Microbial Source Tracking Study in The Grand River

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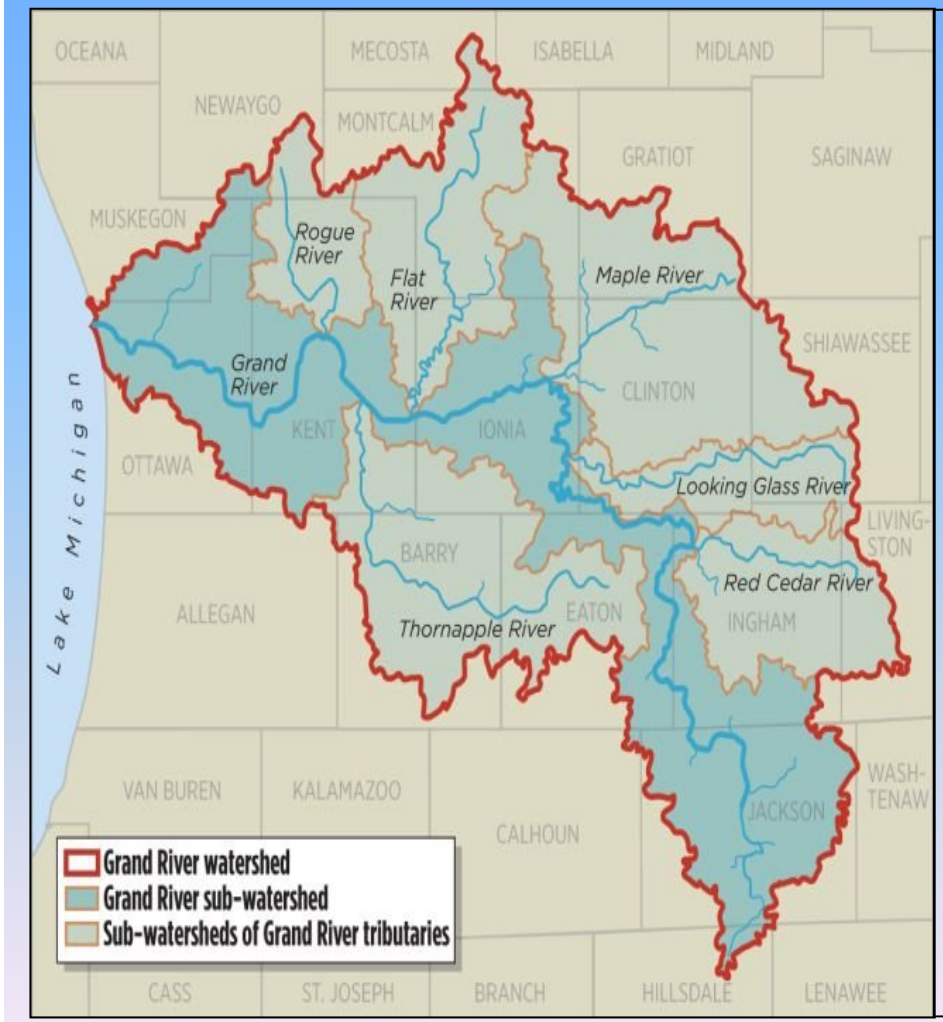
Grand River

- **Served as an important navigational route through the Lower Peninsula of Michigan.**
- **Used for centuries by various Native American tribes and later by explorers, for traders, and white settlers.**
- **The river was called *O-wash-ta-nong*, meaning "Far-away-water" because of its length.**
- **The Grand River is the longest river in the state of Michigan.**

Grand River Watershed



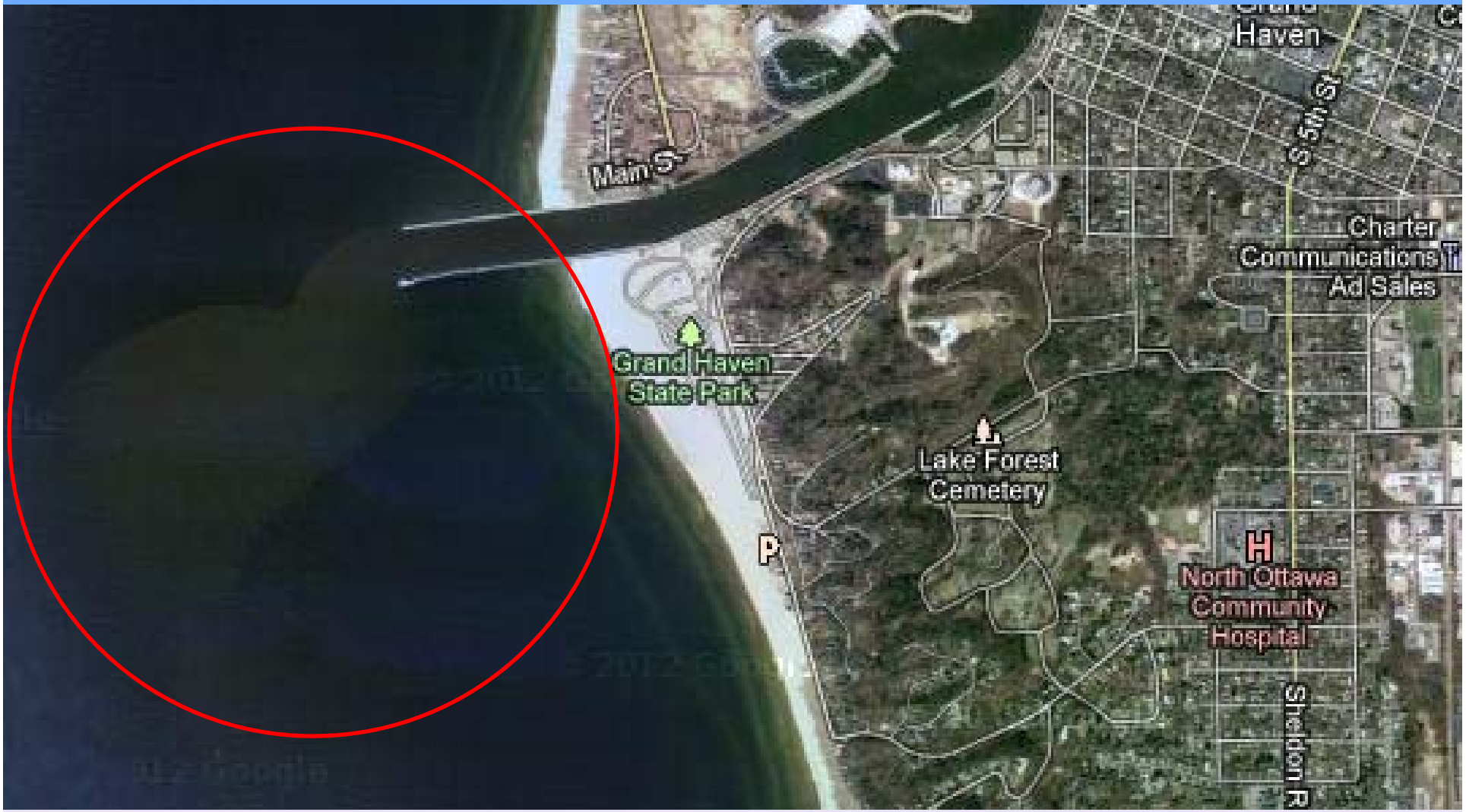
- **Runs 252 miles (406 km)**
- **Through the cities of Jackson, Eaton Rapids, Lansing, Grand Rapids, and Grand Haven and empties into Lake Michigan.**
- **Grand River watershed drains an area of 5,572 square miles (14,430 km^2).**
- **Including 18 counties and 158 townships.**

Grand River
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)

Grand River Discharging into Lake Michigan



EPA Water Quality Standards (WQS) Based on FIB

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

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

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

FOR FRESH WATERS

33 **Enterococci CFU/100 ml**

126 ***E. coli* CFU/100 ml**

FOR MARINE WATERS

35 **Enterococci CFU/100 ml**

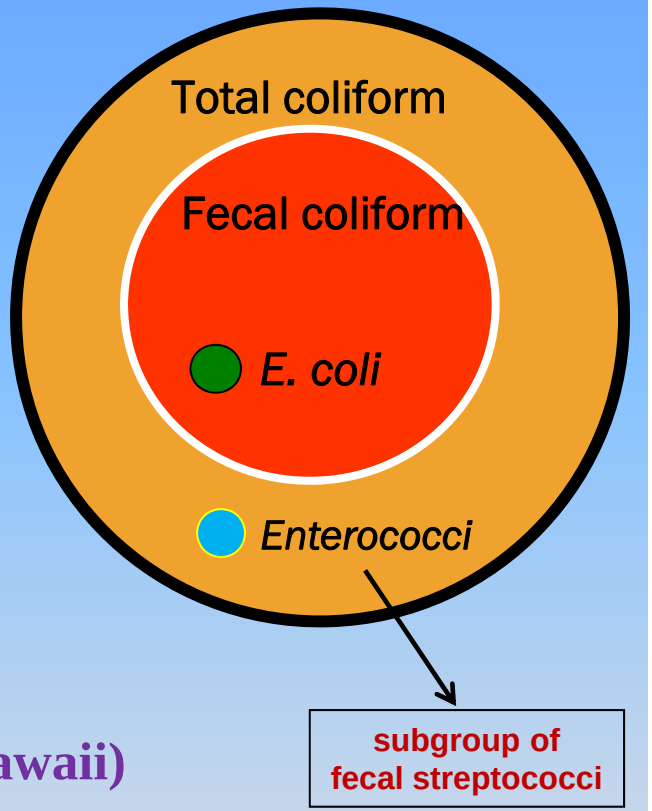
7 **Enterococci CFU/100 ml (Hawaii)**

***Geo mean of at least five samples taken over a period of 30 days**

235 ***E. coli* CFU/100 ml**

61 **Enterococci CFU/100 ml**

**** Single sample regulatory limits**



Michigan Beach Water Quality Standards

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

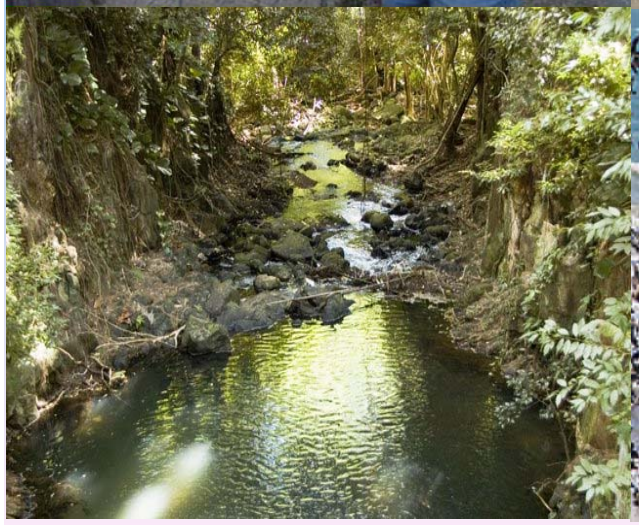
- 130 CFU *E.coli*/ 100 mL
  (monthly geometric mean of at least 5 sampling events)

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

- Below the EPA's acceptable risk level of 1%
  (10 people per 1000 getting sick).

Problem

“FIB such as *E. coli* is not human specific.”



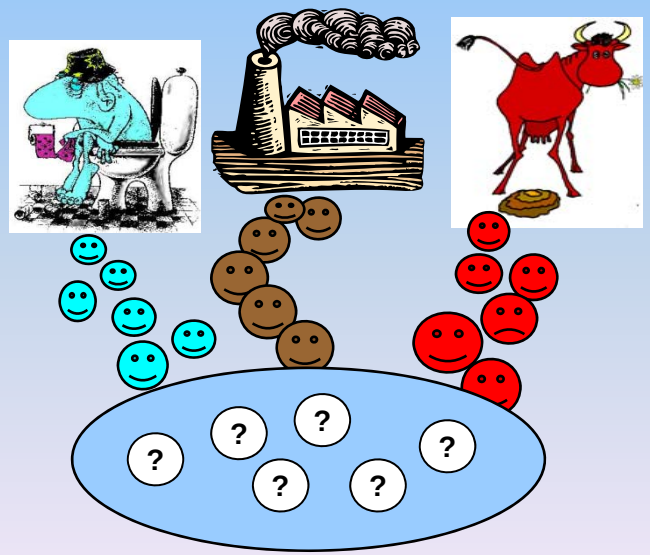
MICROBIAL RISK ASSESSMENT STRATERGY - SOURCE DETERMINES RISK

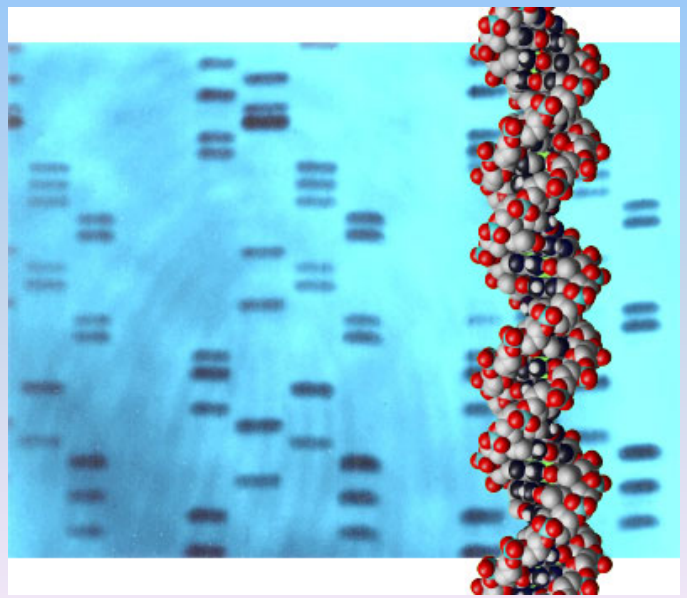
- **Human feces/sewage: High Risk.**
  Human intestine is habitat for growth of all known human enteric pathogens.
- **Non-human/animal feces: Moderate Risk.**
  Majority of human enteric pathogens (human viruses) cannot grow in animal intestines.
- **Environment (soil, plants, sediments): Low Risk.**
  No hard evidence that any human enteric pathogens can grow to any level of risk in the environment.

Solution

- Microbial Source Tracking tools identify the sources of FIB (e.g., human, cattle, bird)

- Helps to provide a more rational solution to recreational water-related disease





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.

The Use of DNA based source tracking is very effective

- Sate Of The Art Technique

- Accurately identifies the target/source

- DNA Finger Printing: Library based method

Challenging Regulatory Standards

- E. Coli Vs Bacteroides

E. coli (Traditional FIB)

- Culture method
- Existing standards are based Epi study
- Well established, regulated and mandated by USEPA.
- Not human specific

Bacteroides spp. (Alternate FIB)

- Molecular method.
- More target (cow, swine, human) specific.
- No Epi study was conducted.
- Approved but not regulated by USEPA.

Grand River Water Sampling

Sampling Period : May to August 2012

E. coli monitoring : Once a week (14 sampling event)

Microbial Source Tracking : Once a Month (4 sampling events)

E. coli -Idexx-Colilert

Bacteroides- qPCR

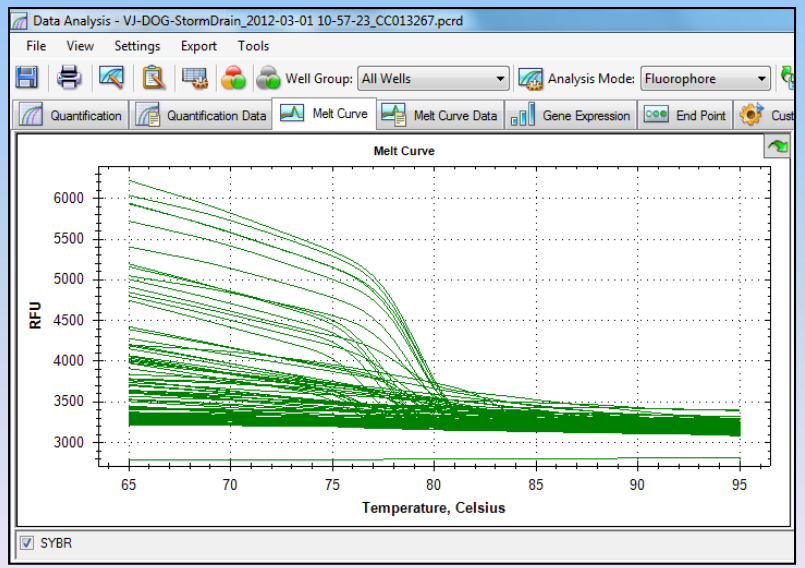
Bacteroides- qPCR

General bacteroides: bac708R, bac32F

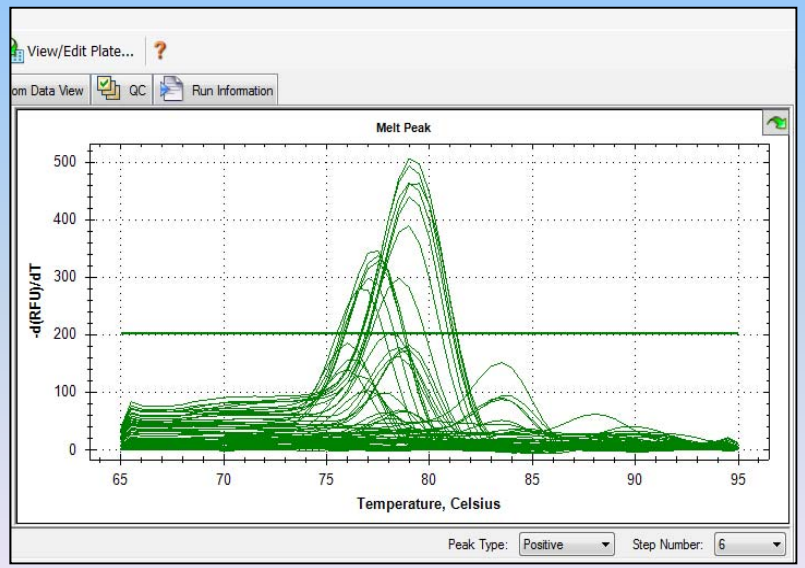
Human: HrevShort, HF183

Cow: HrevShort, CF128

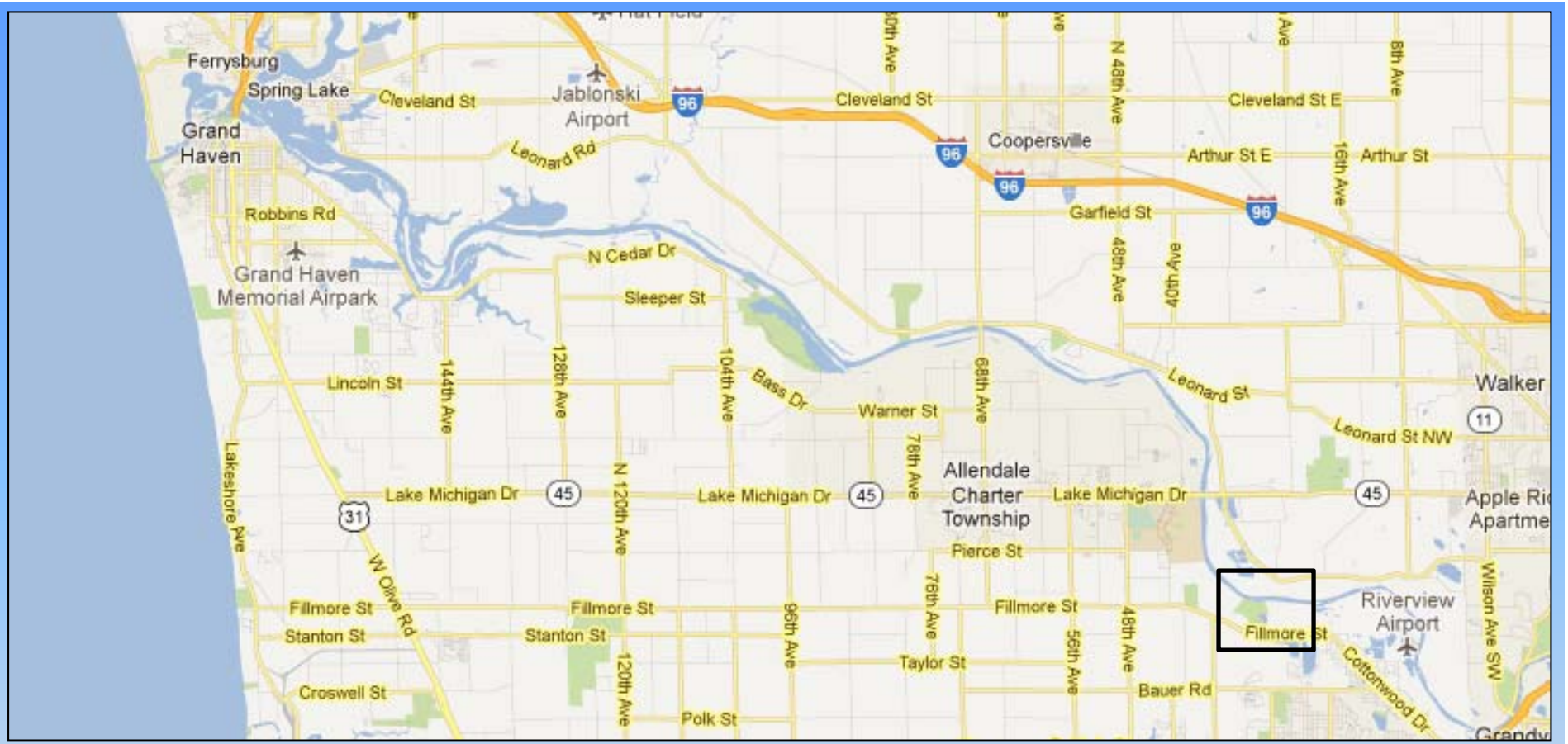
Pig: 708R, PF163F



Cycle Threshold (ct)
- concentration of the target DNA

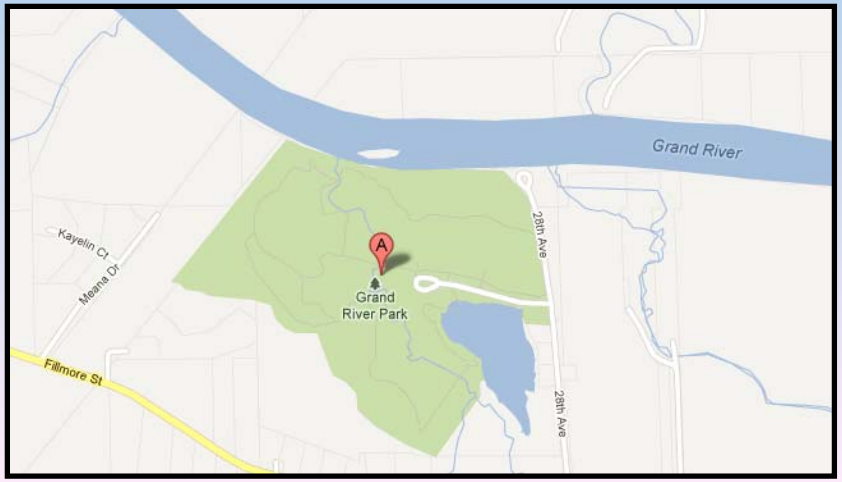


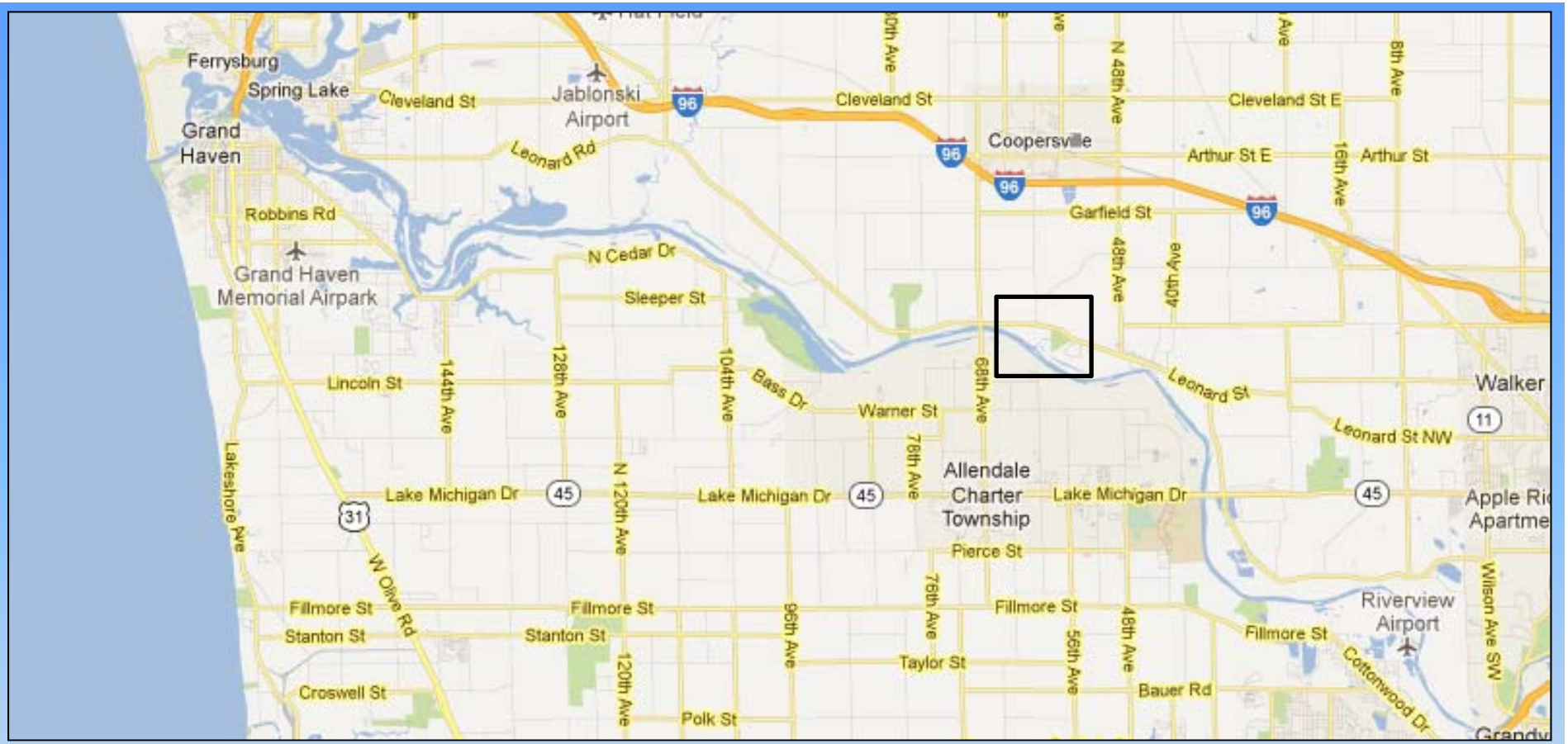
Melt Curve
- characteristics of the target DNA



Grand River Study Sites

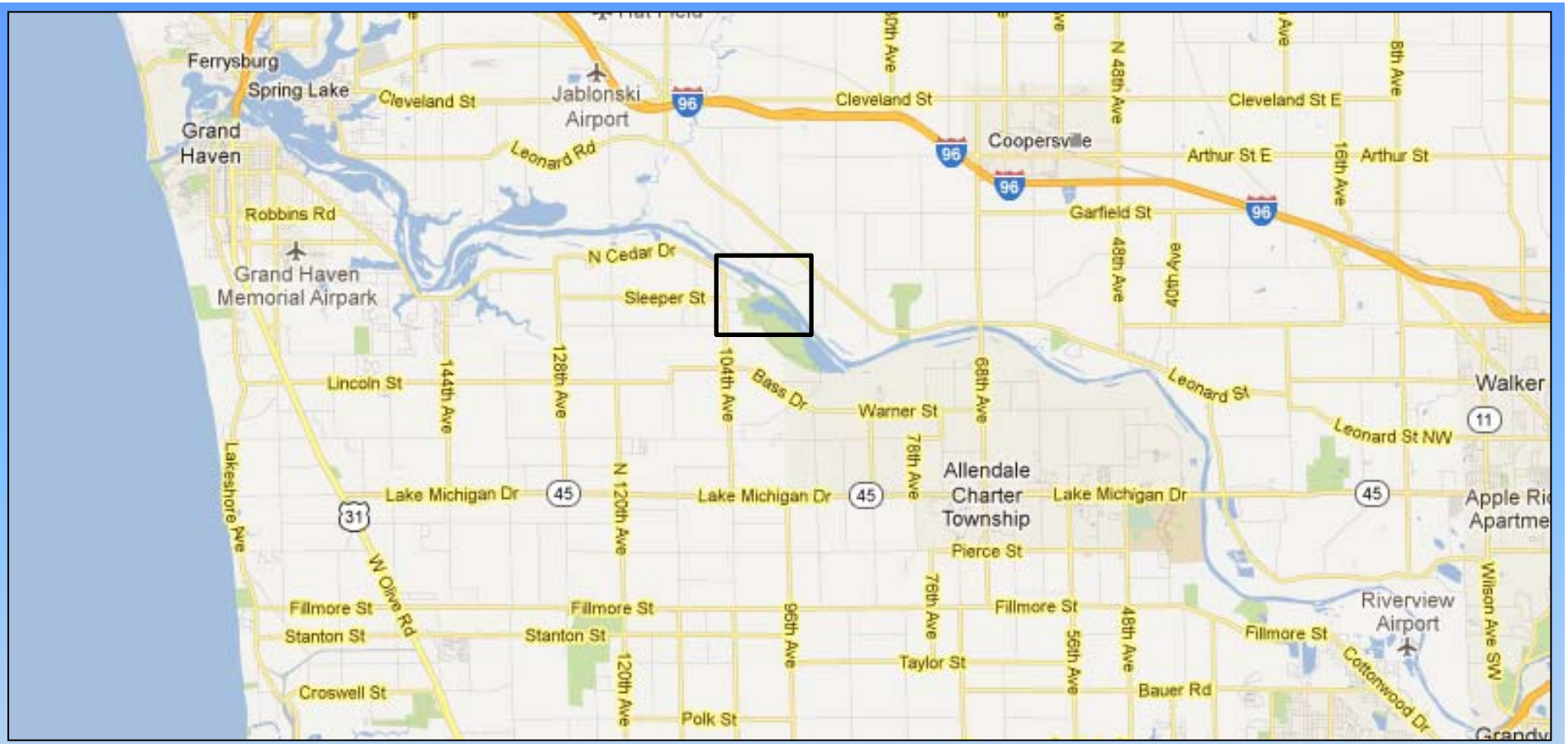
GR1 : Grand River Park (George Town)





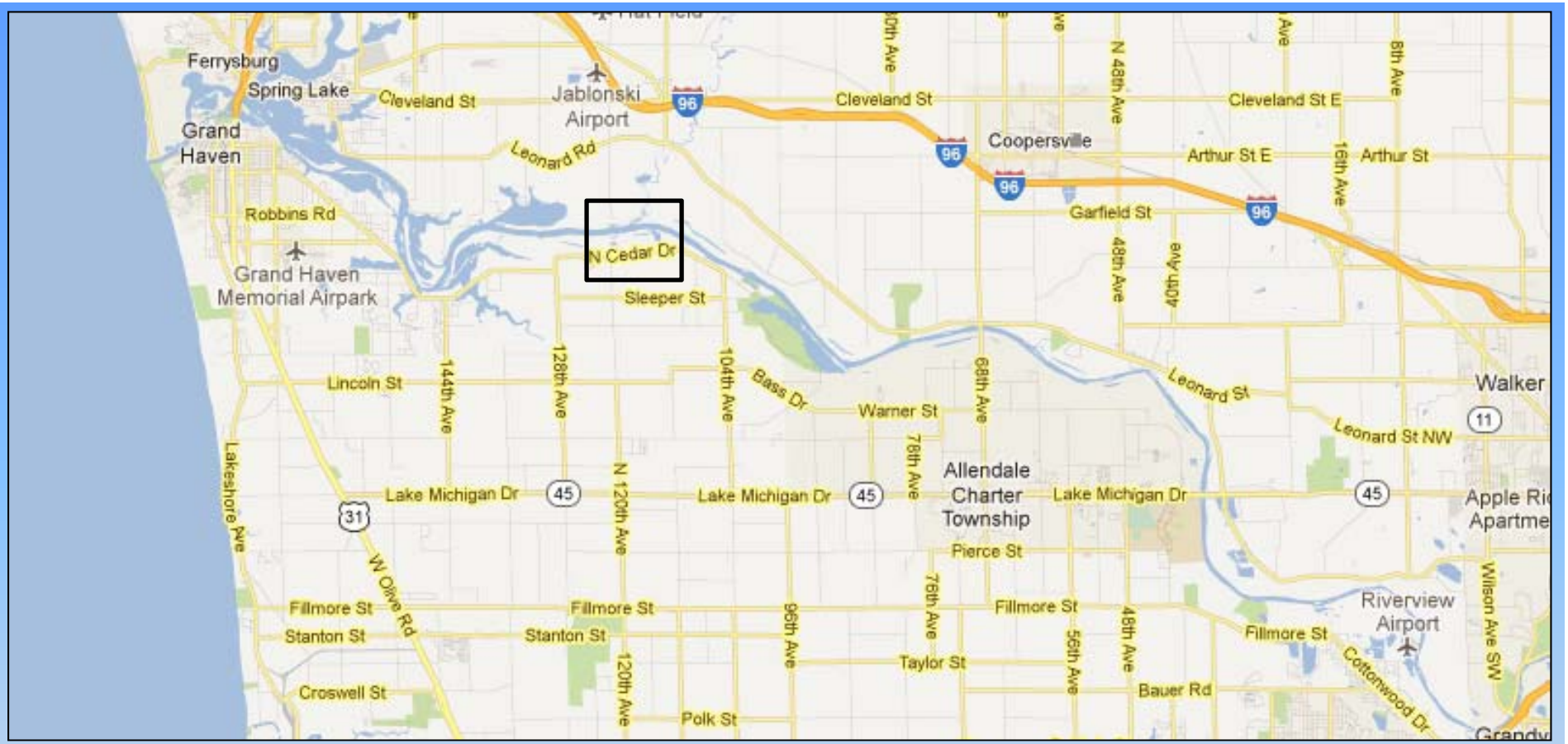
Grand River Study Sites

GR2 : Deer Creek Park (Allendale)



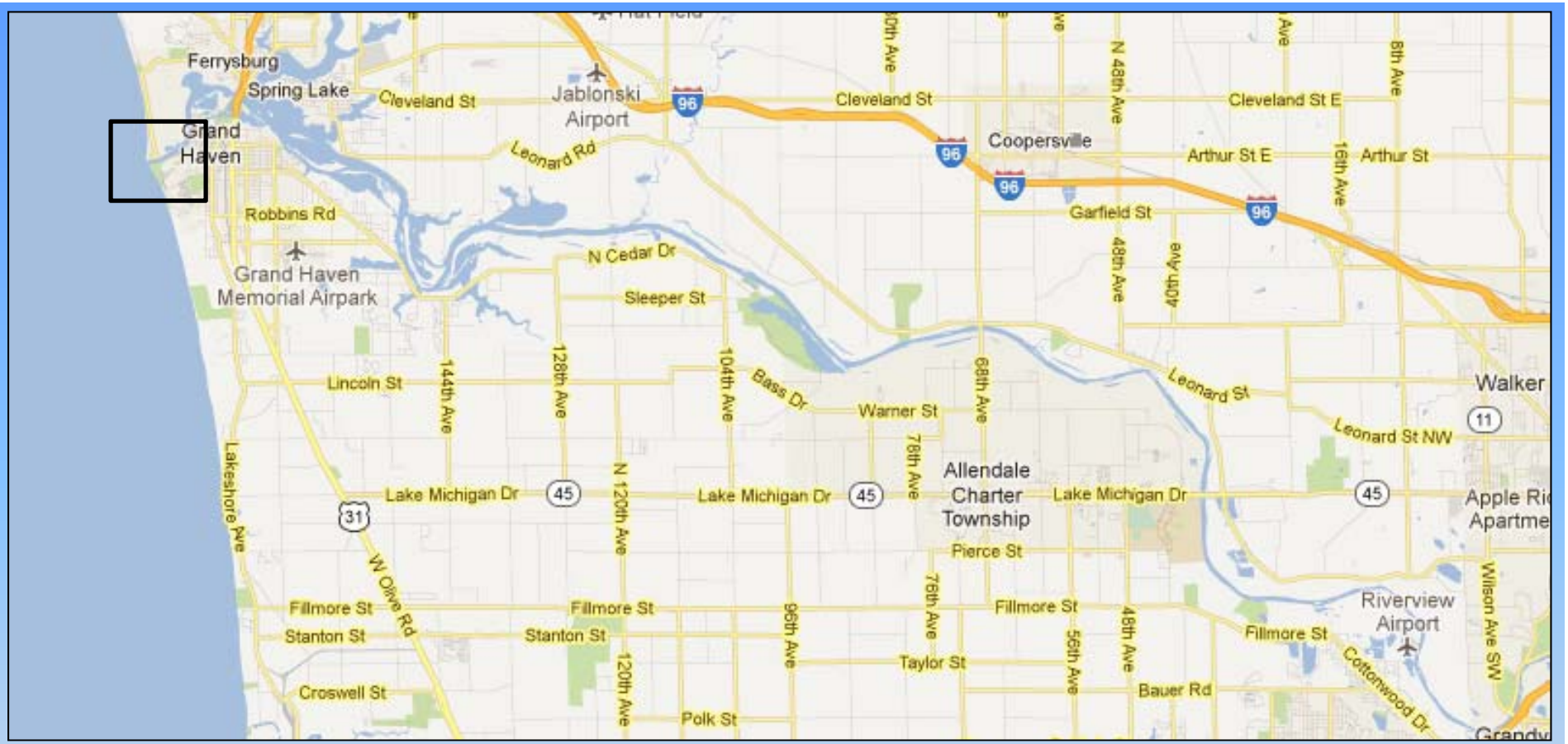
Grand River Study Sites

GR3 : Riverside Park (Robinson)



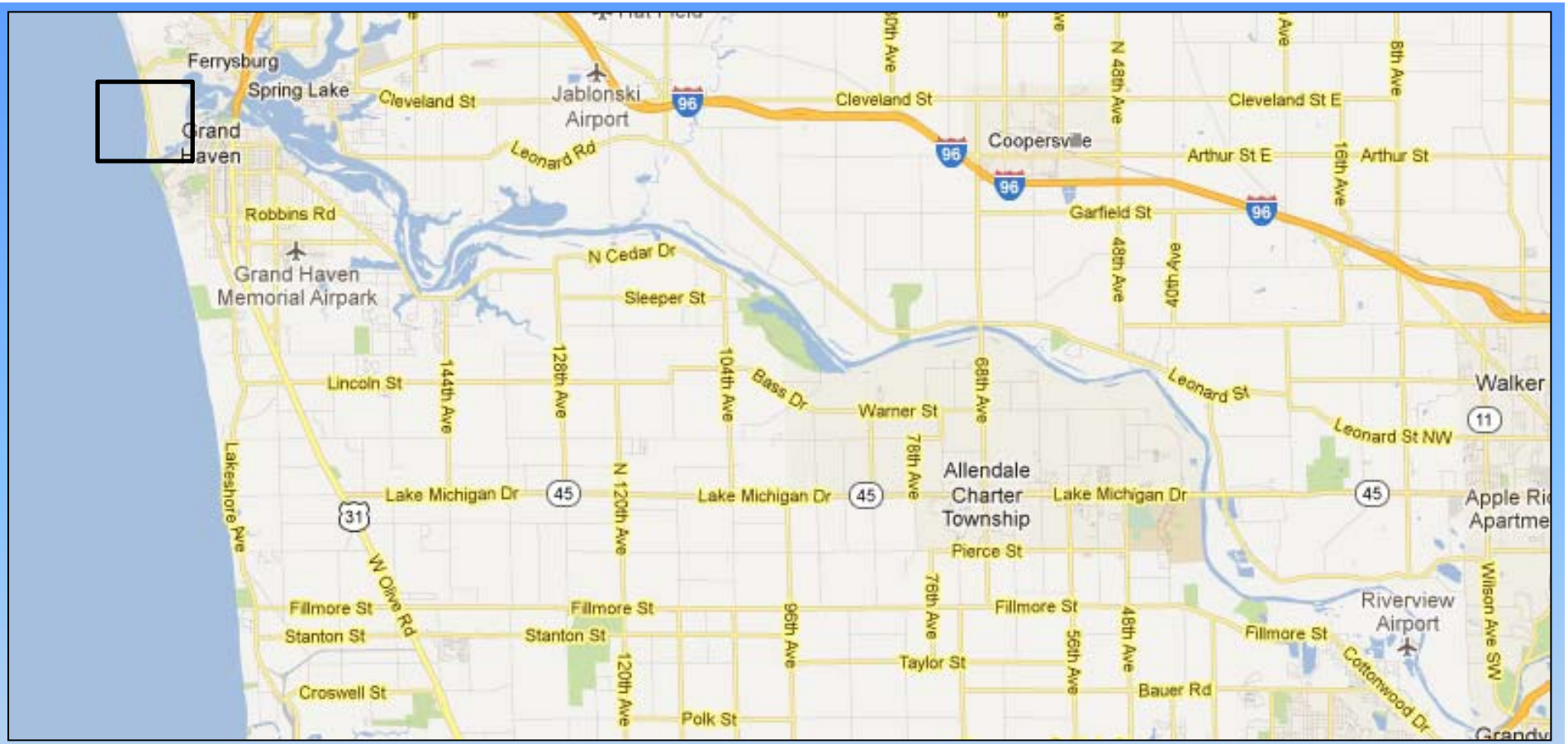
Grand River Study Sites

GR4 : Boat Access Site (Robinson)



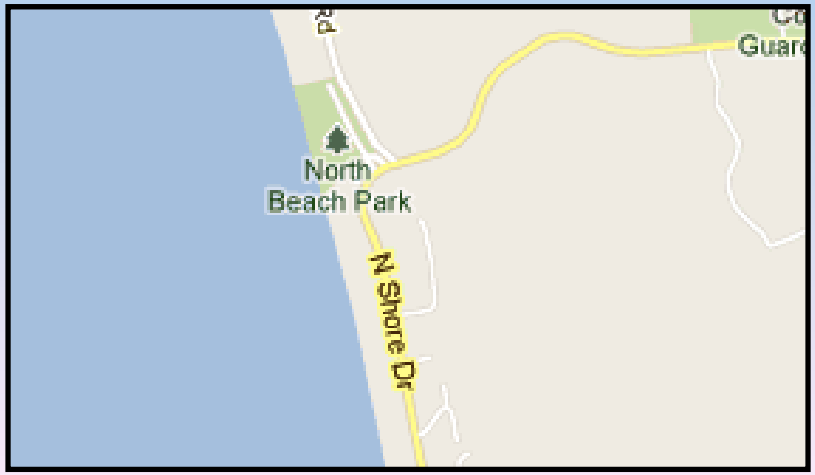
Grand River Study Sites

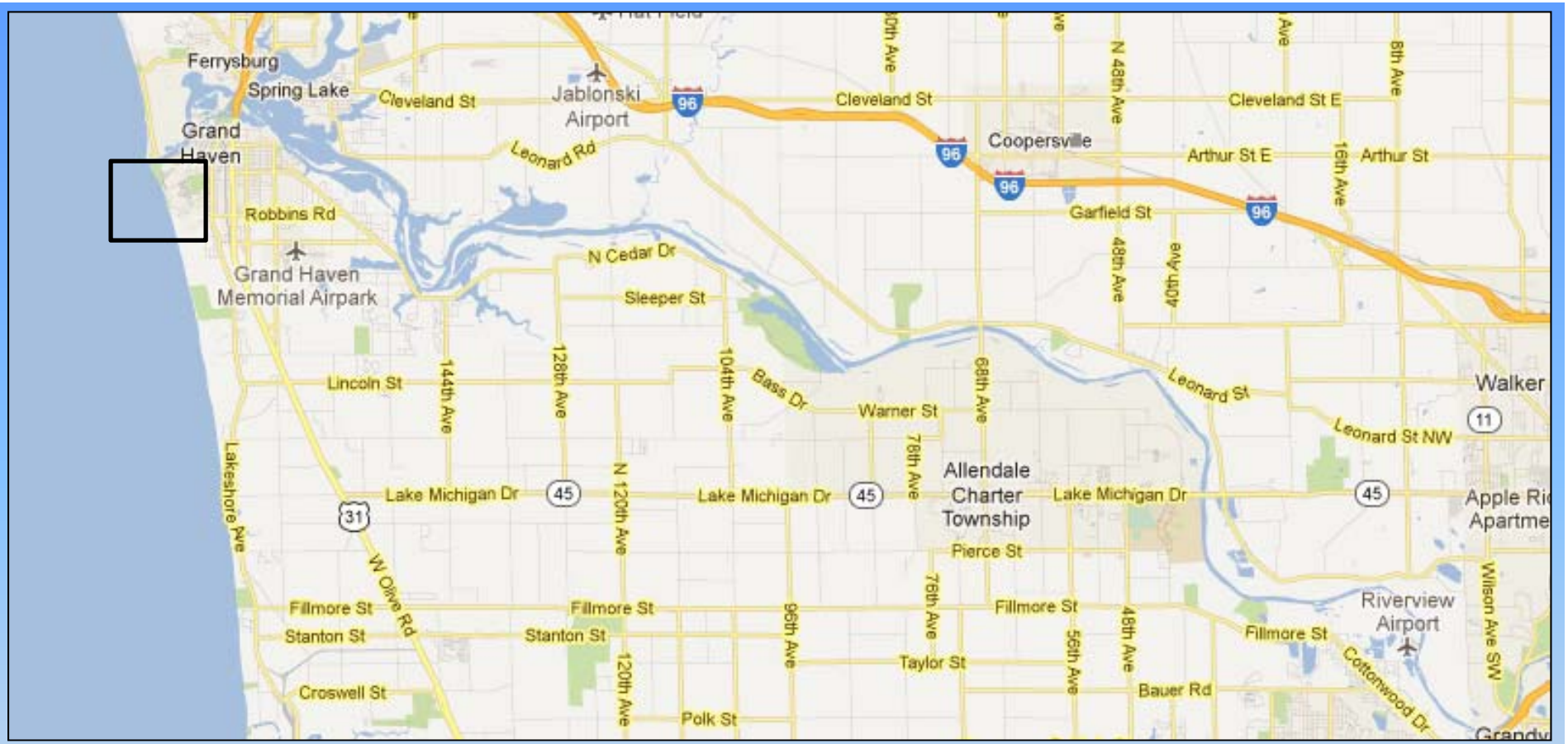
GR5 : Grand River Mouth (Grand Haven)



Grand River Study Sites

LM1 : North Beach Park (Spring Lake)

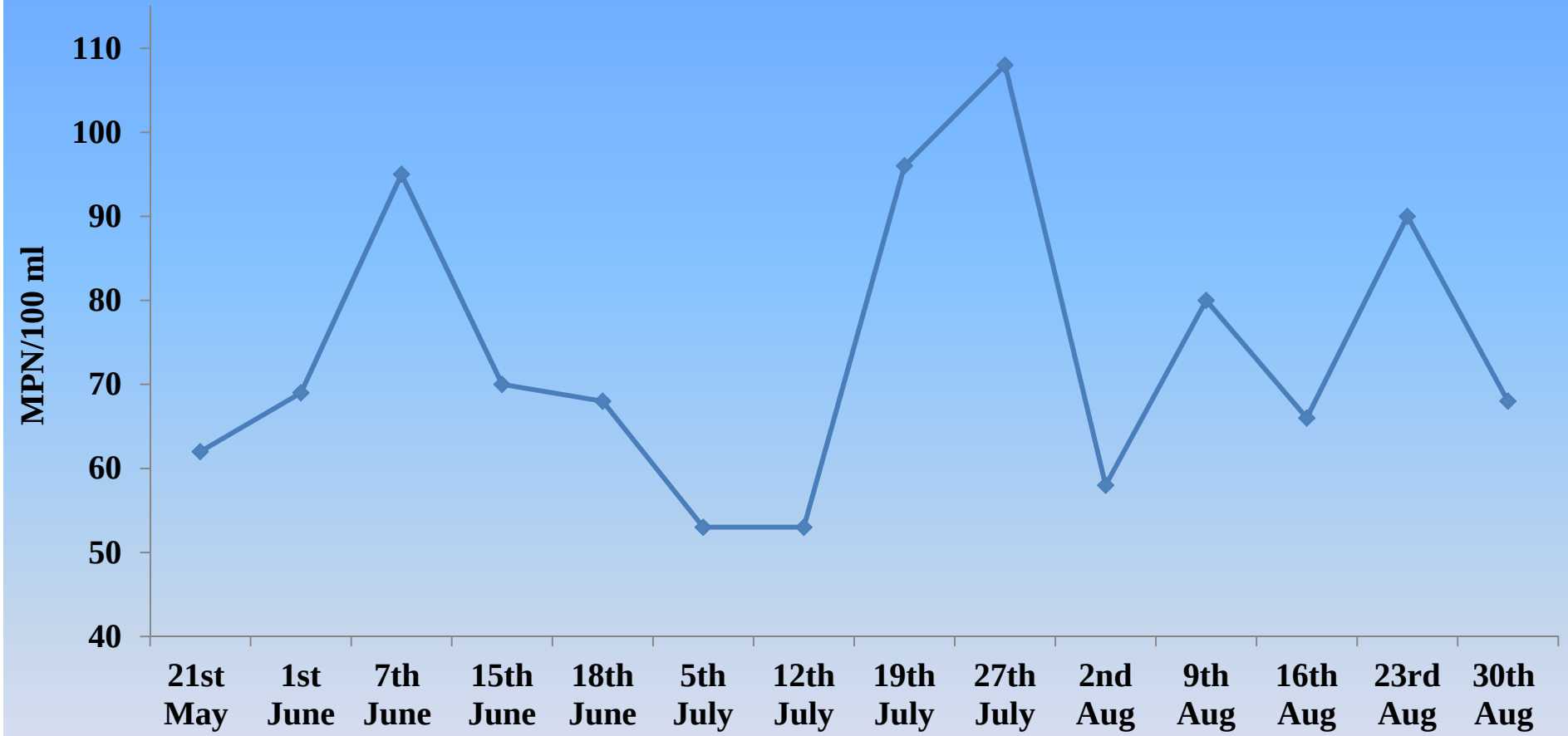




Grand River Study Sites

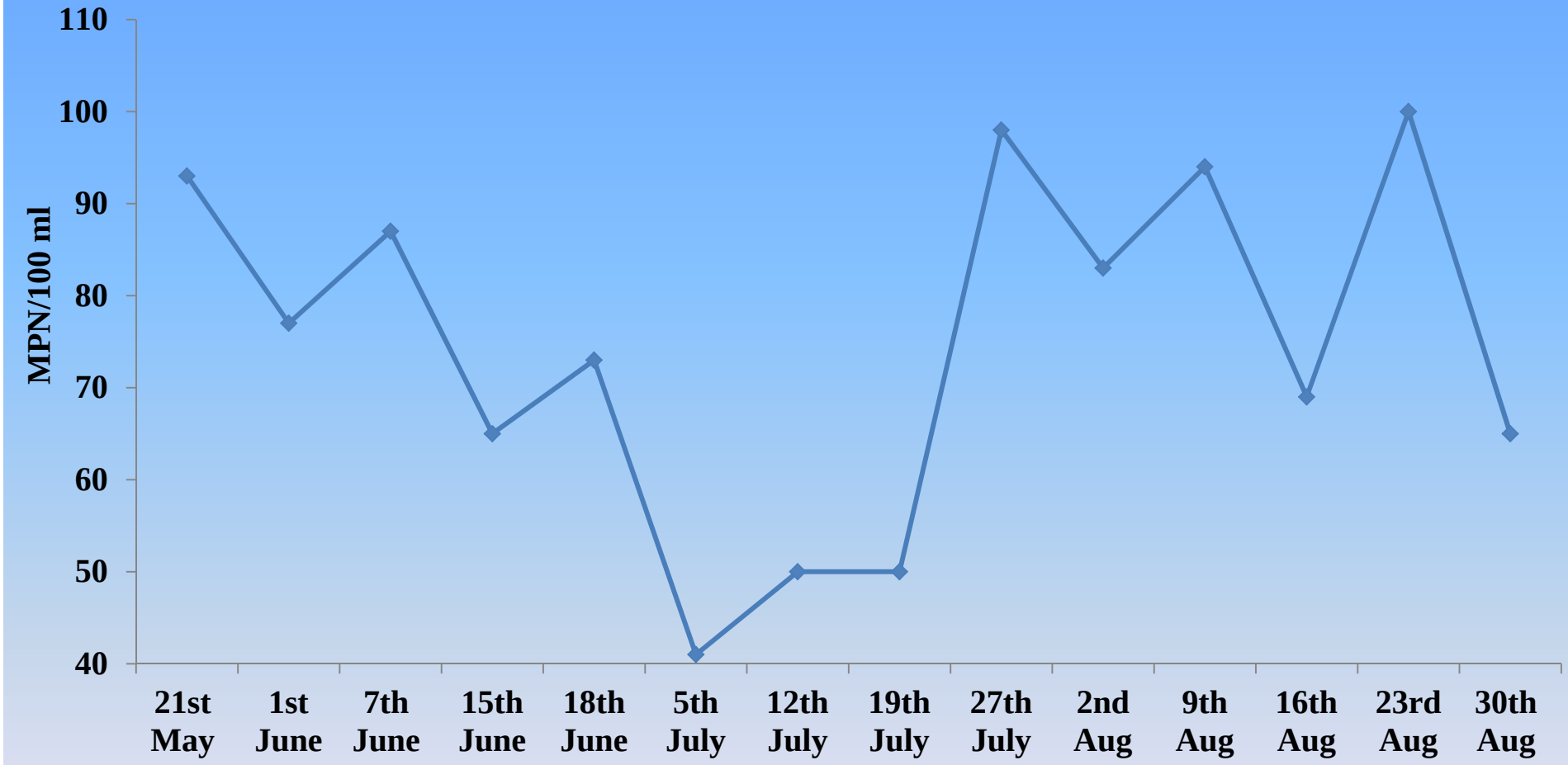
LM2 : Grand Haven State Park (Grand Haven)

E. coli results : GR1



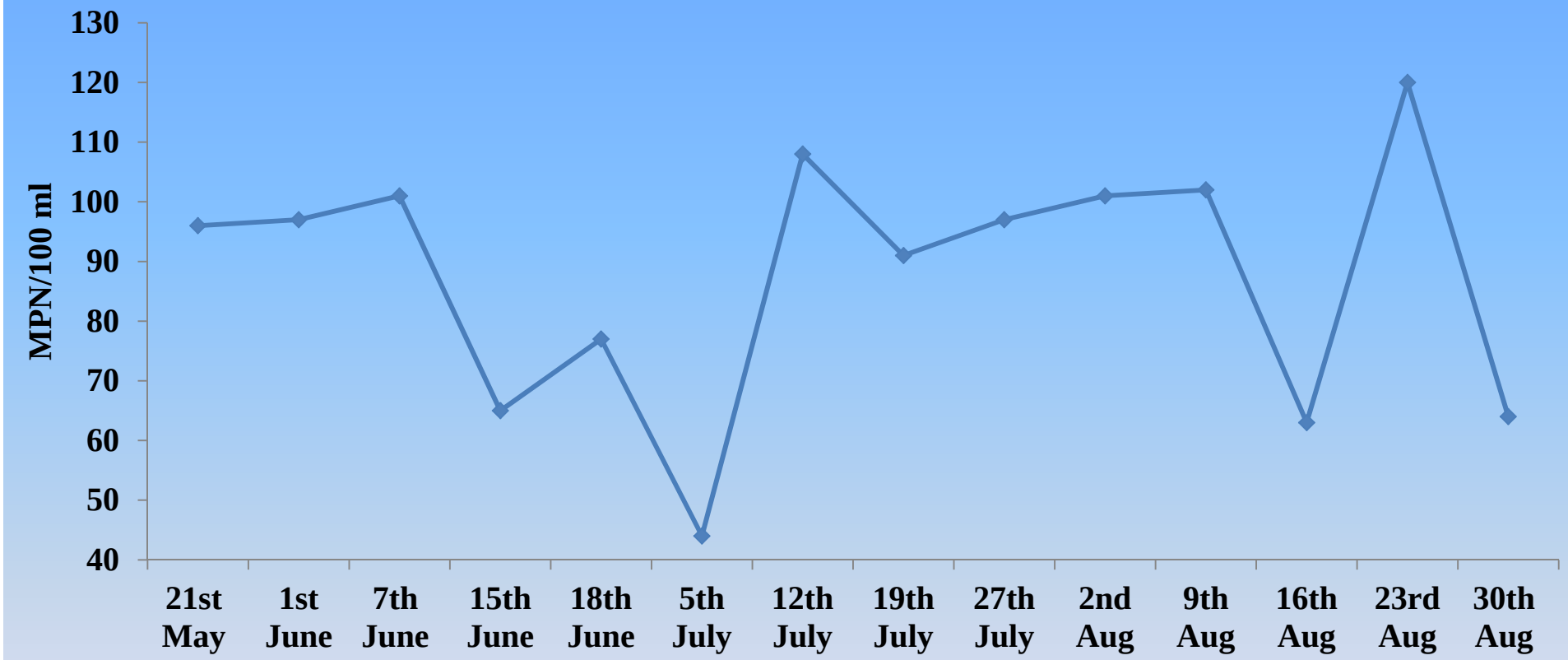
Regulatory Standard for Water Quality Exceedance : 300 MPN/100ml

E. coli results : GR2



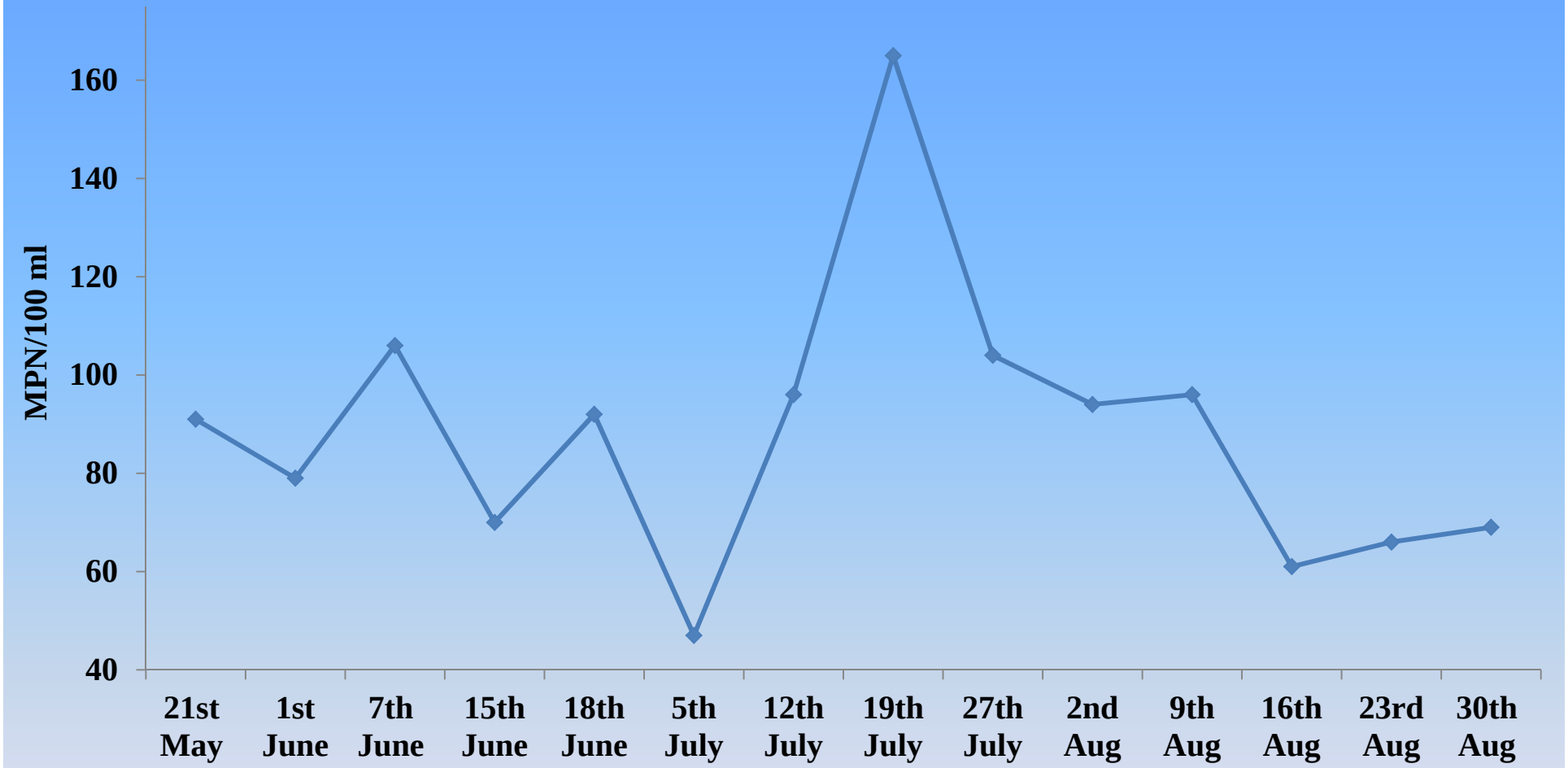
Regulatory Standard for Water Quality Exceedance : 300 MPN/100ml

E. coli results : GR3



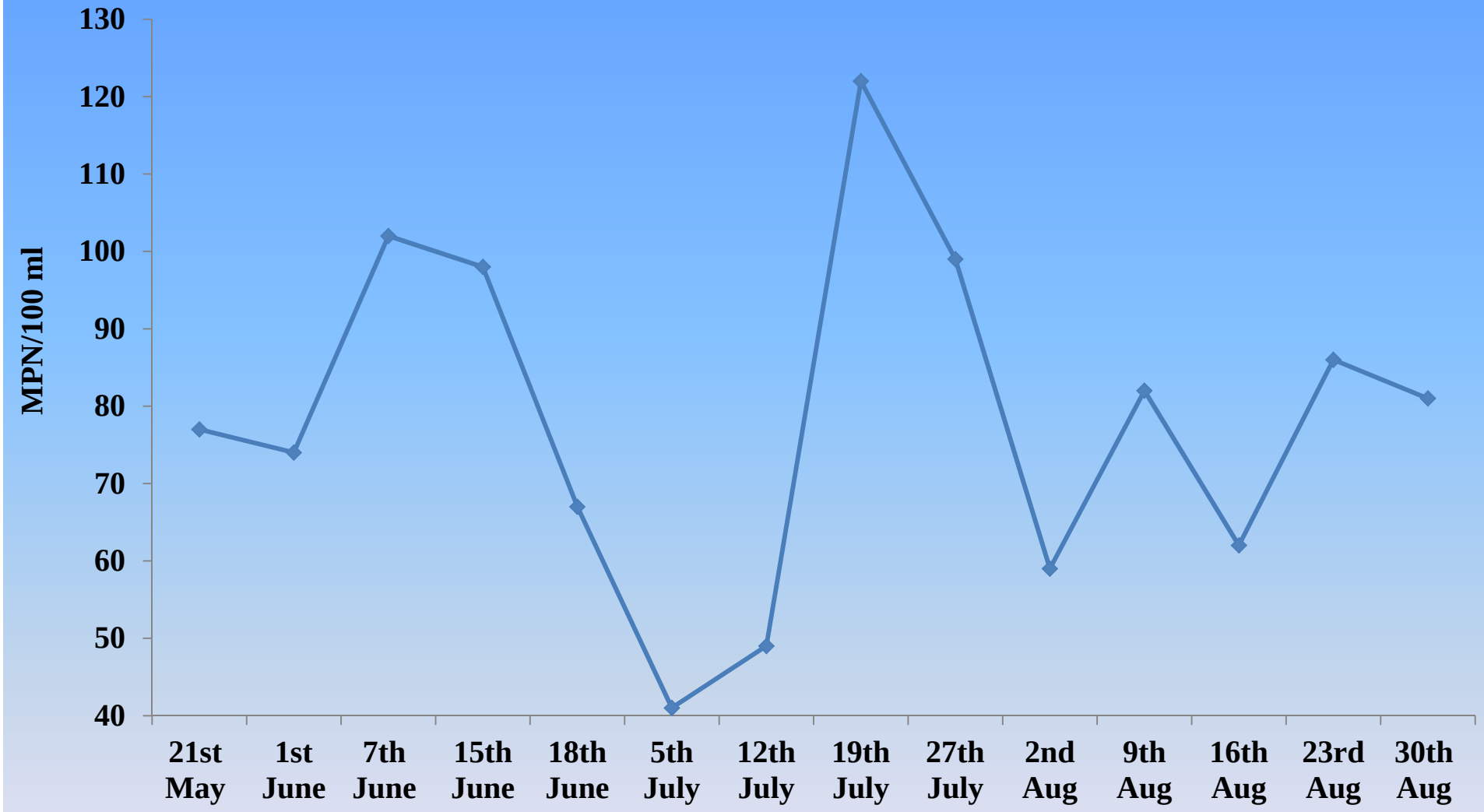
Regulatory Standard for Water Quality Exceedance : 300 MPN/100ml

E. coli results : GR4

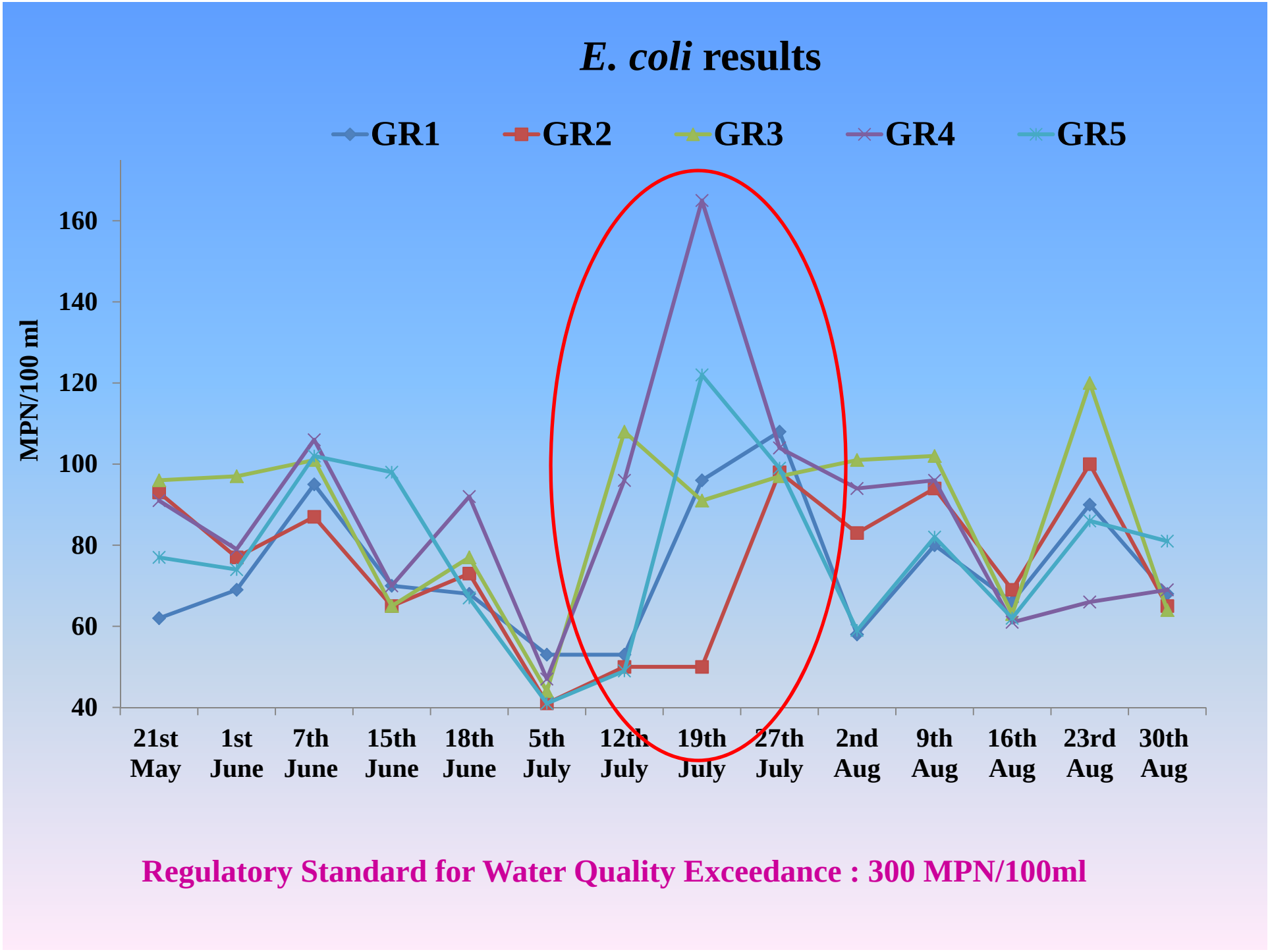


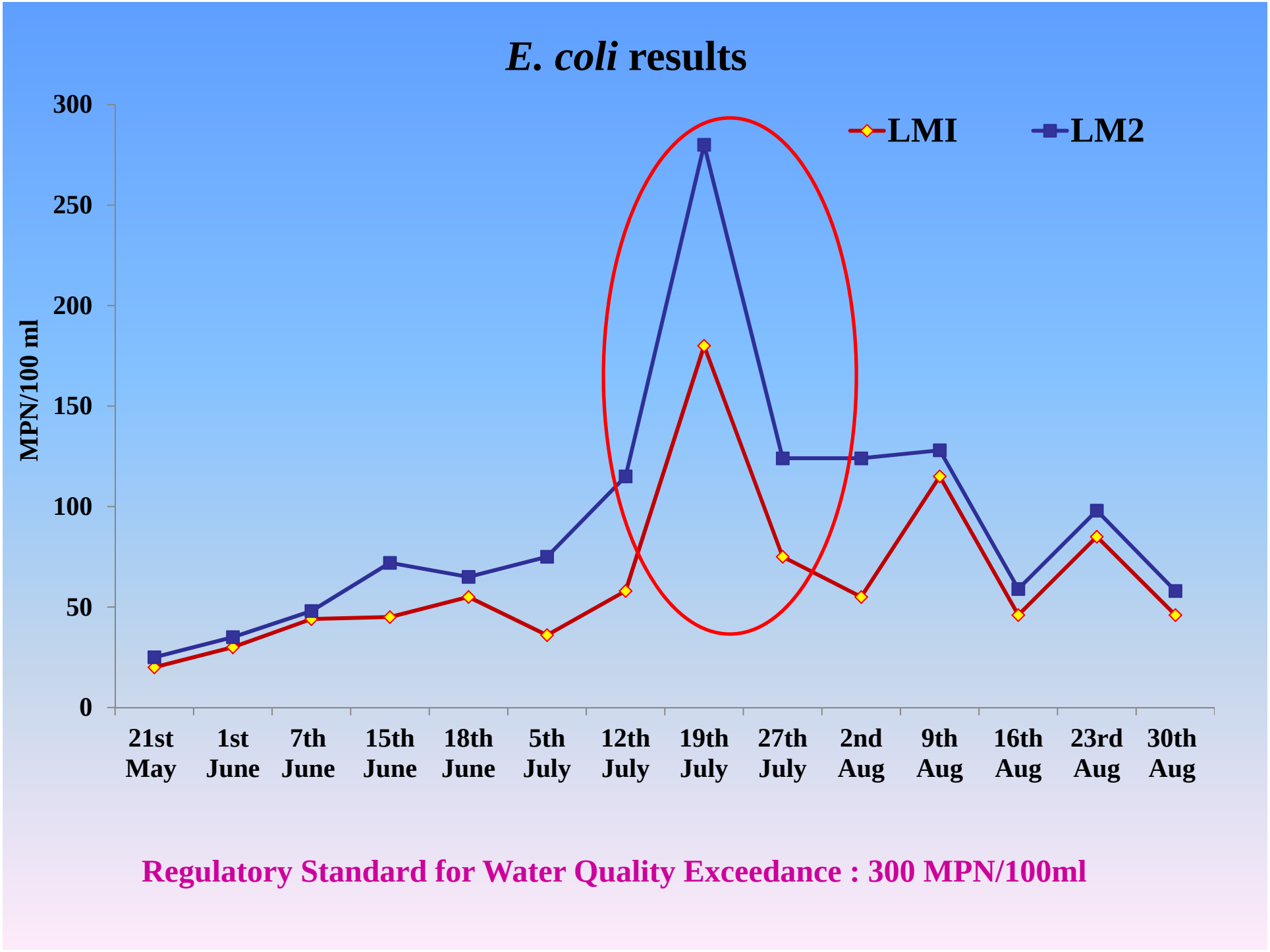
Regulatory Standard for Water Quality Exceedance : 300 MPN/100ml

E. coli results : GR5



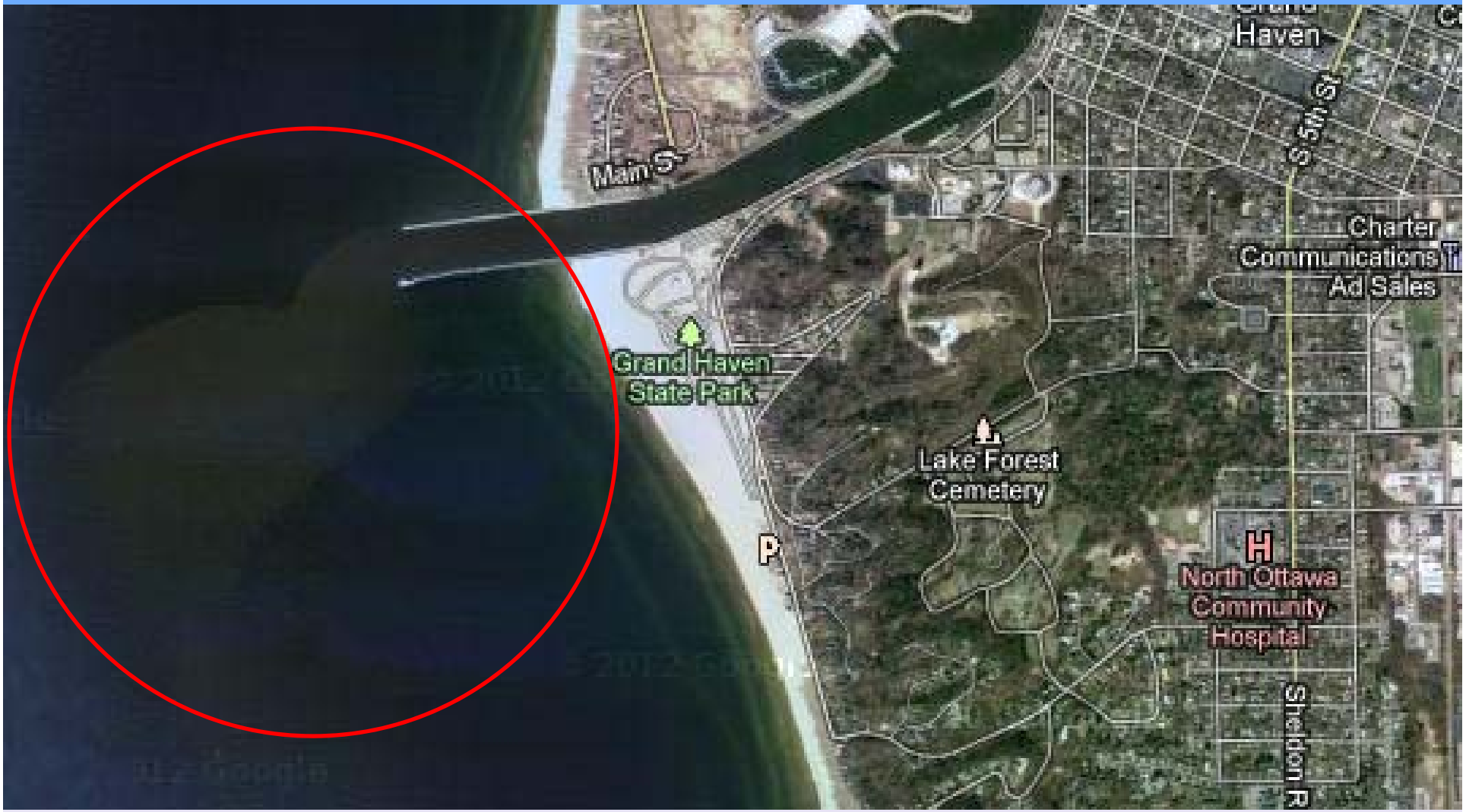
Regulatory Standard for Water Quality Exceedance : 300 MPN/100ml


E. coli results
GR1
GR2
GR3
GR4
GR5
MPN/100 ml
160
140
120
100
80
60
40
21st May
1st June
7th June
15th June
18th June
5th July
12th July
19th July
27th July
2nd Aug
9th Aug
16th Aug
23rd Aug
30th Aug
Regulatory Standard for Water Quality Exceedance : 300 MPN/100ml



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

Grand River Discharging into Lake Michigan



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			

Study Findings: *E. coli*

- Low levels
  - below the state approved regulatory standards.
- Concentration ranged
  - from 20 to 280 MPN/100ml.
- Water quality rating : Good

Study Findings : General Bacteroides

- Were present in all the tested samples
- Comparable to 10^{-3} concentration of sewage.
- Water quality rating : No conclusion can be drawn

Study Findings : Human specific Bacteroides

- Scarcely present.
- Very Low concentrations.
- Comparable to 10^{-6} concentration of sewage.

(GR1 site; August sample comparable to 10^{-3} sewage concentration)

- Water quality rating : Good

Study Findings : Cow specific Bacteroides

- Scarcely present.
- Low concentrations
- Comparable to 10^{-5} concentration of cow feces.
- Water quality rating : Good

Study Findings : Swine specific Bacteroides

- Moderately present.
- Low concentrations
- Comparable to 10^{-4} concentration of swine feces.
- Water quality rating : Moderate to low risk

What's next?

- Extend the source tracking to Geese, and Gulls specific markers.

- The Bacteroides target is not well established.
- Researches are still debating the use of best marker.
- Our research in Hope College confirmed the same (low Bacteroides targets in gulls and goose feaces).
- Will continue to research the best markers for gulls and goose.
- The Grand River DNA samples has been archived for future studies.

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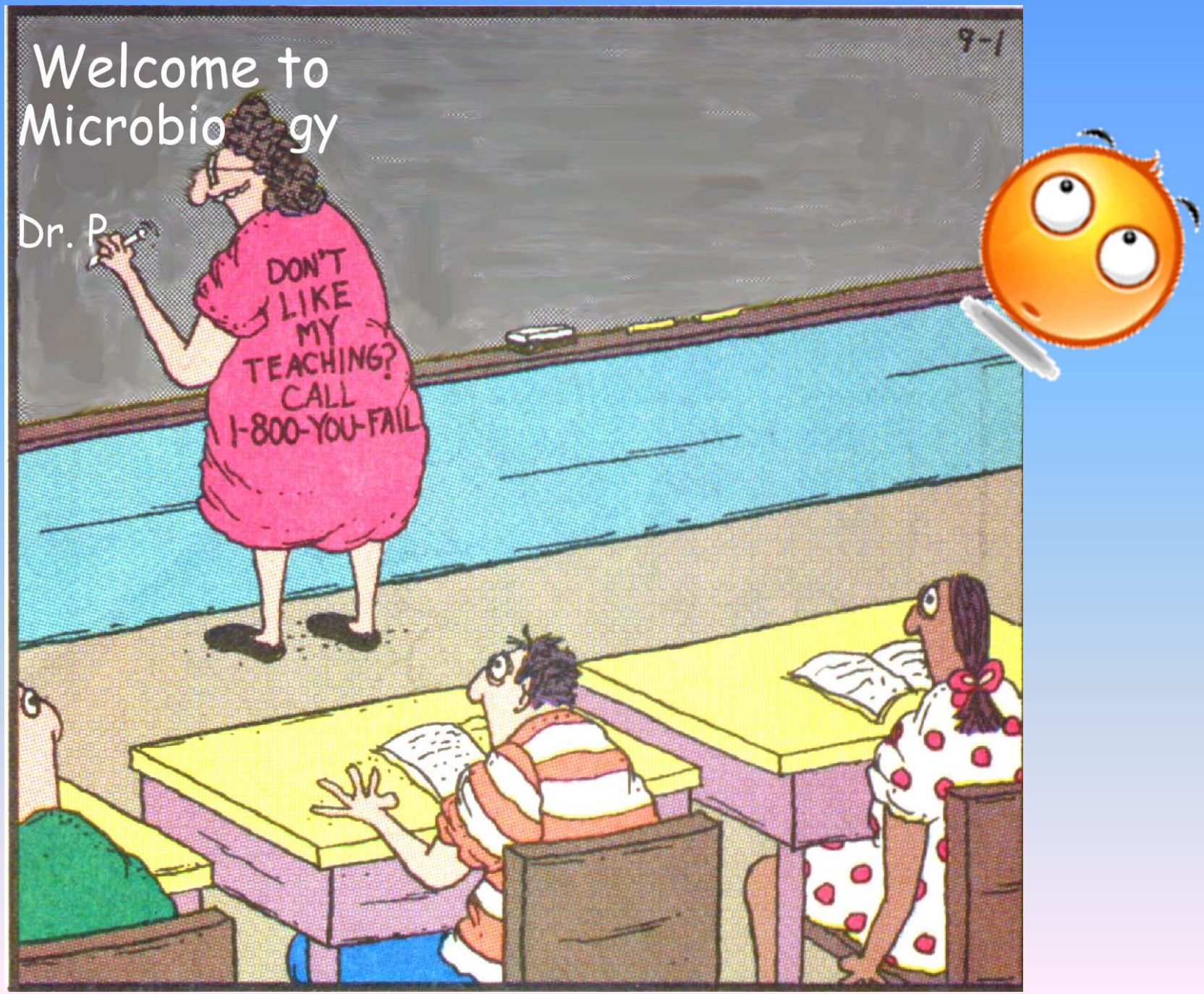
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