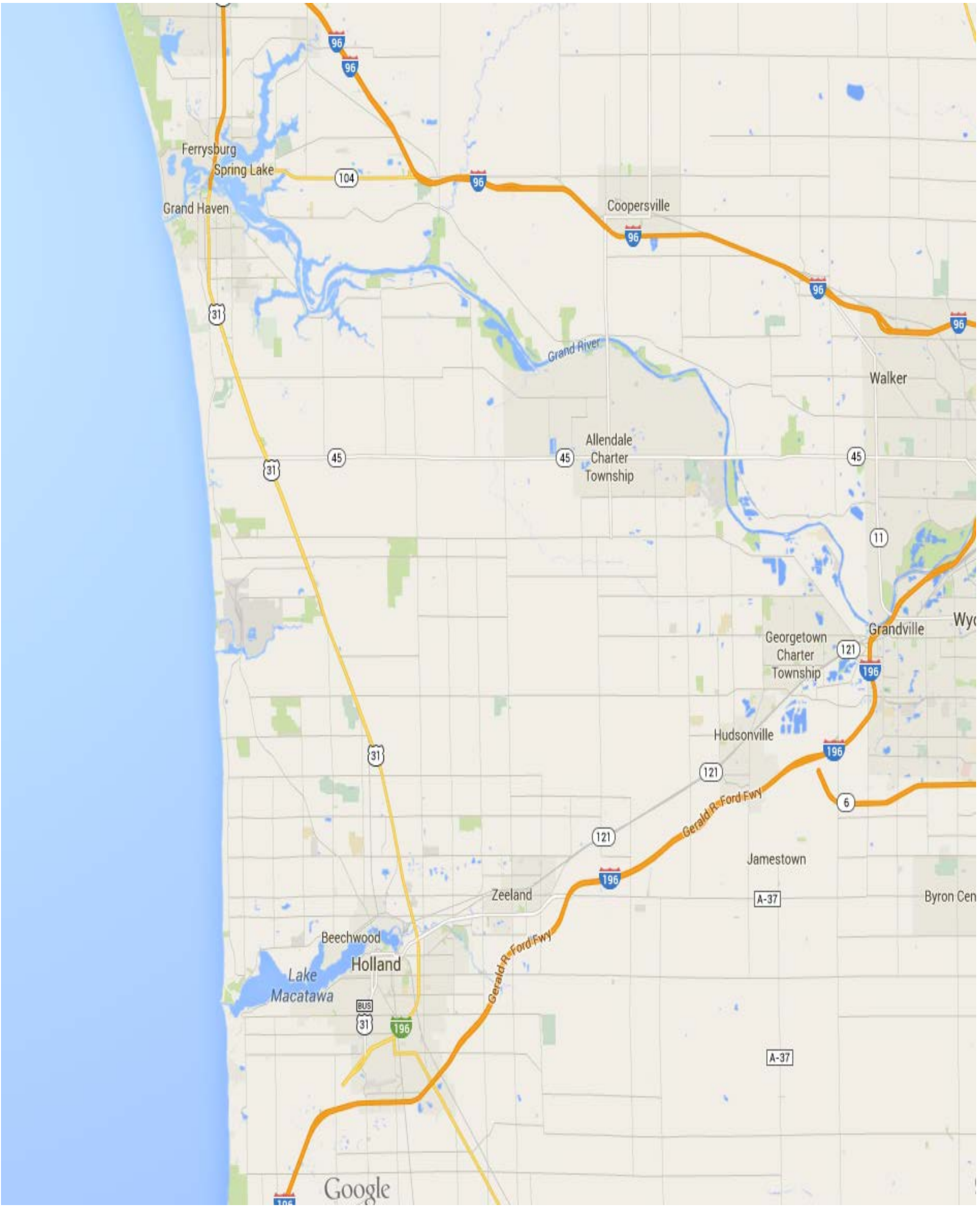
Update on E coli and Source Tracking Monitoring of Grand River and Lake Macatawa Watersheds



Michael Pikaart, PhD

Hope College

Local watershed
Potential Pollution Sources & Pollutants

- Combined Sever Over Flow
- Sanitary Sever Over Flow
- Failing Septic Tanks
- Illicit Discharges
- Storm Water Discharge
- Agriculture Runoff
- Industrial Discharge

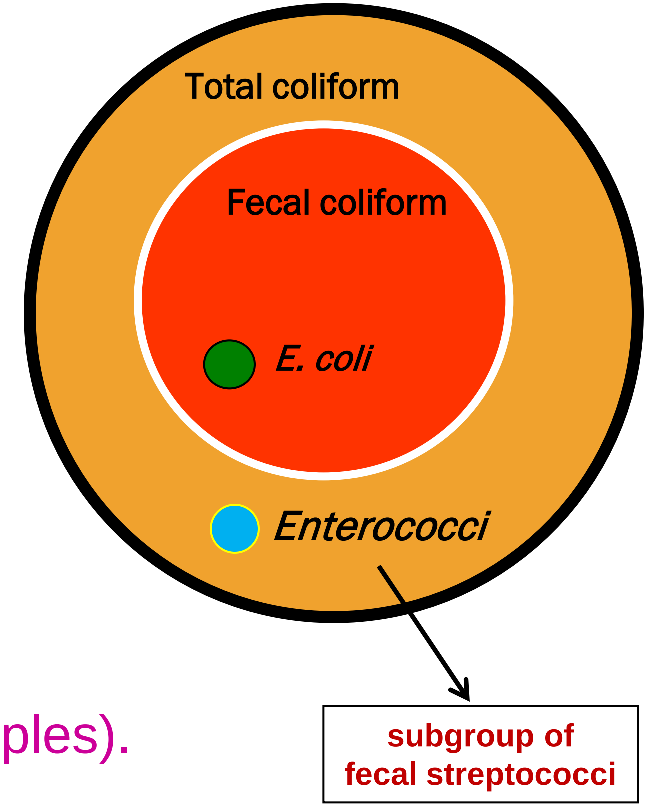
- Pathogens
  (Bacteria, Virus, Protozoans)
- Nutrients
- Sedimentation
- Agricultural chemicals
  (Fertilizer, Pesticides)
- Road Chemicals (Oil, Salt)

Water Quality Standards Based on FIB

1950–1972 **1000 Total Coliform/100 ml**

1972–1986 **200 Fecal Coliform/100 ml**

1986–2012… **Health Based (Epidemiology)**



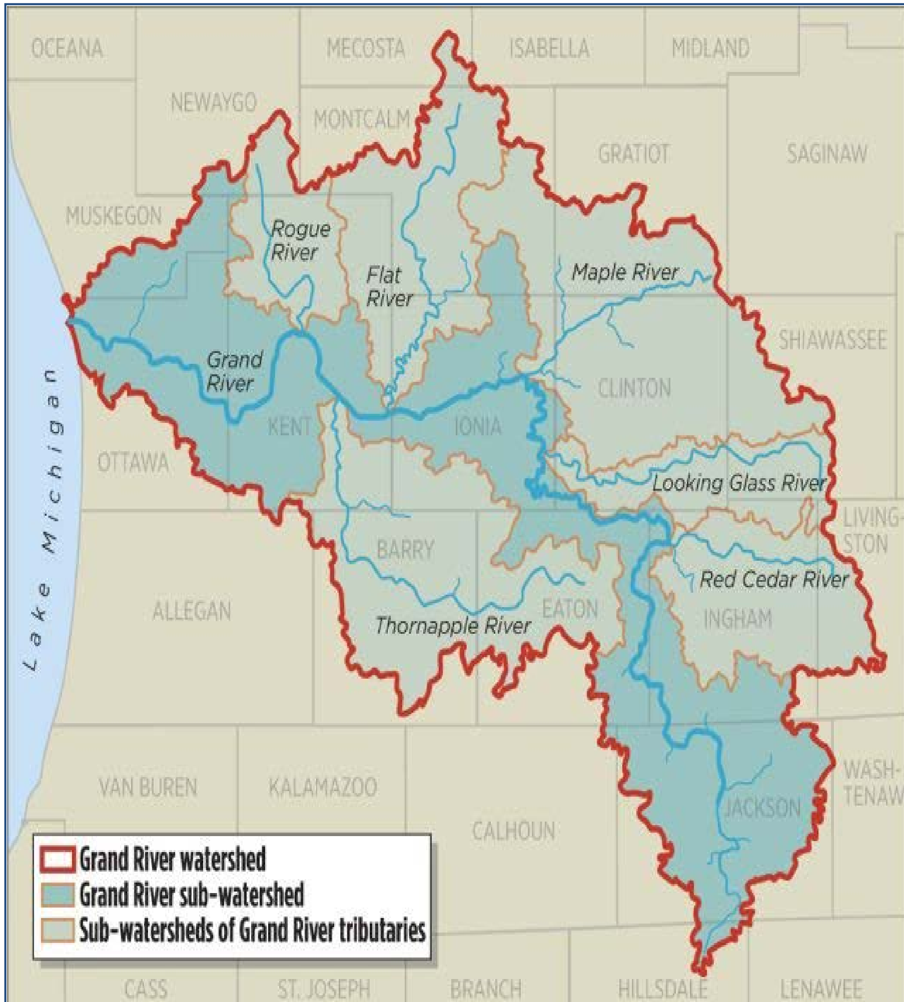
Michigan Beach Water Quality Standards

Single sample standard : 300 CFU/100ml of *E. coli*
(daily geometric mean of at least three samples).

130 CFU *E.coli*/ 100 mL
(monthly geometric mean of at least 5 sampling events)

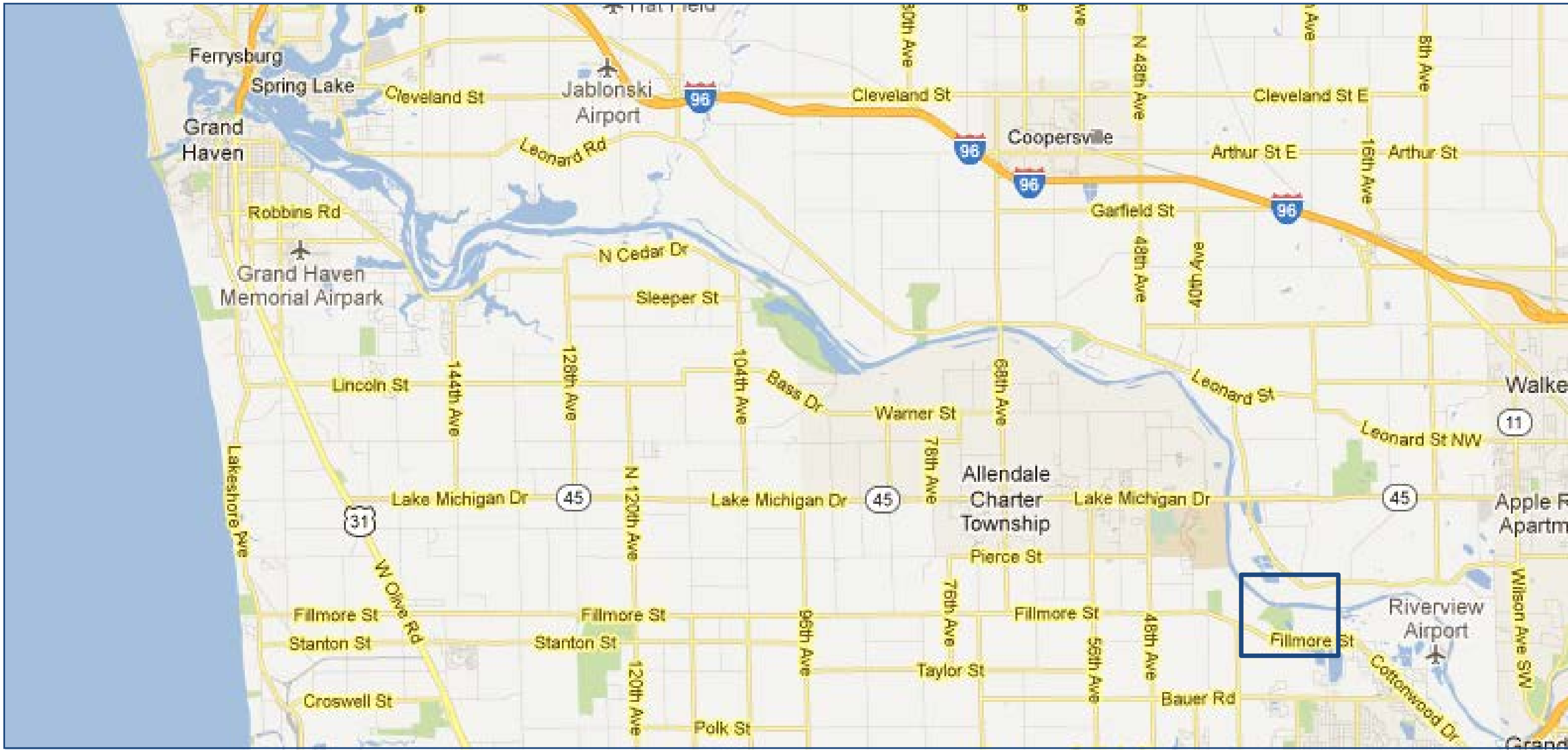
Michigan Water Quality Standards for recreational beaches are slightly different from the EPA's criteria.

Grand River Watershed



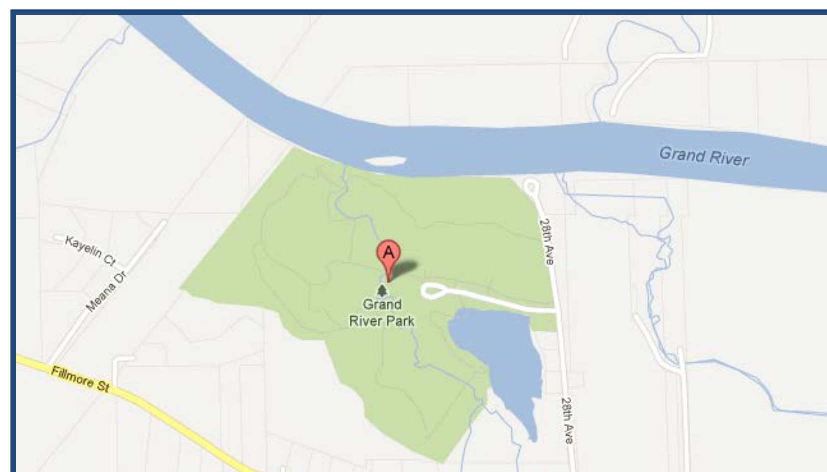
- **Runs 252 miles (406 km)**
- **Through the cities of Jackson, Eaton Rapids, Lansing, Grand Rapids, and Grand Haven and empties into Lake Michigan.**
- **Grand River watershed drains an area of 5,572 square miles (14,430 km^2).**
- **Including 18 counties and 158 townships.**

Samples collected summer 2012 (Vijay Kannappan of OCHD)



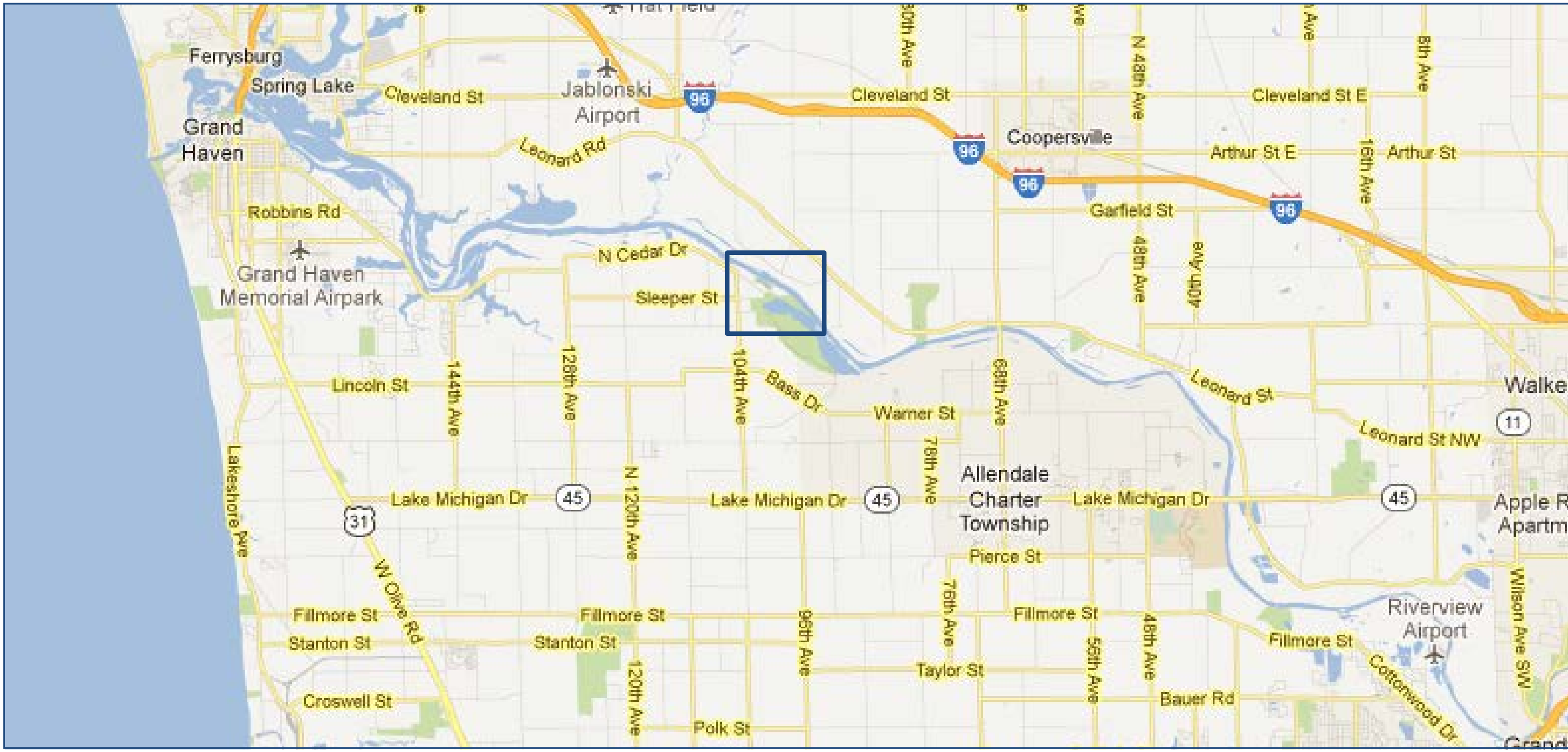
Grand River Study Sites

GR1 : Grand River Park (George Town)



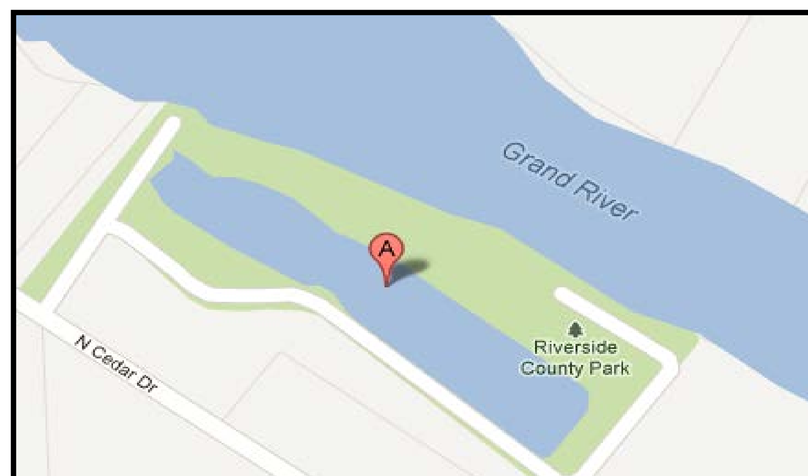
Grand River Study Sites

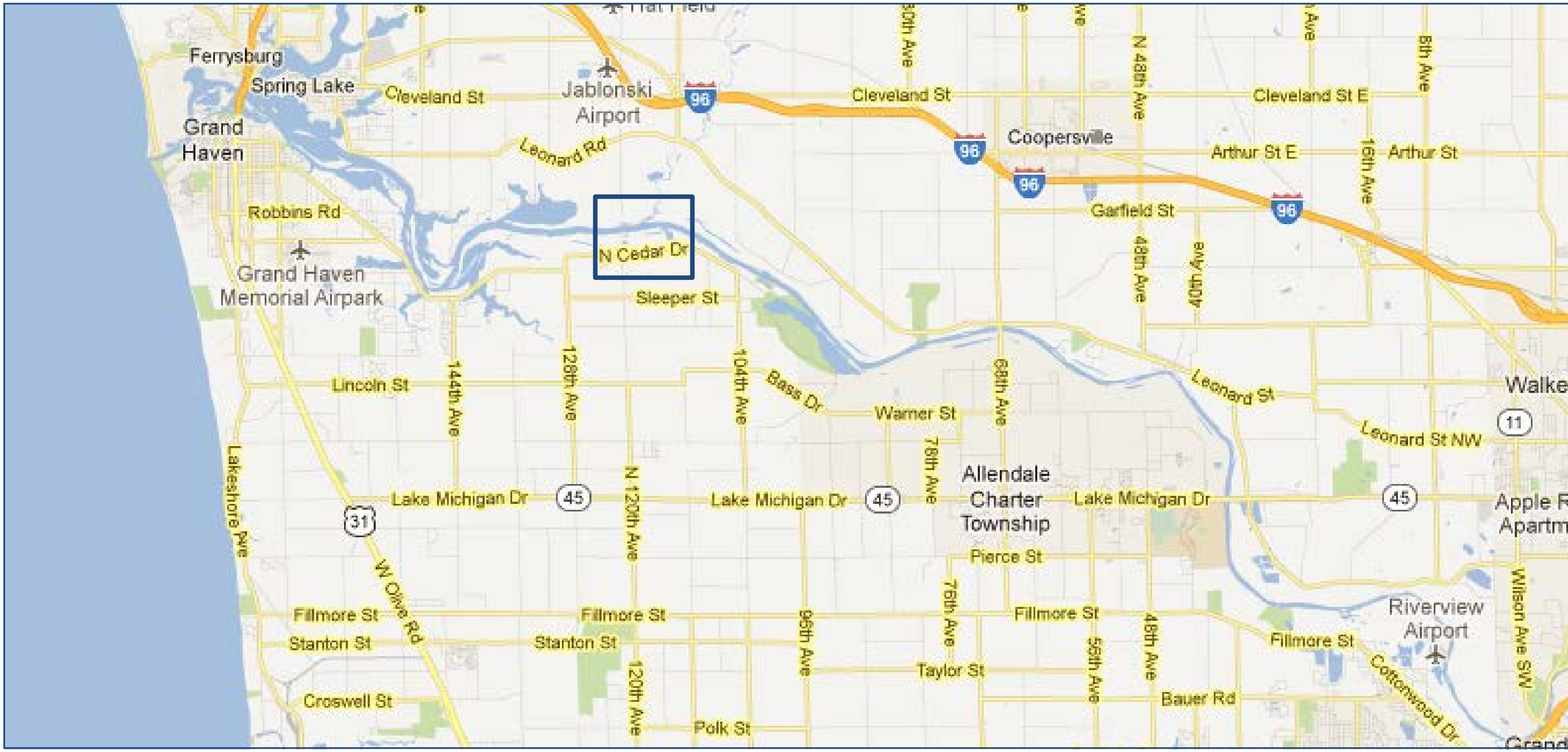
GR2 : Deer Creek Park (Allendale)



Grand River Study Sites

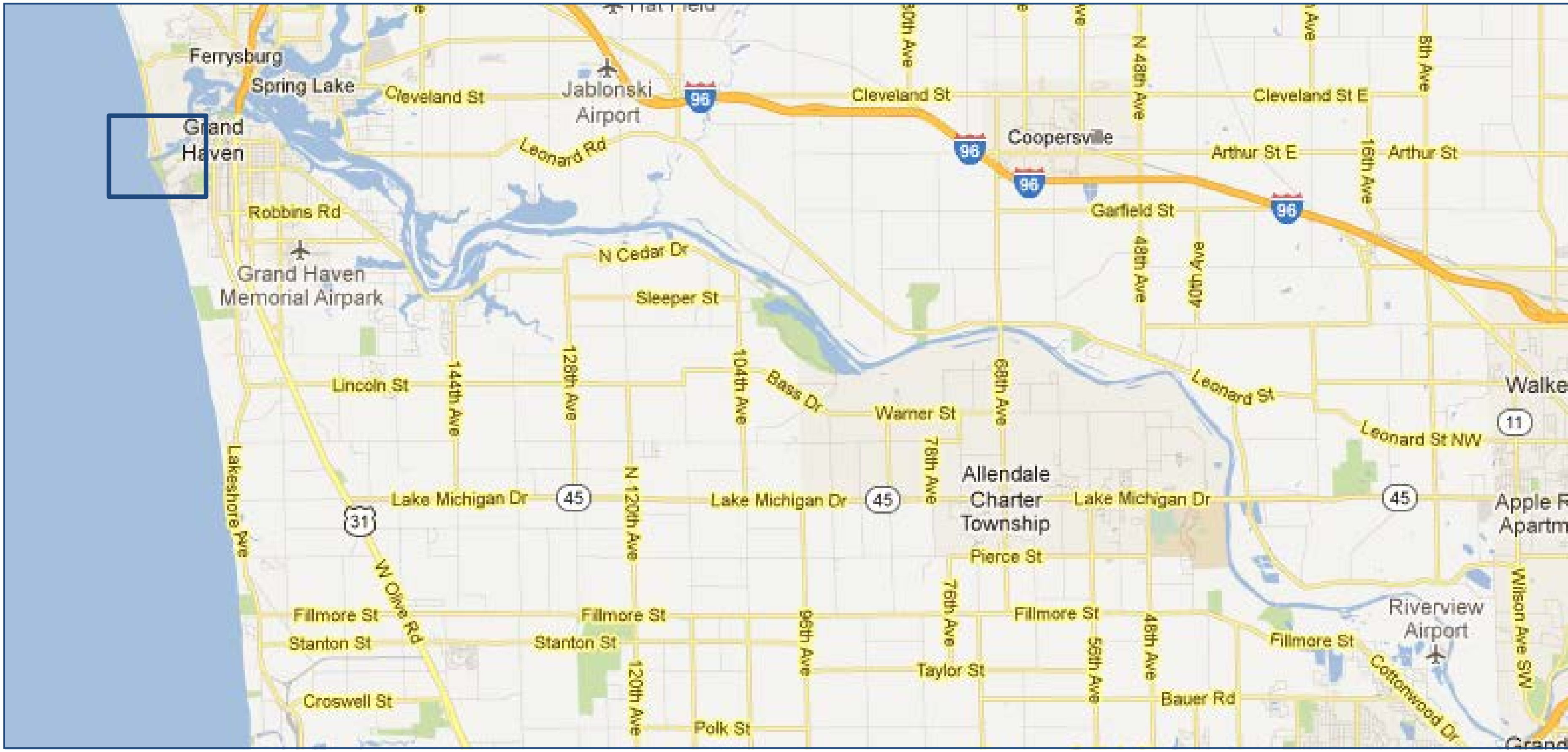
GR3 : Riverside Park (Robinson)





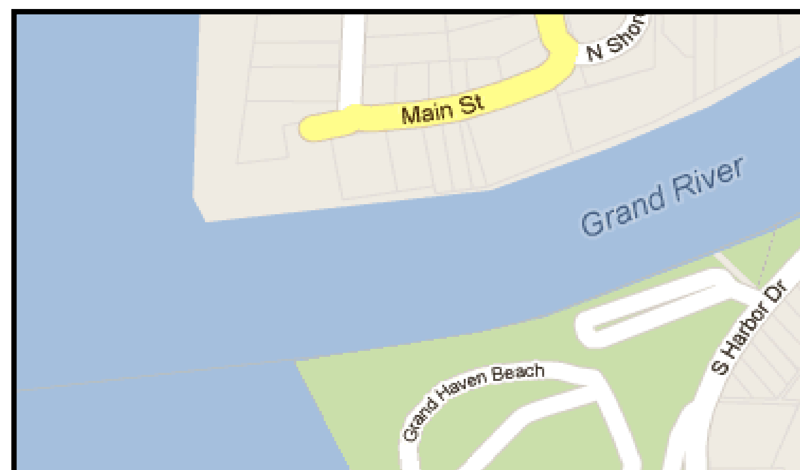
Grand River Study Sites

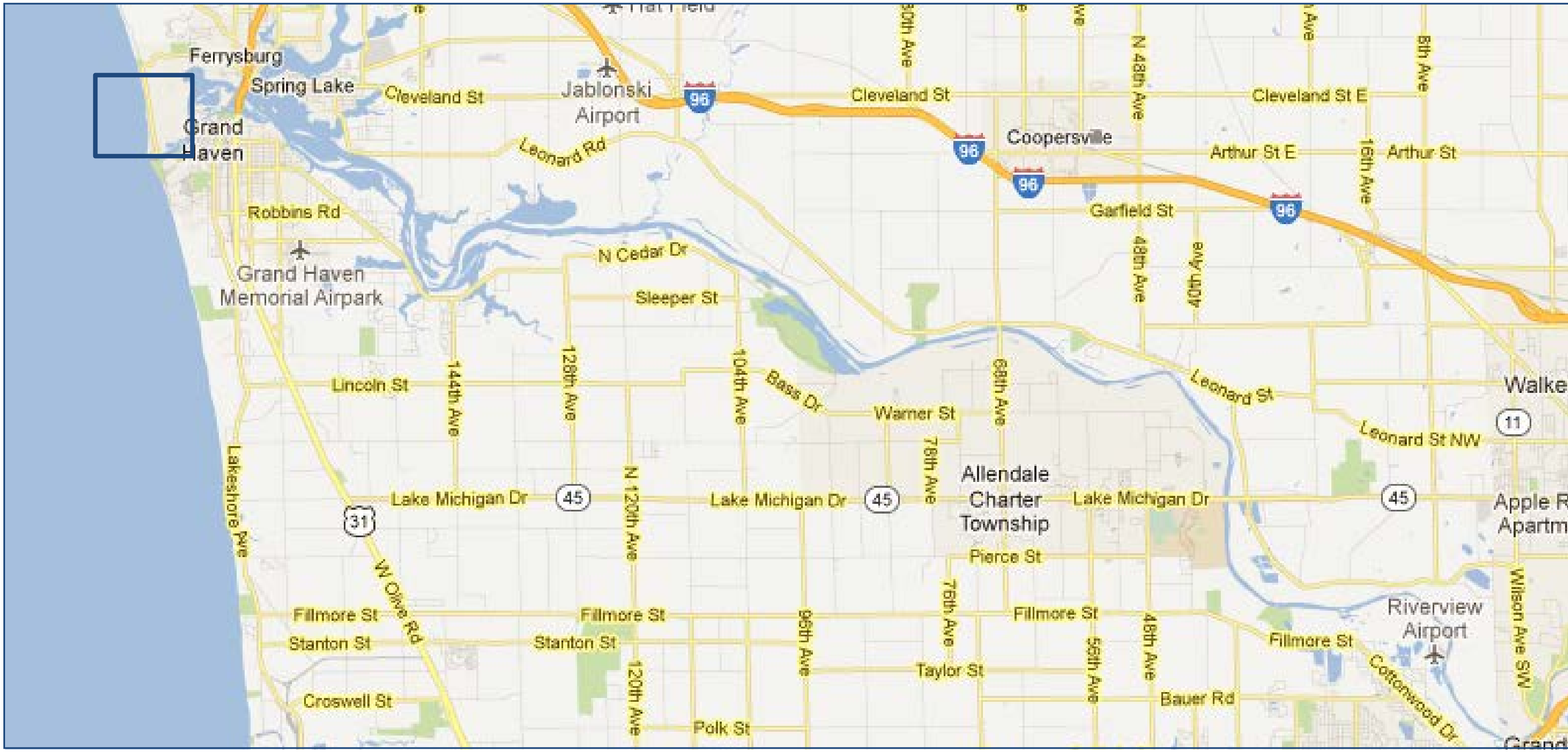
GR4 : Boat Access Site (Robinson)



Grand River Study Sites

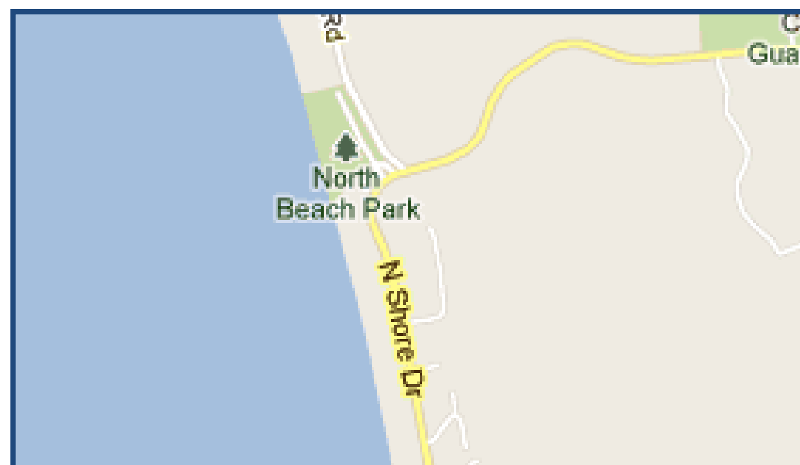
GR5 : Grand River Mouth (Grand Haven)

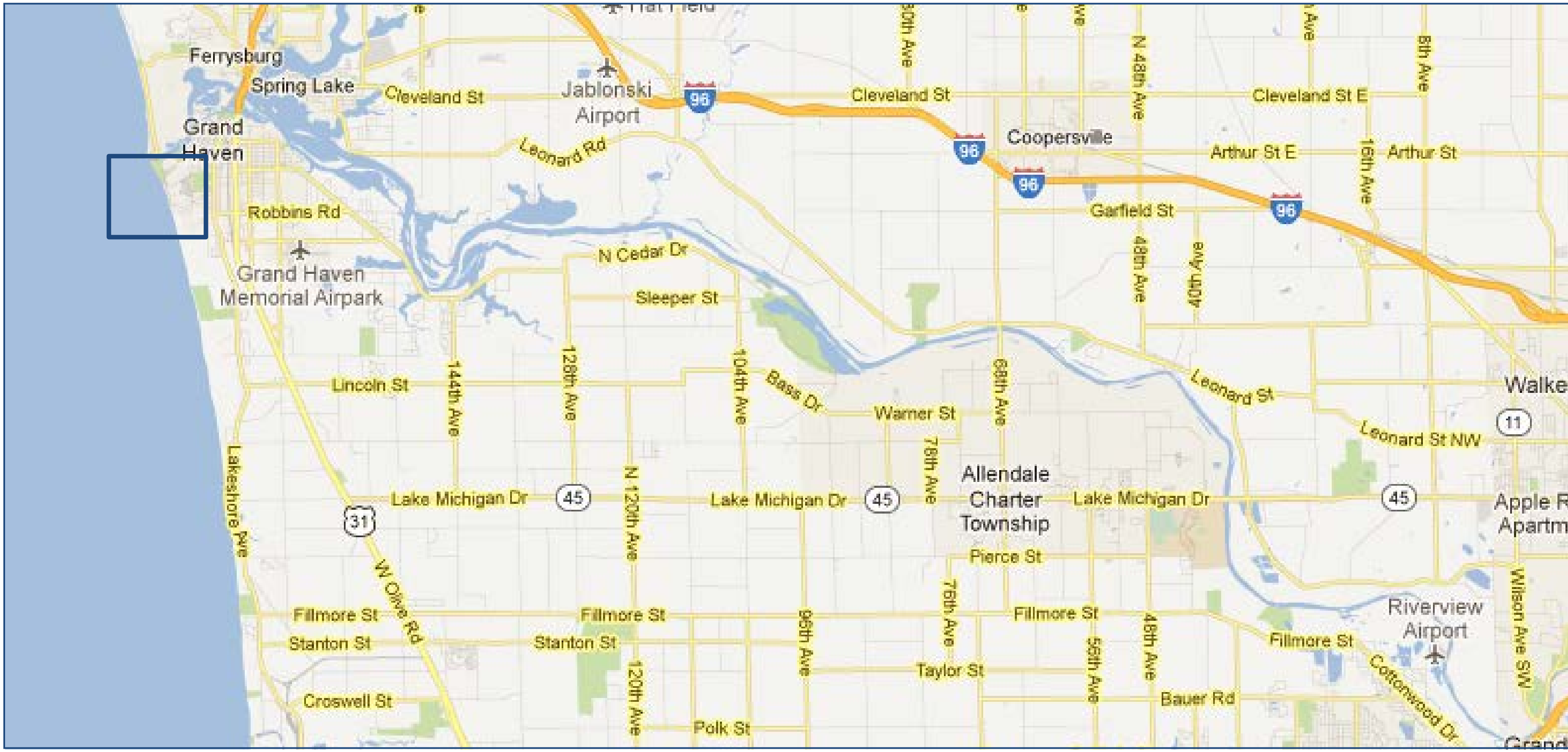




Grand River Study Sites

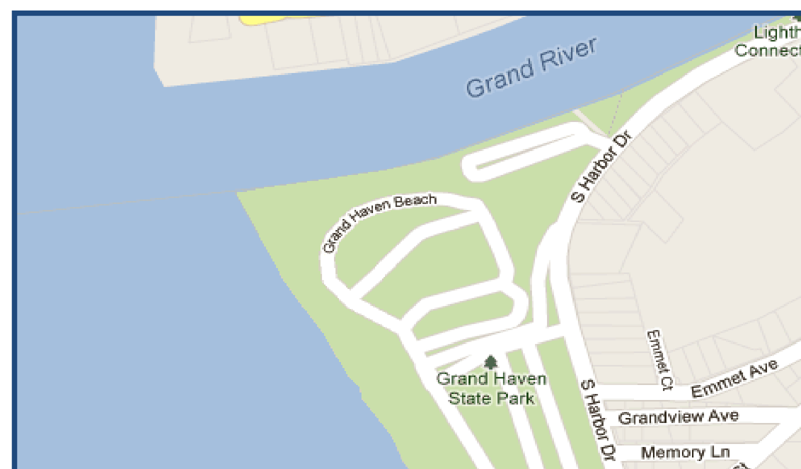
LM1 : North Beach Park (Spring Lake)



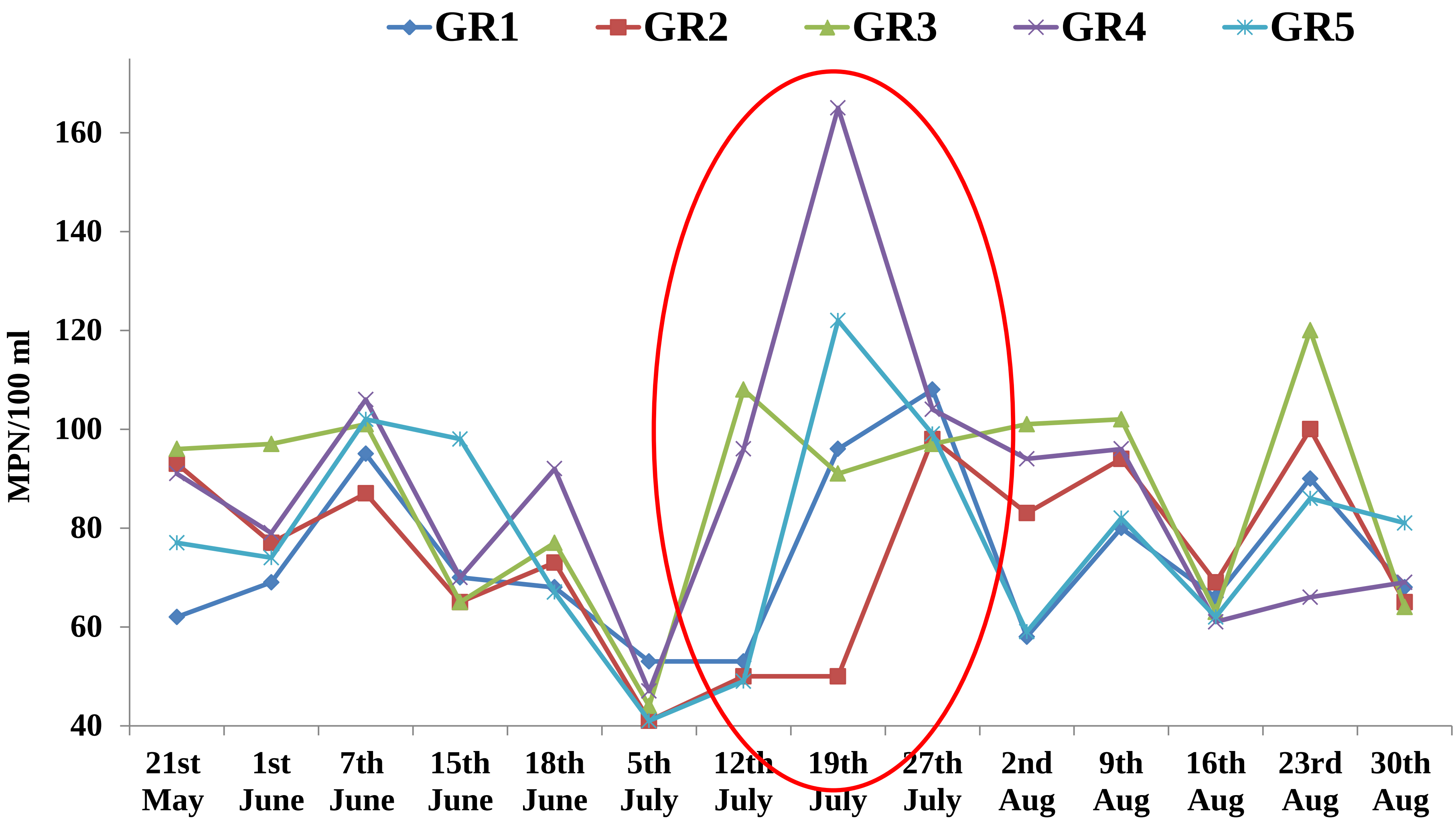


Grand River Study Sites

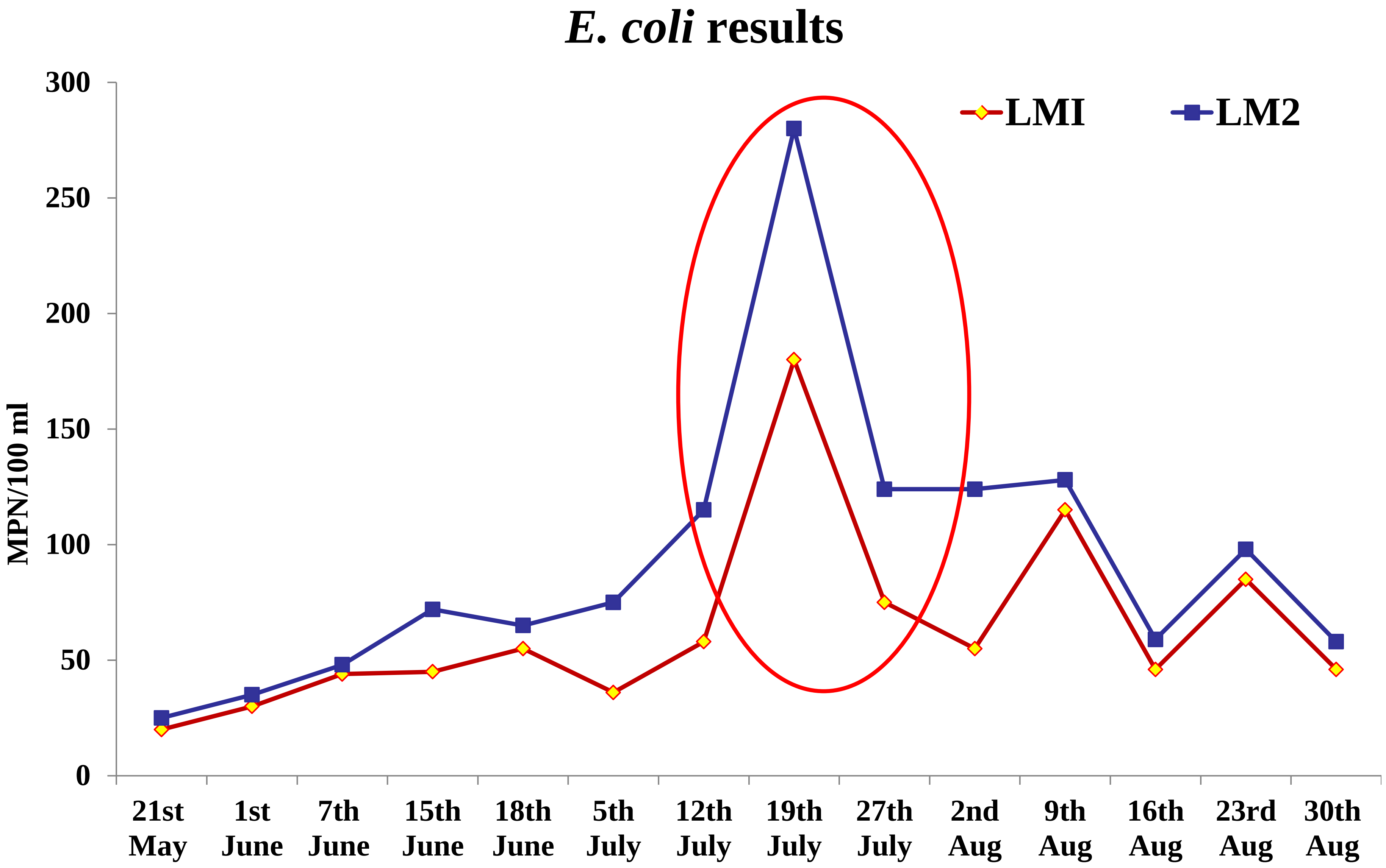
LM2 : Grand Haven State Park (Grand Haven)



E. coli results



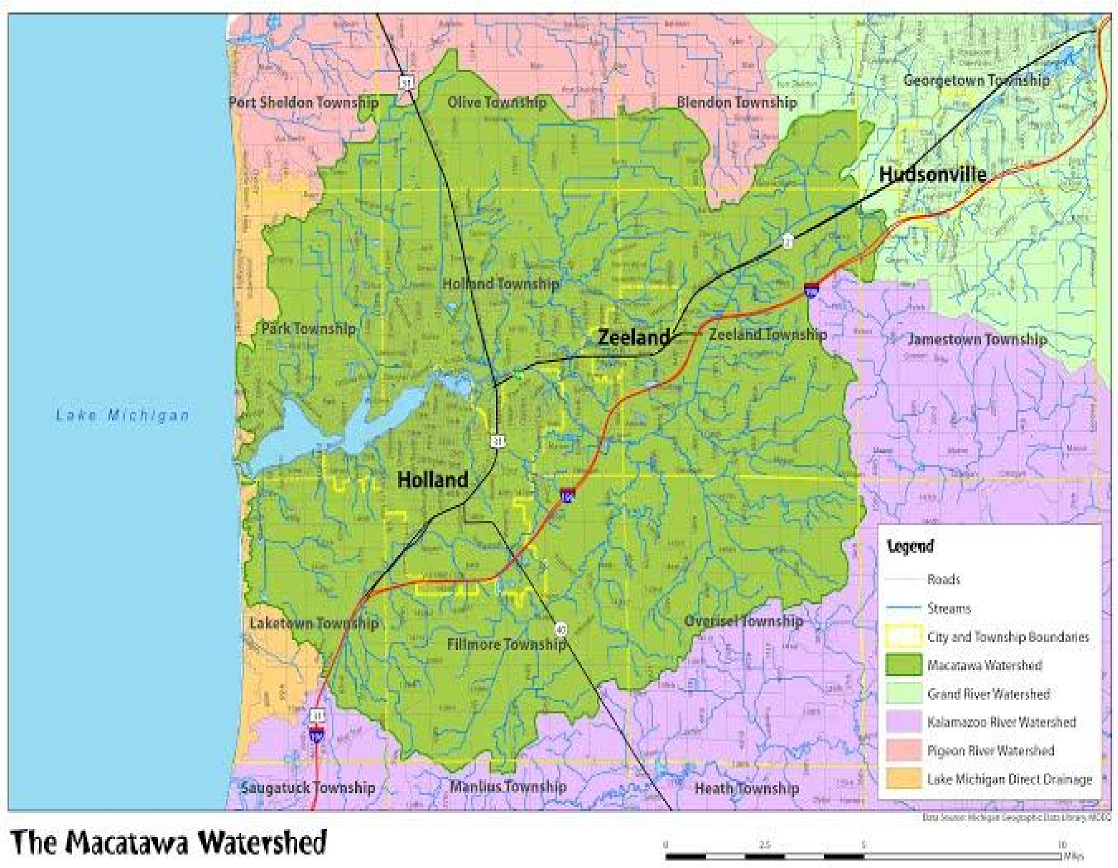
Regulatory Standard for Water Quality Exceedance : 300 MPN/100ml



Regulatory Standard for Water Quality Exceedance : 300 MPN/100ml

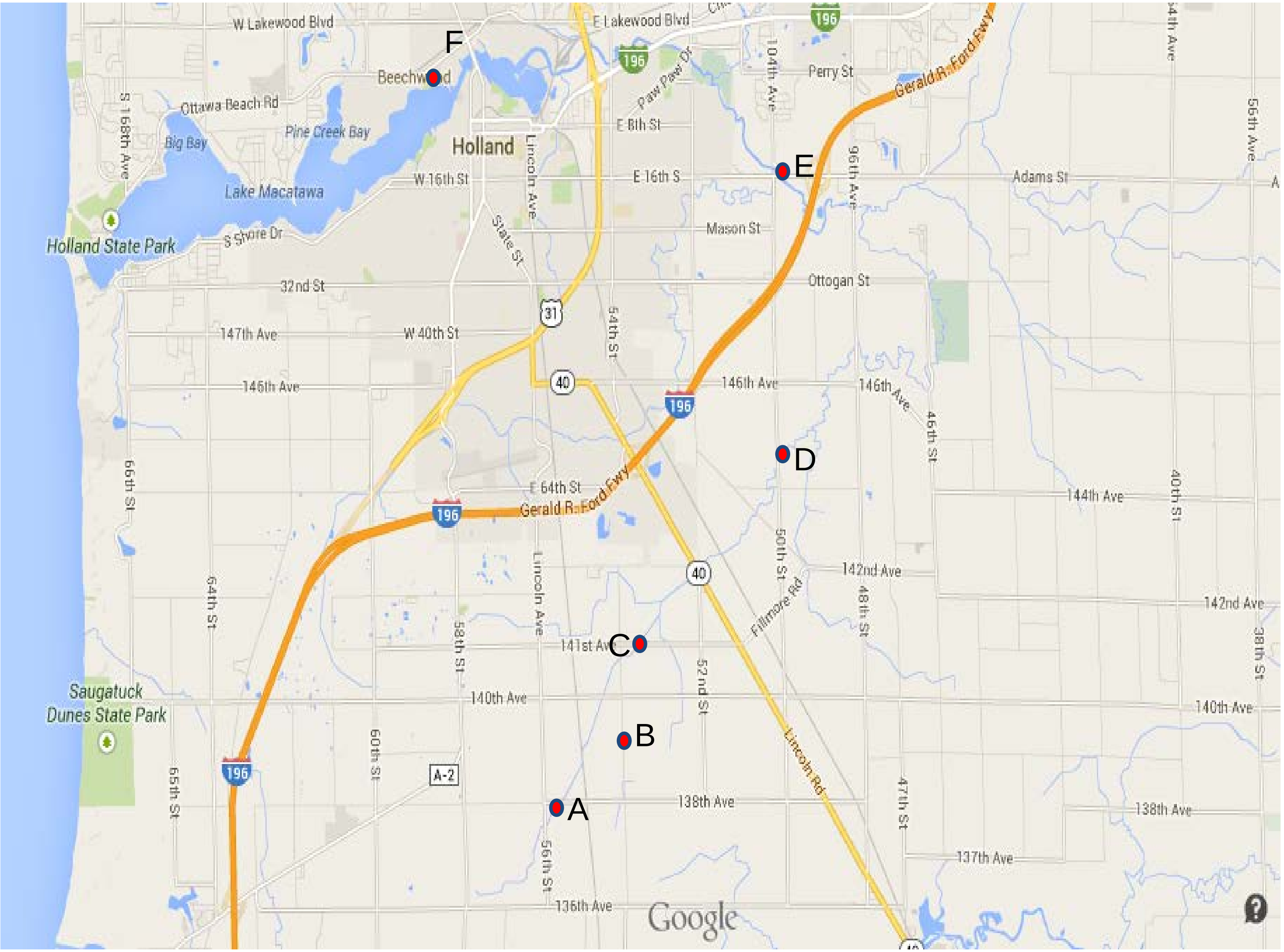
Lake Macatawa Watershed

- Much shorter in length, smaller in area.
- Lower population density.
- Just two counties!



http://www.outdoordiscovery.org/macatawa-greenway/do-you-live-in-the-macatawa-watershed

Macatawa River – South Branch sampling sites



The Tile Drain

June 2, 2014

.01 in precipitation

June 18, 2014

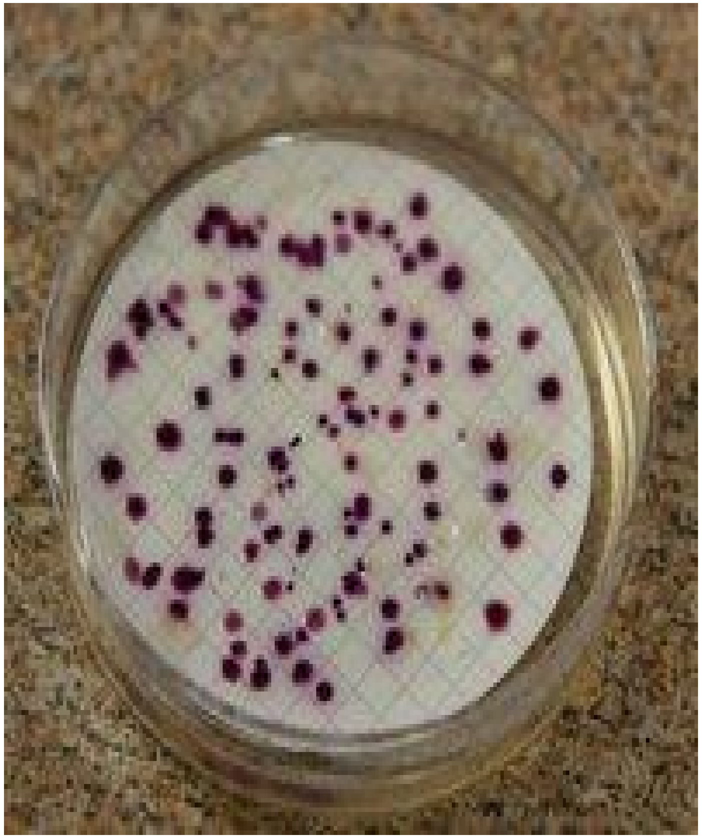
.80 in precipitation

June 20, 2014

.07 in precipitation

Membrane Filtration

- Membrane filtration was used to gather individual colonies of E.coli
- Colonies were then verified using the following tests



Source:
http://www.sccwrp.org/ResearchAreas/ResearchAreasArchive/BeachWaterQuality/ComparisonAmongIDEXXMembraneFiltration.aspx

mTec Counts

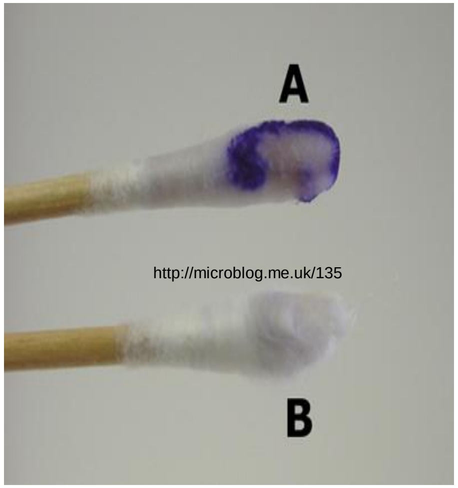
As seen in the tables the post-rain E.coli colony counts were found to be lower than the colony counts from the rain samples. This indicates that E.coli CFUs occur rapidly in the Lake Macatawa watershed.

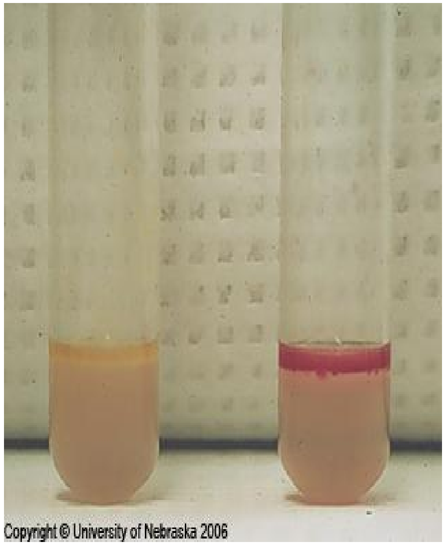
Sample ID	Colony Counts 6/2	Colony Counts 6/18	Colony Counts 6/20
A1	N/A	189	0
A2	N/A	239	2
B1	0	1600	6
B2	0	1700	5
C1	43	166	23
C2	50	149	27
D1	51	152	46
D2	44	230	31
E1	N/A	272	**140**
E2	N/A	364	**125**
F1	7	9	**74**
F2	6	4	**83**

Verification Test

The E.coli tested as expected:

- Oxidase Negative
- Kovac Reagent Positive
- Simmon's Citrate Negative





http://academic.missouriwestern.edu/jcbaker/bio390sec01/bio390_laboratory_study_images.htm

E. coli - Conclusion

- Overall presence of E coli as a fecal indicator bacterium were low in the Grand River over summer 2012.
- Somewhat higher in Macatawa River South Branch summer 2014
  - Associated with a sudden and large rainfall event
  - High in upper stretches – low regular volume, adjacent to cornfields
  - Significantly diluted or die back as rainfall bolus moves downstream.

Where are those bacteria coming from?

MICROBIAL RISK ASSESSMENT STRATERGY
- SOURCE DETERMINES RISK

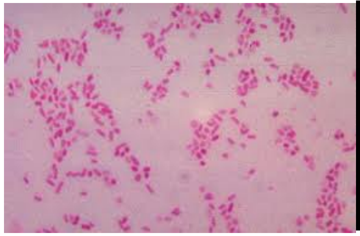
- <u>Human feces/sewage</u>: High Risk.
  Human intestine is habitat of choice for human enteric pathogens.

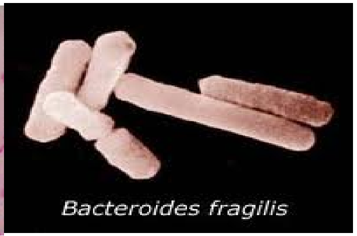
- <u>Non-human/animal feces</u>: Moderate Risk.
  Majority of human enteric pathogens (human viruses) cannot grow in animal intestines.

- <u>Environment (soil, plants, sediments)</u>: Low Risk.
  No hard evidence that any human enteric pathogens can grow to any level of risk in the environment.

Alternate FIB : Bacteroides species

- *Bacteroides* is a genus of Gram-negative, rod-shaped, non- endospore forming anaerobes.
- Normal commensals in mammalian gastro intestinal tract and feces.
- Make up a significant portion of the fecal bacterial population.
- Present in intestine and feces at thousand times greater than *E. coli*.
- Unlike *E. coli*, Bacteroides spp. do not proliferate in the environment
- Bacteroides hosts (human, cow, swine) are well established for environmental application.
- Examples of Bacteroides species :
  B. fragilis, B. thetaiotaomicron, B. uniformis, B. ovatus, B. vulgatus, B. caccae, B. eggerthii.





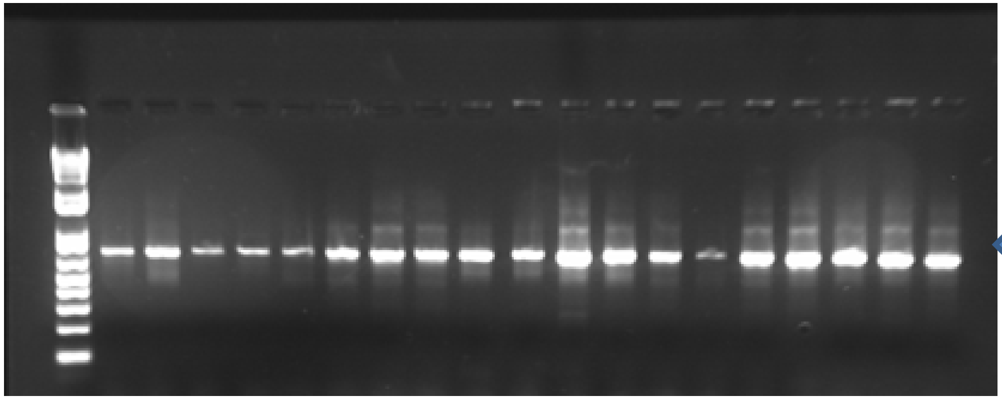
DNA-based source tracking to identify origin of bacteroides in water sample

Purify DNA from sample rather than culture.

Detection is not growth of bacterial colony, but “growth” (actually amplification) of a DNA molecule of a given length and sequence

Depending on the “text” we are looking for, the amplified fingerprint shows presence of:

- DNA from any old type bacteria
- DNA from bacteroides, from any host
- DNA from bacteroides of a known host



General (non-host specific) bacteroides fingerprint present in all samples that had any sort of bacteria present.

Host specific origins we can fingerprint for are:

- Human
- Cow
- Pig
- Goose/avian
- Gull

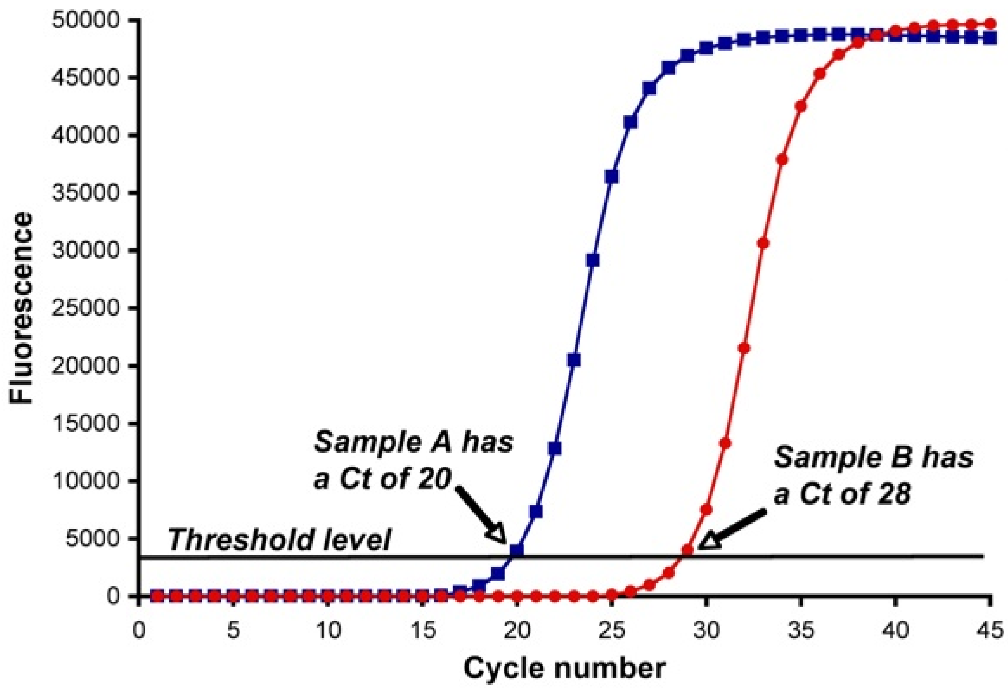
PCR Prep

http://macflow.tradeindia.com/pcr-machine-403460.html

- DNA isolations
- Qiagen method
- Samples ready to use on PCR
- Used SYBR Green and various primers

Quantitative (qPCR) aka Real-time (RT-PCR) monitors appearance of copied DNA product every cycle.

- Since amount of target DNA should double every cycle, more cycles = less DNA in original sample:



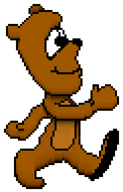
If it takes 8 more cycles for sample B to appear relative to sample,

- That means sample A had 2^8 = 258 times more original target DNA than sample B

Microbial Source Tracking Results

Gen Bacteroides	May (cT)	June (cT)	July (cT)	Aug (cT)
GR1	30	25	29	28
GR2	29	29	31	28
GR3	29	28	29	29
GR4	30	27	28	28
GR5	29	30	30	30
LMI	28	28	28	30
LM2	28	28	29	30
RS10^-1	22	**Risk Level : ?????**		
RS10^-2	25			
RS10^-3	28			
RS10^-4	31			

Microbial Source Tracking Results



Human Bacteroides	May (cT)	June (cT)	July (cT)	Aug (cT)
GR1	NA	34	39	**29**
GR2	NA	NA	NA	37
GR3	NA	NA	NA	39
GR4	NA	NA	38	39
GR5	NA	NA	NA	39
LMI	39	NA	NA	38
LM2	NA	38	NA	NA
RS10^-1	23	**Risk Level : 0**		
RS10^-2	26			
RS10^-3	29			
RS10^-4	31			

Microbial Source Tracking Results

Cow Bacteroides	May (cT)	June (cT)	July (cT)	Aug (cT)
GR1	NA	37	NA	NA
GR2	NA	NA	NA	NA
GR3	NA	NA	NA	NA
GR4	NA	39	34	NA
GR5	NA	40	NA	NA
LMI	NA	NA	NA	NA
LM2	NA	NA	NA	NA
Cow10^-1	26	**Risk Level : 0**		
Cow10^-2	30			
Cow10^-3	32			
Cow10^-4	35			

Microbial Source Tracking Results

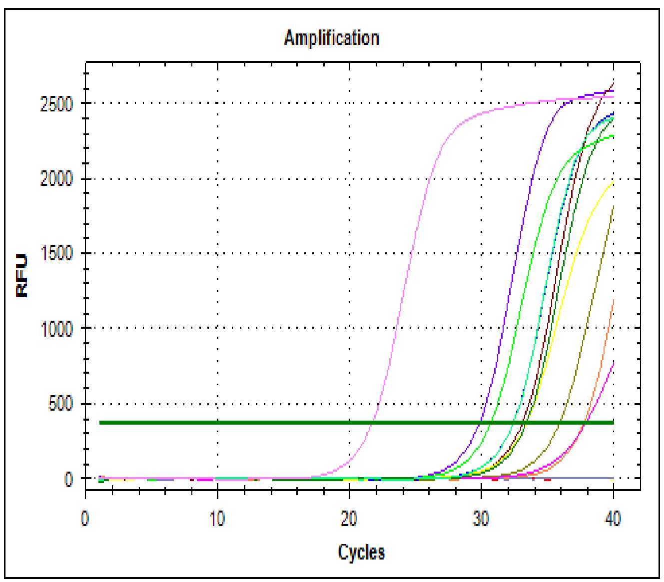
Swine Bacteroides	May (cT)	June (cT)	July (cT)	Aug (cT)
GR1	37	**34**	NA	NA
GR2	39	37	NA	36
GR3	38	39	NA	36
GR4	39	37	NA	36
GR5	39	39	NA	NA
LMI	37	37	39	37
LM2	34	36	NA	NA
Swine 10^-1	27	**Risk Level : Moderate**		
Swine 10^-2	31			
Swine 10^-3	36			
Swine 10^-4	38			

We have recently added avian – goose and gull – to validated primers.

Significant gull marker showed at only one time/location.

		Non-specific		Goose		Gull	
		Ct	Fecal conc (ppm)	Ct	Fecal conc (ppm)	Ct	Fecal conc (ppm)
Month:	100 mg std	17.65		20.4975		20.1	
May	GR1	29.85	0.4243	36.74	0.0258	>40	nd
	GR2	28.79	0.8878	>40	nd	>40	nd
	GR3	29.00	0.7649	>40	nd	>40	nd
	GR4	29.72	0.4660	>40	nd	>40	nd
	GR5	29.21	0.6613	>40	nd	>40	nd
	LM1	27.98	1.5538	>40	nd	>40	nd
	LM2	27.70	1.8866	36.95	0.0224	>40	nd
June	GR1	24.76	14.5030	37.85	0.0120	>40	nd
	GR2	28.46	1.1160	37.72	0.0131	>40	nd
	GR3	28.27	1.2730	39.18	0.0048	>40	nd
	GR4	27.30	2.4851	37.99	0.0109	>40	nd
	GR5	30.04	0.3733	39.21	0.0047	>40	nd
	LM1	27.97	1.5673	37.45	0.0158	>40	nd
	LM2	27.76	1.8160	>40	nd	>40	nd
July	GR1	29.32	0.6159	>40	nd	>40	nd
	GR2	30.92	0.2032	39.24	0.0046	>40	nd
	GR3	29.53	0.5316	>40	nd	>40	nd
	GR4	28.11	1.4199	38.08	0.0102	>40	nd
	GR5	30.06	0.3675	39.85	0.0030	>40	nd
	LM1	28.22	1.3157	37.38	0.0166	>40	nd
	LM2	28.78	0.8940	38.59	0.0072	>40	nd
August	GR1	28.35	1.2002	>40	nd	33.80	0.1503
	GR2	28.01	1.5192	38.89	0.0058	>40	nd
	GR3	28.73	0.9255	>40	nd	>40	nd
	GR4	27.63	1.9804	>40	nd	>40	nd
	GR5	29.92	0.4056	37.97	0.0110	33.30	0.2125
	LM1	29.93	0.4014	>40	nd	>40	nd
	LM2	30.06	0.3681	>40	nd	>40	nd

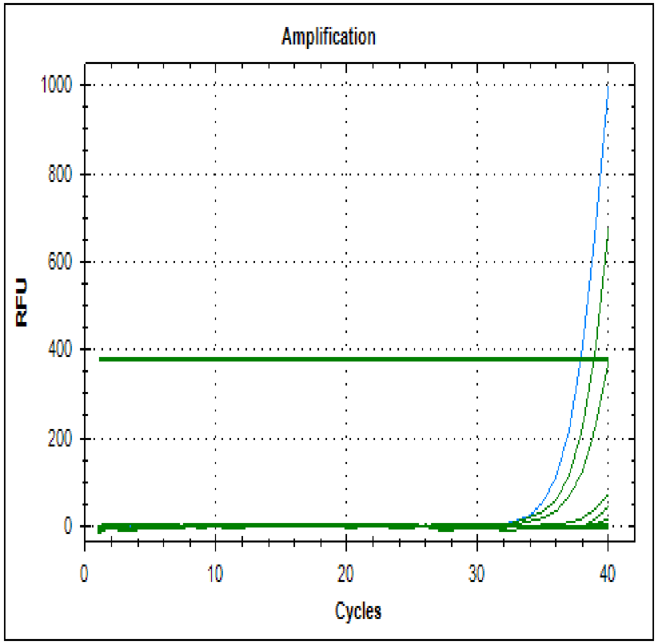
PCR Results: General Bacteroides



Sample	Ct	Melt temp
Water	N/A	None
A 6/18	35.94	86.50
A 6/20	37.73	None
B 6/18	N/A	None
B 6/20	32.36	86.00
C 6/18	N/A	None
C 6/20	29.84	87.50

Sample	Ct	Melt temp
D 6/18	33.37	86.00
D 6/20	32.37	86.50
E 6/18	37.90	None
E 6/20	33.38	86.00
F 6/18	33.10	86.00
F 6/20	30.66	86.00
Duck 6/20	21.70	86.00

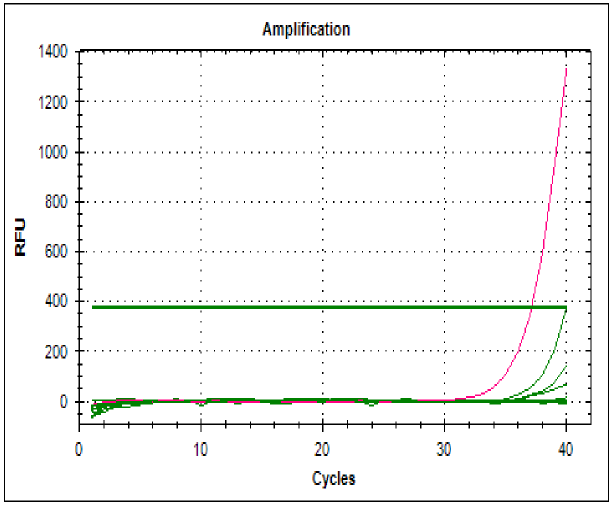
PCR Results:
Human



Sample	Ct	Melt temp
Water	N/A	None
A 6/18	N/A	None
A 6/20	N/A	None
B 6/18	N/A	None
B 6/20	38.87	None
C 6/18	N/A	None
C 6/20	37.86	73.50

Sample	Ct	Melt temp
D 6/18	N/A	None
D 6/20	N/A	None
E 6/18	N/A	None
E 6/20	N/A	None
F 6/18	N/A	None
F 6/20	N/A	None
Duck	N/A	None

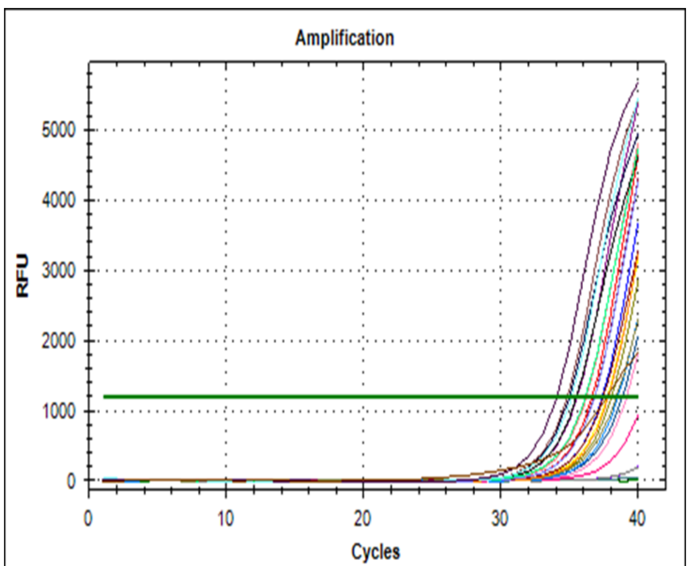
PCR Results:
Cow



Sample	Ct	Melt temp
Water	N/A	None
A 6/18	N/A	None
A 6/20	N/A	None
B 6/18	N/A	None
B 6/20	N/A	None
C 6/18	N/A	None
C 6/20	N/A	None

Sample	Ct	Melt temp
D 6/18	N/A	None
D 6/20	37.12	None
E 6/18	N/A	None
E 6/20	N/A	None
F 6/18	39.98	None
F 6/20	N/A	None
Duck 6/20	N/A	None

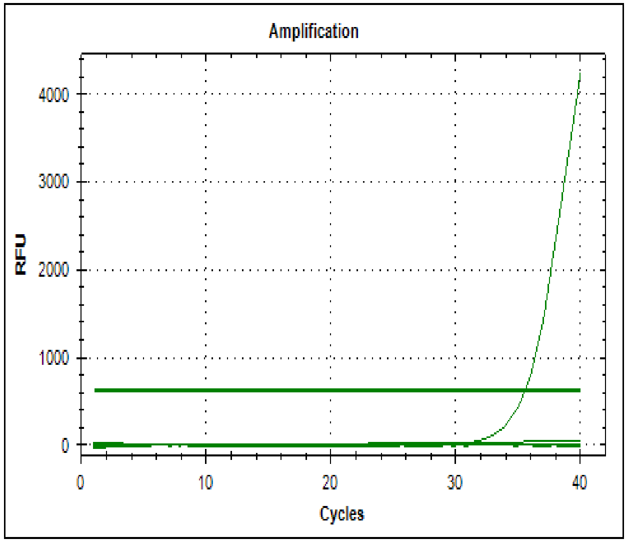
PCR Results: Goose



Sample	Ct	Melt temp
Water	N/A	None
A 6/18	N/A	None
A 6/20	38.59	None
B 6/18	N/A	None
B 6/20	38.27	None
C 6/18	36.97	None
C 6/20	35.86	89.50

Sample	Ct	Melt temp
D 6/18	36.47	89.00
D 6/20	34.59	90.00
E 6/18	38.44	None
E 6/20	37.29	None
F 6/18	35.10	90.00
F 6/20	36.19	None
Duck 6/20	37.68	None

PCR Results: Gull



Sample	Ct	Melt temp
Water	N/A	None
A 6/18	N/A	None
A 6/20	N/A	None
B 6/18	N/A	None
B 6/20	N/A	None
C 6/18	N/A	None
C 6/20	N/A	None

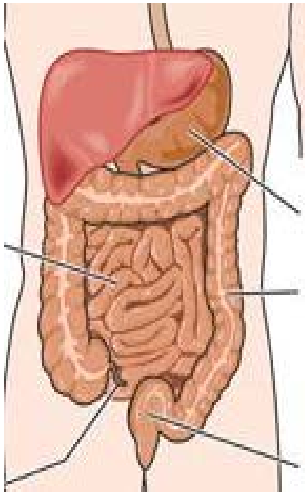
Sample	Ct	Melt temp
D 6/18	N/A	None
D 6/20	N/A	None
E 6/18	N/A	None
E 6/20	N/A	None
F 6/18	35.52	87.50
F 6/20	N/A	None
Duck 6/20	N/A	None

PCR Conclusion

- Only sporadic presence of identifiable host origin. Particularly worrisome organisms (from human or domestic animals) do not appear to be a major source

http://health.howstuffworks.com/skin-care/beauty/sun-care/uv-radiation.htm

- E.coli source yet to be determined
  - Where is it coming from?
  - How does it behave?
    - Environmental E.coli different from fecal E.coli?



http://www.socallaparoscopy.com/small_intestine_laparoscopic_procedures.html

Acknowledgements

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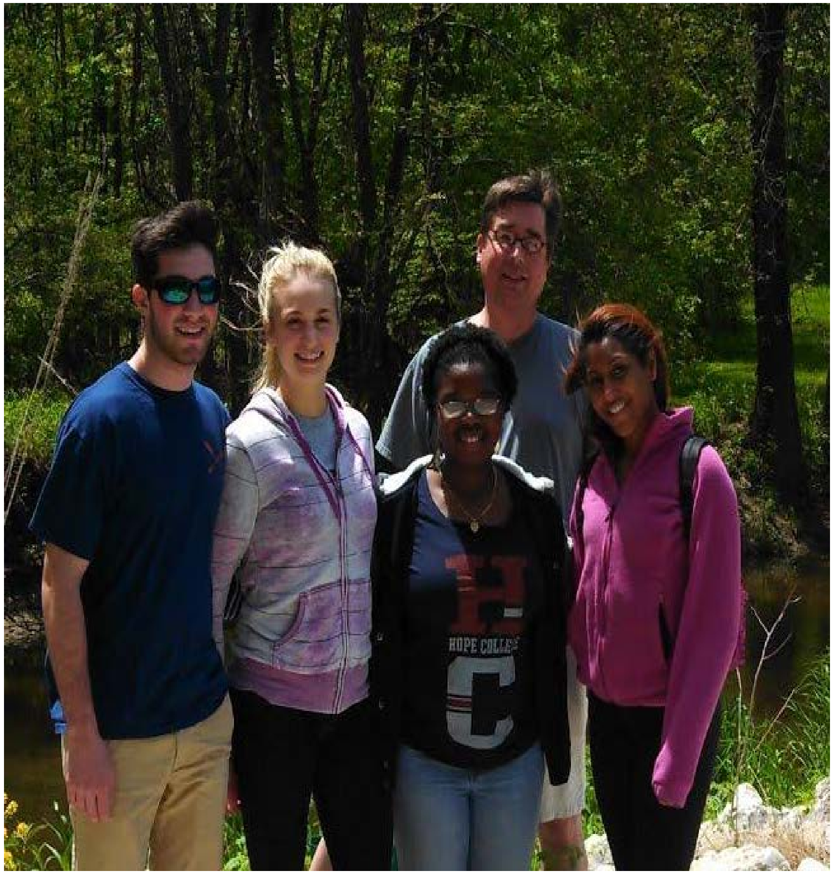
- Ottawa County Health Dept



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