Macatawa Watershed Water Quality Research Project

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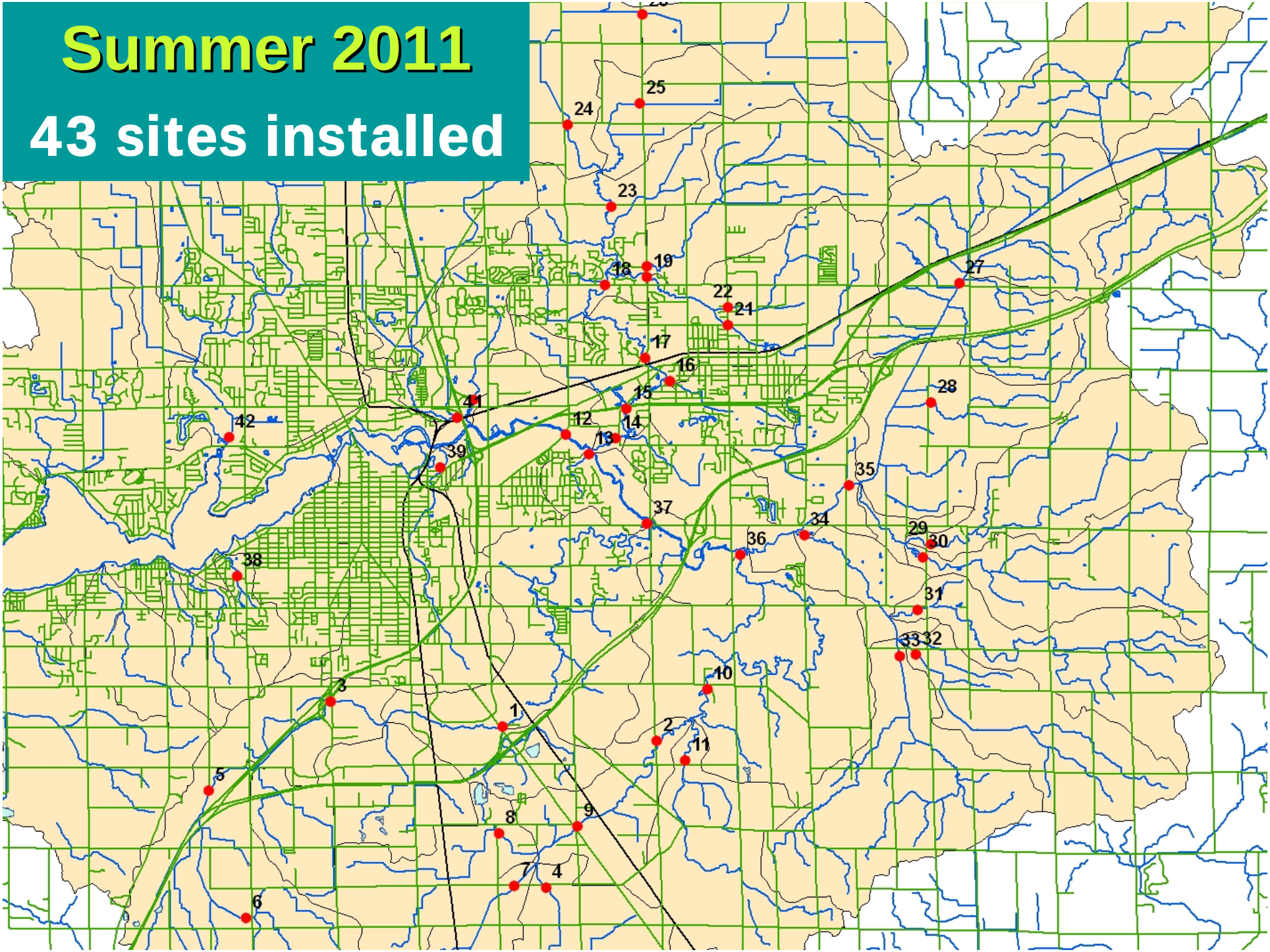
Hope College Chemistry Dept.

November 4, 2011

Summer 2011

43 sites installed

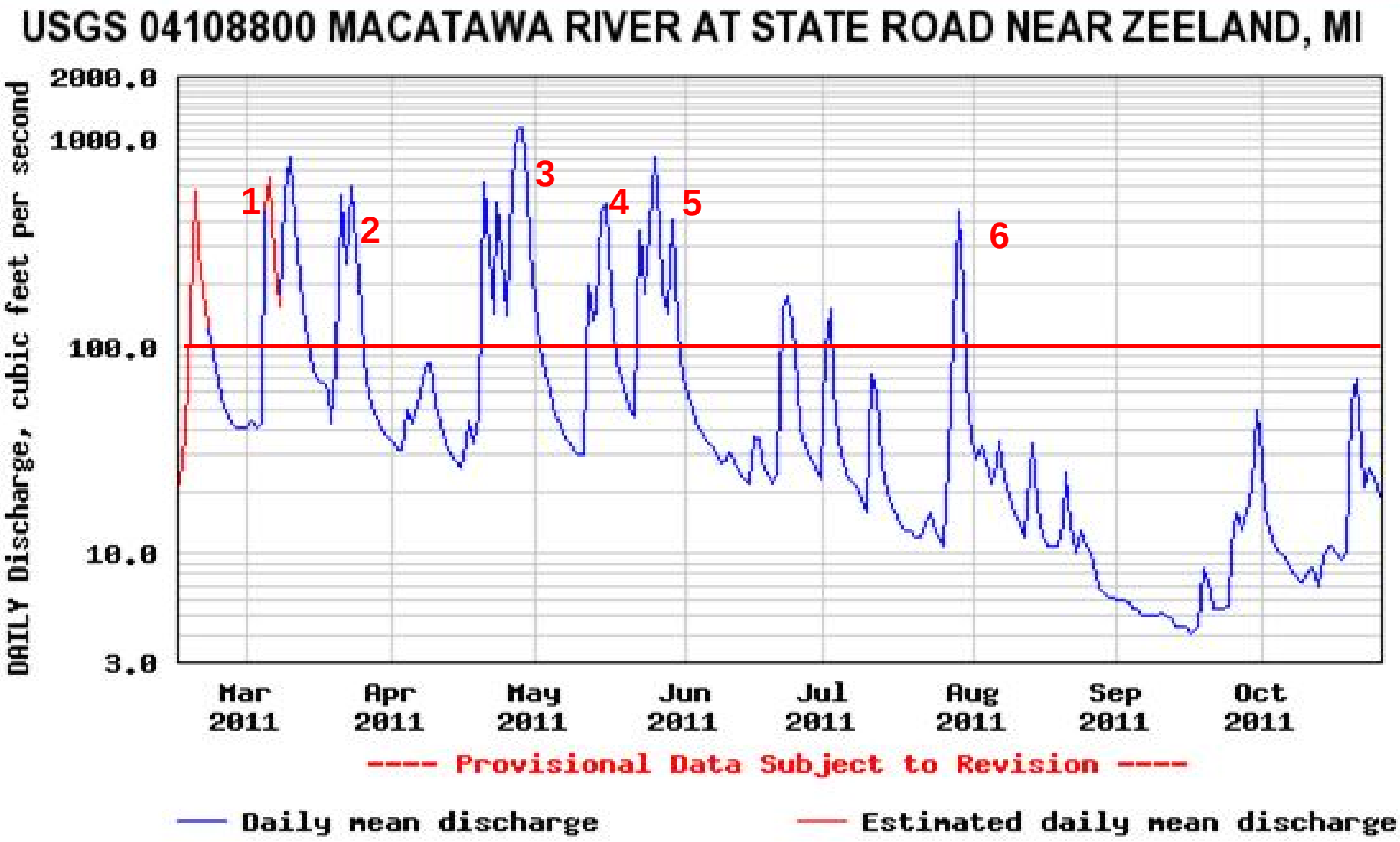



Please Do Not
Disturb This Equipment
This equipment is sampling the suspended sediment content of the stream and is part of a watershed monitoring effort conducted by the Macatawa Area Coordinating Council and Hope College.
If you have any questions about this sampler, please contact:
Dr. Graham Peaslee peaslee@hope.edu
616-395-7117 OR
Mary Fales mfales@the-macc.org
616-395-2688
45U

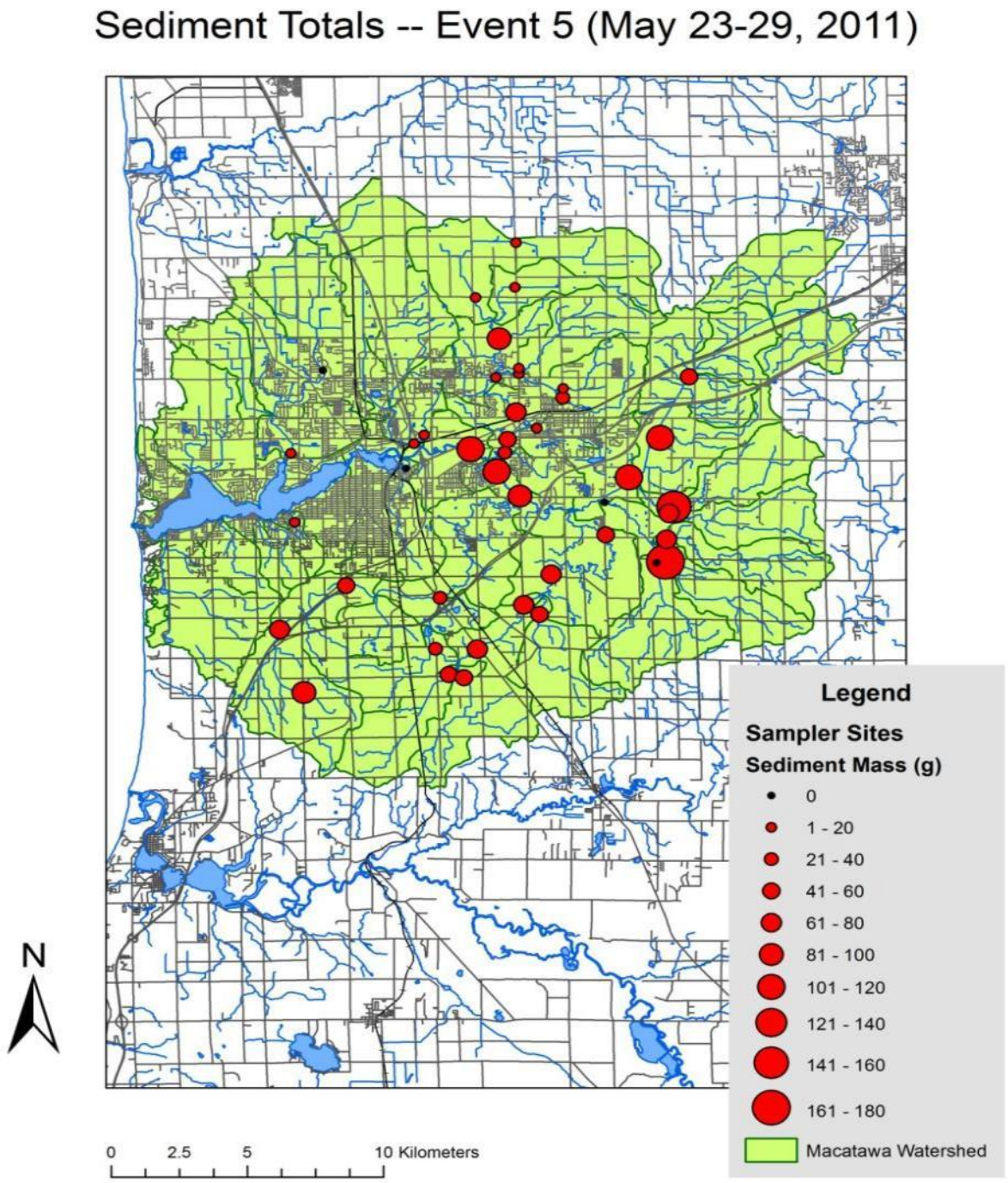
Summary

Sampling - sediment

USGS There have been six good sediment events



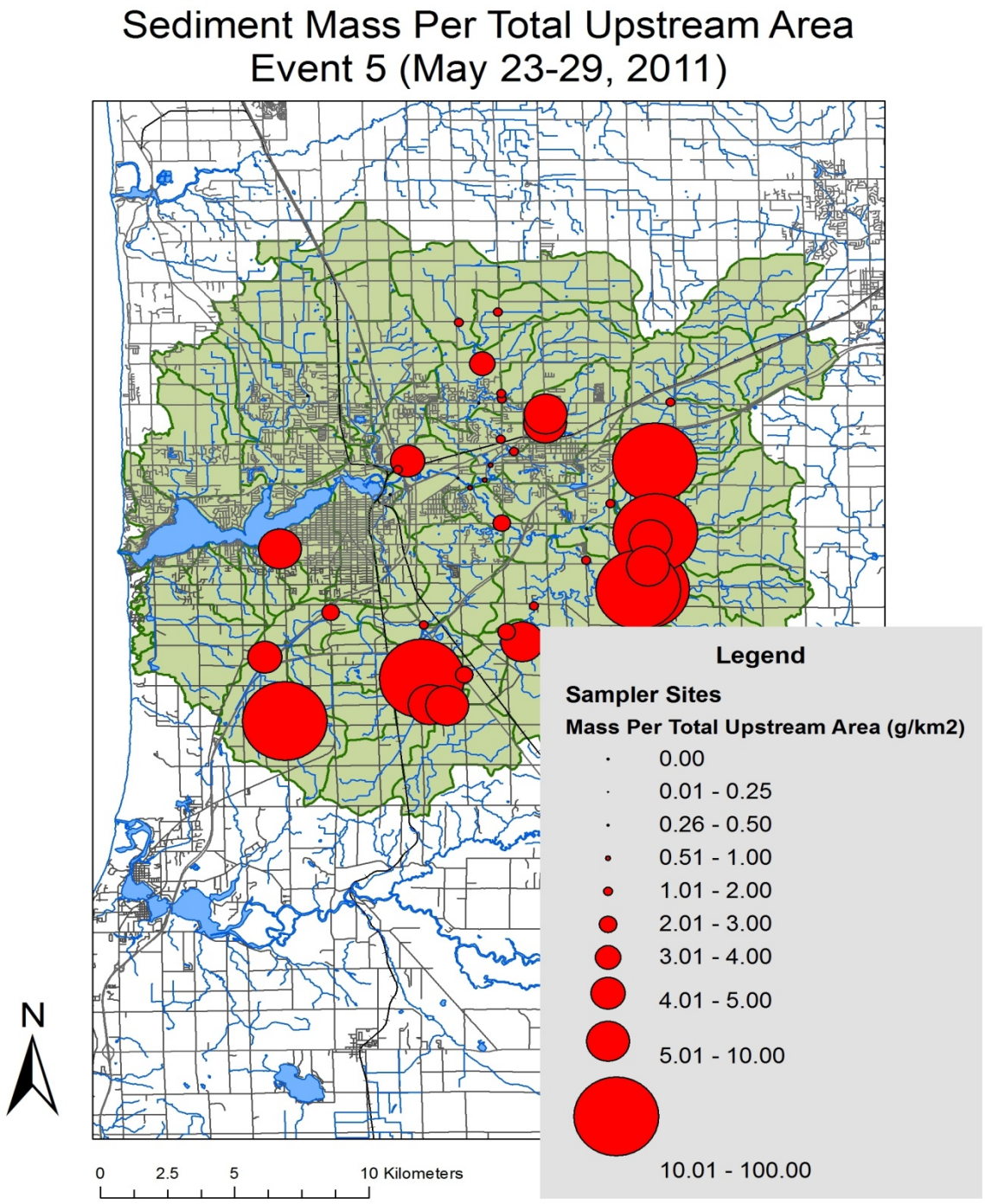
Sample Collection



Preliminary Results:

A typical "event"

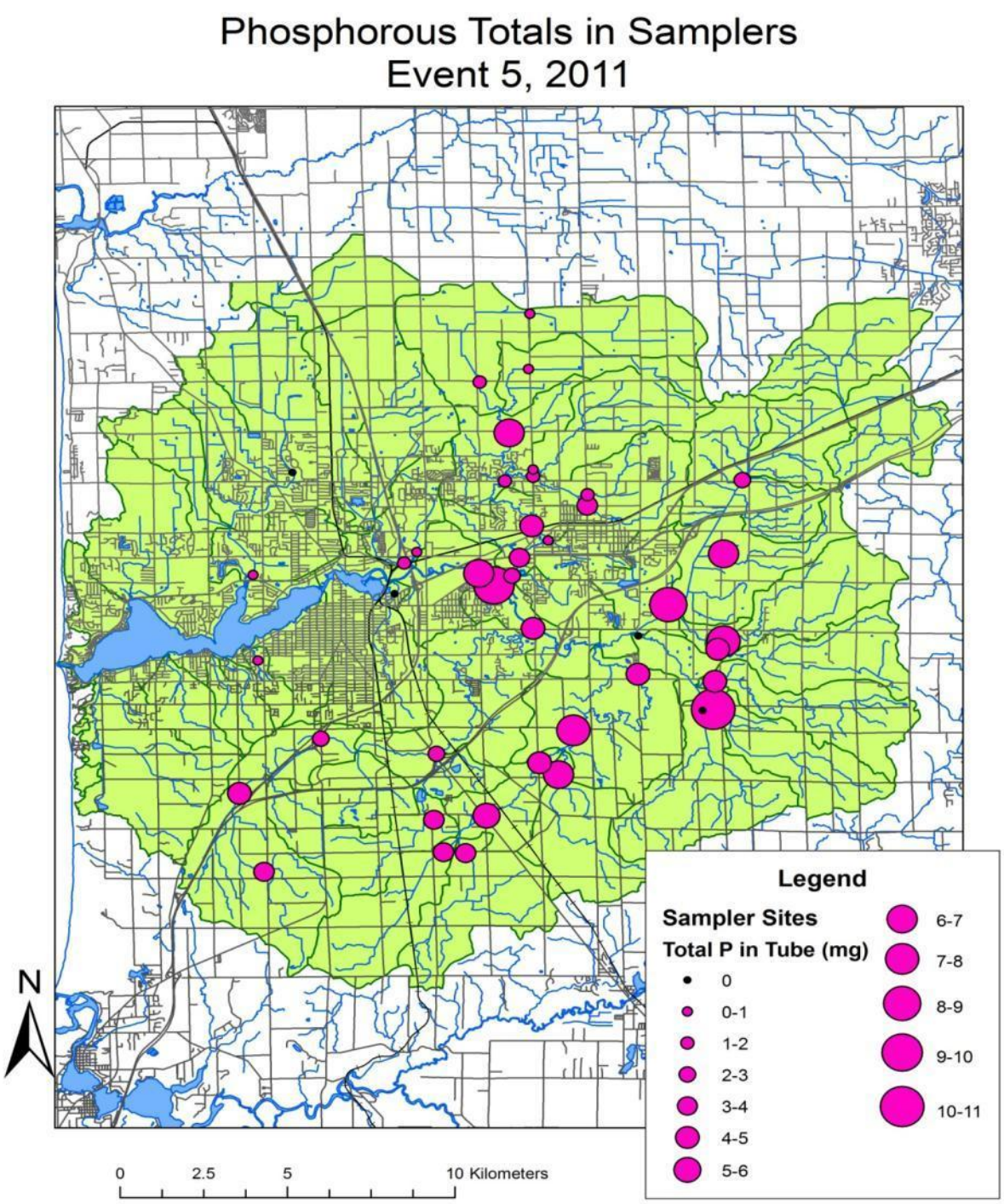
Sediment



Preliminary Results:

A typical "event"

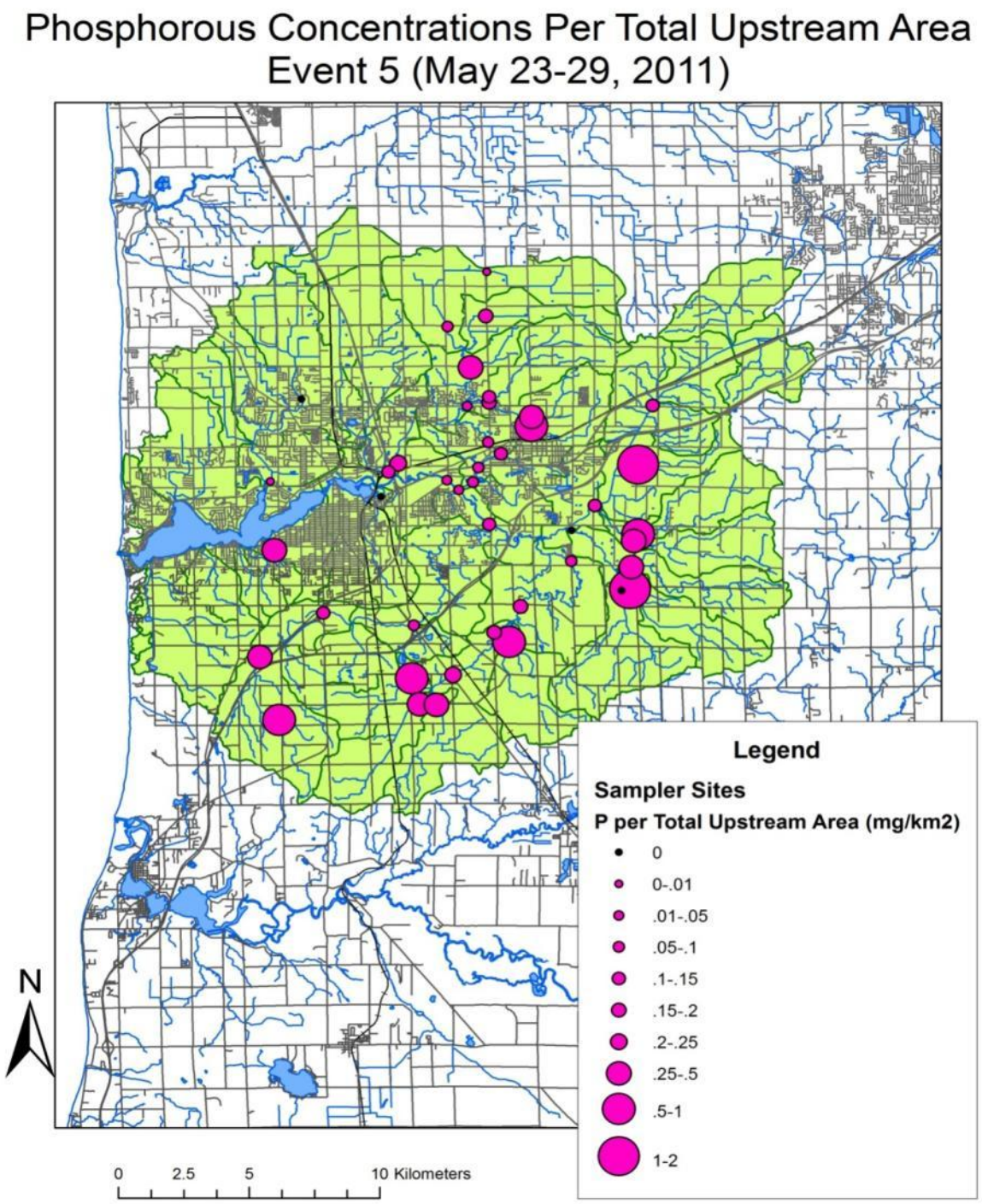
Sediment/km^2



Preliminary Results:

A typical "event"

Phosphorus



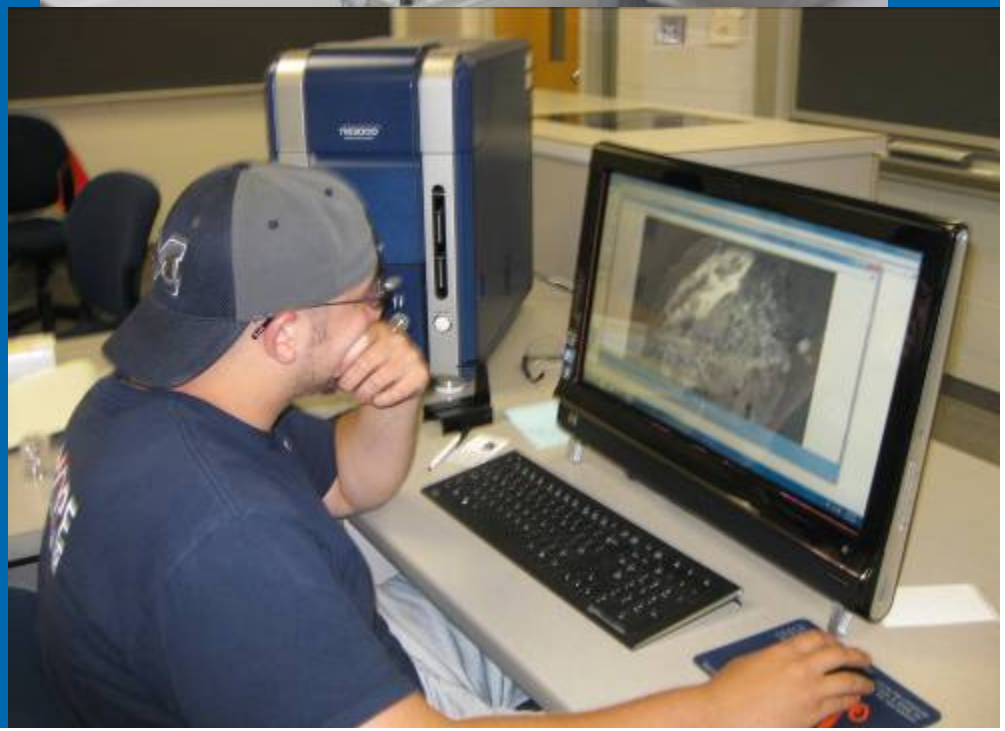
Preliminary Results:

A typical "event"

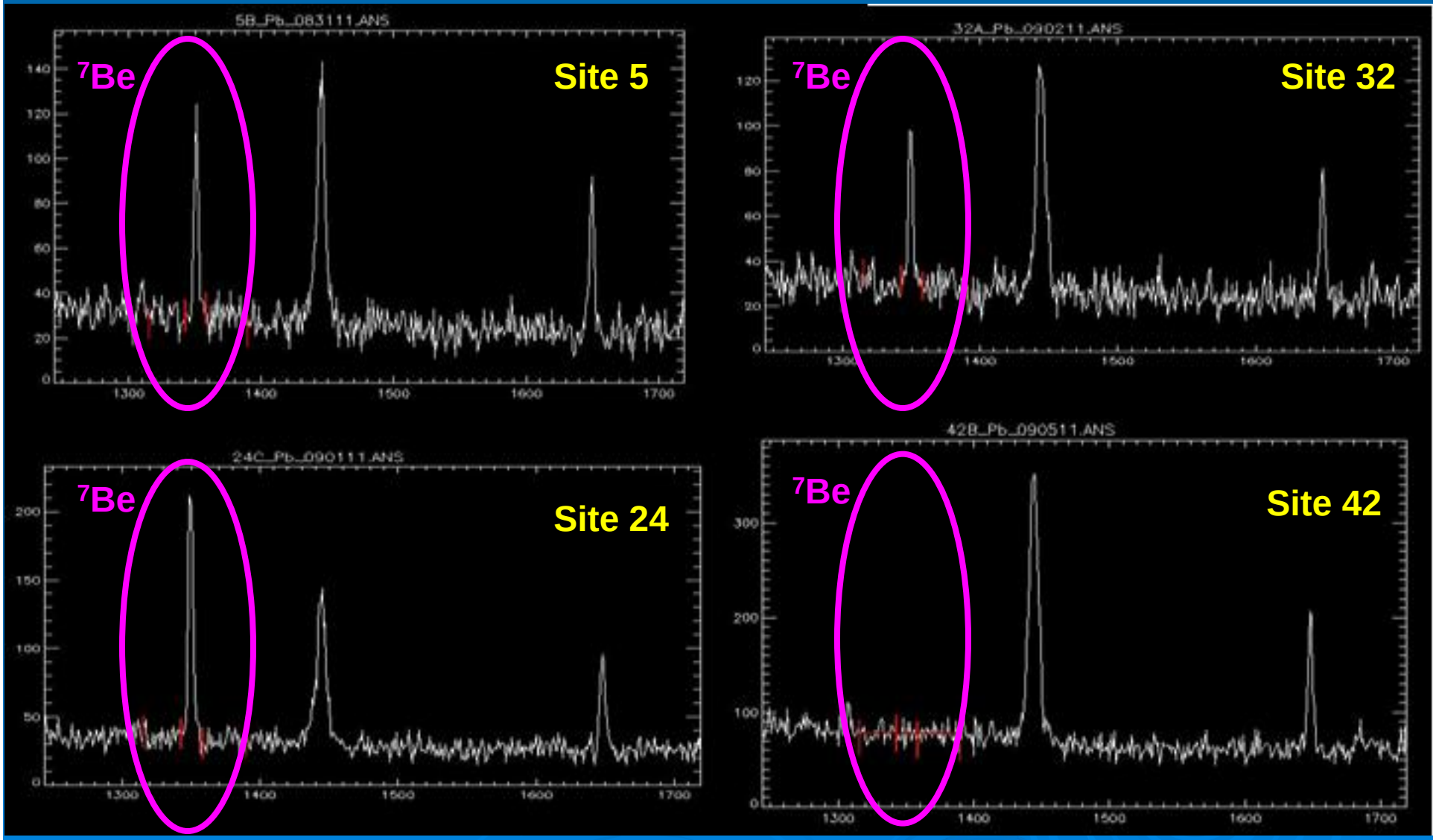
Phosphorus

Sediment Fingerprinting Methods

- Autoanalyzer: PO_4
- Scanning electron microscope (size, shape)
- Cathodoluminescence
- Color
- Elemental analysis
- Radiometric Dating

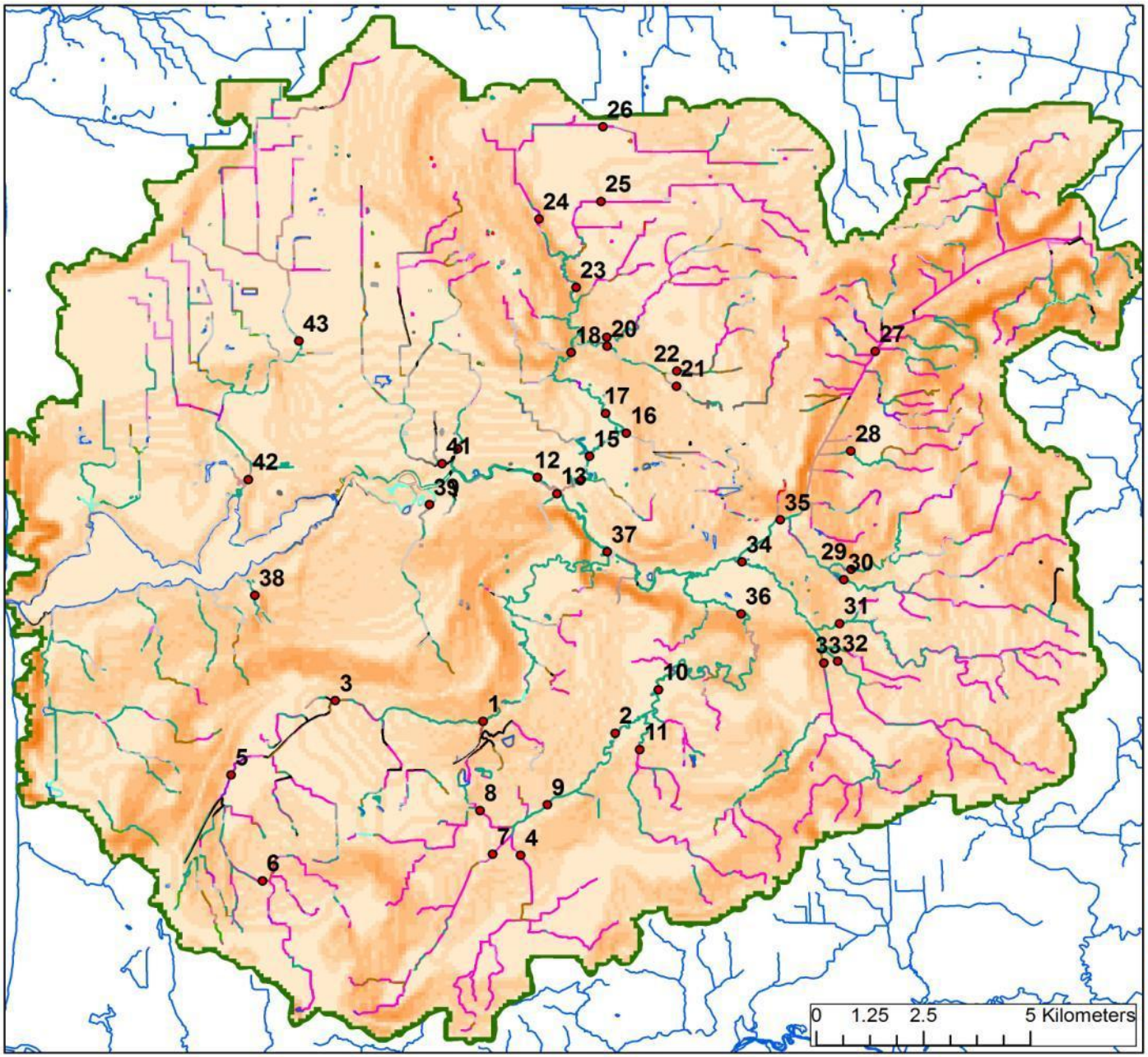


First ^{7}Be Radiodating Results



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

Ground Cover within 30m of Streams -- 2009



Legend

- Sampler Sites

Land Cover

Within 30m of Streams

- Coniferous Forest
- Mixed Conifer-Broadleaved Forest
- Deciduous Forest
- Cropland
- Confined Feeding
- Permanent Pasture
- Orchards and Other Specialty Crop
- Other Agricultural Land
- Grasses and Forbs
- Open Land and Other
- Shrub Open Field
- Forested Wetland
- Non-Forested Wetland
- Lakes/Ponds
- Reservoir
- Residential
- Commercial, Services, Institutional
- Industrial
- Transportation, Communication, Utilities

Gradient (degrees)

- 0-.14
- .14-.47
- .47-.8
- .8-1.19
- 1.19-1.68
- 1.68-2.37
- 2.37-3.31
- 3.31-4.41
- 4.41-7.03
- Macatawa Watershed

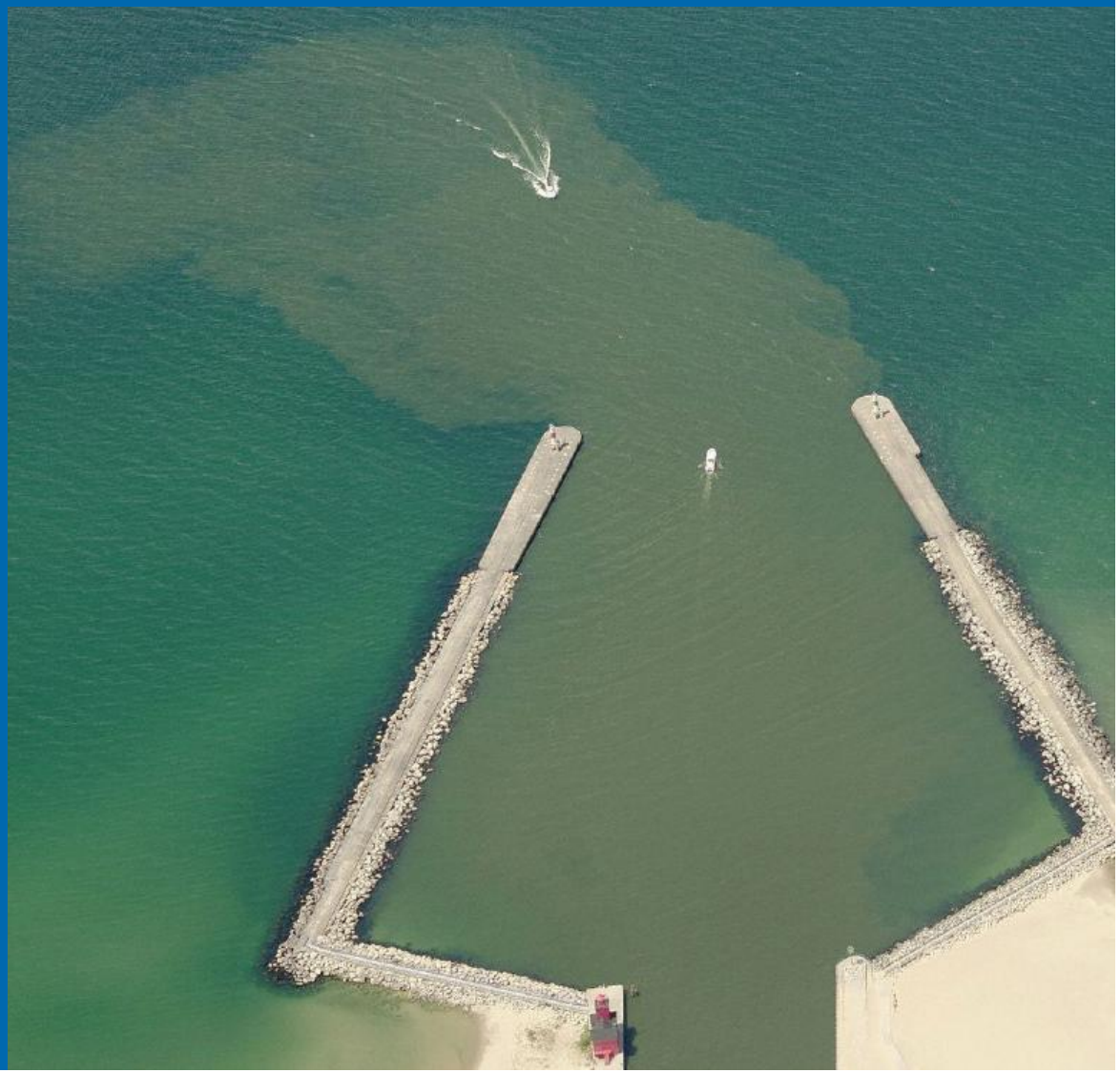
How much top soil are we losing?

>500,000 tons/yr

~1500 tons/day

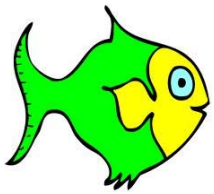
~$7.5M / yr

~ $20,000 / day

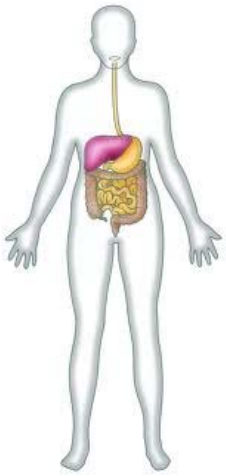


Some microbiology terms:

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



Fecal coliform: Rod-shaped, Gram-negative, lactose-fermenting, acid-producing AND grow at 44°C.

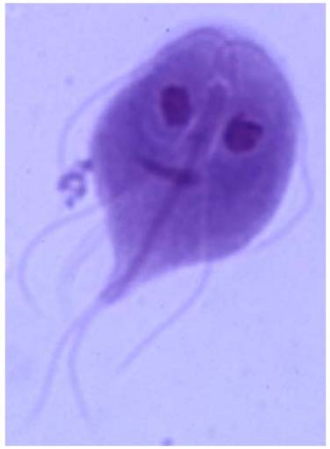


E. coli: A particular genus/species found in normal gut microorganisms.

EC O157H7, O104H4

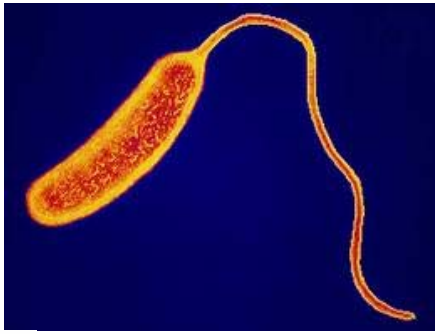
Enterococci: A class of related organisms found in normal gut.

The trouble is, none of these guys (except the few E. coli strains that are pathogenic) can actually make you sick! Some of the real bad actors include…



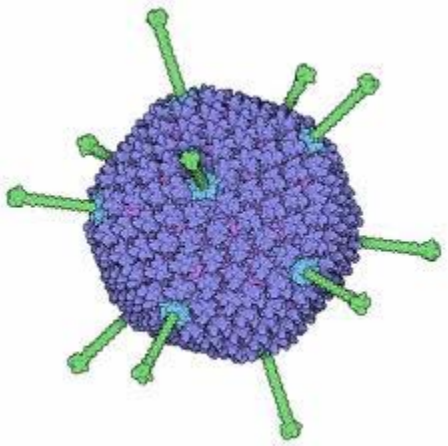
Protozoans like:

- Entamoeba
- Cryptosporidium
- Giardia



Bad bacteria like:

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



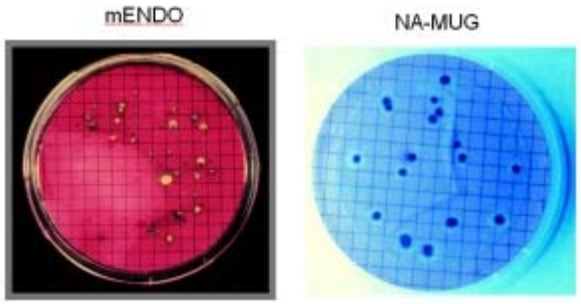
Viruses like:

- 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. With good reason, but let’s remember what we’re really concerned about….

Don’t poop in the water! Every culture throughout history knows this.

For example:

“As part of your equipment have something to dig with, and when you relieve yourself, dig a hole and cover up your excrement.” - Deuteronomy 23:13

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VIRUS CLOSES HOPE CAMPUS

• Health department orders school shut down until Tuesday • More than 130 students report symptoms of norovirus • Students asked to stay near Hope to avoid spreading sickness

Photos Zoom

By JEREMY GONSIOR
The Holland Sentinel
Posted Nov 07, 2008 @ 06:15 PM

Recommend Be the first of your friends to recommend this.

Holland, MI — Katelyn Hemmeke was gone for most of Thursday, but when she returned to her Hope College dorm room she got the bad news.

Possible sources of historically high FIB counts at Dunton Park, Lake Macatawa:

Point source –
•Ineffective municipal treatment
•Illicit discharge
•Bad septic tank nearby
•*(Probably not combined sewer overflow)*

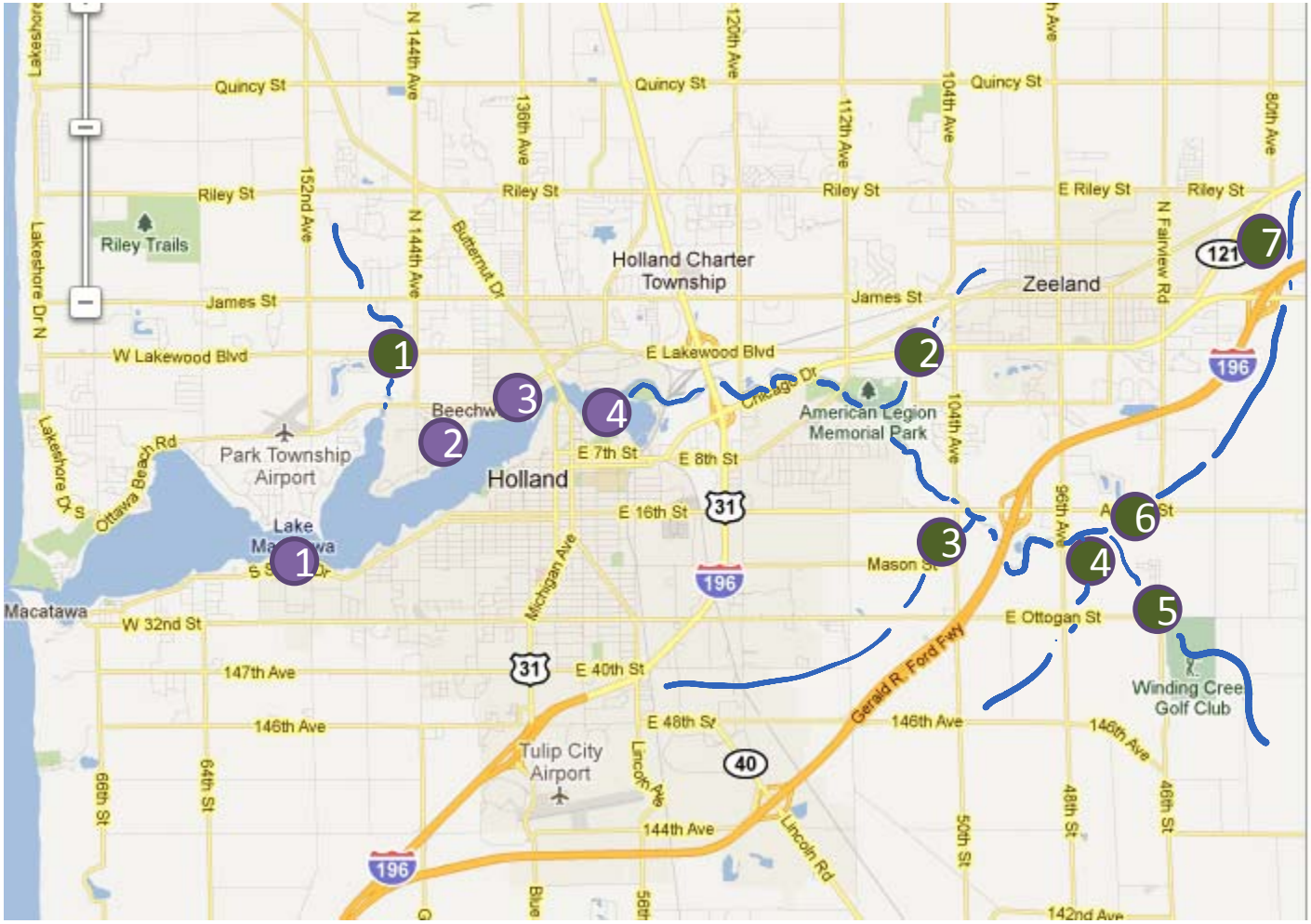
Non-point source –
•Upstream septic tank or sewerage leakage
•Agricultural (either animal facility or manure spread on fields)
•Wildlife
•Persistent indigenous growth in environment

Current study, phase I – survey FIB in broader Lake Mac watershed.

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

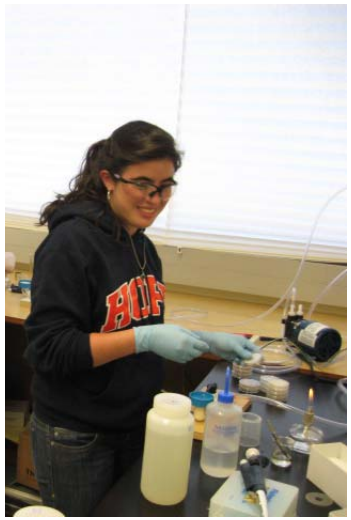
Narrows
Keizer dock
Dunton Park
River Ave

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

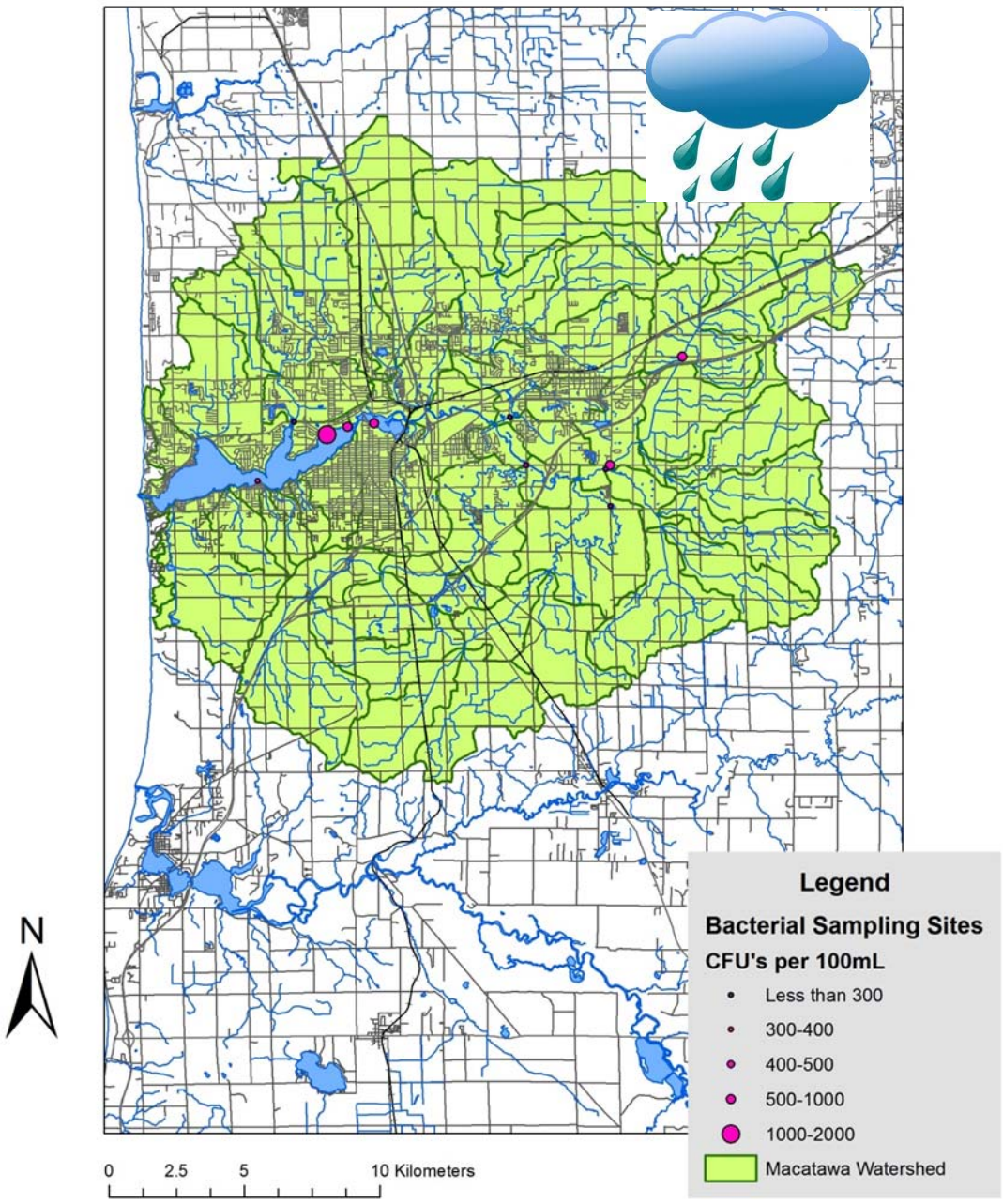


Methodology – membrane filtration followed by colony enumeration:

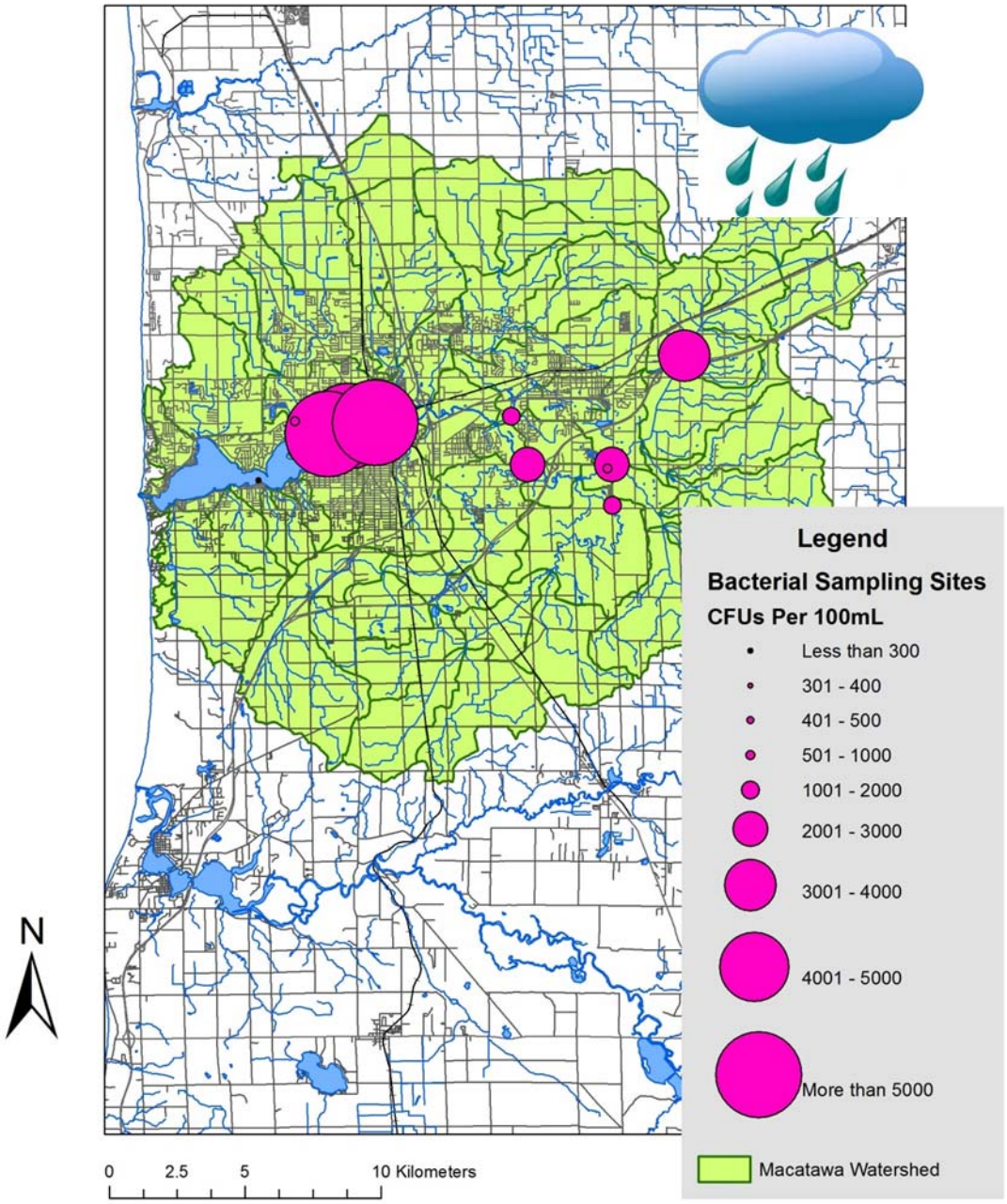
•Samples were collected once a week for eight weeks, late May – mid July.
•In biological triplicates.
•Three dilutions of each replicate applied to membrane filter and placed on modified mTEC and on mEI (E coli and entercocci).
•EPA method 1600 and 1603.
•Arithmetic means calculated from triplicates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



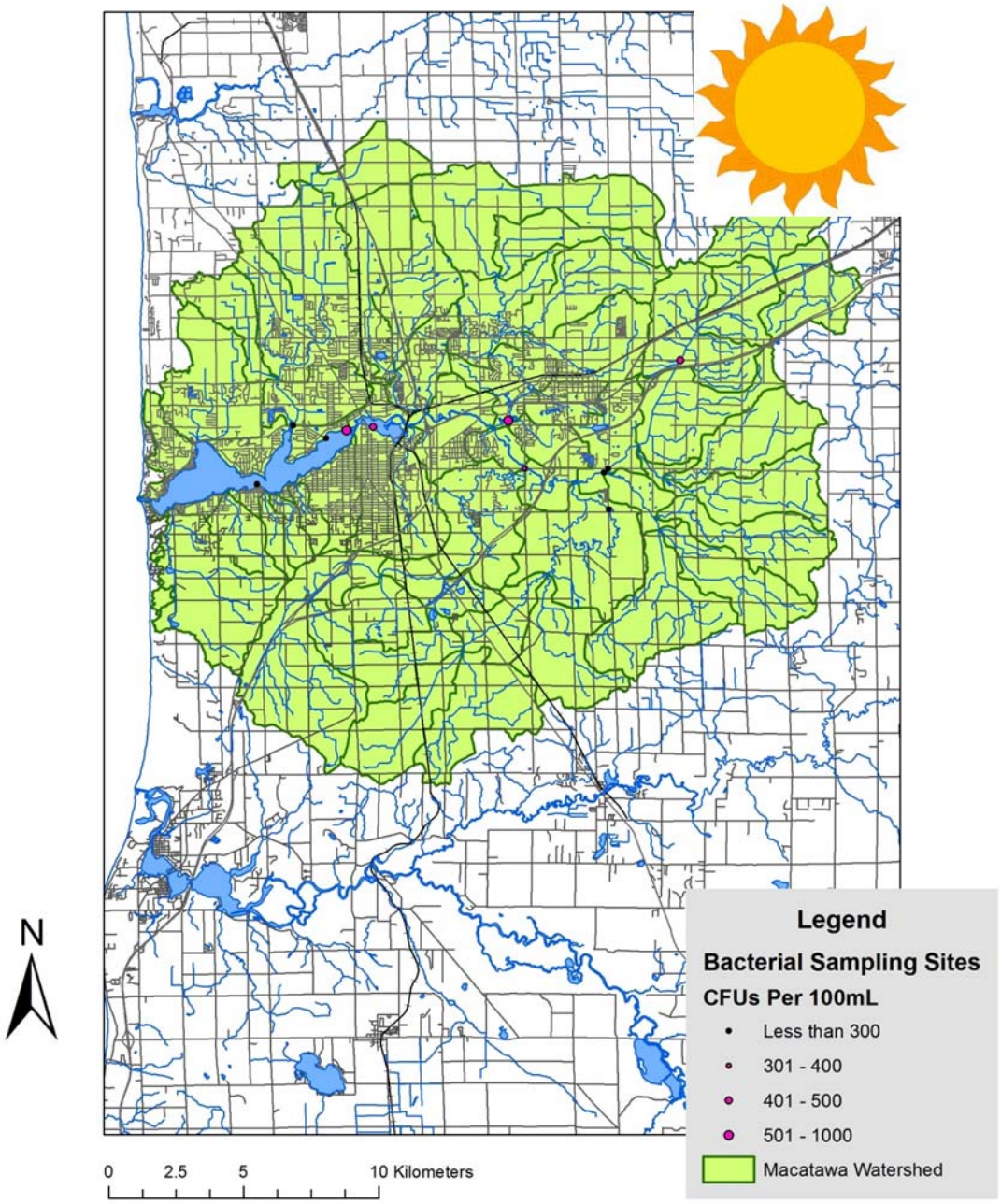
E. Coli Levels -- 18 May, 2011



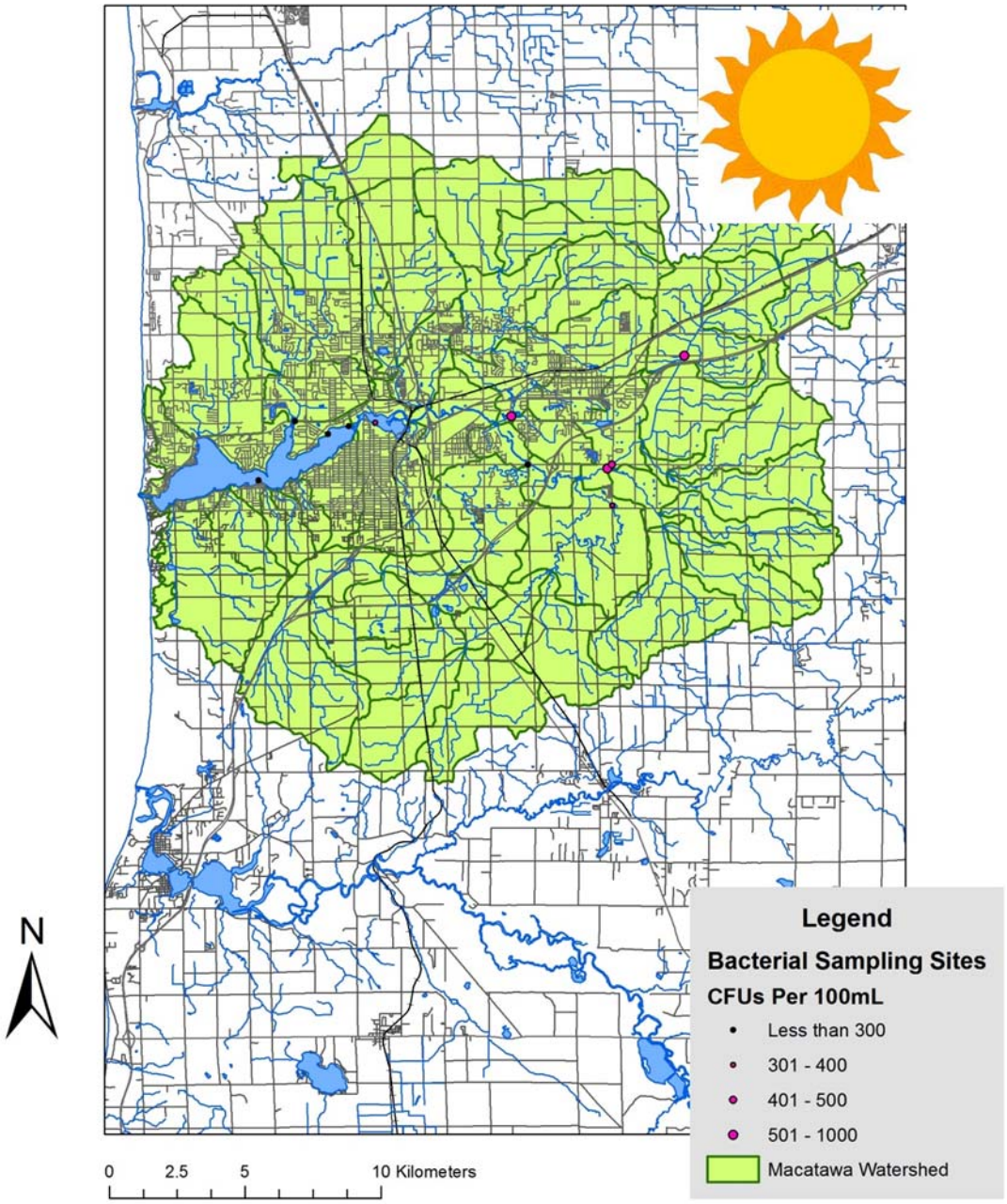
E. Coli Levels -- 24 May, 2011



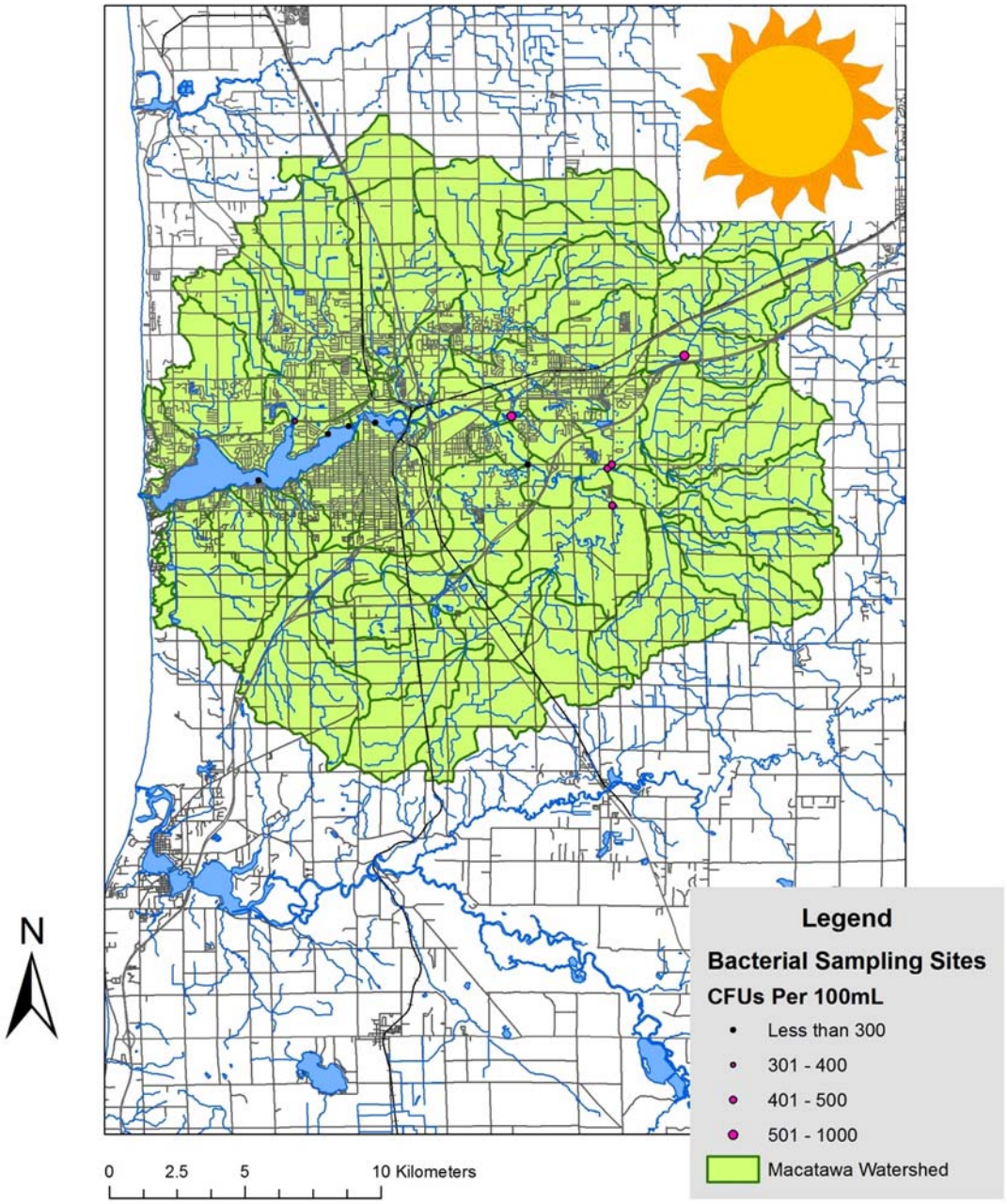
E. Coli Levels -- 1 June, 2011



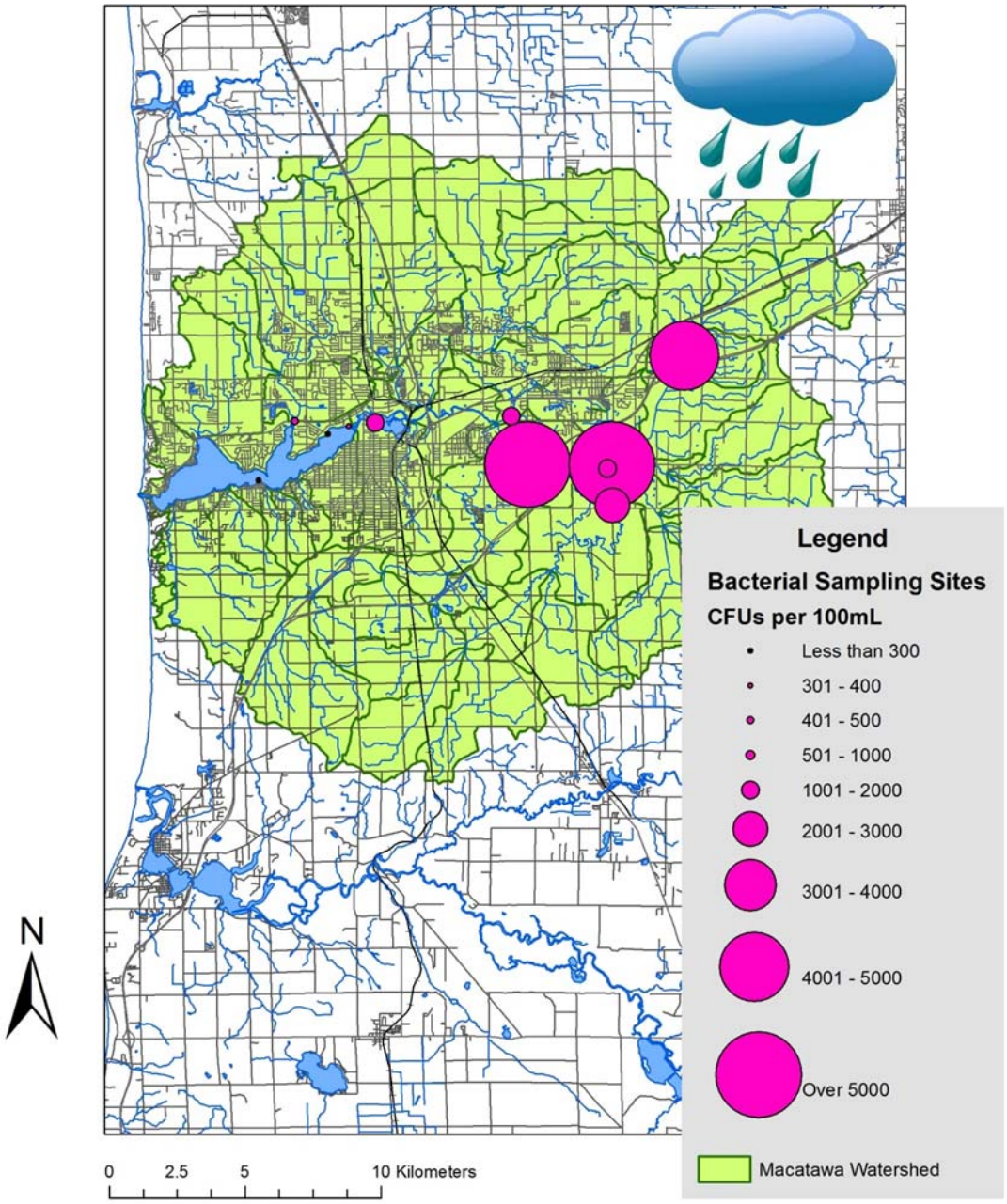
E. Coli Levels -- 7 June, 2011



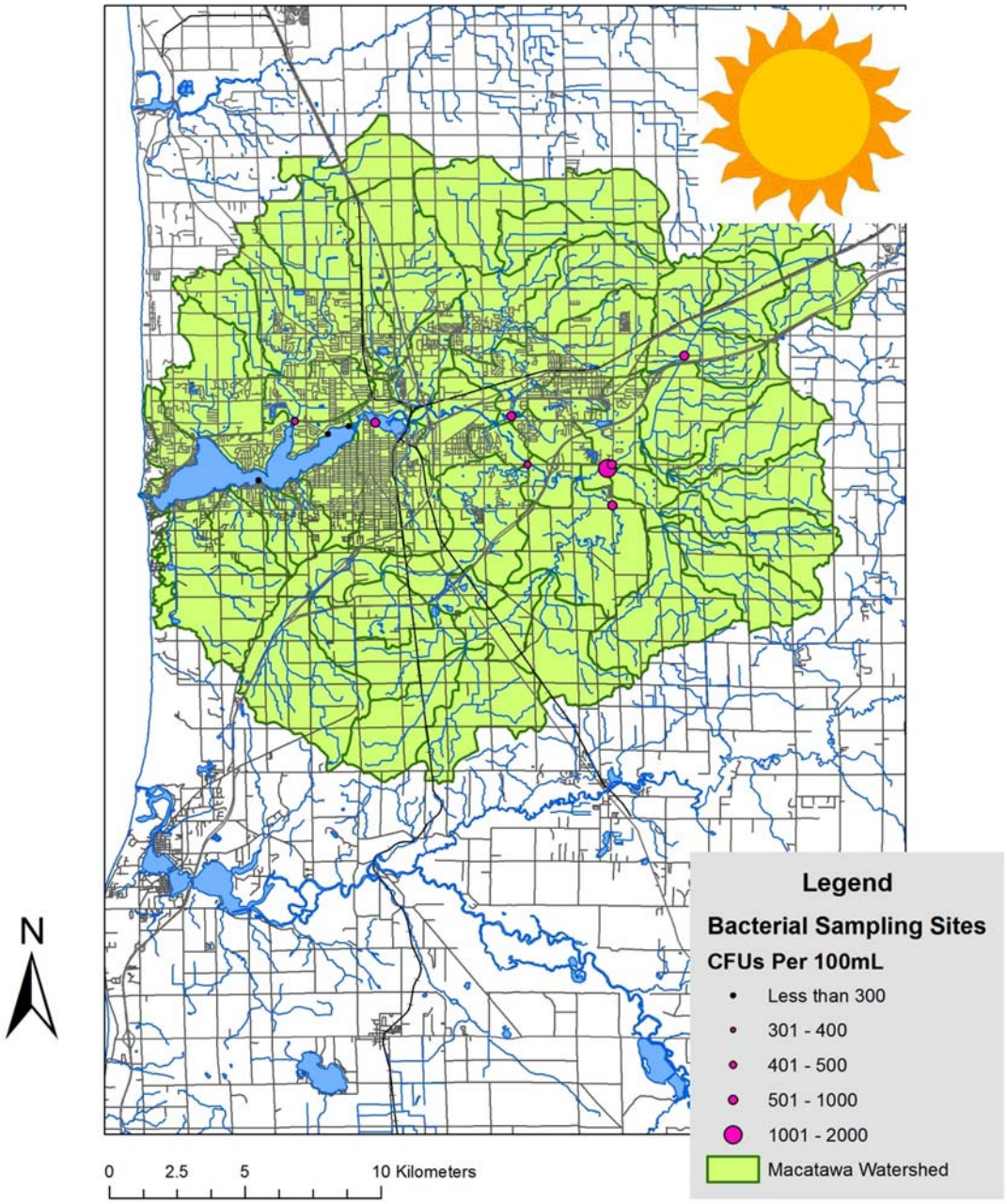
E. Coli Levels -- 16 June, 2011



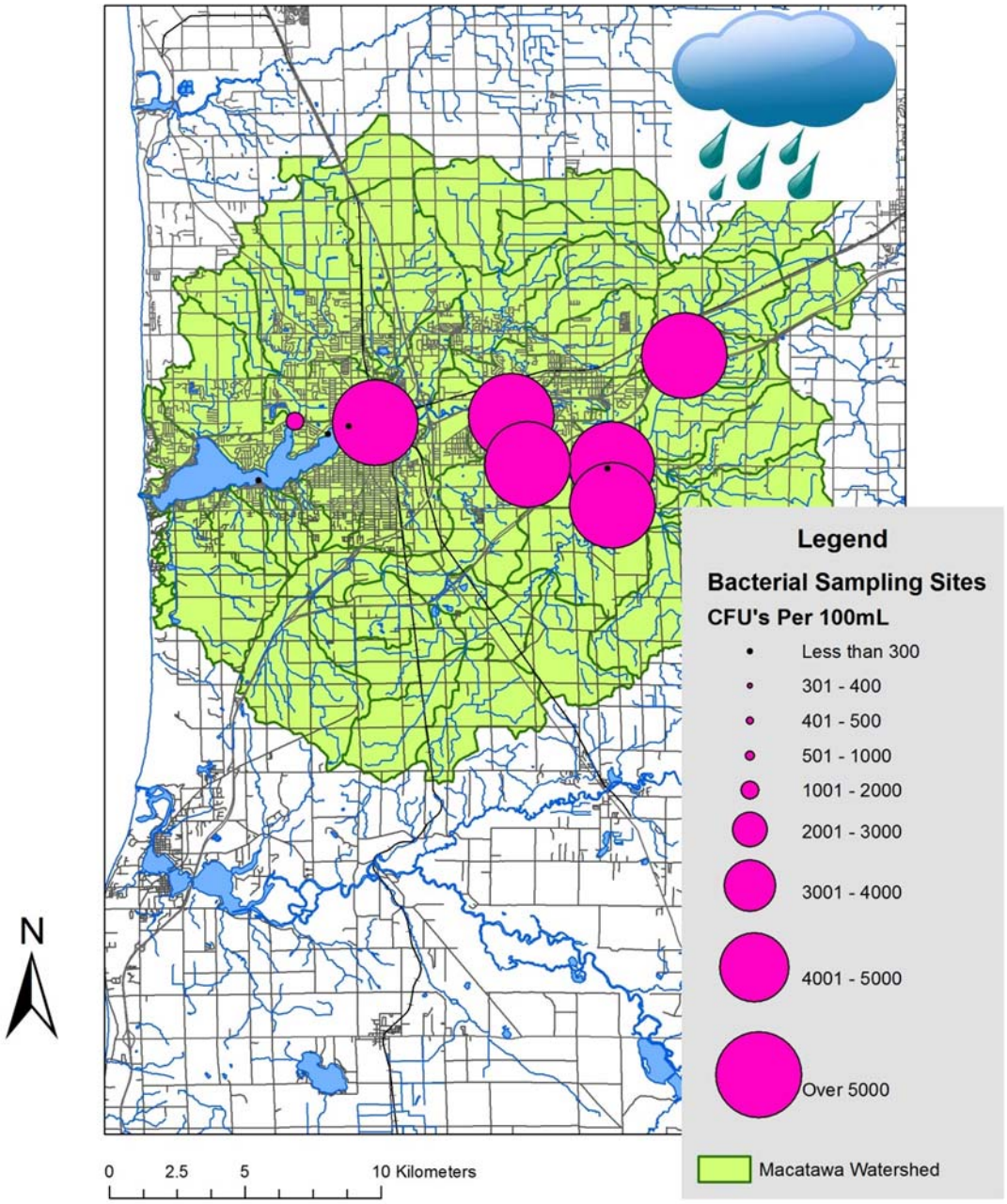
E. Coli Levels -- 21 June, 2011



E. Coli Levels -- 6 July, 2011

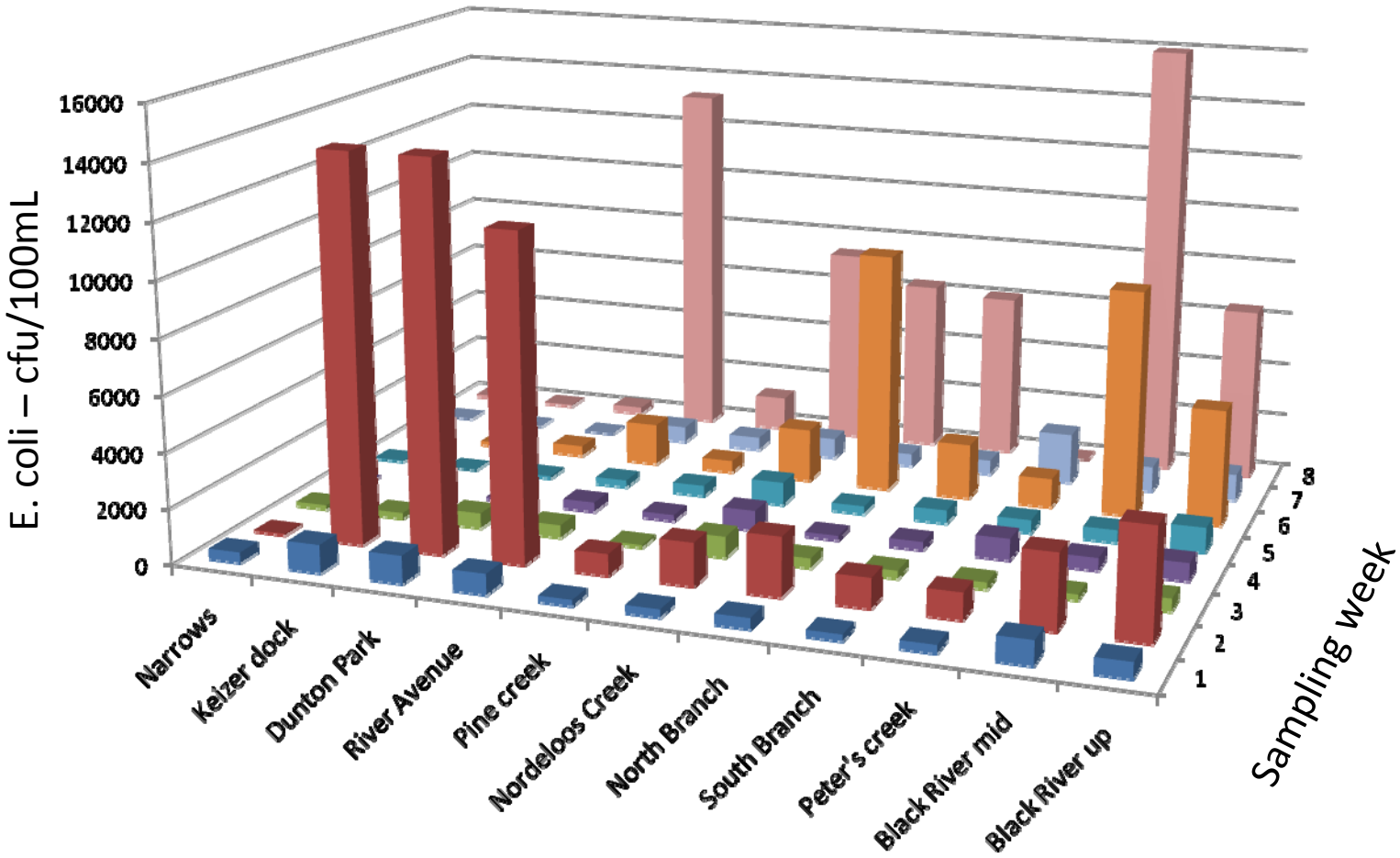


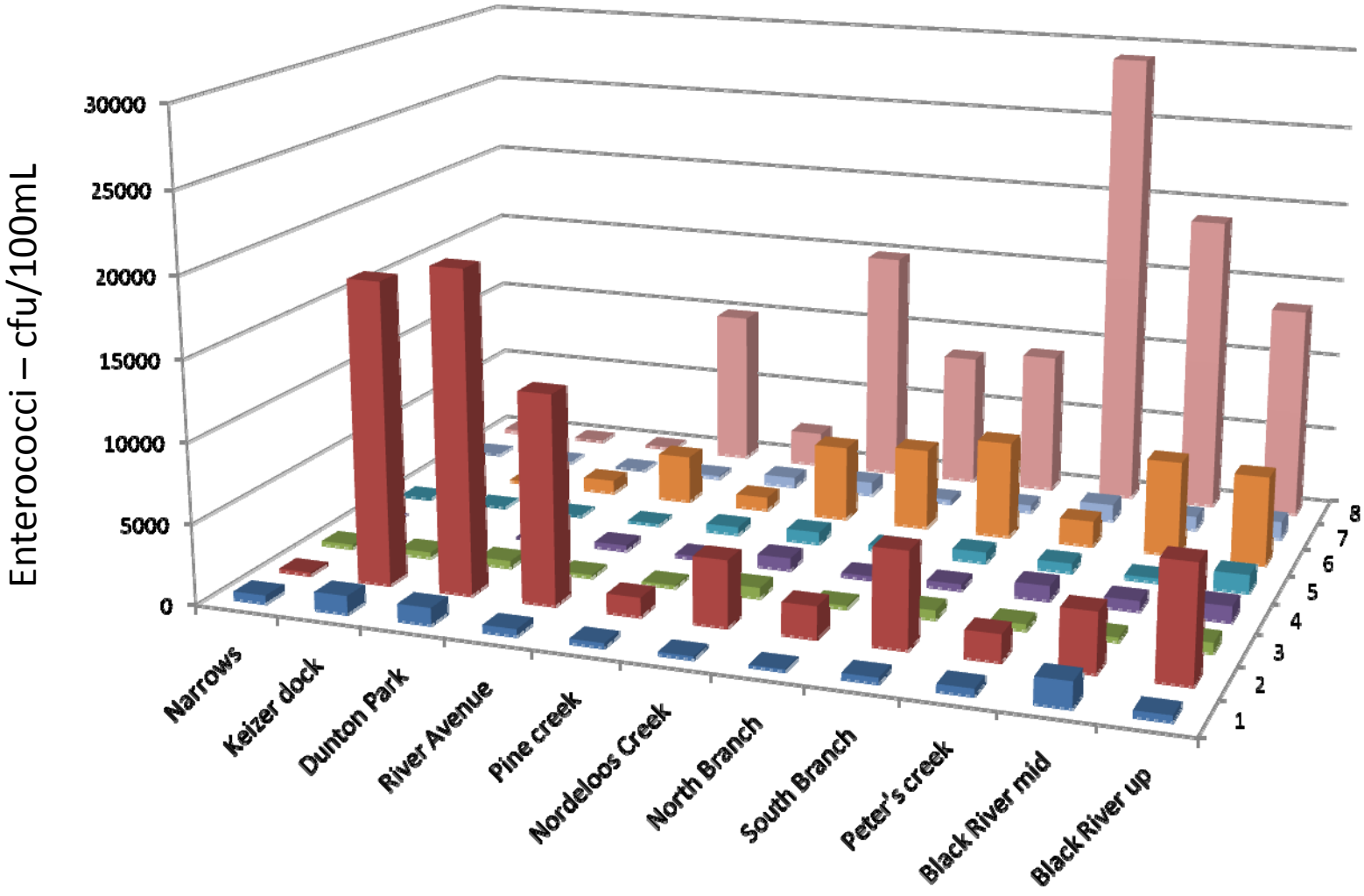
E. Coli Levels -- 12 July, 2011



Weeks 1,2, 6, and 8 = Rainy

Weeks 3, 4, 5, and 7 = Dry




Enterococci – cfu/100mL
30000
25000
20000
15000
10000
5000
0
Narrows
Keizer dock
Dunton Park
River Avenue
Pine creek
Nordeloos Creek
North Branch
South Branch
Peter's creek
Black River mid
Black River up
1
2
3
4
5
6
7
8

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.

But what's the source?

- Are live feces continually creeping in to the upstream watershed, perhaps from septic tanks, leaky sewerage, agriculture, or wildlife?
- Or – have FIB have established themselves as indigenously-growing organisms in the upstream areas?

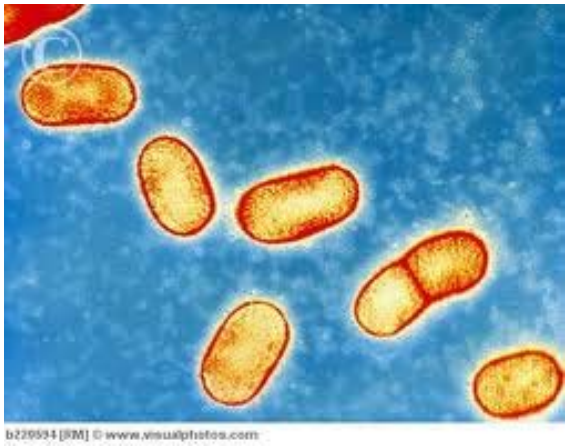
Microbial study – Phase II… Source Tracking (or, who's pooping in the water?)

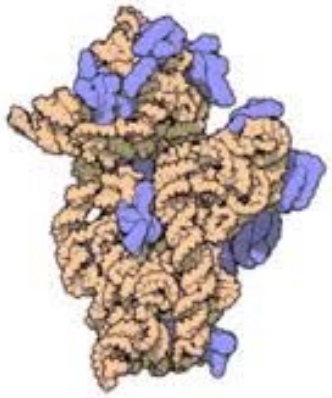
- The idea – the gut biome is a complex place.
- You have 10's of trillions of bacterial cells in your gut, against ~ 1 trillion cells of YOU! Of those bacteria, there are countless species and strains each adapted to its host.
- In particular, different *host species* are populated by different *bacterial species.*
- **Connecting a particular type of bacteria to a known host = source tracking (Bacterial Source Tracking/BST; Microbial Source Tracking/MST).**
- We have begun using a molecular genetics approach to genotype material found in environmental samples.

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

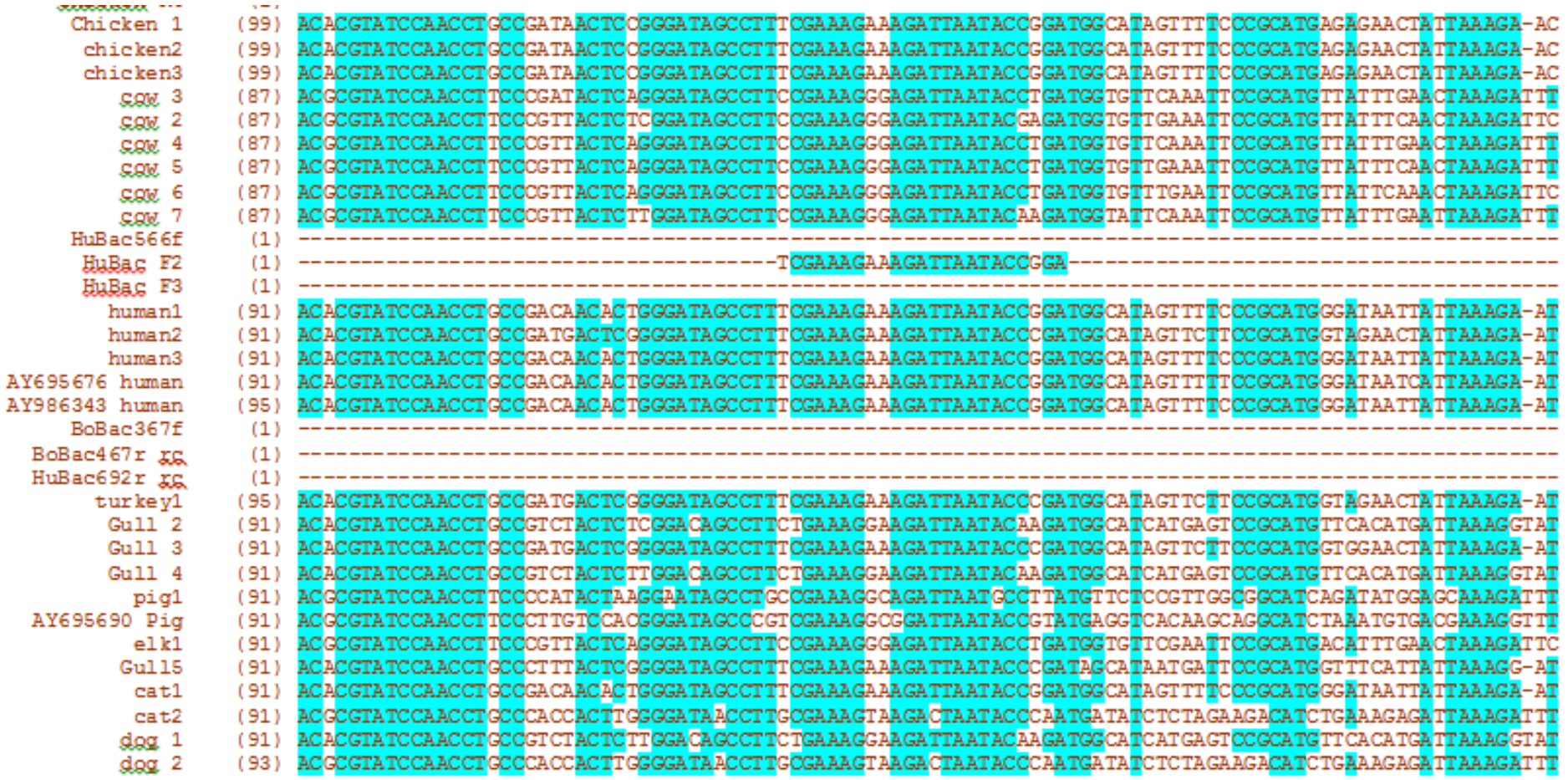
Bacteroides is an anaerobic organism – can't grow outside the gut. Makes up about %50 of the mass of the gut biome.

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





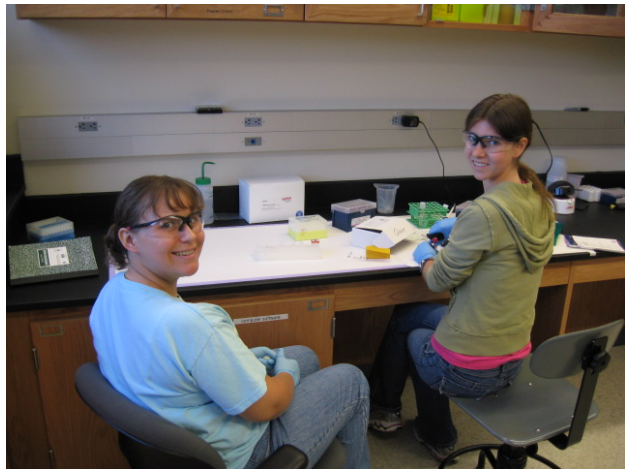
Some 16S RNA genes from bacteroides of various animals:

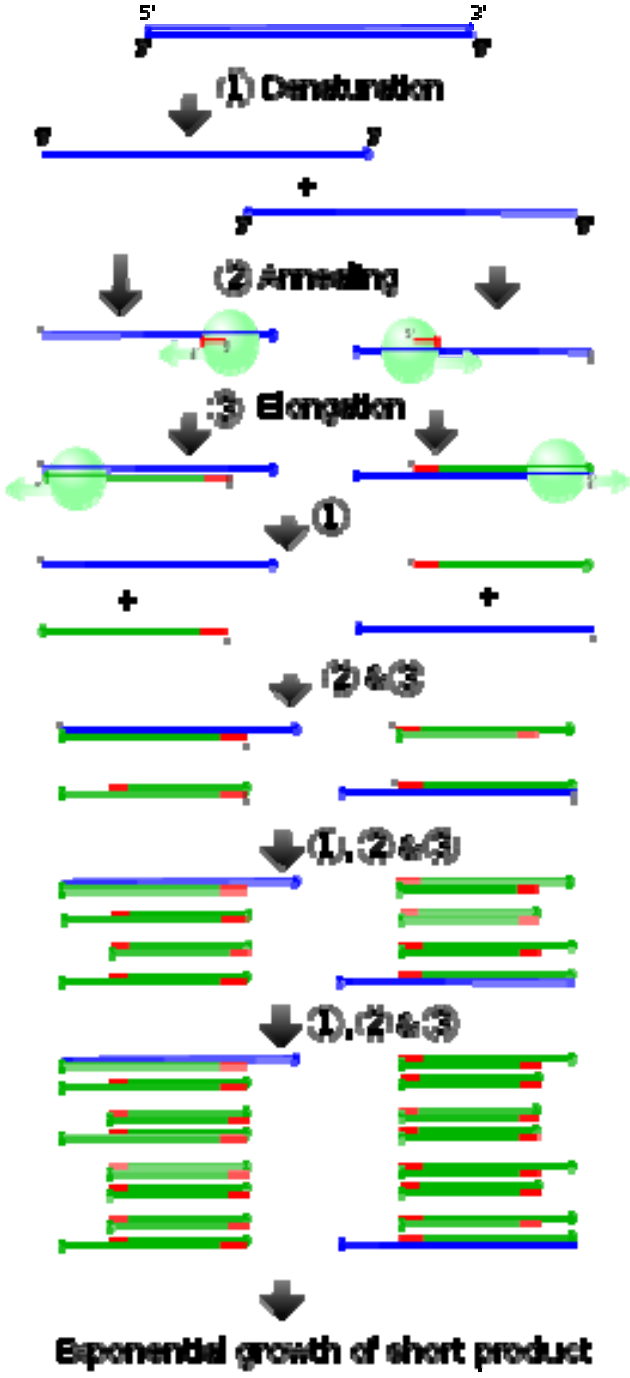


Polymerase Chain Reaction: If you can design a pair of primers that flank a sequence of interest, you can use PCR to make a large amount from as little as one molecule of starting DNA. PCR allows you to:

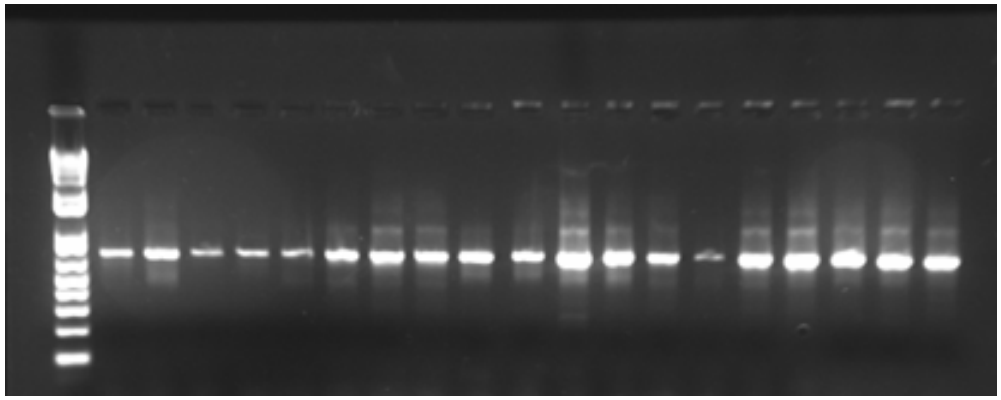
- Measure amount of target sequence
- Clone amplified DNA
- Sequence it

- 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 some samples
- strongly present municipal sewage.

Pig-specific:

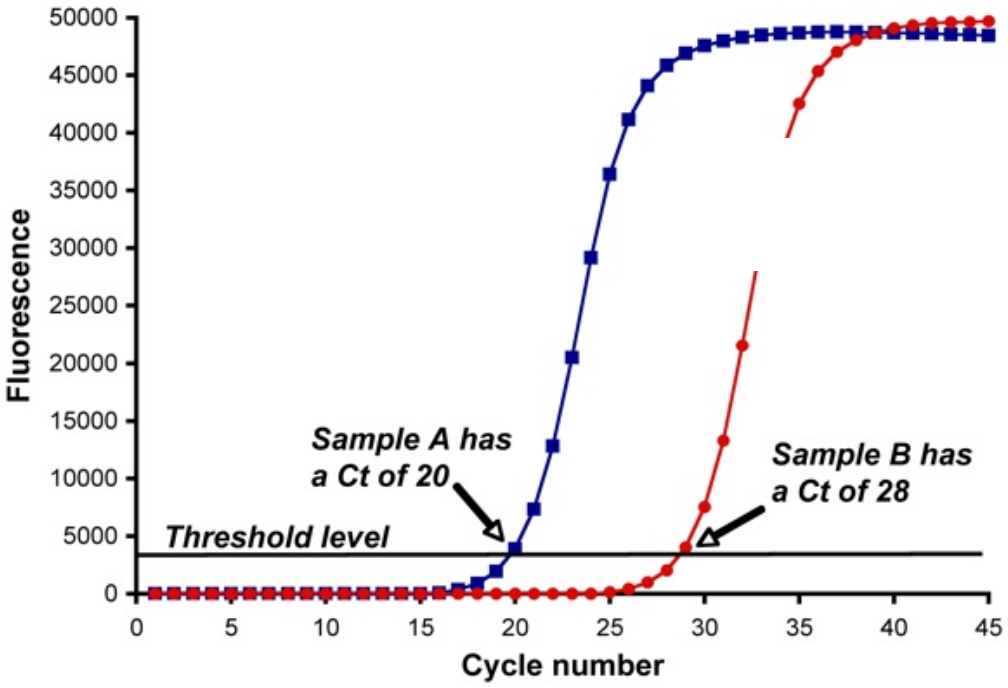
- present in pig feces;
- in drainage surrounding a local hog farm;
- in days following pig manure spill immediately downstream
- *Completely absent in routine samples*

Cow-specific

- Present in cow feces
- Otherwise absent in routine samples

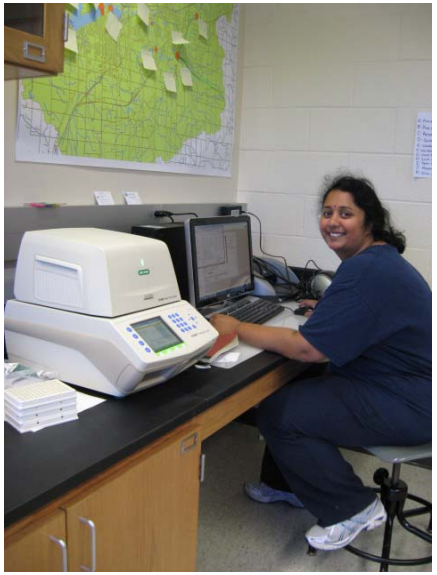
Quantitative (qPCR) aka Real-time (RT-PCR) monitors appearance of copied DNA product every cycle.

•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



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:

$$\left(\frac{Bac_{human}}{Bac_{total}}\right)_{sample}$$

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

Conclusion: Human fecal fingerprint appears in some samples but not all.

Questions and further experimentation:

What's going into Dunton Park that's not elsewhere?
•Collaborating with Vijay and the County to correlate dog-sniffing data with molecular fingerprint data.

If only some is human, and none is pig or cow, what is the origin of the rest of the bacteroides DNA?
•Chicken PCR assay conditions exist but are not well-validated.
•DNA samples will be sequenced to look for any matches.

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