

Macatawa Watershed Water Quality Research Project

An aerial photograph of the Macatawa Watershed. The central feature is a large, irregularly shaped lake with a deep blue-green hue. The lake is surrounded by dense green forest and some residential or commercial buildings. In the foreground, a concrete dam structure extends from the shore into the lake, creating a smaller body of water. The background shows a vast expanse of land with a mix of forest and open fields under a clear blue sky.

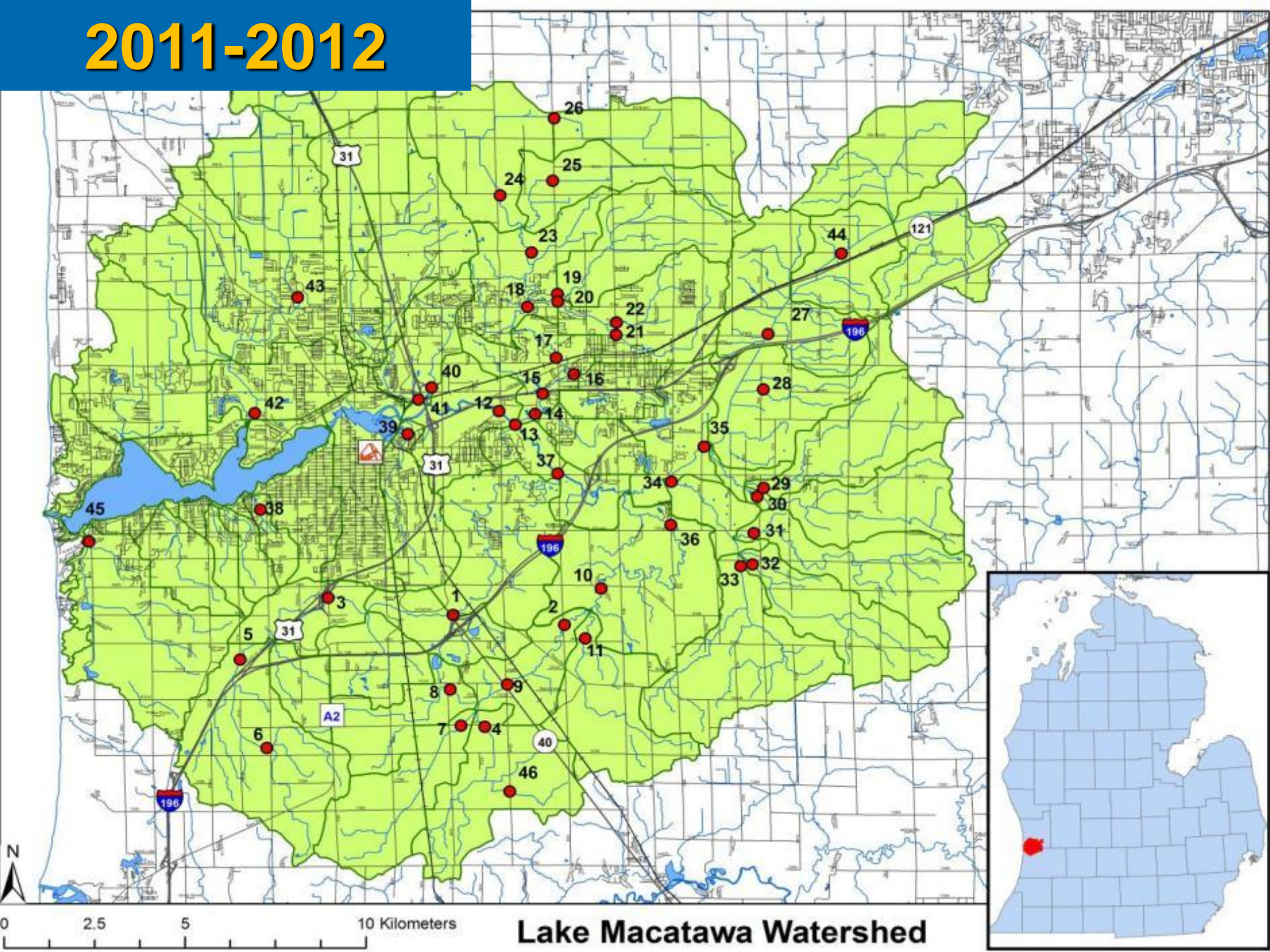
Graham F. Peaslee

Michael J. Pikaart

Hope College Chemistry Dept.

Nov. 1 2012

2011-2012



Lake Macatawa Watershed

**Please Do Not
Disturb This Equipment**

This equipment is sampling the suspended sediment content of the stream and is part of a watershed monitoring effort conducted by the Macatawa Area Coordinating Council and Hope College.

If you have any questions about this sampler, please contact:

Dr. Graham Peaslee peaslee@hope.edu
616-395-7117 OR

Mary Fales mfales@mac-macc.org
616-395-2688







Site 1



Site 7



Site 14

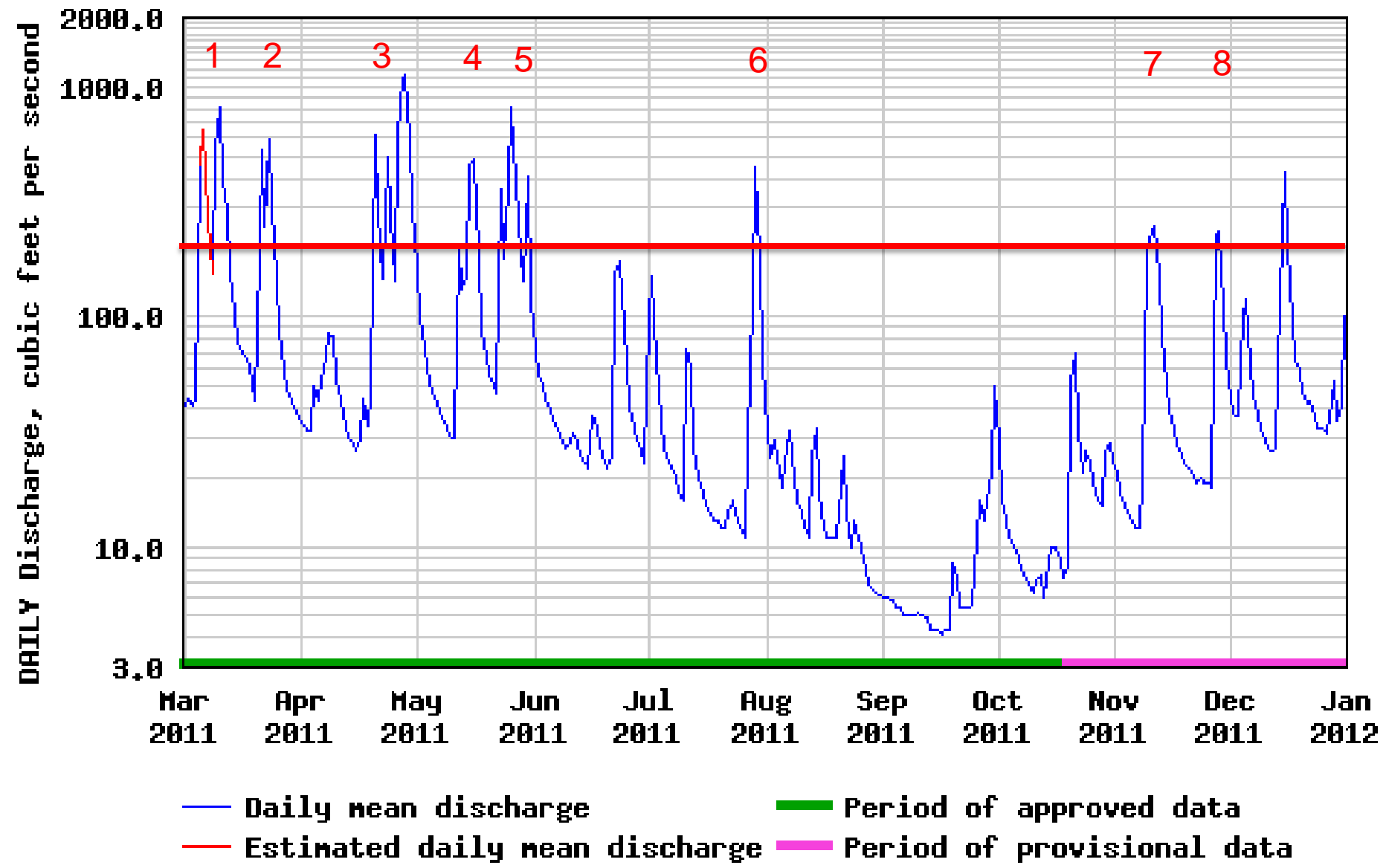


Site 27

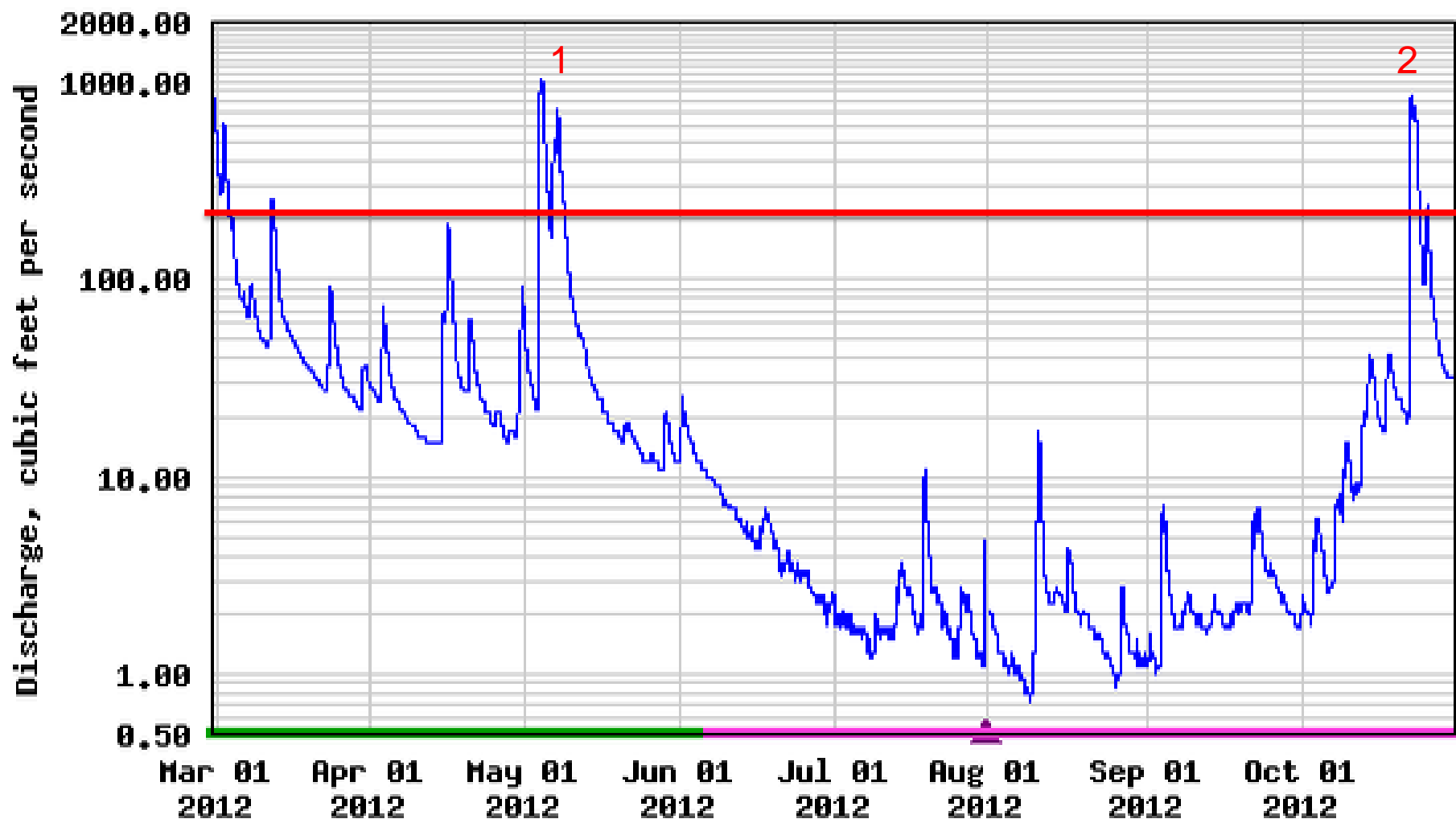
Sample Collection



USGS 04108800 MACATAWA RIVER AT STATE ROAD NEAR ZEELAND, MI



USGS 04108800 MACATAWA RIVER AT STATE ROAD NEAR ZEELAND, MI



— Discharge

— Period of approved data

▲ Equipment malfunction

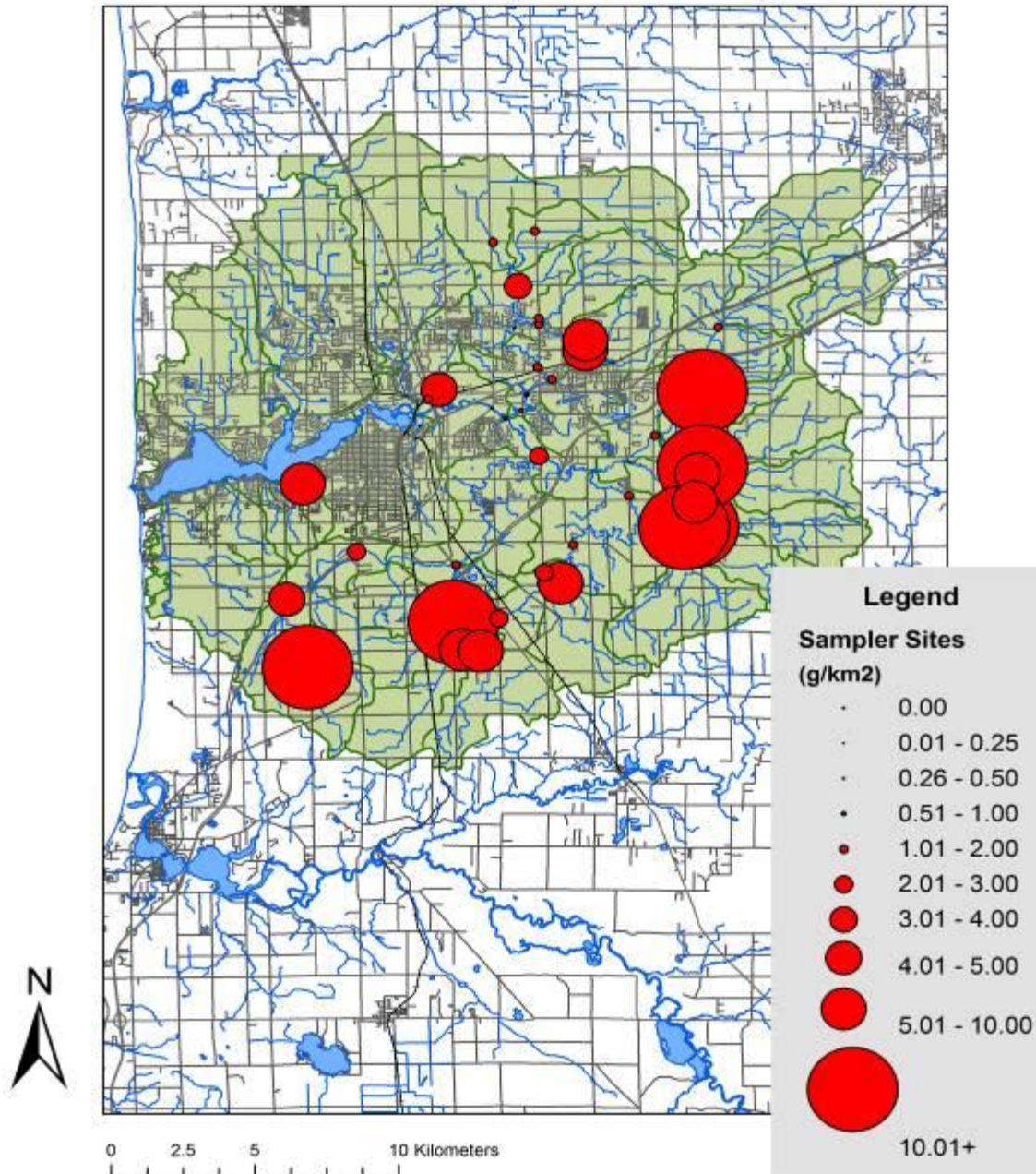
— Period of provisional data

Sediment Mass Per Total Upstream Area Event 5 (May 23-29, 2011)

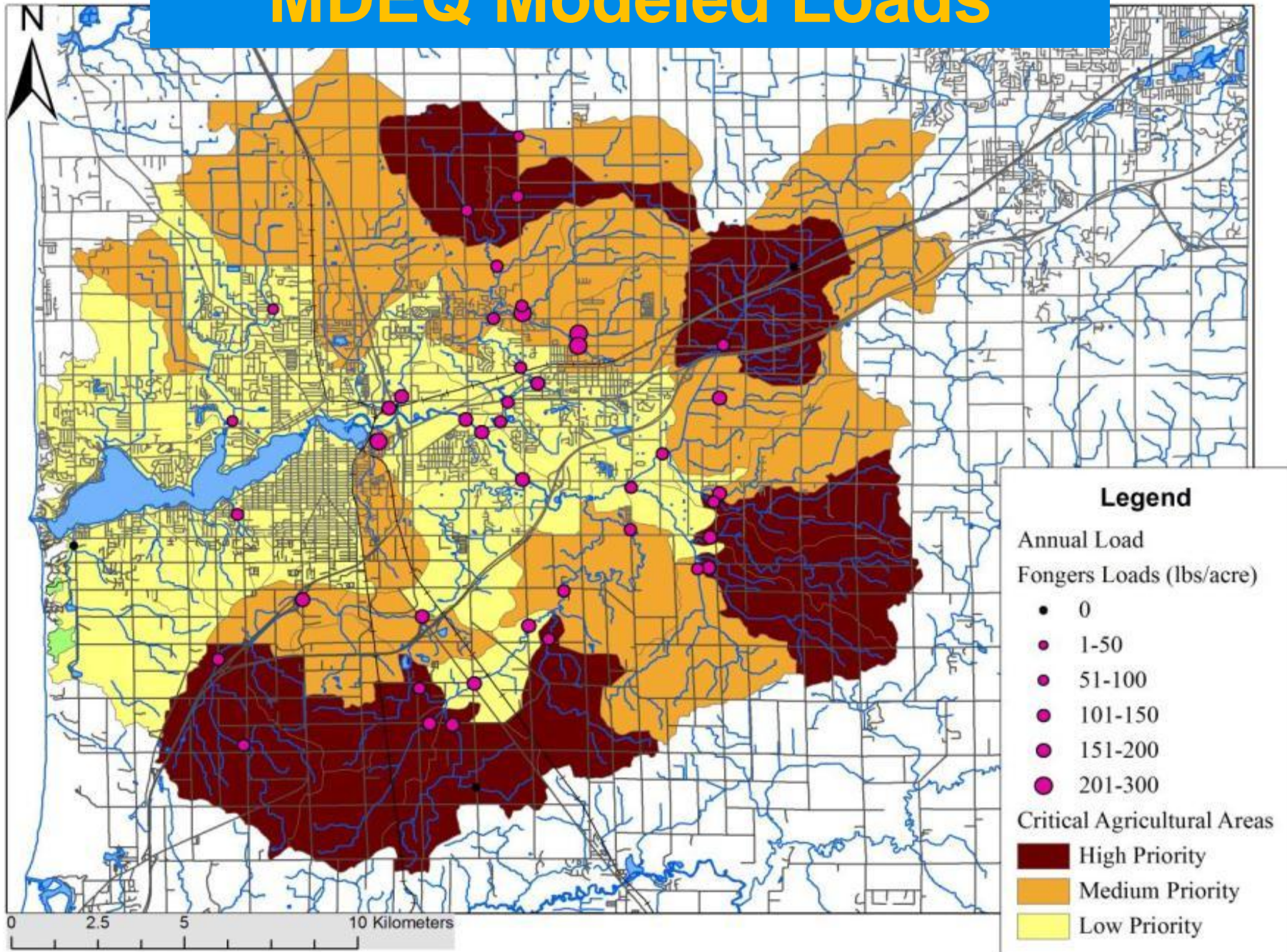
Preliminary Results:

A typical “event”

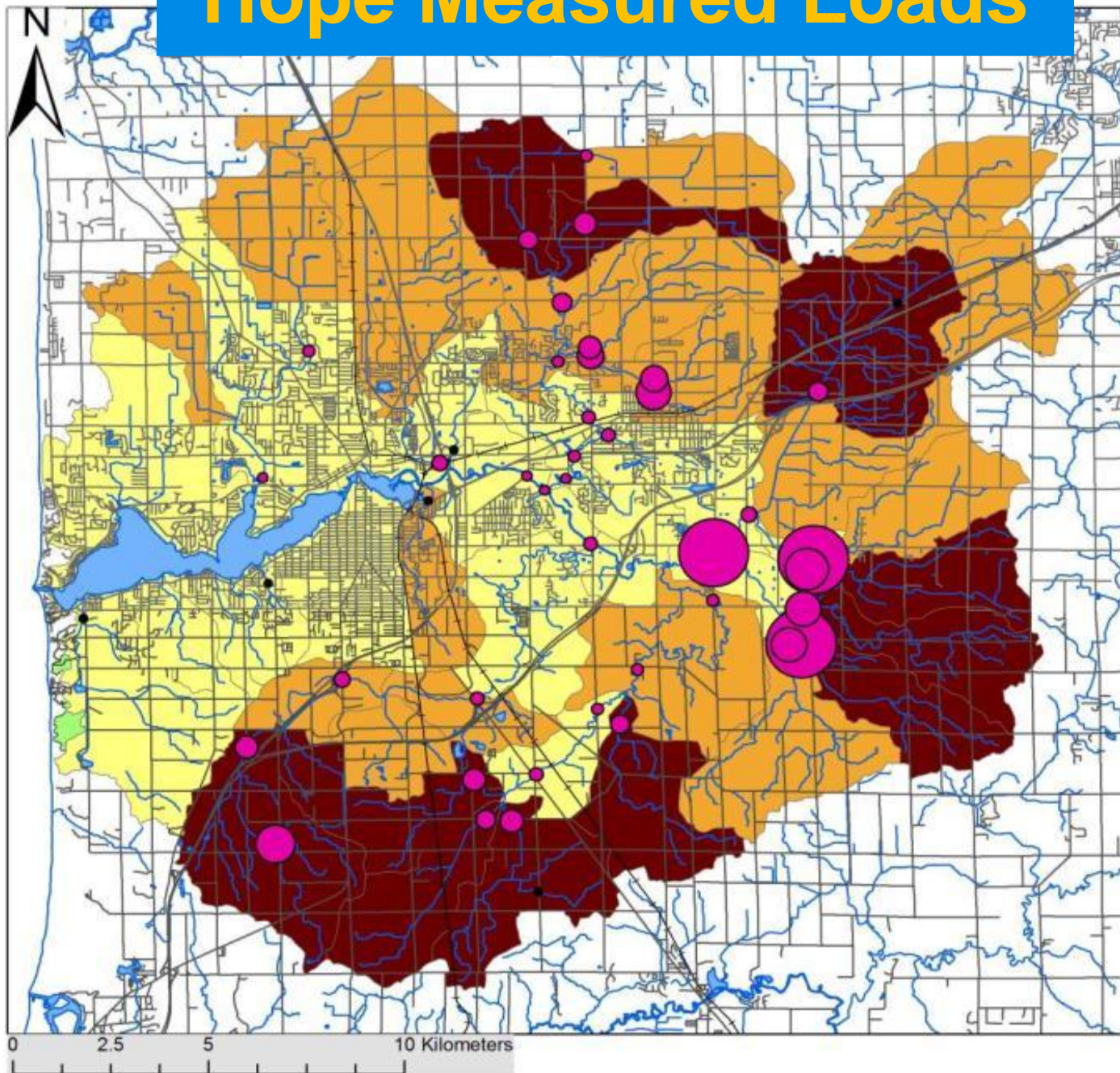
Sediment/km²



MDEQ Modeled Loads



Hope Measured Loads



Legend

Annual Load

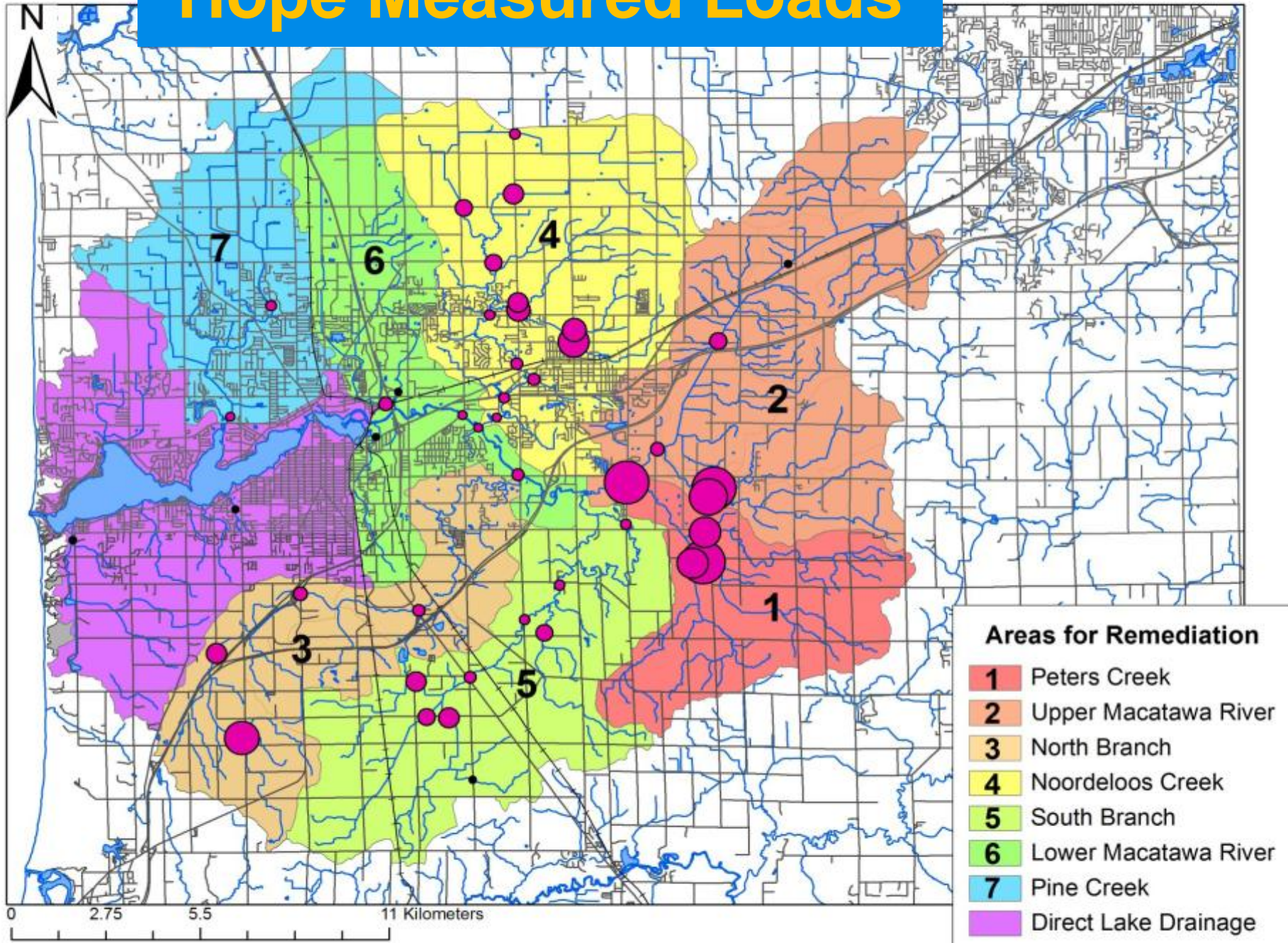
Hope Loads (lbs/acre)

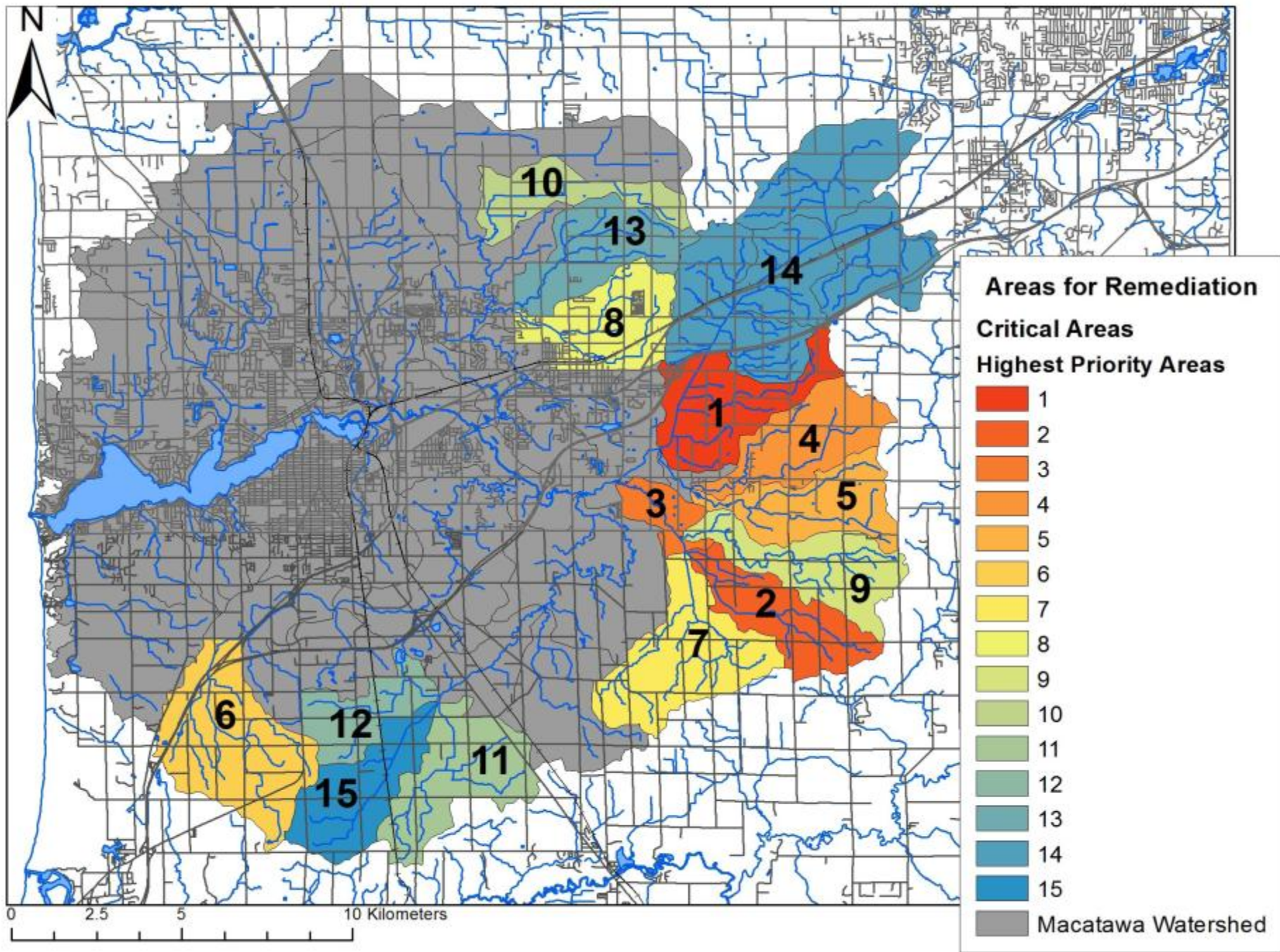
- 0
- 1 - 50
- 51 - 100
- 101 - 150
- 151 - 200
- 201 - 300
- 301 - 400
- 401 - 500
- 501 - 600
- 601 - 700
- 701 - 800
- 801 - 900
- 901 - 1000
- 1001 - 2000
- 2001 - 3000

Critical Agricultural Areas

- High Priority
- Medium Priority
- Low Priority

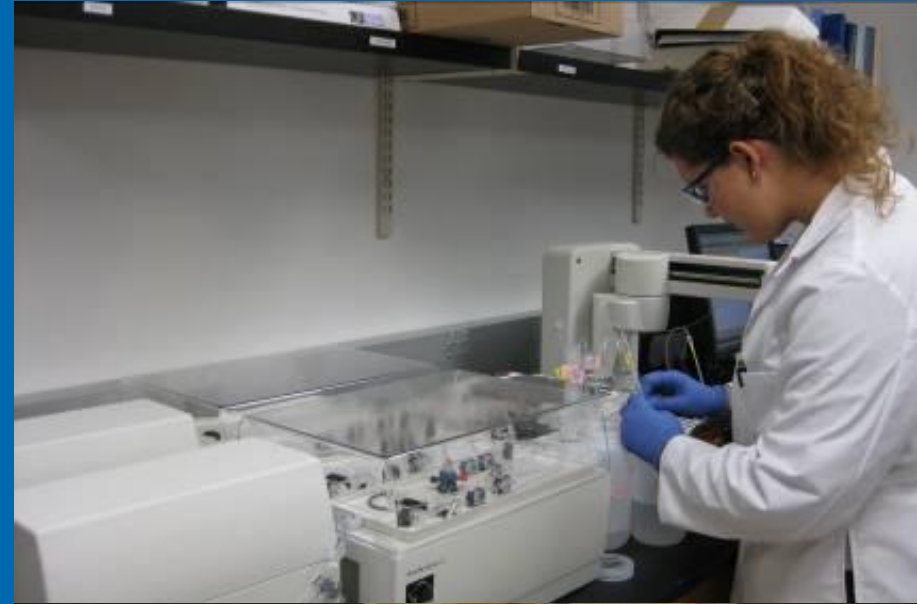
Hope Measured Loads



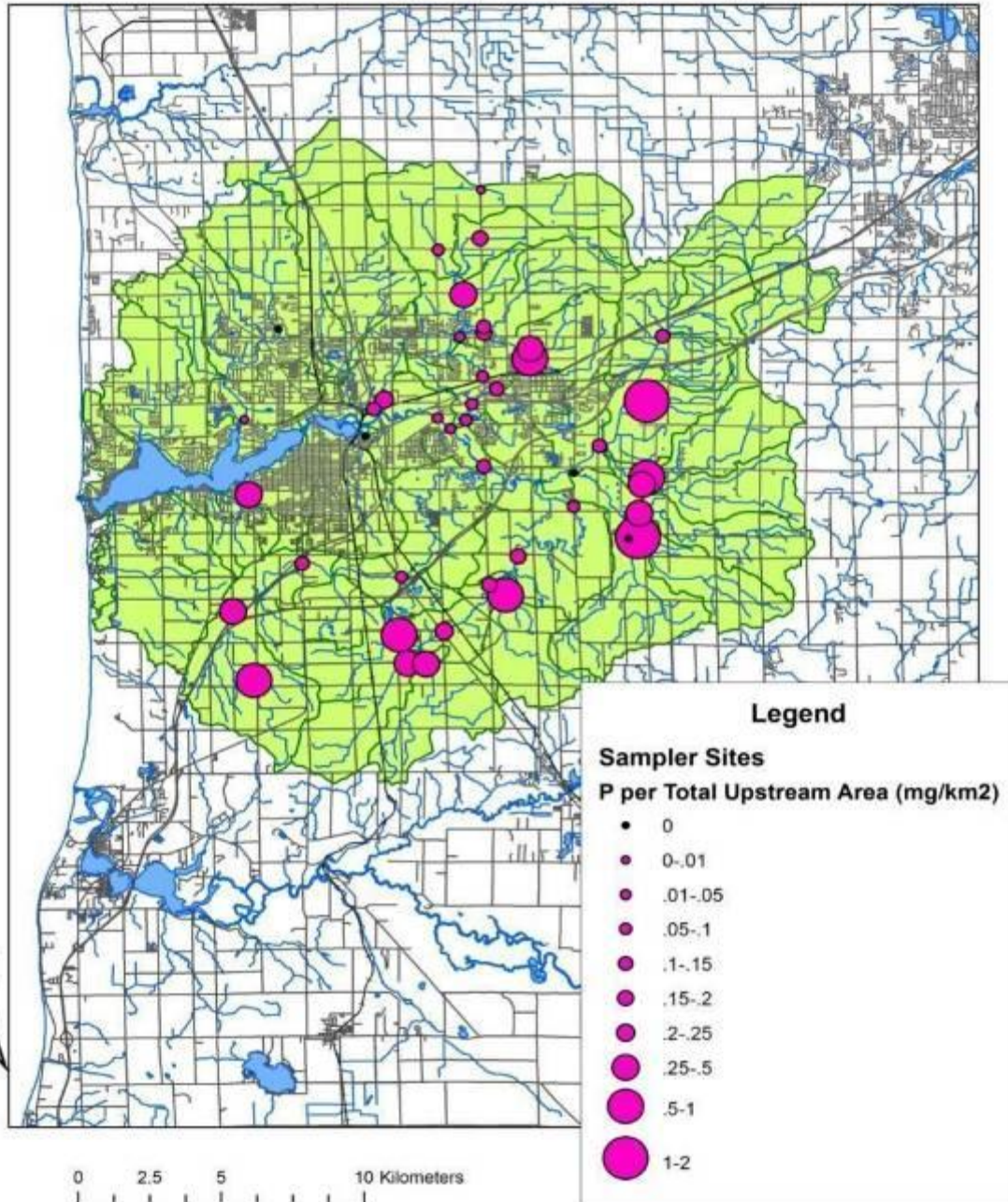


Sediment Fingerprinting Methods

- PO_4 Analysis
- Reflected light: Color
- Elemental analysis
- Radiometric Dating
- Biologicals: Pollen



Phosphorous Concentrations Per Total Upstream Area Event 5 (May 23-29, 2011)

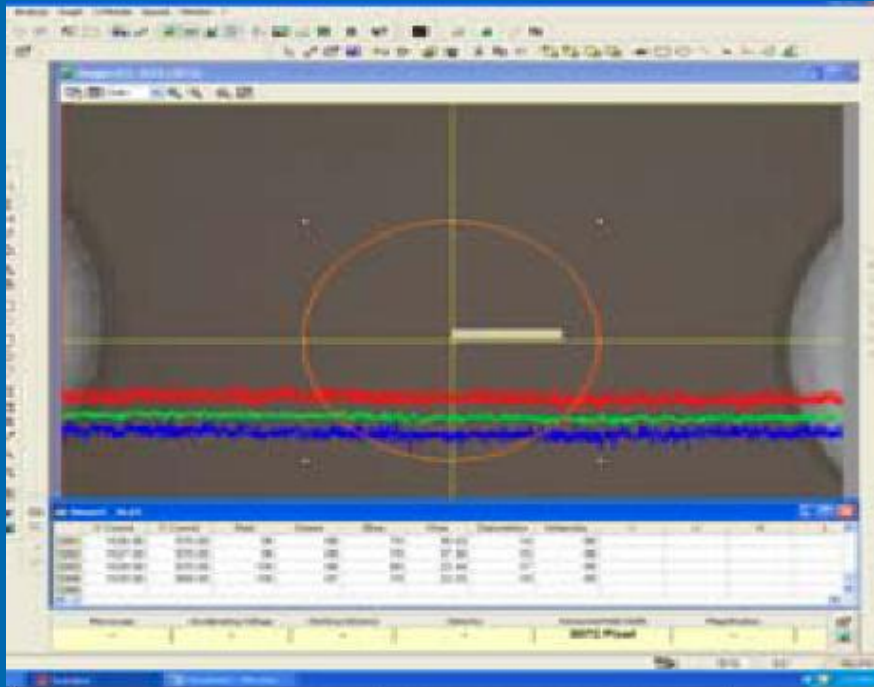


Phosphates:

A typical
“event”

Soluble PO_4
Fe-bound PO_4
Ca-bound PO_4

RGB Color Analysis



16C

42C

27A

38B

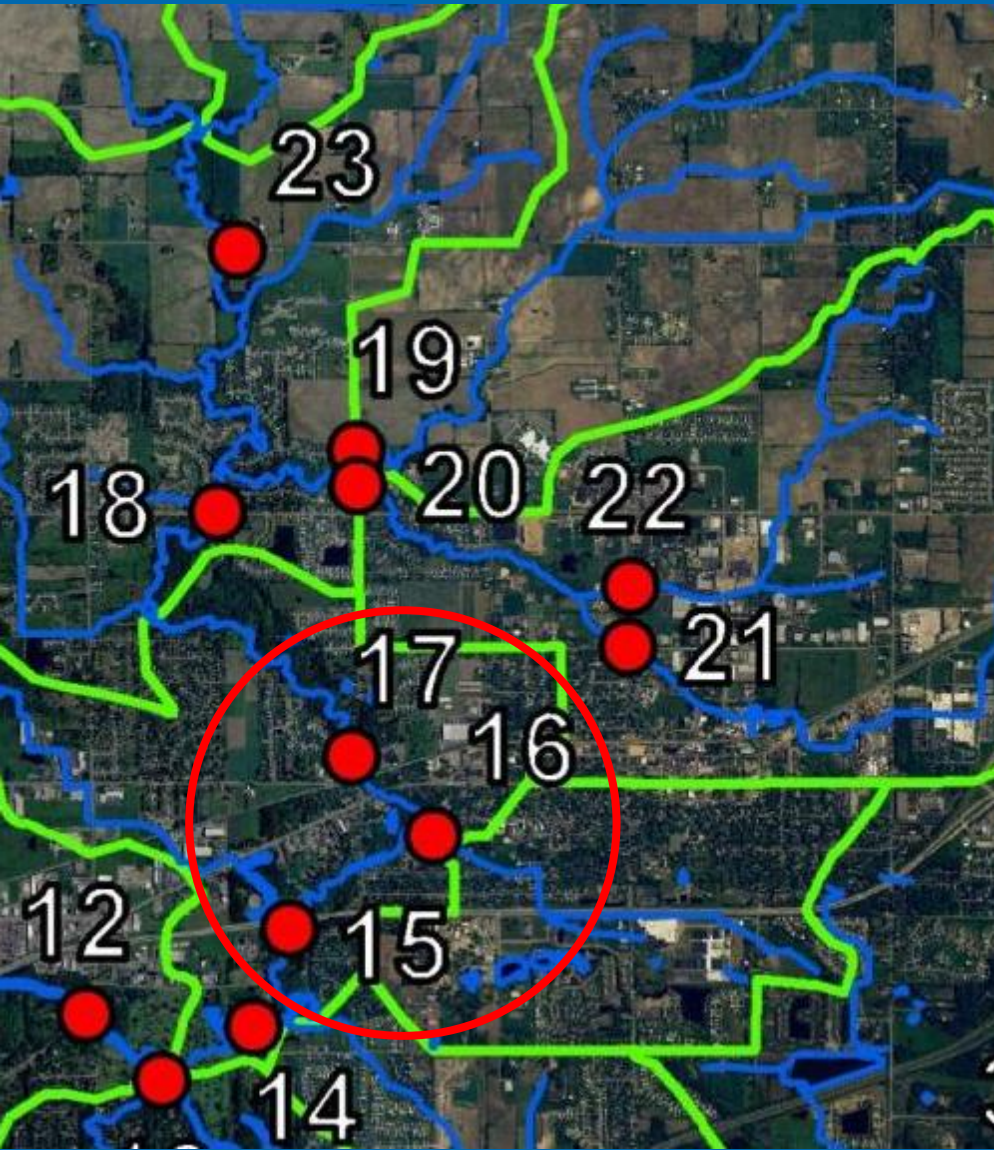
6C

14Ax

43A

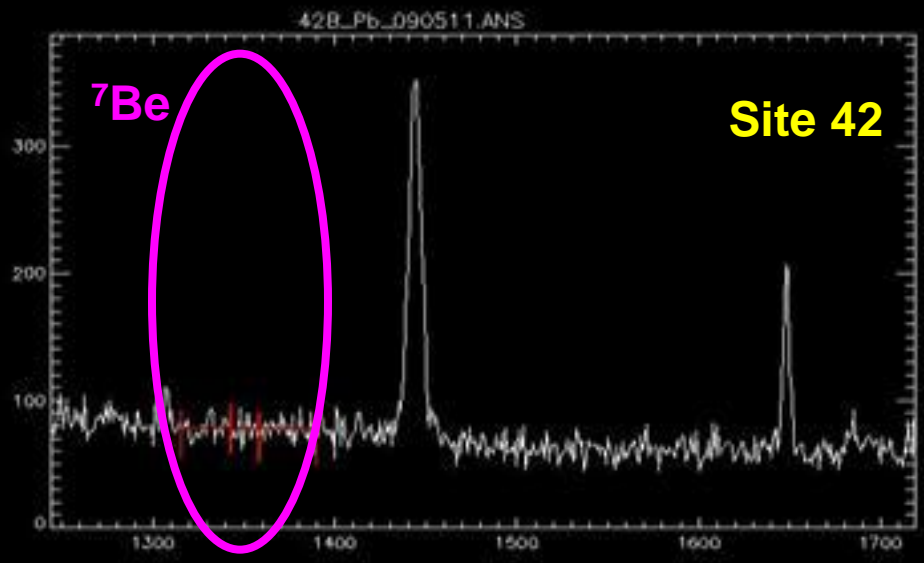
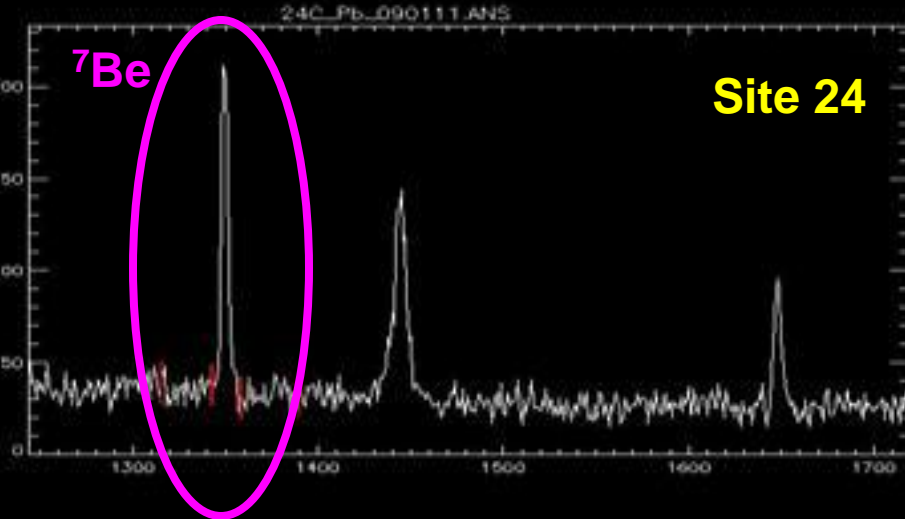
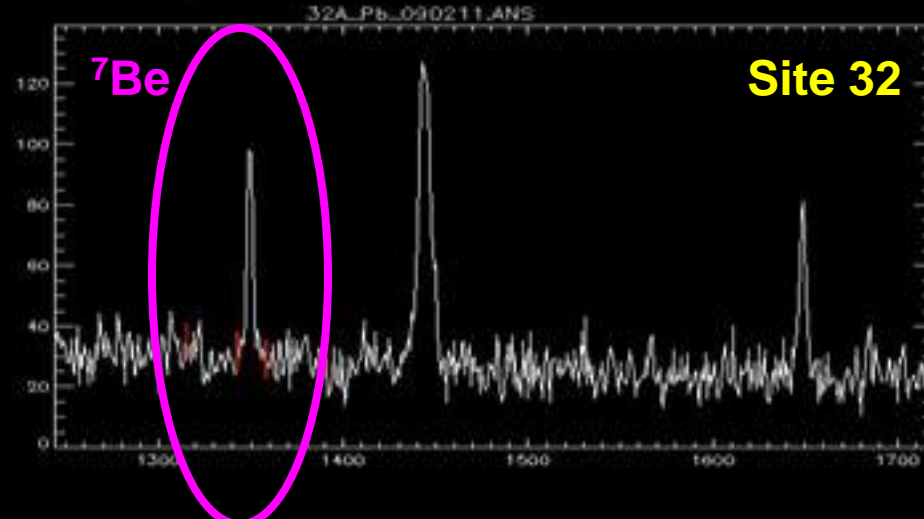
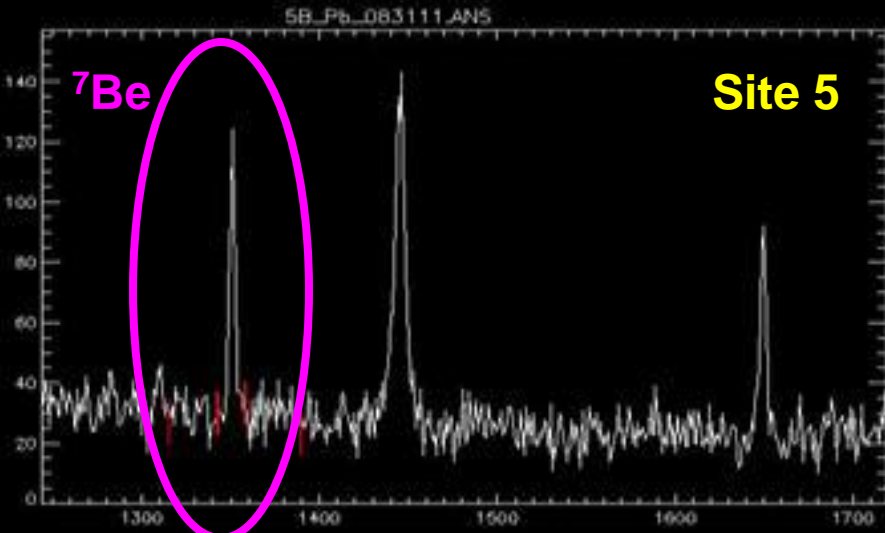
32C

Elemental Analysis



- **Sediment Ca content:**
 - Site 16: 7.85% Ca
 - Site 17: 1.89% Ca
 - Site 15: 2.17% Ca
- **Weighted average:**
 - Site 16 = 4.9%
 - Site 17 = 95.1%
- **Modeled load estimates:**
 - Site 16 = 6.2%
 - Site 17 = 93.7%

^7Be Radiodating Results



Most sediment collected is topsoil (<1 yr old) = Run-off

The result of a dry year...



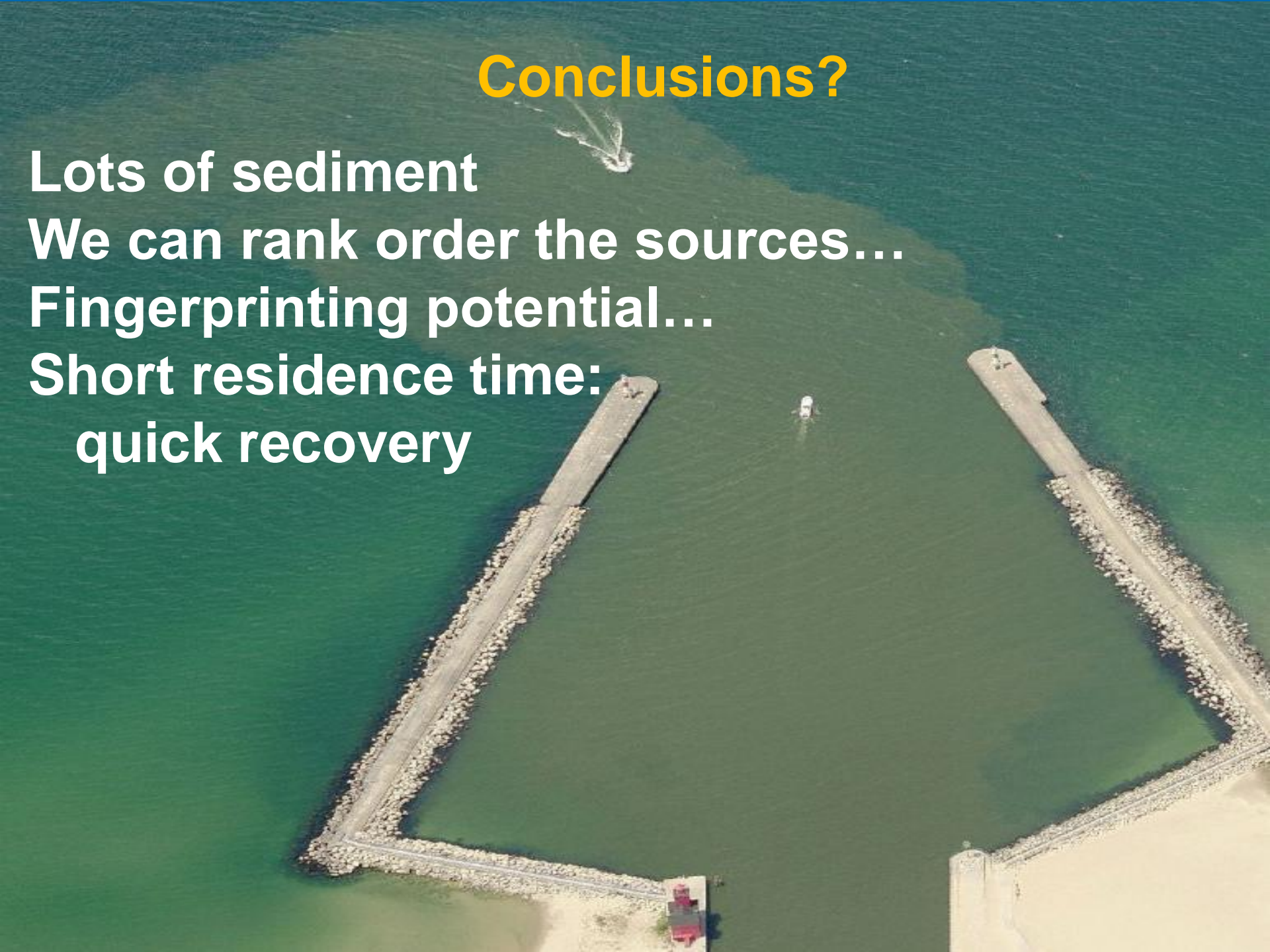
Conclusions?

Lots of sediment

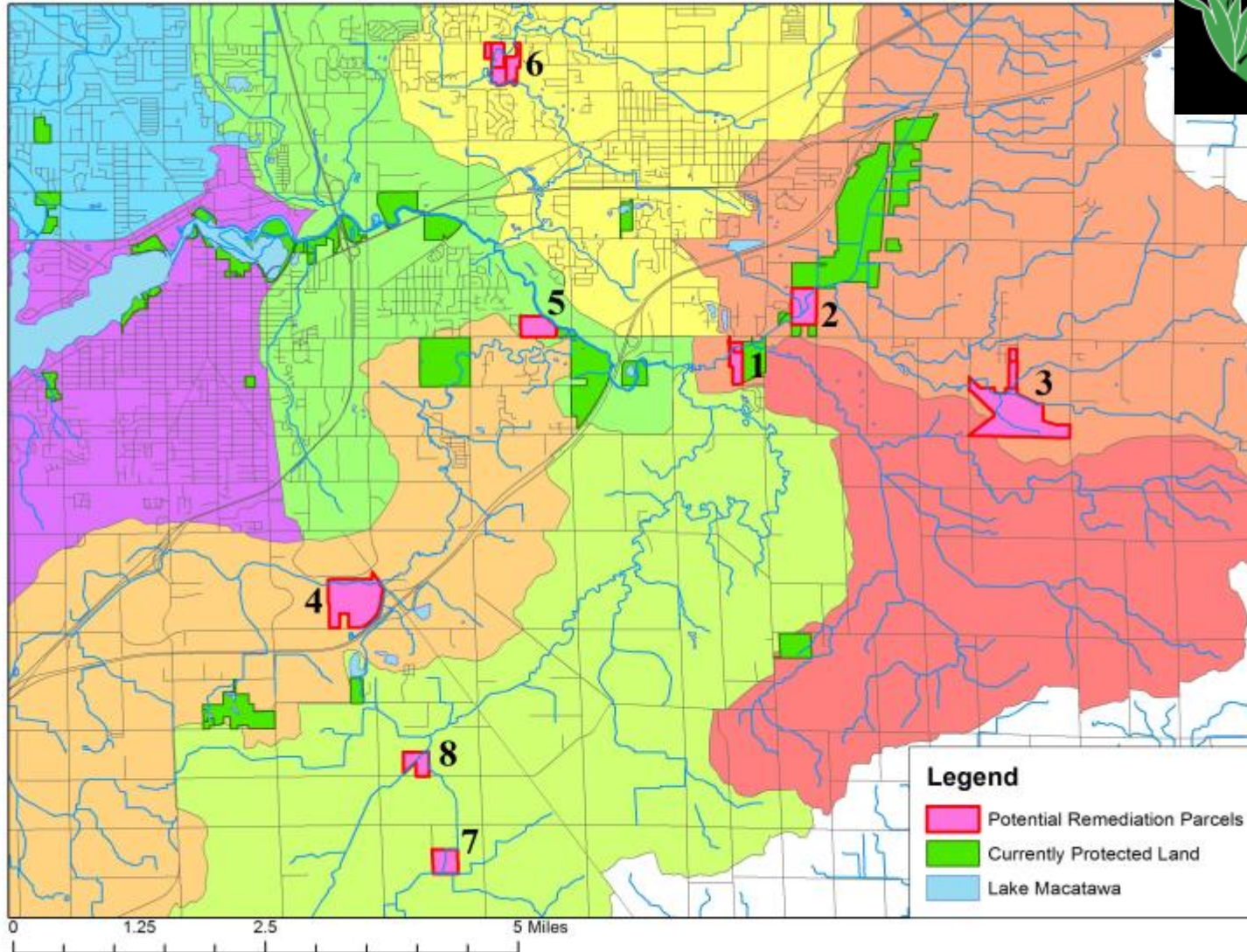
We can rank order the sources...

Fingerprinting potential...

Short residence time:
quick recovery



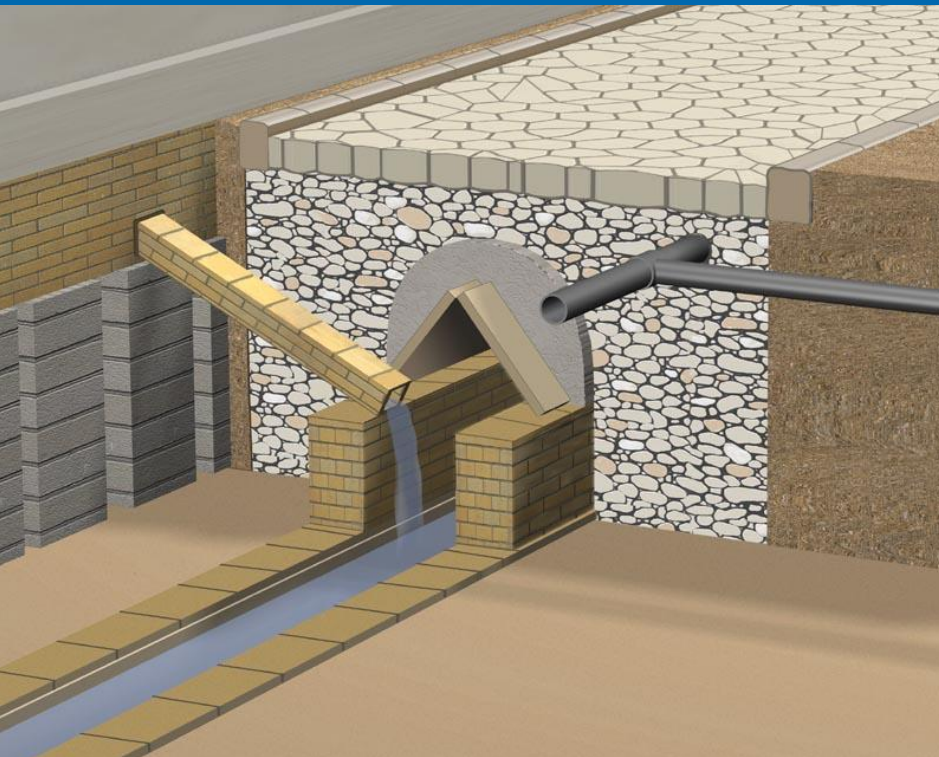
Next Steps...



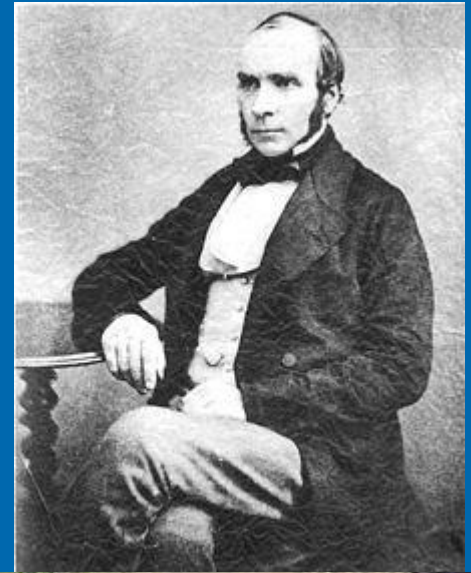
That mountain stream looks pretty clean, but what's upstream?



Best public health invention ever:
separation of sewage from
drinking water.



Dr. John Snow – first modern epidemiology investigation; tracked cholera outbreak to a contaminated pump.





Modern sanitation removes risk of waterborne pathogens in developed countries:



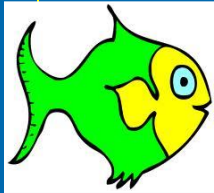
- Sterilization of water supply
- Hygiene
- Sanitary sewers (more effective if separate from storm sewers)
- Sewage treatment prior to discharge
- **Testing and monitoring**

Microbiological culturing techniques going back to 1920's led to "Total coliform" concept.

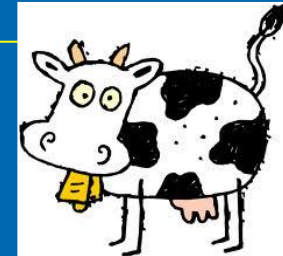
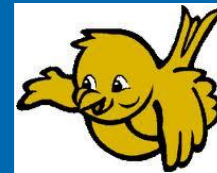
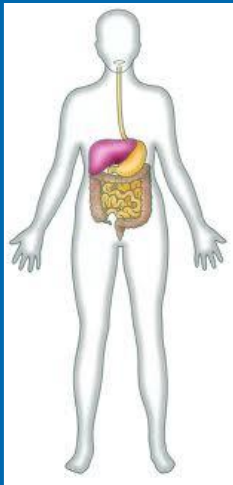
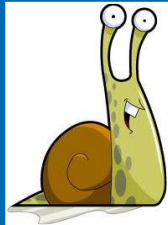
Established in law with the Safe Drinking Water Act of 1974

Some microbiology terms:

Total coliform: Rod-shaped, Gram-negative, lactose-fermenting, acid-



Fecal coliform: Rod-shaped, Gram-negative, lactose-fermenting, acid-producing AND grow at



E. coli: A particular genus/species found in normal gut microorganisms.

EC O157H7, O104H4

Enterococci: A class of related organisms found in normal gut.

The trouble is, coliforms (except the few *E. coli* strains that are pathogenic) do not actually make you sick!

Some of the real bad actors include...

Protozoans like:

- Entamoeba
- Cryptosporidium
- Giardia



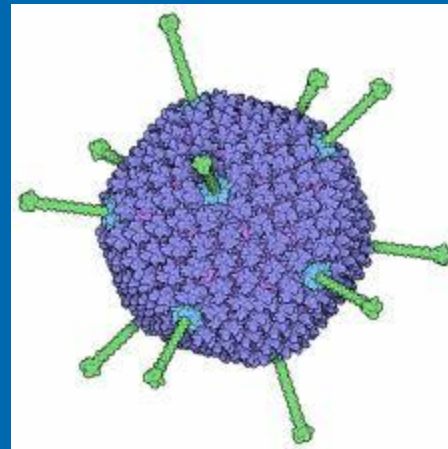
Bad bacteria like:

- *C. botulinum*
- *Campylobacter*
- *V. cholerae*
- *Shigella*
- *Salmonella*



Viruses like:

- Adeno, parvo, corona
- Hepatitis A
- Polio

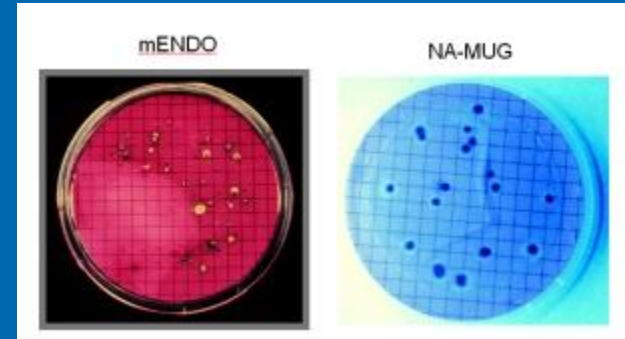


So why do we bother with coliform (or *E. coli*, entero) “counts”?

- Because we can, using classical microbiology culture.



Plate assays give us colony-forming units (cfu) per 100mL of water sample.



“Colisure” tray cultures give us essentially the same thing (technically a “most-probable number” of cells per 100mL)



These are “Fecal Indicator Bacteria.” Current drinking and recreational water standards are based on these methods.

How are we doing with
Lake Mac?

It looks cloudy...is that
bad?

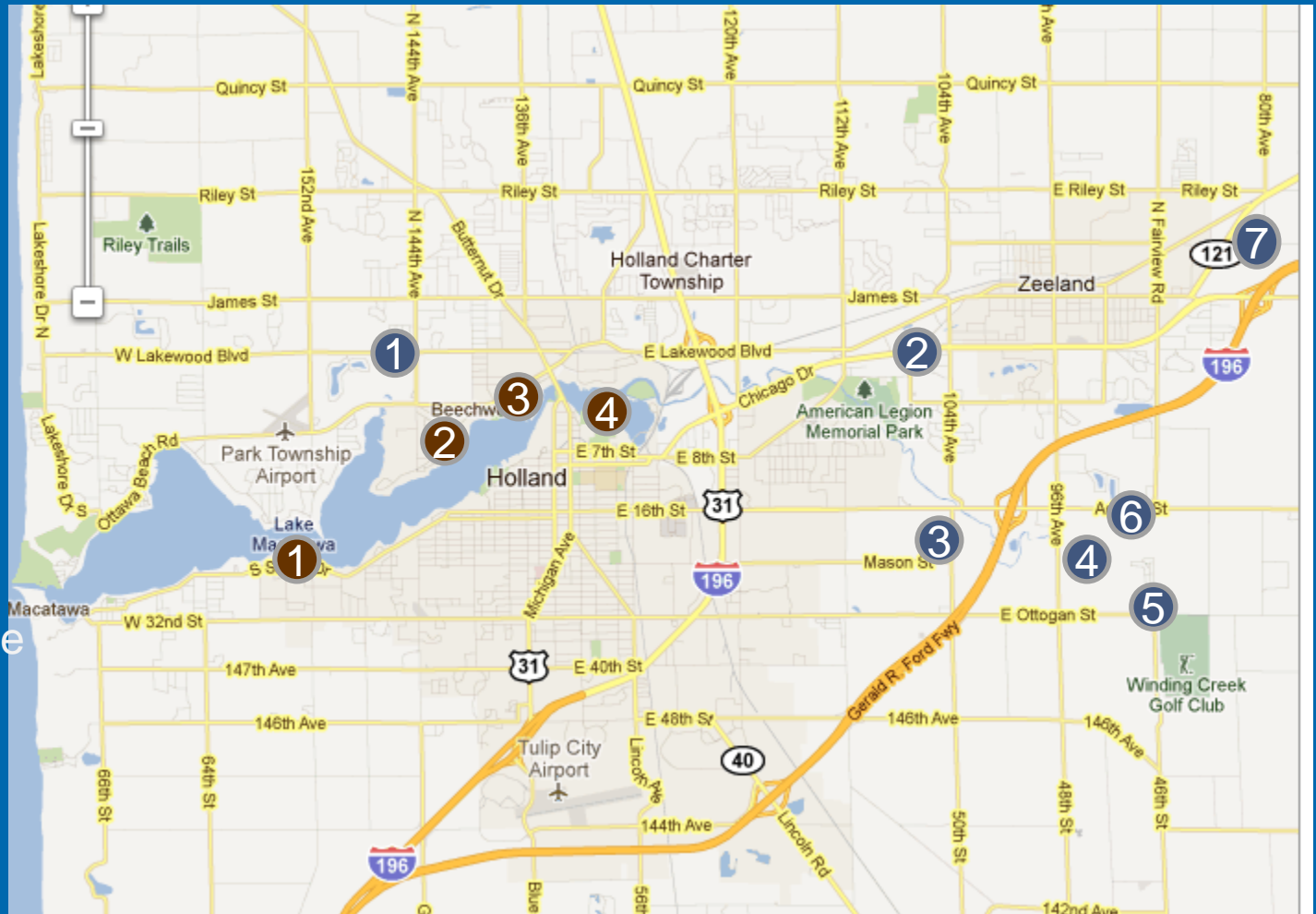


Phase I – survey FIB in broader Lake Mac watershed.

Eleven sites were selected; four on the lake, seven in various upstream sites.

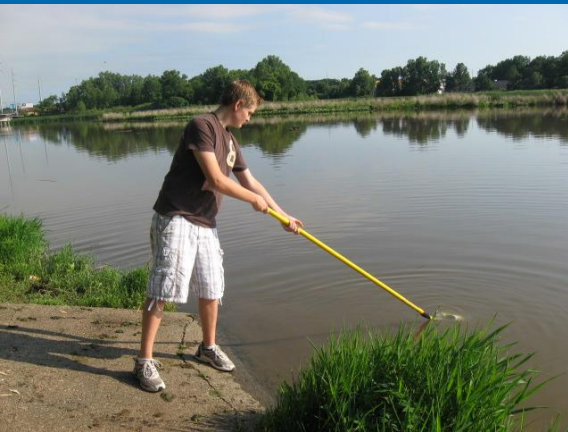
Narrows
No. Shore Dr.
Dunton Park
River Ave

Pine creek
Nordeloos creek
North branch
South branch
Peters creek
Black river – middle
Black river -
upstream

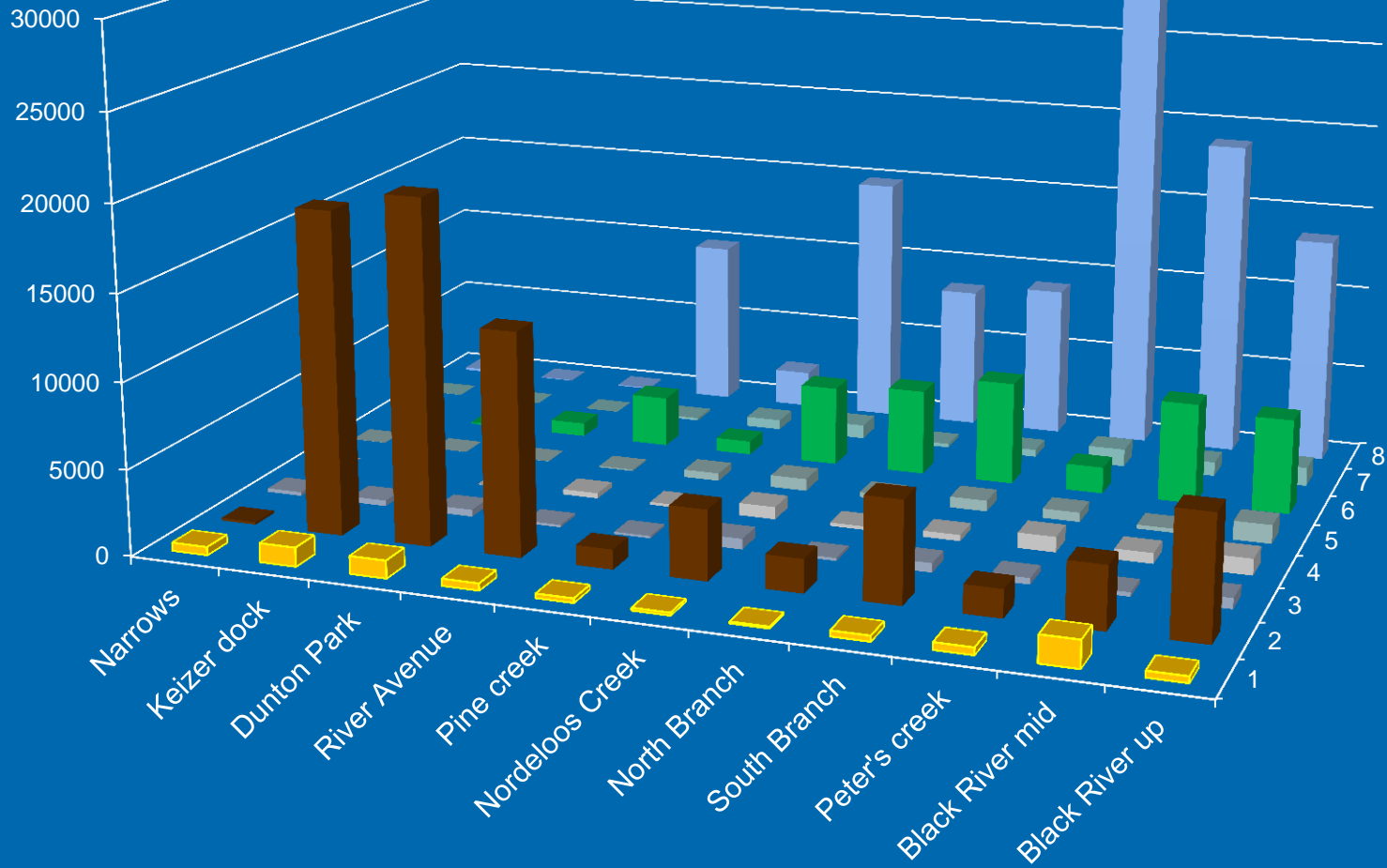


Methodology – membrane filtration followed by colony enumeration:

- Samples were collected once a week for eight weeks, late May – mid July.
- In biological triplicates.
- Three dilutions of each replicate applied to membrane filter and placed on modified mTEC and on mEI (E coli and enterococci).
- EPA method 1600 and 1603.
- Arithmetic means calculated from triplicates of dilution giving suitable colony density for counting.



Enterococci – cfu/100mL



Conclusions from microbiology analysis:

Tributary and east end of Lake Mac (including Dunton Park) levels spike very high FIB (sometimes **>10,000** cfu/100mL, about 1% the level in raw sewage) during rains.

FIB levels are much lower during dry spells (**<300**) throughout.

Further west in Lake Mac, levels never reached **>300** cfu/100 mL.

Two tributary sites, Pine creek and Peters creek, remained moderate all summer (**1000's** but not 10's of thousands cfu/100mL).

Thus, FIB seem to be coming from *widely distributed* upstream sources whose presence is continually replenished, and washed downstream during rains. These appear to pool in the Dunton Park area.

Microbial study – Phase II... Source Tracking (or, who's pooping in the water?)

- **Connecting a particular type of bacteria to a known host = source tracking (Bacterial Source Tracking/BST; Microbial Source Tracking/MST).**
- Molecular fingerprinting approaches allow us to distinguish host species among certain target hosts (some more specifically than others).

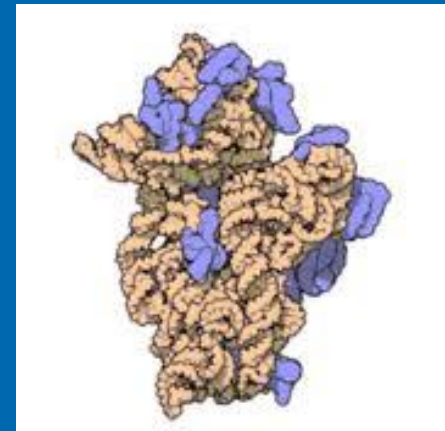
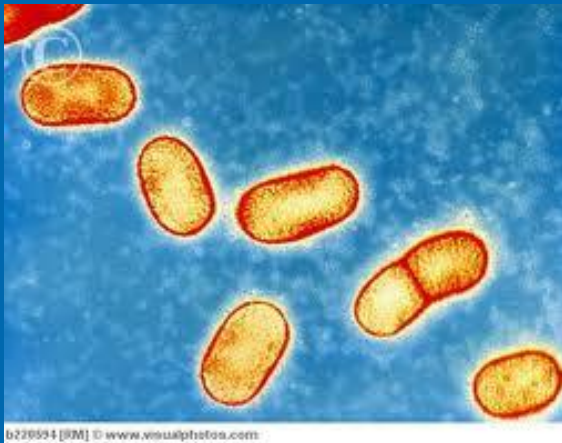


Most work to date is based on Bacteroides 16S RNA gene sequence

Bacteroides is an anaerobic organism – can't grow outside the gut.

Makes up about %50 of the mass of the gut biome.

“16S RNA” = part of the protein factory...every cell has to have it, so it's a good place to look for genomic fingerprints.



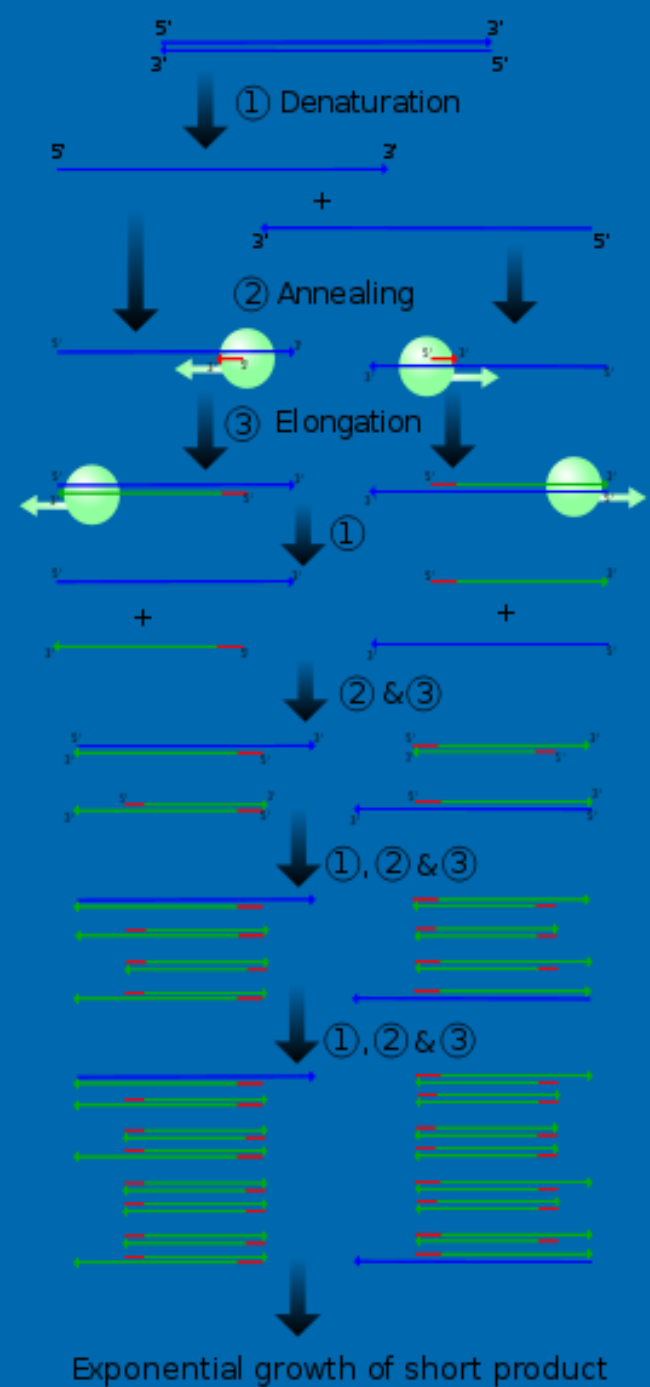
Some 16S RNA genes from bacteroides of various animals:

Chicken 1	(99)	ACACGTATCCAACCTGCCGATAA	CTC	CGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGAGAGAACTATTAAAGA	-AC	
chicken2	(99)	ACACGTATCCAACCTGCCGATAA	CTC	CGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGAGAGAACTATTAAAGA	-AC	
chicken3	(99)	ACACGTATCCAACCTGCCGATAA	CTC	CGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGAGAGAACTATTAAAGA	-AC	
cow 3	(87)	ACGCGTATCCAACCTTCCCGATA	CTC	AGGGA	TAGCCTTC	CGAAAGGGGAGATTAATACCTGA	TGG	TGTT	CAAAT	CCGCA	TGTTATTTGAACTAAAGATTT	
cow 2	(87)	ACGCGTATCCAACCTTCCCGTTA	CTC	CGGGA	TAGCCTTC	CGAAAGGGGAGATTAATACGAGA	TGG	TGTT	GAAAT	CCGCA	TGTTATTTCAACTAAAGATTC	
cow 4	(87)	ACGCGTATCCAACCTTCCCGTTA	CTC	AGGGA	TAGCCTTC	CGAAAGGGGAGATTAATACCTGA	TGG	TGTT	CAAAT	CCGCA	TGTTATTTGAACTAAAGATTT	
cow 5	(87)	ACGCGTATCCAACCTTCCCGTTA	CTC	AGGGA	TAGCCTTC	CGAAAGGGGAGATTAATACCTGA	TGG	TGTT	GAAAT	CCGCA	TGTTATTTCAACTAAAGATTT	
cow 6	(87)	ACGCGTATCCAACCTTCCCGTTA	CTC	AGGGA	TAGCCTTC	CGAAAGGGGAGATTAATACCTGA	TGG	TGTT	GAAAT	CCGCA	TGTTATTTCAACTAAAGATTC	
cow 7	(87)	ACGCGTATCCAACCTTCCCGTTA	CTC	CGGGA	TAGCCTTC	CGAAAGGGGAGATTAATACAAGA	TGG	TAT	CAAAT	CCGCA	TGTTATTTGAACTAAAGATTT	
HuBac566f	(1)	-----										
HuBac F2	(1)	-----T CGAAAGAAAGATTAATACCGGA-----										
HuBac F3	(1)	-----										
human1	(91)	ACACGTATCCAACCTGCCGACA	ACAC	TGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGGGATAATTAATTAAGA	-AT	
human2	(91)	ACACGTATCCAACCTGCCGATG	ACTC	GGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGGTAGAACTATTAAAGA	-AT	
human3	(91)	ACACGTATCCAACCTGCCGACA	ACAC	TGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGGGATAATTAATTAAGA	-AT	
AY695676 human	(91)	ACACGTATCCAACCTGCCGACA	ACAC	TGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGGGATAATTAATTAAGA	-AT	
AY986343 human	(95)	ACACGTATCCAACCTGCCGACA	ACAC	TGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGGGATAATTAATTAAGA	-AT	
BoBac367f	(1)	-----										
BoBac467r	SS	-----										
HuBac692r	SS	-----										
turkey1	(95)	ACACGTATCCAACCTGCCGATG	ACTC	GGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGGTAGAACTATTAAAGA	-AT	
Gull 2	(91)	ACACGTATCCAACCTGCCGCTA	CTACTC	CGGGA	TAGCCTTC	TGAAAGGGGAGATTAATACAAGA	TGG	CAT	CAT	GAGT	CCGCA	TGTTACATGATTAAGGTAT
Gull 3	(91)	ACACGTATCCAACCTGCCGATG	ACTC	GGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGGTAGAACTATTAAAGA	-AT	
Gull 4	(91)	ACACGTATCCAACCTGCCGCTA	CTACTC	TGGGA	TAGCCTTC	TGAAAGGGGAGATTAATACAAGA	TGG	CAT	CAT	GAGT	CCGCA	TGTTACATGATTAAGGTAT
pig1	(91)	ACGCGTATCCAACCTTCCCCATA	CTAAGG	GA	TAGCCTTC	CGAAAGGGGAGATTAATACCTTA	TGT	TCT	CCGTT	GG	CCGCA	TCAGTATGGAGCAAAGATTT
AY695690 Pig	(91)	ACGCGTATCCAACCTTCCCTTG	TC	CACGGGA	TAGCCGT	CGAAAGGGGAGATTAATACCGTA	TGAG	TC	CACA	GC	AGGCA	TCTAATGTGACGAAAGGTTT
elk1	(91)	ACGCGTATCCAACCTTCCCGTTA	CTC	AGGGA	TAGCCTTC	CGAAAGGGGAGATTAATACCTGA	TGG	TGTT	CGAA	TT	CCGCA	TGACATTTGAACTAAAGATTC
Gull15	(91)	ACACGTATCCAACCTGCCCTT	ACTC	GGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATG	CA	TAA	TGAT	TT	CCGCA	TGTTTCAATTAATTAAGG-AT
cat1	(91)	ACACGTATCCAACCTGCCGACA	ACAC	TGGGA	TAGCCTTT	CGAAAGAAAGATTAATACCGGATGG	CA	TAGT	TTTC	CCGCATGGGATAATTAATTAAGA	-AT	
cat2	(91)	ACGCGTATCCAACCTGCCACCA	CTTGGG	GA	TAGCCTTC	CGAAAGTAAAGCTAATACCCAA	TGATA	TCT	TAGA	AGACA	TCTG	AAAGAGATTAAGATTT
dog 1	(91)	ACACGTATCCAACCTGCCGCTA	CTACTC	TGGGA	TAGCCTTC	TGAAAGGGGAGATTAATACAAGA	TGG	CAT	CAT	GAGT	CCGCA	TGTTACATGATTAAGGTAT
dog 2	(93)	ACGCGTATCCAACCTGCCACCA	CTTGGG	GA	TAGCCTTC	CGAAAGTAAAGCTAATACCCAA	TGATA	TCT	TAGA	AGACA	TCTG	AAAGAGATTAAGATTT

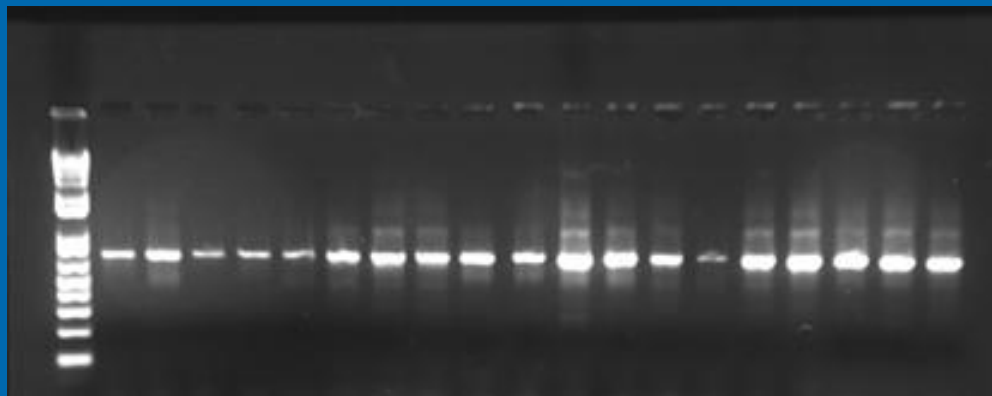
Polymerase Chain Reaction: If you can design a pair of primers that flank a sequence of interest, you can use PCR to make a large amount from as little as one molecule of starting DNA. PCR allows you to:

- Measure amount of target sequence
- Clone amplified DNA
- Sequence it

• <http://www.dnalc.org/ddnalc/resources/shockwave/pcranwhole.html>



End-point analysis (presence/absence) uses electrophoresis (separation of DNA molecules by size) and staining to detect PCR reaction product:



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

Human-specific bacteroides fingerprint:

- present in samples originating near Dunton Park
- strongly present municipal sewage.

Pig-specific:

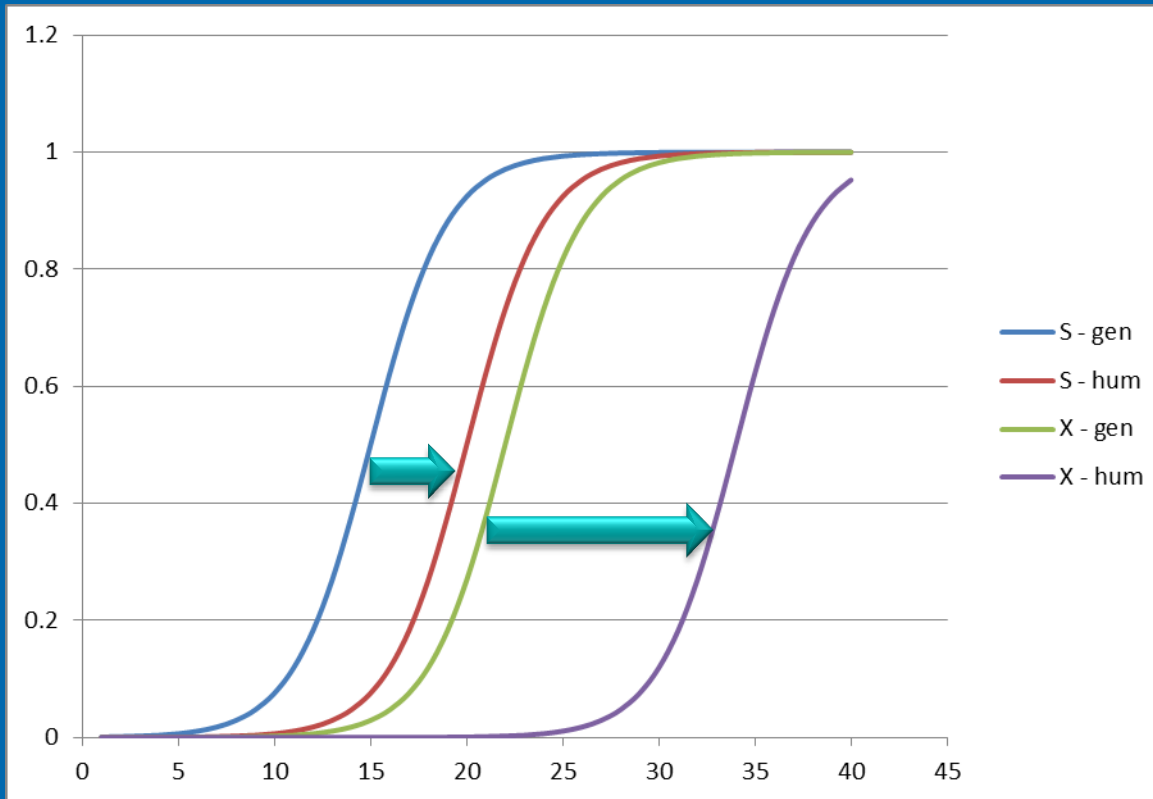
- present in pig feces;
- in drainage surrounding a local hog farm;
- in days following pig manure spill immediately downstream
- *Completely absent in routine samples*

Cow-specific

- Present in cow feces
- Positive at some sites

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,

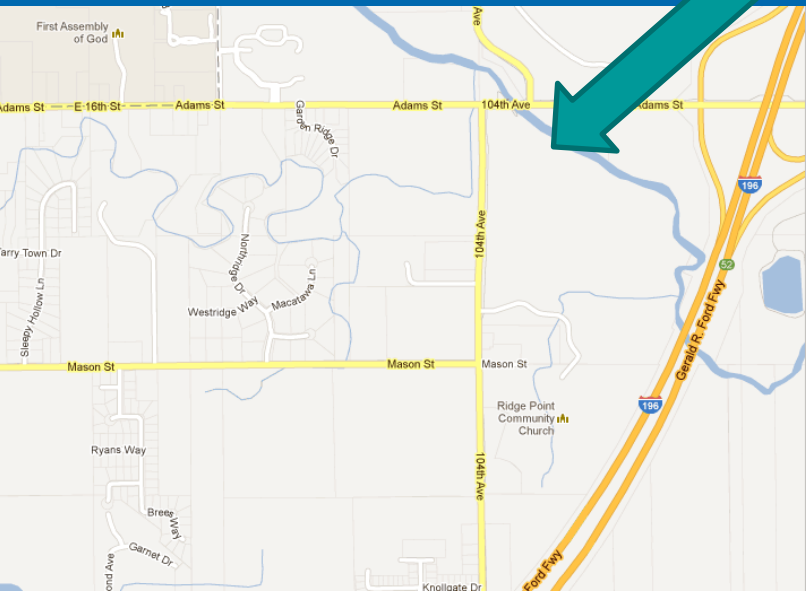
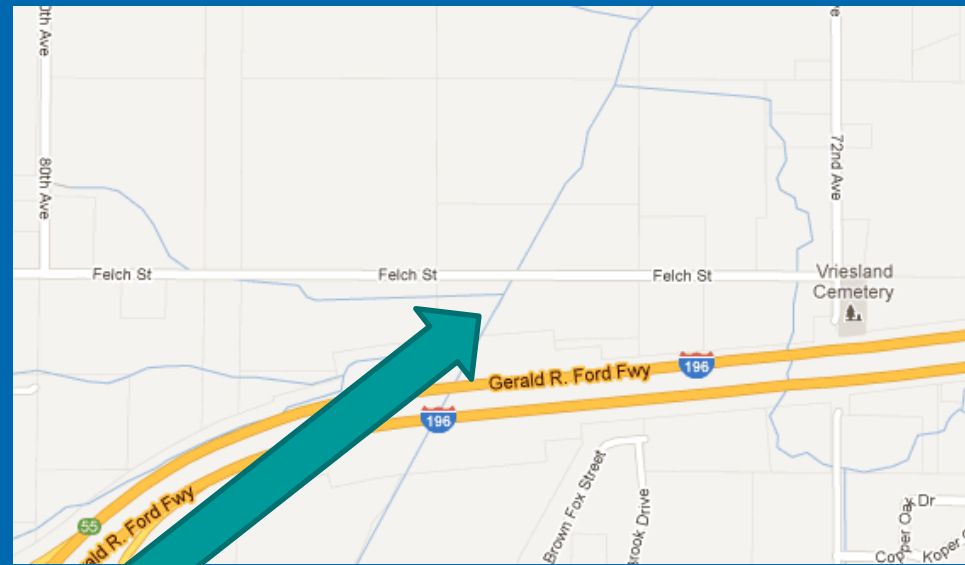
- That means sample A had $2^8 = 256$ times more original target DNA than sample B



Cow-specific probes:

- Conventional PCR
- qPCR (bacteroides and non-bacteroides)
- Specificity – does cross react with other livestock (horse, sheep).
- Low abundance at:

Main Branch Black River
North Branch Black River



Pig-specific probes:

- qPCR bacteroides
- High specificity for pig
- None detected!

After spending some time validating appropriate conditions, we are now able to compare total bacteroides to human bacteroides in our environmental samples.

	Total Bac	Human Bac
Black River upstream (1)	26.07	35.72
Black River midstream (1)	29.94	39.155
River Avenue (2)	ND	ND
Dunton Park (2)	31.67	37.06
South Branch (4)	30.545	ND
Nordeloos Creek (4)	28.21	32.2
Peters Creek (4)	26.915	36.51
River Avenue (6)	27.58	38.66
Dunton Park (6)	27.23	32.495
Peters Creek (7)	26.765	37.415
Dunton Park (7)	31.115	35.195
Black River upstream (8)	ND	ND
Black River midstream (8)	ND	ND
Pig feces	13.815	ND
Cow feces	15.54	ND
Sewage	20.68	24.045

Remember:
Less is
more!!

(and ND
means none
detected)

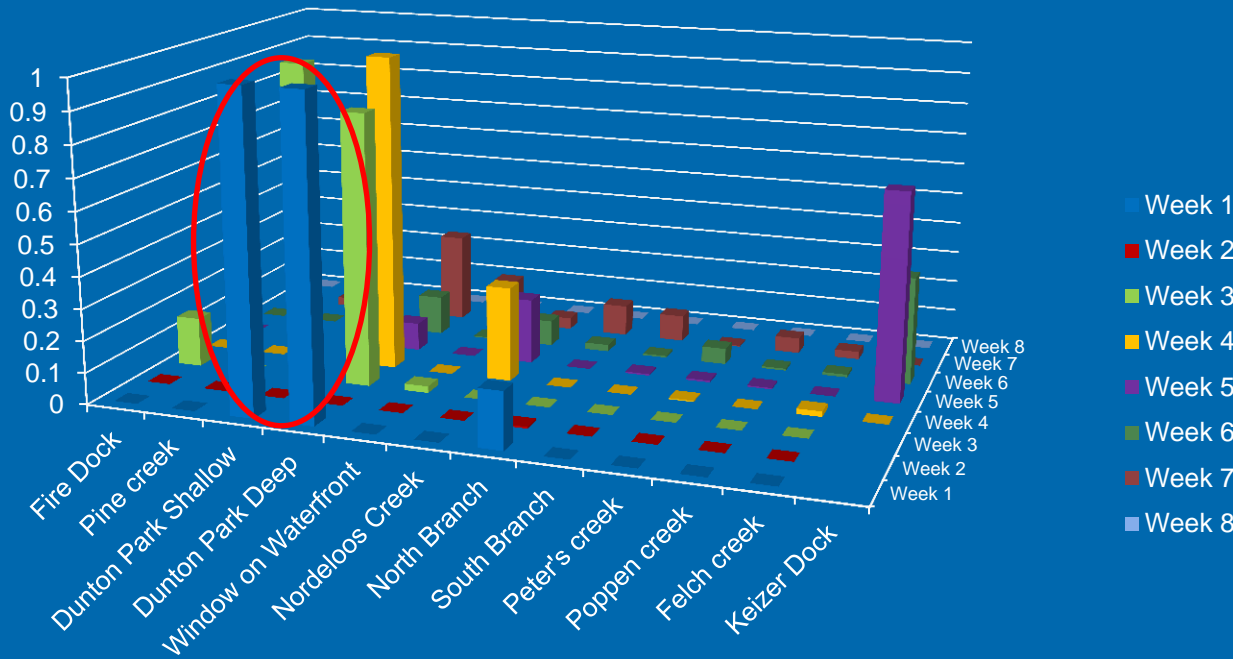
Even better, we can turn that into a ratio of human compared to total; let's call that:

$$\left(\frac{\quad}{\quad} \right)$$

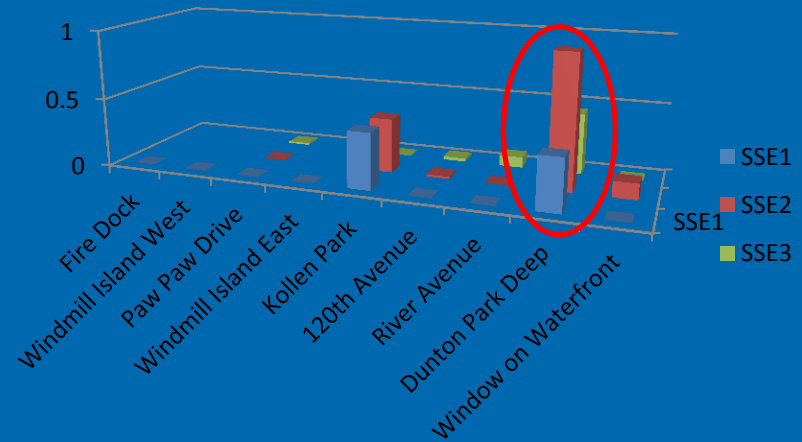
By comparing the ratio in our environmental sample to that in sewage (presumably entirely human in origin), we can calculate the fraction of bacteroides present in a given sample attributable to human sewage:

	Fraction of human origin:
Black River upstream (1)	0.01
Black River midstream (1)	0.02
River Avenue (2)	ND
Dunton Park (2)	0.25
South Branch (4)	0.01
Nordeloos Creek (4)	0.65
Peters Creek (4)	0.01
River Avenue (6)	0.00
Dunton Park (6)	0.27
Peters Creek (7)	0.01
Dunton Park (7)	0.61
Black River upstream (8)	ND
Black River midstream (8)	ND
Pig feces	ND
Cow feces	ND
Sewage	1.00

Human/Total levels – by site



Sewage spill at Lakewood/120th
in June 2011 →



Problem areas for human-origin sewage?

- Through most of Lake Mac, no.
- Only sporadic in tributaries.
- Dunton Park area ... consistently shows significant human sewage.
 - Catch basin from any upstream contamination?
 - Local point source?
 - Dog testing → molecular source tracking

Pinpoint: Duntun Park



So where are all the bacteria coming from?

Live coliform (or E.coli or enterobacteria) may indicate recent fecal contamination, BUT...

May also represent an ongoing environmental population, associated with:

Infrastructure

(e.g. drain tiles...uniform temperature, wet, dark!)

Periodic application of manure

Sediment-associated

Because drain tiles seem a likely host, samples are currently being analyzed for:

Bacterial counts

– where are live (maybe dormant) cells harbored?

Genome sequencing

– can we use metagenomic analysis to identify?



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