conditions.



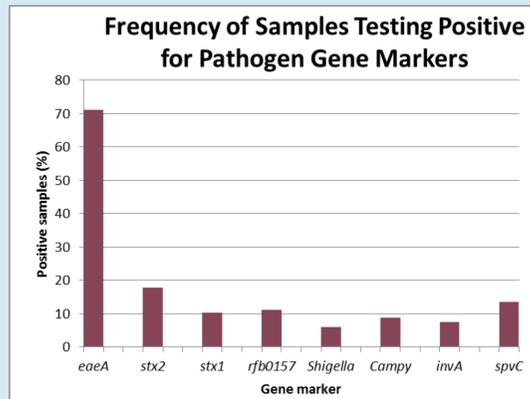
Hydrologic conditions were determined based on mean daily discharge at each USGS station on the date of collection, calculated from date of first record to current, and evaluated such that ≥75th percentile was considered high flow, and <75th percentile was considered normal/low flow.

There was a significant difference in median FIB concentrations between all 21 sampling locations (Kruskal-Wallis). Also, the median densities of FIB were significantly higher in samples collected during high flow than in those collected during normal/low flow at the same sites, as identified by the Kruskal-Wallis rank test (SYSTAT 13).

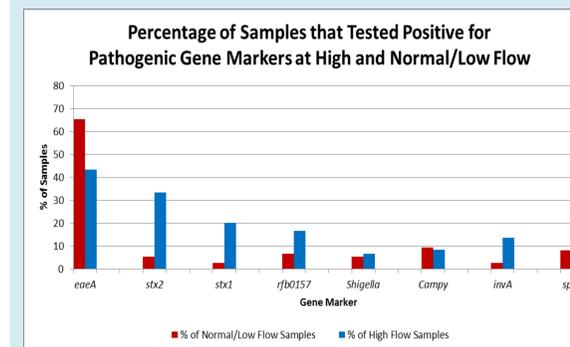
The lowest *E. coli* concentrations were recorded at the Au Sable River near Au Sable, MI (during high and normal/low flows), and the St. Joseph River at Niles, MI (during normal/low flow). The highest *E. coli* concentrations were recorded at the River Rouge at Detroit, MI (during high flow). The lowest enterococci concentrations were recorded at the Au Sable River near Au Sable, MI (during high and normal/low flows), and the Oswego River at Lock 7 at Oswego, NY (during high flow). The highest enterococci concentrations were recorded at the River Rouge at Detroit, MI (during high flow).



Data Analysis & Results - Pathogen Testing



Pathogen frequency was analyzed in relation to hydrologic condition among all sites as illustrated in the following graph.



Overall, the occurrence of the *eaeA*, *rfb0157*, *ipaH* (*Shigella*), and *Campy* genes were not significantly different between samples collected at high and normal/low flow conditions. However, there was a significantly higher frequency of *stx2*, *stx1*, *spvC*, and *invA* genes identified in high flow versus normal/low flow conditions.

Percentage of Pathogen Gene Markers by Hydrologic Condition

Hydrologic Condition	n	<i>eaeA</i>	<i>stx2</i>	<i>stx1</i>	<i>rfb0157</i>	<i>ipaH</i>	<i>Campy</i>	<i>invA</i>	<i>spvC</i>
High Flow	60	43.33	33.33	20.00	16.67	6.67	8.33	13.56	20.34
Normal/Low Flow	75	65.33	5.33	2.67	6.67	5.33	9.33	2.67	8.00
p-value	—	ns	<.05	<.05	ns	ns	ns	<.05	<.05

ns=not significant
p-value < 0.05 indicates significant difference between groups

The frequency of pathogen gene occurrence in samples that met and exceeded the RWQC for *E. coli* and enterococci was compared between all 21 sites. The percentage of samples that exceeded the RWQC are identified in the table below.

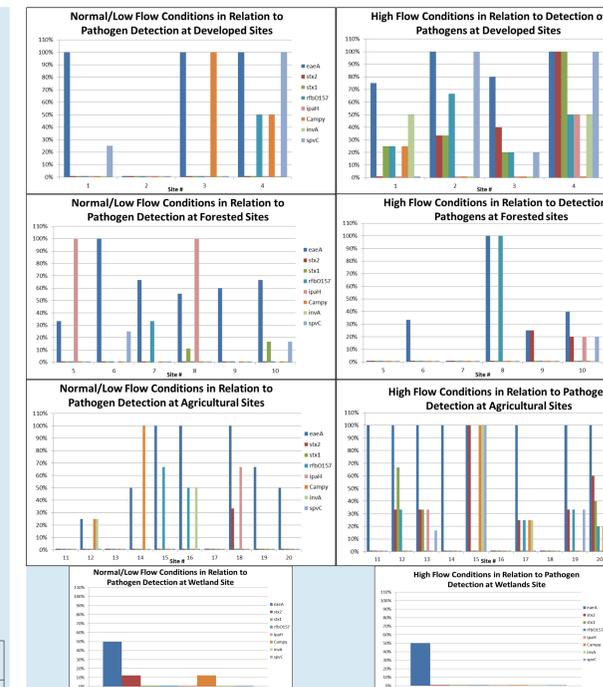
Percentage of Pathogen Gene Markers by RWQC

RWQC	n	<i>eaeA</i>	<i>stx2</i>	<i>stx1</i>	<i>rfb0157</i>	<i>ipaH</i>	<i>Campy</i>	<i>invA</i>	<i>spvC</i>
Meet <i>E. coli</i> stds	109	.65	.11	.04	.06	.06	.05	.02	.08
Exceed <i>E. coli</i> stds	25	.96	.48	.40	.32	.04	.24	.32	.36
p-value	—	<.05	<.05	<.05	<.05	ns	<.05	<.05	<.05

ns=not significant
RWQC = Recreational Water Quality Criteria for Moderate Full Body Contact
p-value < 0.05 indicates significant difference between groups

Overall, there was a greater occurrence of pathogens in samples that exceeded the RWQC for both *E. coli* and enterococci. However, there was no significant difference between the occurrence of the *ipaH* gene in relation to the RWQC.

The relation between land cover and the frequency of occurrence of pathogenic bacteria was also analyzed. The pathogenic bacterial gene concentrations varied greatly based on hydrologic event and land cover, as can be seen in the following graphs.



Conclusions & Future Work

- The analysis indicated that the median densities of FIB were significantly higher in samples collected during high flow conditions than in those collected during normal/low flow under different land cover classifications, as well as amongst all sampling locations.
- Based on land cover, enterococci concentrations more often exceeded the RWQC during normal/base flow conditions than did *E. coli*.
- Overall, there was a greater occurrence of pathogens in samples that exceeded the RWQC for both *E. coli* and enterococci.
- The pathogenic bacterial genes indicating toxin producing *E. coli* and pathogenic *Salmonella* (*invA* & *spvC*) were more prevalent in samples collected under high flow conditions. The flow related gene frequencies were affected variably by land cover.

The results of this study will be used to improve the understanding of microbiological water quality in Great Lakes tributaries with the future goal of determining the relations between the occurrence of bacterial pathogens, FIB, seasonality, water chemistry, and hydrology.

Results of the study will also help determine if there is a correlation between beach closures and FIB concentrations, pathogenic bacterial concentrations, and loads on those rivers that enter the Great Lakes near public beaches.

Great Lakes resource managers may also use the results of this study to determine the risks to human health through recreational activities in rivers, as well as develop future monitoring plans for pathogens.

References

¹U.S. Environmental Protection Agency, 1989, Drinking water-National Primary Drinking Water Regulations - Total coliforms (including fecal coliforms and *E. coli*). Federal Register, v. 54, no. 124, p. 27544-27568
²U.S. Environmental Protection Agency, 2006a, Method 1603 "Escherichia coli in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar". Washington, D.C., EPA-821-R-06-009, p. x-x
³U.S. Environmental Protection Agency, 2006b, Method 1600 "Enterococci in water by membrane filtration using membrane-Enterococcus indoxyl-β-D-glucoside agar (MTE)". Washington, D.C., EPA-821-R-06-009, p. x-x
⁴Shelton, L.R., 1994, Field guide for collecting and processing stream-water samples of the National Water Quality Assessment Program. U.S. Geological Survey Open File Report 94-455, 42 p.
⁵Center for Disease Control and Prevention: Escherichia coli, 1986-Ambient Water Quality Criteria for Bacteria: Washington, DC, EPA 440/5-84-002/
⁶Dufour, A.P., and Ballantine, R.K., 1986-Ambient Water Quality Criteria for Bacteria: Washington, DC, EPA 440/5-84-002/
⁷U.S. Geological Survey, 2011, NLCD 2006 Land Cover, accessed April 3, 2012, at http://www.mdc.gov/nlcd06_data.php.