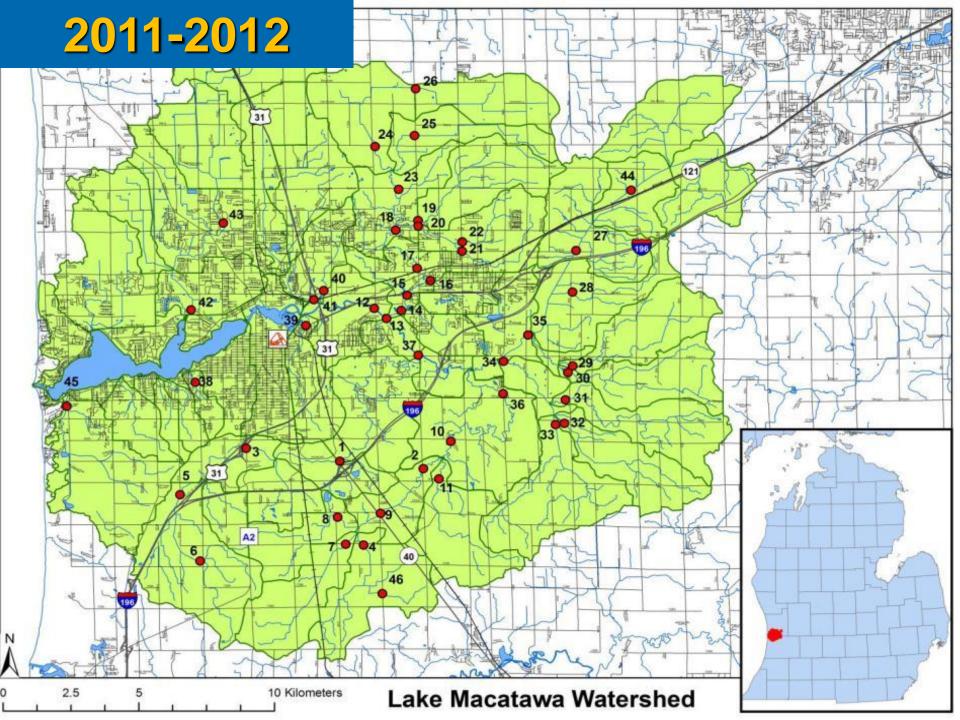
Macatawa Watershed Water Quality Research Project

Graham F. Peaslee Michael J. Pikaart Hope College Chemistry Dept.

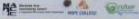
Nov. 1 2012



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 cooducted by the Macontawa Area Coordinating Council and Hope College.

If you have any questions about this sampler, please contact: Dr. Oraham Pessies <u>pensive/tope edu</u> 616-395-7117 OR Mary Fales <u>indures the mace org</u> 616-395-3688



WARANT 45U





Sample Collection

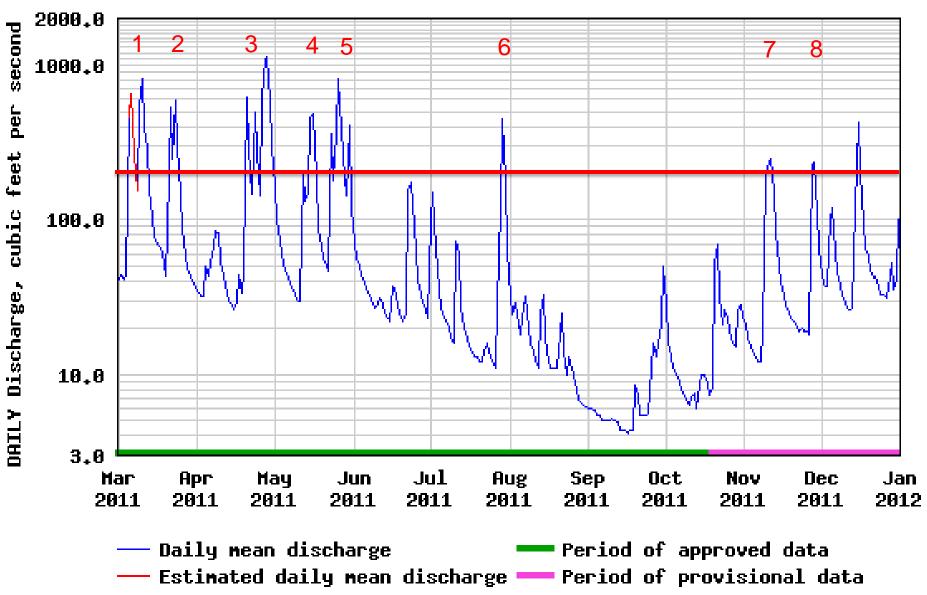




2011 Sampling Events

≊USGS

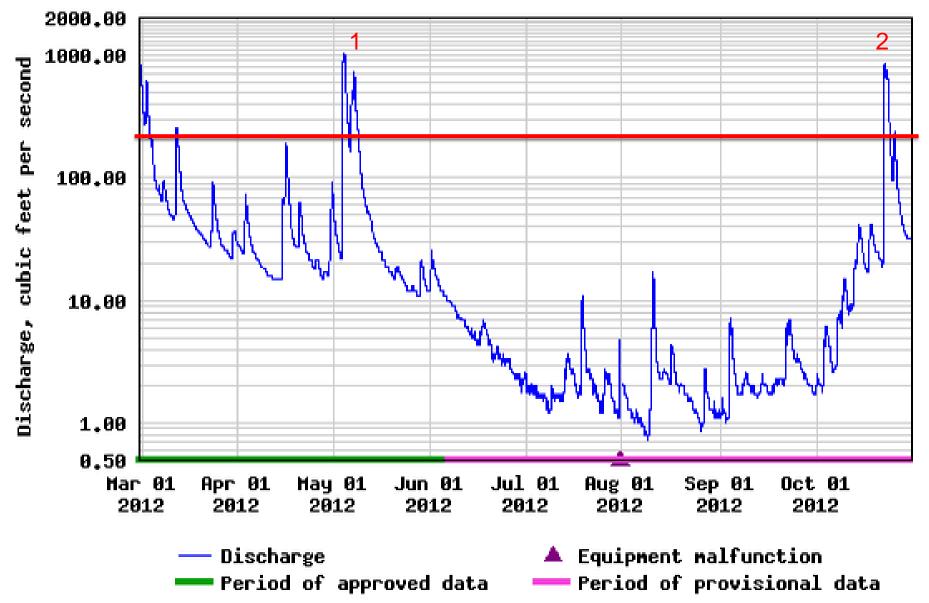
USGS 04108800 MACATAWA RIVER AT STATE ROAD NEAR ZEELAND, MI



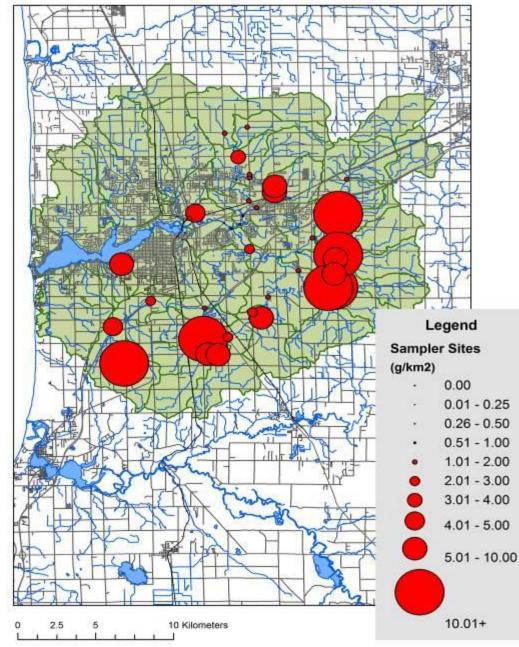
2012 Sampling Events

≊USGS

USGS 04108800 MACATAWA RIVER AT STATE ROAD NEAR ZEELAND, MI



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

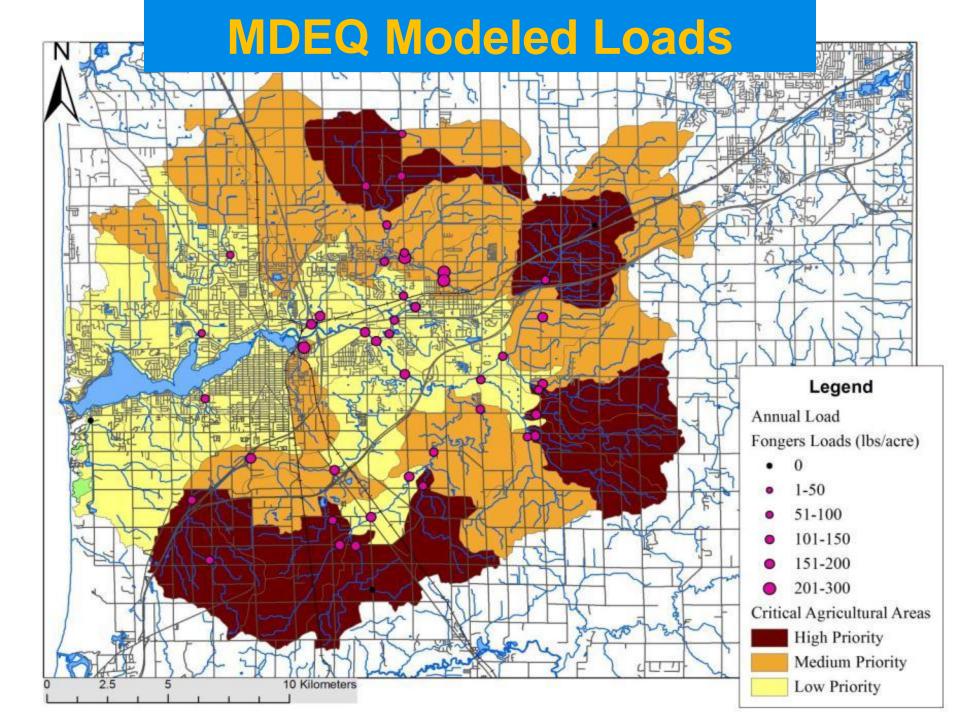


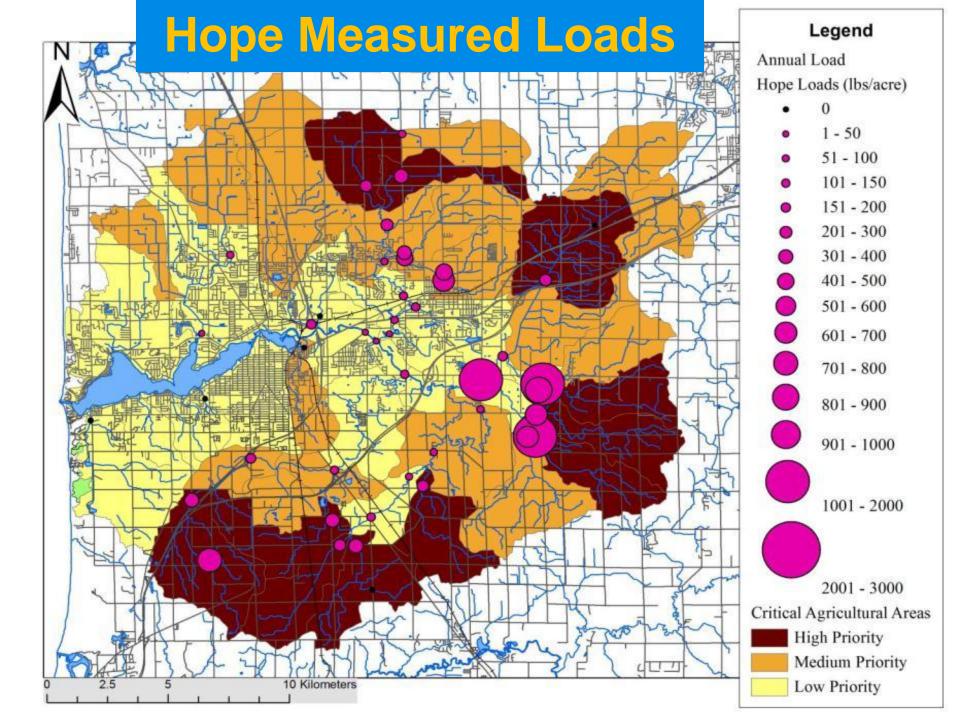
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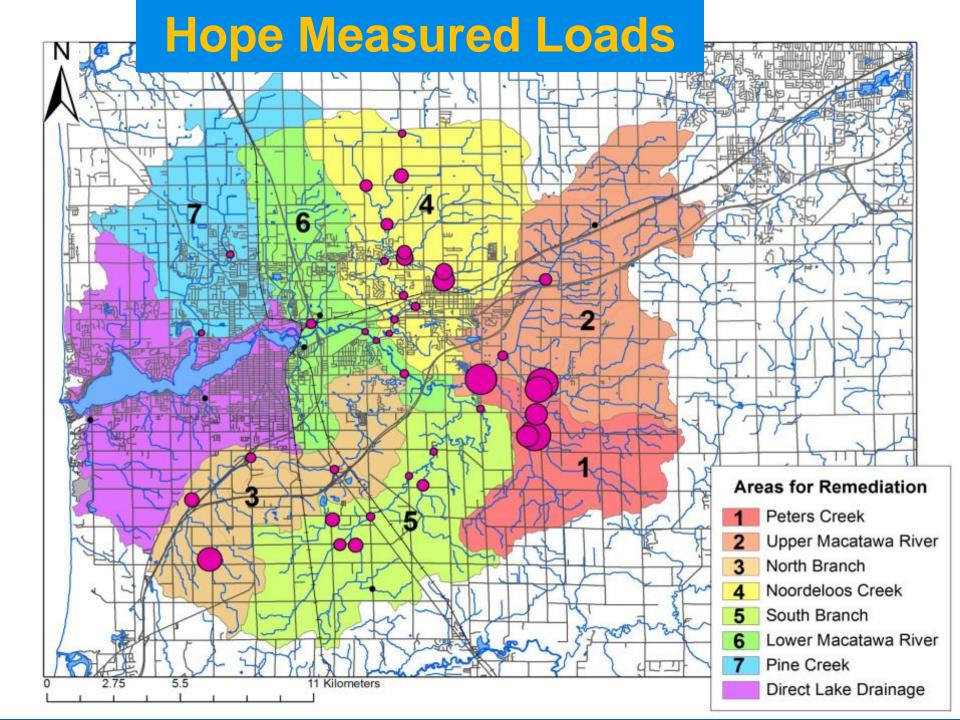
Preliminary Results:

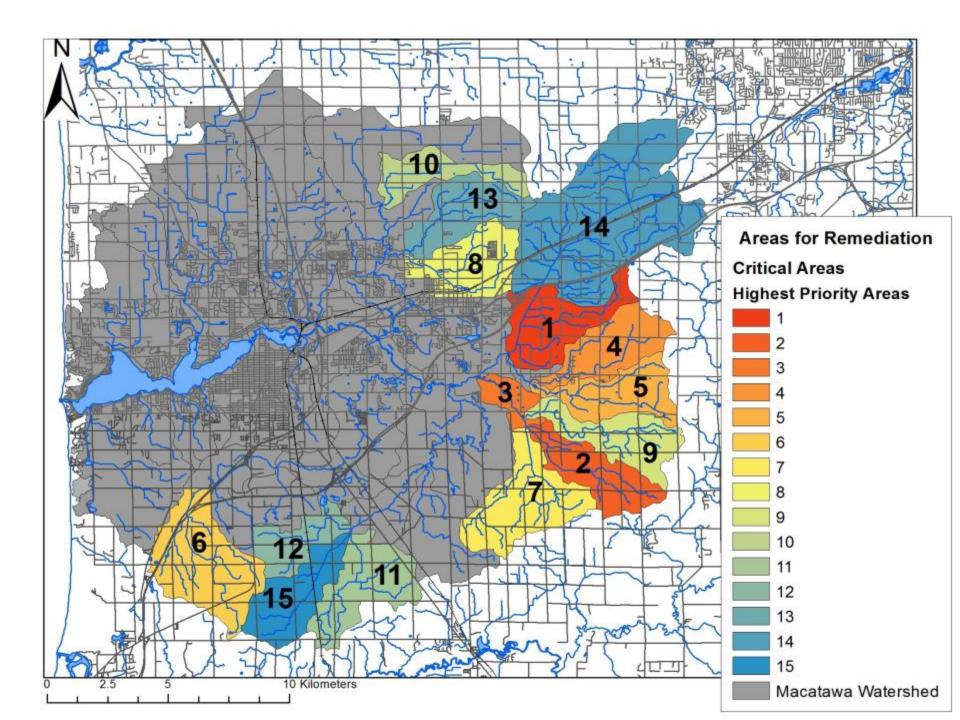
> A typical "event"

Sediment/km²







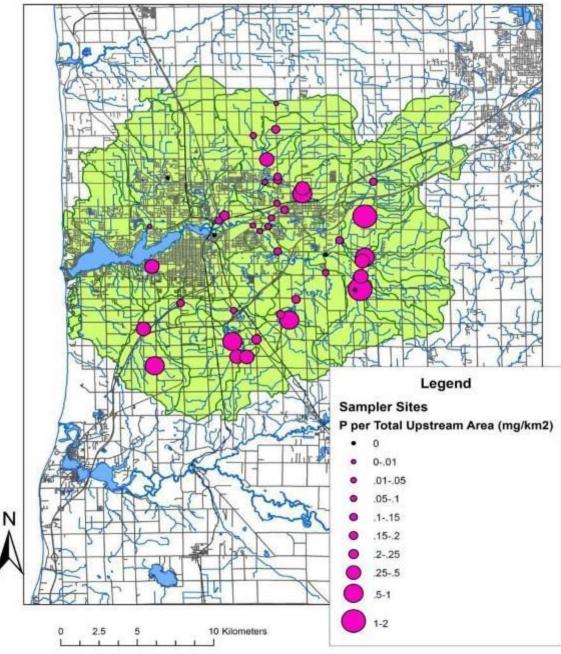


Sediment Fingerprinting Methods

- PO₄ Analysis
- Reflected light: Color
- Elemental analysis
- Radiometric Dating
- Biologicals: Pollen



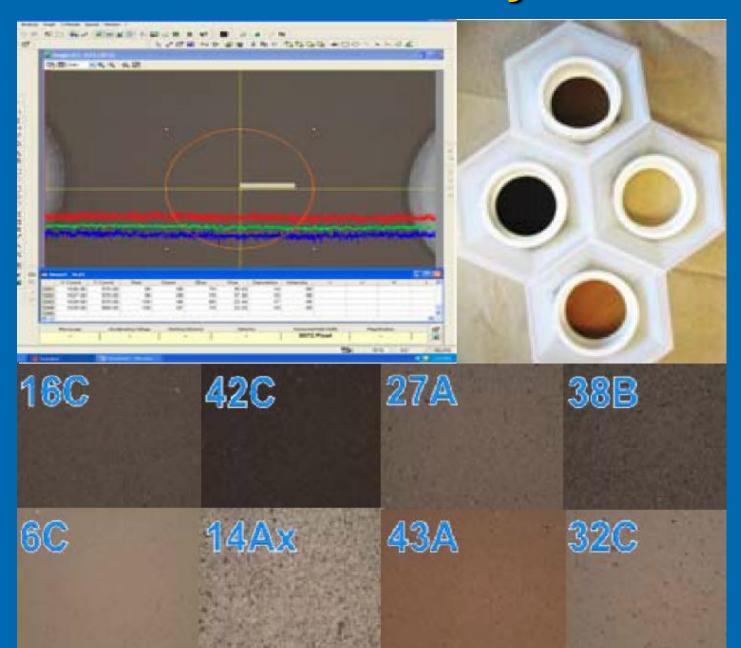
Phosphorous Concentrations Per Total Upstream Area Event 5 (May 23-29, 2011)



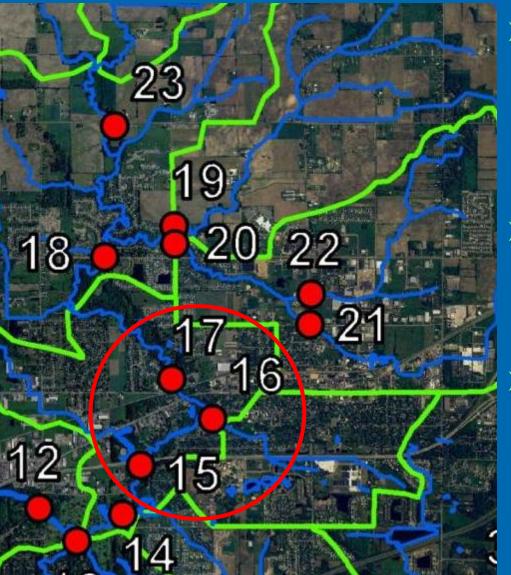
Phosphates: A typical "event"

Soluble PO₄ Fe-bound PO₄ Ca-bound PO₄

RGB Color Analysis

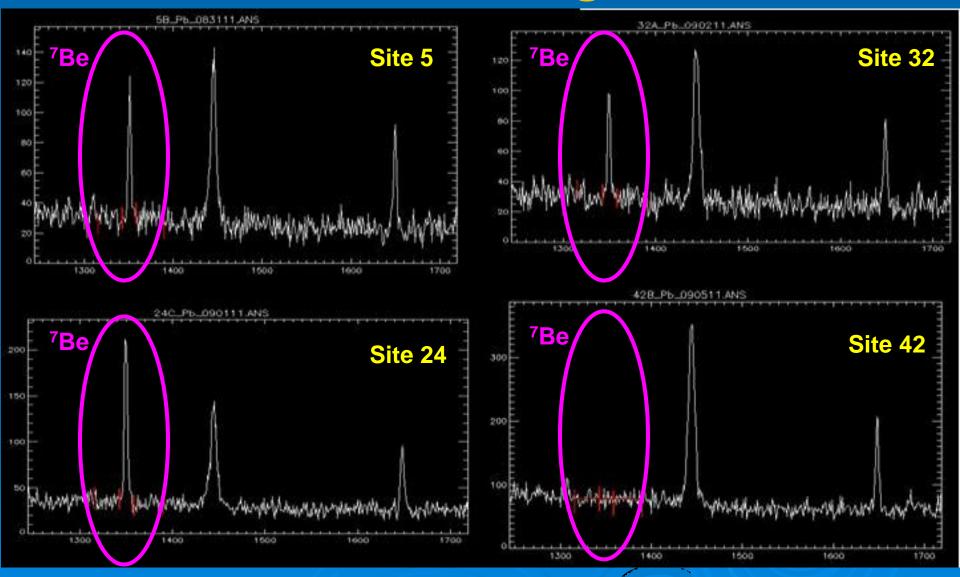


Elemental Analysis



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...



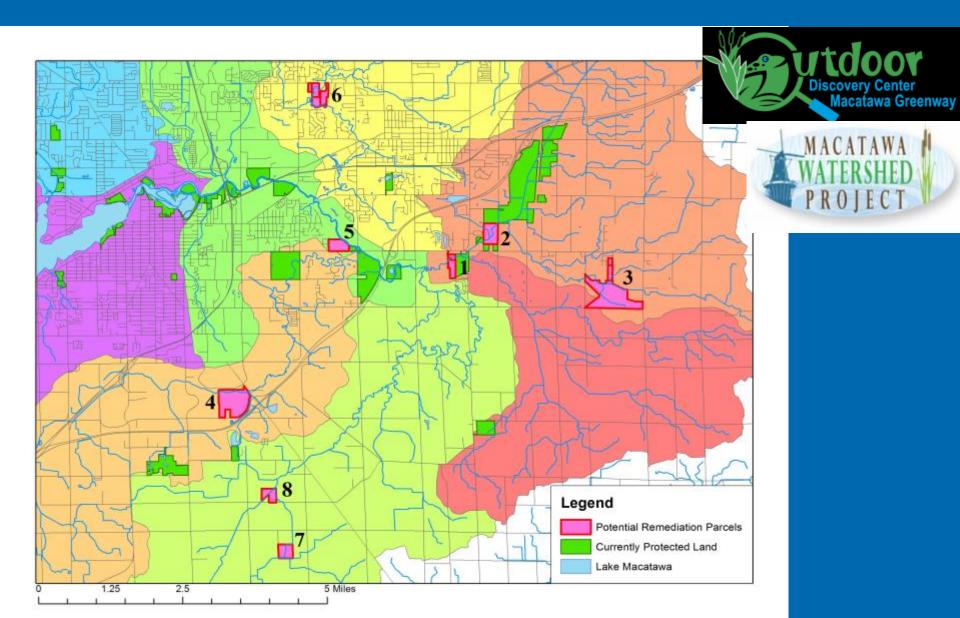




Conclusions?

Lots of sediment We can rank order the sources... Fingerprinting potential... Short residence time: quick recovery

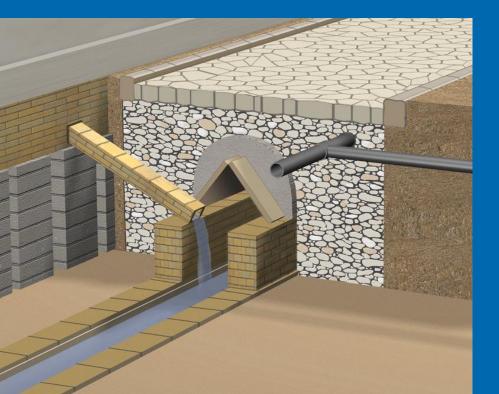
Next Steps...

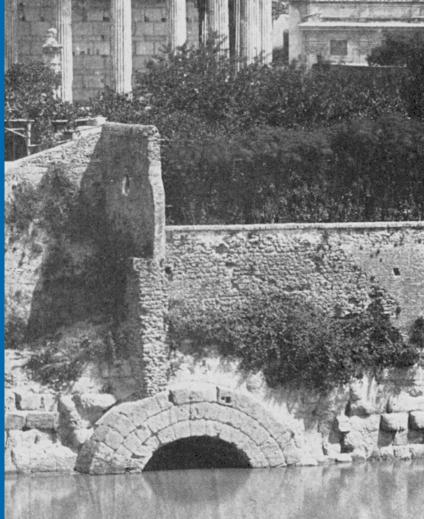


That mountain stream looks pretty clean, but what's upstream?

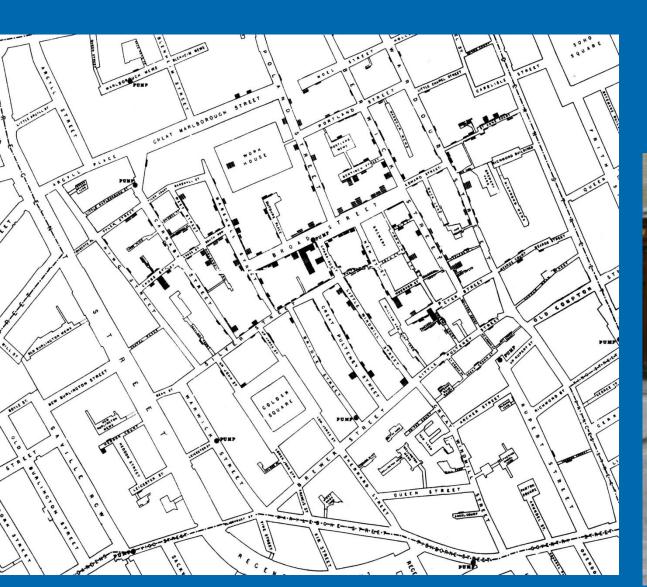


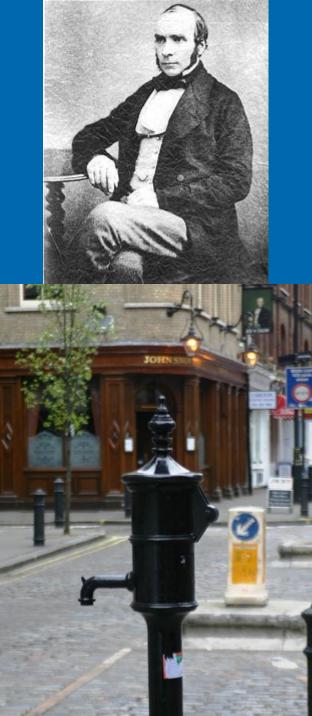
Best public health invention ever: separation of sewage from drinking water.





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







Modern sanitation removes risk of waterborne pathogens in developed countries:

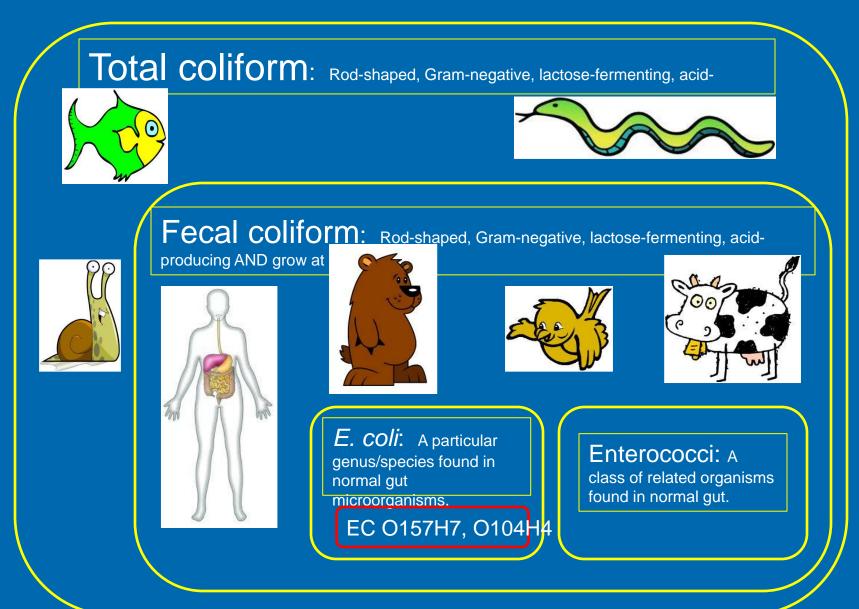


- Sterilization of water supply
- Hygeine
- Sanitary sewers (more effective if separate from storm sewers)
- Sewage treatment prior to discharge
- Testing and monitoring

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:



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:

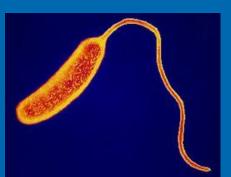
- C. botulinum
- Campylobacter
- V. cholerae
- Shigella
- Salmonella

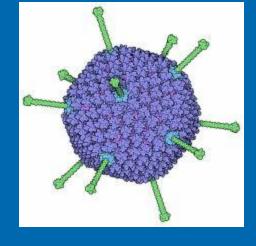
Viruses like:

- 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 "mostprobable 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?

•

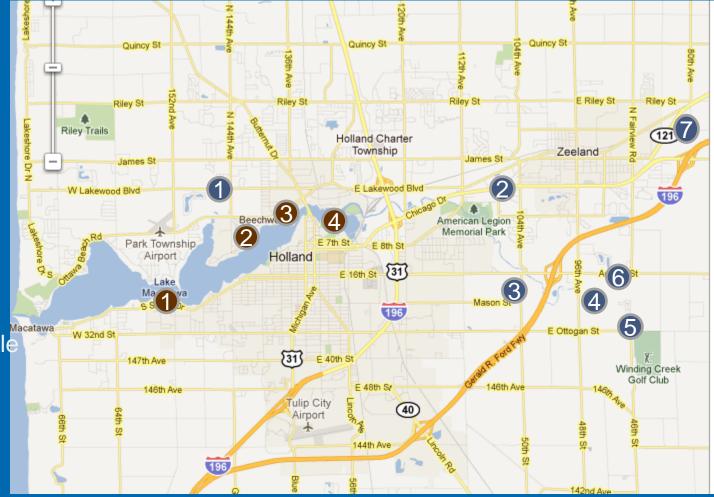


Phase I – survey FIB in broader Lake Mac watershed.

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 entercocci).
- EPA method 1600 and 1603.
- Arithmetic means calculated from triplicates of dilution giving suitable colony density for counting.

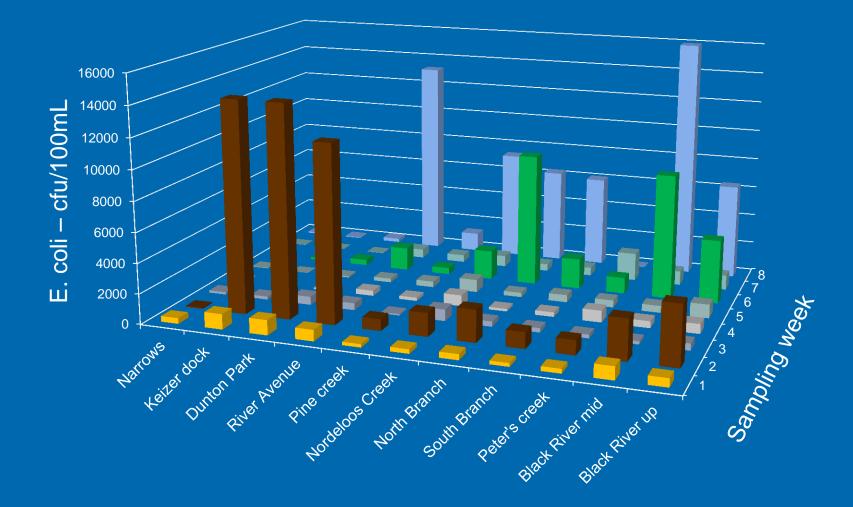


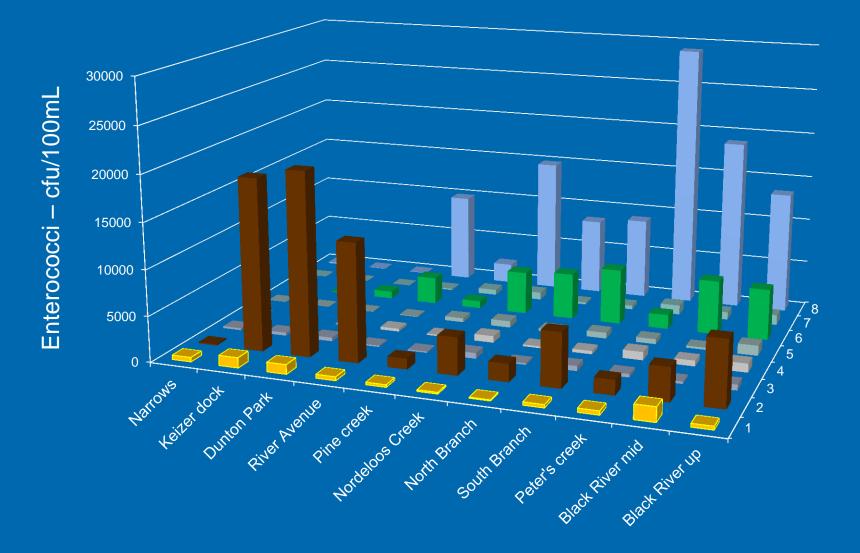






Weeks 1,2, 6, and 8 = Rainy Weeks 3, 4, 5, and 7 = Dry





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?)

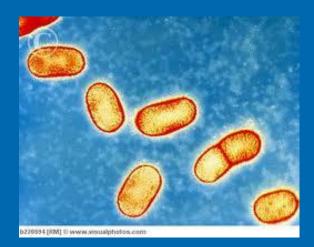
- Connecting a particular type of bacteria to a known host = source tracking (Bacterial Source Tracking/BST; Microbial Source Tracking/MST).
- Molecular fingerprinting approaches allow us to distinguish host species among certain target hosts (some more specifically than others).



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) ACAGTATCCAACCTCCCCATAACTCCGGGATAGCCTTCCGAAGGAACATTAATACCCGATGCCATGCTTTCCCCCATGAGAGAACTATAAGGACA chicken3 (99) ACAGTATCCAACCTCCCCATAACTCCGGGATAGCCTTCCGAAGGAACATTAATACCCGATGCCATGCTTTCCCCCATGAGAGAACTATAAGGA-AC chicken3 (97) ACAGTATCCAACCTCCCGATAACTCCGGGATAGCCTTCCGAAGGAACATTAATACCCGATGCCATGCTCCGAAGTCCCATGCAGAGACTTATAAGG-AC cg82 (97) ACAGTATCCAACCTTCCCGTTACTCGGGATAGCCTTCCGAAGGGACATTAATACCGATGGCATGATGTTCCCCCATGAGAGACTATTATAGCAAGTCC cg82 (97) ACAGTATCCAACCTTCCCGTTACTCGGGATAGCCTTCCGAAGGGACATTAATACCGATGGTGTGAAATTCCCCATGTTTTTCAACTAAAGTTC cg82 (97) ACAGTATCCAACCTTCCCGTTACTCAGGGATAGCCTTCCGAAGGGACATTAATACGGATGGTGTGAAATTCCCCATGTTATTTCCACTAAGGTT cg82 (97) ACAGTATCCAACCTTCCCGTTACTCAGGGATAGCCTTCCGAAGGGACATTAATACCGATGGTGTTGAATTCCCCATGTTATTTCCACTAAAGATTC cg82 (97) ACAGTATCCAACCTTCCCGTTACTCAGGGATAGCCTTCCGAAGGGACATTAATACCGATGGTGTTGAATTCCCCATGTTATTTCCACTAAAGATT cg82 (97) ACAGTATCCAACCTTCCCGTTACTCAGGGATAGCCTTCCGAAGGGACATTAATACCGATGGTTGCAAATTCCCCATGTTATTTCCACTAAAGATT cg82 (97) ACAGTATCCAACCTTCCCGTTACTCAGGGATAGCCTTCCGAAGGGACATTAATACCGATGGTGTTGAATTCCCCATGTTATTCCACTGAAGATT cg82 (97) ACAGTATCCAACCTTCCCGTCCGAAGGGATACCTTCCGAAGGGACATTAATACCGATGGTGTTGAATTCCCCATGTTATTCACTGAAGATT cg82 (97) ACAGTATCCAACCTCCCGCCACAGCACTGGGATAGCCTTCCGAAGGGACATTAATACCGATGCCATGCGTTGCAATTCCCCATGGTATATTCACTGAAGATT cg83 (97) ACAGTATCCAACCTCCCCCCACACACTGGGATAGCCTTTCGAAAGGAAGATTAATACCGATGCCATGCGTAGCTTTCCGACGGATGACTTTTCAACTGAAGGATAGATT human1 (91) ACAGTATCCAACCTCCCCCACACACTGGGATAGCCTTTCGAAAGAACATTAATACCGATGCCATGCTGTTCCTCCCCCTGGGATAATTATTAAAGA-AT human3 (91) ACACGTATCCAACCTCCCCCACACACTGGGATAGCCTTTCGAAAGAACATTAATACCCGATGCCATGCTGTTCCTGGGATAACTTATATAGAAGATT AY995675 human (95) ACACGTATCCAACCTCCCCACACACTGGGATAGCCTTTCGAAAGAACATTAATACCCGATGCCATGTTCCTGCGATGGTAACTTATATAGAAGATA AY995679 human (91) ACACGTATCCAACCTCCCCACACACTGGGATAGCCTTTCGAAAGAACATTAATACCCGATGCCATGTTCCTGCGATGGTGGATACTTATATAGAAGATA AY995679 human (95) ACACGTATCCAACCTCCCCACACACTGGGATAGCCTTTCGAAAGAACATTAATACCCGATGGCACATGATGTTCCTGCGGATAACTTATATAGAGATA AY995679 human (95) ACACGTATCCAACCTCC			
chicken3 (99) MARCEATCOARCE COCGATA CTC COCGATACCT COGAAGCACTTA CALCOUNT COALAGAAGA TAATACCCGA TOG CATAGATT COCCATGAAGACAACACTTATAAGA - AC SQR 3 (87) ACCCTATCCAACCT TCCCATC TTCCAACCT COCGAACCCT TCCGAAAGCACACTTAATACCTGA TOG TG TCGAAAT COCCCATGATTTTCCAACTAAGACT SQR 4 (87) ACCCTATCCAACCT TCCCATTCT TCCGATACCT TCCGAAAGCACACTTAATACCTGA TOG TG TTCGAAT TCCCCATGATTTTCCAACTAAGATT SQR 6 (87) ACCCTATCCAACCT TCCCGTTACTCAGGA TACCT TCCGAAAGCACACTTAATACCTGA TOG TG TTGAAT TOCCCATGATTTTCCAACTAAGATT SQR 6 (87) ACCCTATCCAACCT TCCCGTTACTCAGGA TACCT TCCGAAAGCACACTTAATACTGA TOG TG TTGAAT TOCCCATGATTTTCCAACTAAGATT SQR 6 (87) ACCCTATCCAACCT TCCCGTTACTCAGGA TACCT TCCGAAAGCACACTTAATACTGA TOG TG TTGAAT TOCCCATGATTTTCCAACTAAGATT SQR 7 (87) ACCCTATCCAACCT TCCCGTTACTTCTGAGGA TACCT TCCGAAAGCACACTTAATACTGA TOG TG TTGAAT TOCCCATGATTTTTCCAACTAAGATT SQR 7 (87) ACCCTATCCAACCT TCCCGTTACTTCTGAGGA TACTTCCGAAAGCACACTTAATACTGC TA TOG TG TTGAAT TOCCCATGATTTTTCCAACTAAGATT HUBAC FE (1) 	Chicken 1	(99)	ACACGTATCCAACCTGCCGATAACTCOGGGATAGCCTTTOGAAAGAAAGATTAATACCGGATGGCATAGTTTTCCCCGCATGAGAGAACTATTAAAGA-AC
 SSR: 3 (87) KC CGTATOCAACCT TOCCATACT AGGGATACCCTT C GAAAGGGAGATTAATACGAGATG TTTTGAAT TOCCATG TTTTTTTGACTAAAGATT SSR: 4 (87) KC CGTATOCAACCT TOCCGTT KCTCAGGGATACCCTT C GAAAGGGAGATTAATACGAGATG TCTTGAAT TOCCATG TT ATTTGACTAAAGATT SSR: 5 (87) KC CGTATOCAACCT TOCCGTT KCTCAGGGATACCCTT C GAAAGGGAGATTAATACGAGATG TCTTGAAT TOCCATG TT ATTTGACTAAAGATT SSR: 5 (87) KC CGTATOCAACCT TOCCGTT KCTCAGGGATACCCTT C GAAAGGGAGATTAATACGAGATG TCTTGAAT TOCCATG TT ATTTGACTAAAGATT SSR: 6 (87) KC CGTATOCAACCT TOCCGTT KCTCAGGGATACCCTT C GAAAGGGAGATTAATACGT GATG TCTTGAAT TOCCATG TT ATTTGACTAAAGATT SSR: 7 (87) KC CGTATOCAACCT TOCCGTT KCTCAGGGATACCCTT C GAAAGGGAGATTAATACGT GATG TCTTGAATTCCGATG TT ATTTGAATAAAGATT HUBac5666 (1)	chicken2	(99)	ACACGTATCCAACCTGCCGATAACTCOGGGATAGCCTTTOGAAAGAAAGATTAATACOGGATGGCATAGTTTTCCCCGCATGAGAGAACTATTAAAGA-AC
 Seve 2 (87) ACCCUTATECAACCITECCETACT COGATACCITE CGAAAGCGAGATAATACGACATEST STITEGAAATT COGATEST ATTITEAACTAAAGATTA Seve 4 (87) ACCCUTATECAACCITECCUTATEACTAGGGATACCETT CGAAAGCGAGATTAATACETGATOST STITAATACCATEST ATTITEGACTAAAGATT Seve 6 (87) ACCCUTATECAACCITECCUTATEACTAGGGATACCETT CGAAAGCGAGATTAATACETGATOST STITEGAAATT CGCCATESTTATTECAACTAAAGATTA Seve 6 (87) ACCCUTATECAACCITECCUTATEACTAGGGATACECTT CGAAAGCGAGATTAATACETGATOST STITAATTACATCATAAACTAAACTAAACTAAACTAAACT	chicken3	(99)	
 Sevent 4 (87) ACCCUTATECAACCI TEOCOTIACTOAGGATACCETTE CGAAAGCGAGATTAATACE TATES ISTICAAAT TO CCCATGITA IT TIGAA CTAAAGAT TI SEVEN 6 (87) ACCCUTACCATECI TEOCOTIACTOAGGATACECTIC CGAAAGCGAGATTAATACET GATES ISTICAAAT TO CCCATGITA IT TITCAACI TAAAGAT TI SEVEN 7 (87) ACCCUTACCATECTICOCOTIACTOAGGATACECTIC CGAAAGCGAGATTAATACEAT COTTOGING TIGAAT TO CCCATGITA IT TITCAACI TAAAGATTI ALAGAAT TI CAAGATACET COCATGITA CTAAAGCAGATTAATACEAAGATGITA TICAAAT COCCATGITA IT TITCAACI TAAAGATTI ALAGAAT TICAAGGAGATTAATACEAAGATGITA TICAAAT COCCATGITA IT TITCAATACI TAAAGATATI TIAAAGAATTI ALAGAAGATTAATACEAGATGITA TICAAAGAAGATTAATACEAGATGITA TICAAAT COCCATGITA IT TICAATACACATATAAAAGAATATI ALAGAAGATTAATACEAGATGITA IT CAAGATTI COCCATGITA ATATATAAAGAAAATATI TIAAAGAAATATATAAAAACAATAAAGAAGATTAATACCCGATGITA IT CAAGAACATTATAAAGAACATATATAAAGAAGATTAATACCCGATGICAAAGATAATATATATAAAGAAAAAAAATAAAAAAAAAA	<u></u>	(87)	ACGCGTATCCAACCTTCCCGATACTCAGGGATAGCCTTCCGAAAGGGAGATTAATACCTGATGGTGTTCAAATTCCGCATGTTATTTGAACTAAAGATTT
<pre>Set 5 (87) MC CCTATCCAACCT TOCOGTTACTO AGGATACCCTTC OGAAAGGGAGA TTAATACTCGATOGTGTTGAAATTOCCCATG TTATTTCAACTAAAGATTT SEX 6 (87) MC CCTATCCAACCT TOCOGTTACTO AGGATACCCTTC OGAAAGGGAGATTAATACTGATOGTGTTTGAATTOCCCATG TTATTTCAACTAAAGATT HuBacS66f (1) HuBacS66f (1) Human1 (91) MC ACGTATCCAACCT COCGACAGCATGCCTT COGAAGGAGATTAATACCGATOGTATTCCAAATTOCCCATGTTATTTCAACTAAAGATT Human1 (91) MC ACGTATCCAACCT COCGACAACAG TGGATACCCTT COGAAGGAGATTAATACCGGATOGCATGTTCTCCOCCATGGGATAATTATAAAGAAT Human1 (91) MC ACGTATCCAACCT COCGACAACAG TGGATACCCTTT COGAAGAAGATTAATACCGGATOGCATGTTCTCCOCCATGGGATAATTATTAAAGAAT Human3 (91) MC ACGTATCCAACCT COCGACAACAG TGGATACCCTTT COGAAGAAGATTAATACCGGATOGCATGTTCTCCOCCATGGGATAATTATTAAAGAAT Human3 (91) MC ACGTATCCAACCT COCGACAACAG TGGGATACCCTTT COGAAGAAGATTAATACCGGATOGCATAGTTTCCCOCCATGGGATAATTATTAAAGAAT Human3 (91) MC ACGTATCCAACCT COCGACAACAG TGGGATACCCTTT COGAAGAAGATTAATACCGGATOGCATAGTTTCCCOCCATGGGATAATTATTAAAGAAT Human3 (91) MC ACGTATCCAACCT COCGACAACAG TGGGATACCCTTT COGAAGAAGATTAATACCGGATOGCATAGTTTTCCCCCATGGATAATTATTAAAGAAA AY996543 human (91) MC ACGTATCCAACCT COCGACAACAG TGGGATACCCTTT COGAAGAAAGATTAATACCGGATOGCATAGTTTTCCCCCATGGATAATTATTAAAGAAAT BOBac367f (1) HuBac6527 gg (1) HuBac6527 gg (1) HuBac6527 gg (1) HuBac6527 gg (1) HuBac6537 gg (2) HuCCGTATCCAACCTCCCGCGATGACTCGGGATACCCTTTCGAAAGAAGATTAATACCGGATOGCATAGTTTTCCCCGCATGGGATACTTTAAAGAAAT AY695690 Pig (91) MC ACGTATCCAACCTCCCGCGATGACTCTTCGAAGAACGTTTATATACCGGATOGCATAGTTCTTCCCGCATGGTGGAACTATTAAAGAAAT AY695690 Pig (91) MC ACGTATCCAACCTCCCGCGTCACGTCCGGGATACCCTTCGAAGAGAGATTAATACCGGATGGCACTACGGCATCATGTAAAGAACATTAAAGAACATTAACCGGATGCCATGTTCGACAACGCCATCTAACGAGAGACATTAATACCAGAGCCATCAACGCCATCAACGCCATCTAACGTAGGACCAACGCCATCTAACGAGAGACATTAATACCGGATGCACCACCGCCATCGAACGACCATCCAACGCCATCTACATGTTAAGAGAAGATTAATACCGGATGACCATCGTACGATCAACGACGACTATGAACGACGACTATGACCAACGCCATCTAACGACGACGCATCTAACGACGACTATGAAGAACGATTAATACCGGATGACCATCGTACGACGACCACCGCATCGAAGAGATTAAAAGCACGACGACTATGA</pre>	<u>.c.gw</u> 2		
<pre>Gene 6 (87) ACCCGTATCCAACCT TCCCGTTACTC AGGA TACCCT TC GGAAGGGGGA TTAAT ACTATACT GATGE TG TTGAAT TC COCATG TTA TTGAAC TTAAAGAT TC GENE 7 (87) ACCCGTATCCAACCT TCCCGTTACTC TGGATAGCCT TC GGAAGGGGGATTAAT ACAAGATGG TA TTGAAT TC COCATG TTA TTGAA TTAAAGATTT HuBac566f (1) HUBAGE F2 (1) HUBAGE F3 (1) HUBAGE 73 (1) HUBAGE 73 (1) HUBAGE 74 (1) HUBAGE 74 (1) HUBAGE 75 (1</pre>			
CGW 7 (87) MCGCGTATCCAACCT TCCCGTTACTCTTGGATAGCCTTCCGAAAGGGAGATTAATACAAGATCGTATTCAAATTCCCCATGTTATTTGAATTACAAGATTA HuBacS6 6f	<u>.c.gw</u> 5	(87)	
HuBac566f (1) HuBac576 (1) HuBac576 (1) human1 (91) ACACGTATCCAACCTCCCCGCACAGACTCGCGCATAGCCTTCCGAAAGAAGATTAATACCCGATGCATAGTTCTCCCCCATGCGATAGACTATTATTAAAAGAAT human2 (91) ACACGTATCCAACCTCCCGCGACAACATGGCGTTCGAAGAACGATTAATACCCGATGCATAGTTCTTCCCCCATGCGTAGAACTATTATTAAAAGAAT human3 (91) ACACGTATCCAACCTCCCGCGACAACATGGCGTAGCCTTCCGAAAGAAA	<u></u>	(87)	ACGCGTATCCAACCTTCCCGTTACTCAGGGATAGCCTTCCGAAAGGGAGATTAATACCTGATGGTGTTTGAATTCCGCATGTTATTCAAACTAAAGATTC
HuBac F2 (1)	S.S. 7	(87)	ACCCCTATCCAACCTTCCCCGTTACTCTTGGATAGCCTTCCGAAAGGGAGATTAATACAAGATGGTATTCAAATTCCGCATGTTATTTGAATTAAAGATTT
BuBag F3 (1) human1 (91) MCACGTATCCAACCT GCCGACACAT TGGGATAGCCTTT GGAAGAAGATTAATACCGATGGATAGTTTTC CCGCATGGCATAGTATTATAAAGA-AT human2 (91) MCACGTATCCAACCT GCCGACACACAT TGGGATAGCCTTT GGAAGAAGATTAATACCGATGGCATAGTTTC CCGCATGGCATAGTATTATAAAGA-AT human3 (91) MCACGTATCCAACCT GCCGACACACAT TGGGATAGCCTTT GGAAAGAAGATTAATACCGATGGCATAGTTTTC CCGCATGGCATAGTATTATAAAGA-AT human3 (91) MCACGTATCCAACCT GCCGACACACAT TGGGATAGCCTTT GGAAAGAAGATTAATACCGATGGCATAGTTTTTC CCGCATGGGATAATCATTAAAGA-AT AY985476 AY985431 human (95) MCACGTATCCAACCT GCCGACACACATGGGATAGCCTTT GGAAAGAAGATTAATACCGGATGGCATAGTTTT CCGCGATGGGATAATCATTAAAGA-AT BoBac367f (1) BoBac467r (56) MCACGTATCCAACCT GCCGATGCTC TGGGGATAGCCTTT GGAAAGAAGATTAATACCCGATGGCATAGTCTT CCGCATGGT GGAACTAATTATAAAGA-AT BoBac467r (1) HuBac692r (1)		(1)	
human1 (91) ACACGTATCCAACCTGCCGACACAC TGGGATAGCCTTTCGAAGAAGATTAATACCGGATGCCATAGTTTCCCCGCATGGATAATTATTAAAGA-AT human2 (91) ACACGTATCCAACCTGCCGATGACTGGGGATAGCCTTTCGAAAGAAGATTAATACCGGATGCCATAGTTCTCCCGCATGGATAACTATAAAGA-AT human3 (91) ACACGTATCCAACCTGCCGACAACACTGGGGATAGCCTTTCGAAAGAAGATTAATACCGGATGGCATAGTTCTCCCGCATGGGATAACTATTAAAAGA-AT AY695676 human3 (91) ACACGTATCCAACCTGCCGACAACACTGGGATAGCCTTTCGAAAGAAGATTAATACCGGATGGCATAGTTTTCCCCATGGATAACTATTAAAAGA-AT AY695676 human (91) ACACGTATCCAACCTGCCGACAACACTGGGATAGCCTTTCGAAAGAAGATTAATACCGGATGGCATAGTTTTCCCCGATGGGATAACTATTAAAAGA-AT BoBaca677 (1)	HuBac F2	(1)	T <mark>CGAAAG</mark> AA <mark>AGATTAATACC</mark> G <mark>GA</mark>
human2 (91) ACACGTATCCAACCTCCCCGATGACTCGGGGATAGCCTTTCGAAGGAAG	HuBac F3	(1)	
human3 (91) ACACGTATCCAACCTGCCGACAACACTGGGATAGCCTTTGGAAAGAAGATTAATACCGGATGGCATAGTTTTCCCGCATGGGATAATTATTAAAGA-AT AY695676 (91) ACACGTATCCAACCTGCCGACAACACTGGGATAGCCTTTGGAAAGAAGATTAATACCGGATGGCATAGTTTTCCCGCATGGGATAATTATTAAAGA-AT AY986343 (95) ACACGTATCCAACCTGCCGACAACACTGGGATAGCCTTTGGAAAGAATTAATACCGGATGGCATAGTTTTCCCGCATGGGATAATTATTAAAGA-AT BoBac367f (1)	human1	(91)	
AY695676 human (91) ACACGTATCCAACCT GCCGACAACAC TEGGA TAGCCTT TOGAAAGAAAGATTAATACCGGA TGGCATAGTTT TOCCGA TGGGATAATCA TTAAAGA-AT AY986343 human (95) MCACGTATCCAACCT GCCGACAACAC TEGGATAGCCTT OGAAAGAAAGATTAATACCGGATGGCATAGTTT TOCCGCATGGGATAATCA TTAAAGA-AT BOBac367f (1)	human2	(91)	
AY986343 human (95) ACACGTATCCAACCT GCCGACAACAC TGGGATAGCCTTT CGAAAGAAGATTAATACCGGATGGCATAGTTTTCCCCGCATGGGATAATTA TTAAAGA-AT BoBac367f (1) BoBac467r gg (1) HuBac692r gg (1) turkey1 (95) ACACGTATCCAACCT GCCGATGACTCGGGGATAGCCTTT CGAAAGAAGATTAATACCCGATGGCATAGTTCTT CCGCATGGTAGAACTA TTAAAGA-AT Gull 2 (91) ACACGTATCCAACCT GCCGATGACTCGGGGATAGCCTTT CGAAAGAAGATTAATACCCGATGGCATGATCTTCGCGATGGTCGCATGTTCACATGATTAAAGA-AT Gull 4 (91) ACACGTATCCAACCT GCCGATGACTCGGGGATAGCCTTT CGAAAGAAGATTAATACCCGATGGCATGATCTTCGCGATGGTCGCATGTTCACATGATTAAAGA-AT Gull 4 (91) ACACGTATCCAACCT GCCGATGACTCGGGGATAGCCTTT CGAAAGAAGATTAATACCCGATGGCATCATGAGT CCGCATG GTCACATGATTAAAAGA-AT pig1 (91) ACACGTATCCAACCT GCCGATGACTCGGGGATAGCCTT CTGAAAGGAAGATTAATACCGATGGATCATGAGT CCGCATGGTCACAAGATTT AY695690 Pig (91) ACGCGTATCCAACCT TCCCTTGTCCACGGGATAGCCCT CCGAAAGGCGGATTAATACCGTTTGGAAGGCCACAACGAGGCGCATCTAAAGGACGCGCGCATCAAGAATTCCGGGGCGCATAGGCAAGGATTT AY695690 Pig (91) ACGCGTATCCAACCT TCCGCGATAGCCCT TCCGAAGGCAGATTAATACCGTTG GACAAGGACGCACACGAGGCGCATCATGATATCCGATGGTCTCAAGGACGCACATCGAACGACGCAGCTTTAATACCGTATGGCCACACGCACG		(91)	
BoBac 367f (1) BoBac 467r XG (1) HuBac 692r XG (1) turkey1 (95) ACACGTATCCAACCTCCCCGATGACTCCGGGGATAGCCTTTCGAAAGAAGATTAATACCCGATGGCATAGTTCTTCCGCATGGTAGAACTATTAAAGA-AT Gull 2 (91) ACACGTATCCAACCTCCCCGATGACTCCGGGGATAGCCTTTCGAAAGAAGATTAATACCCGATGGCATCATGAGTCCCCCATGTTCACAACGATGATT Gull 4 (91) ACACGTATCCAACCTCCCCGCGTCTACTCTGGGGATAGCCTTTCGAAAGGAAGATTAATACCCGATGGCATCATGAGTCCCCCATGTTCACAACGATTAAAGA-AT Gull 4 (91) ACACGTATCCAACCTCCCCCATGACTCCGGGGATAGCCTTCCGAAAGGAAGATTAATACCCGATGGCACCATGGTCGCGCATCTACATGATTAAAGGTAT pig1 (91) ACGCGTATCCAACCTTCCCCATGTCACCTCTCGGGGATAGCCTTCCGAAAGGCAGATTAATACCCTATGGCGCCATCATGGCGCCATGATATGGGACGAAAGGTTT AY695690 Pig elk1 (91) ACGCGTATCCAACCTTCCCCTTGCCACGGGATAGCCTTCCGAAAGGCAGATTAATACCTGAGGTCACAAGCAGCAGCATCTAAATGTGAGCAAAGGTTT AY695690 Pig (91) ACGCGTATCCAACCTTCCCCTTGCCACGGGATAGCCTTCCGAAAGGCGGATTAATACCTGAGGTCTACGAGCAGCAGCAGCATCTAAATGTCGCAGAGACTATGGAGCAGACTTTGAAAGAATTCGAAGCAGCATCTGAAGCAGCAGCATCCAAGCTGCCGCATGAAGCACGCGTCCGAAAGGCAGACTATAATACCGAAGGTTTCCCCAACGTGCCCATGAACGACGATCCAACGTGCCCATGAAGGAAG			
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elk1 (91) ACCCGTATCCAACCTTCCCGTTACTCAGGGATAGCCTTCCGAAAGGGAGATTAATACCTGATGGTGTTCGAATTCCGCATGACATTTGAACTAAAGATTC Gull5 (91) ACACGTATCCAACCTGCCCTTTACTCGGGGATAGCCTTTCGAAAGAAGATTAATACCCGATGGCATAGTTCCGCATGGTTTCATTATTAAAGG-AT cat1 (91) ACACGTATCCAACCTGCCGACAACACTGGGATAGCCTTTCGAAAGAAA			
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300 B	cat2		
dog 2 (93) ACCCGTATCCAACCTCCCCCCCCCCCTCCCCCCTCCCCCCCTCCCCCAACCTAACACCTAATACCCCAATCATATCCTCTAGAAGACATCTCGAAAGACATTAAAGATTT			
	dog 2	(93)	ACCCGTATCCAACCTCCCCCCCCCTCCGCGATAACCTTCCCGAAACTAACACTAATACCCAATGATATCTCTAGAAGACATCTGAAAGACATTAAAGATTT

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:

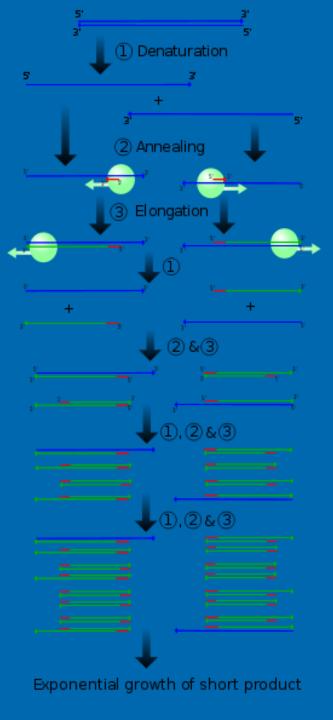
•Measure amount of target sequence

Clone amplified DNA

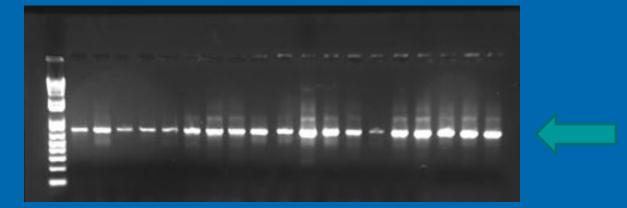
Sequence it

•<u>http://www.dnalc.org/ddnalc/resources/sh</u> ockwave/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 orginating near Dunton Park
- strongly present municipal sewage.

Pig-specific:

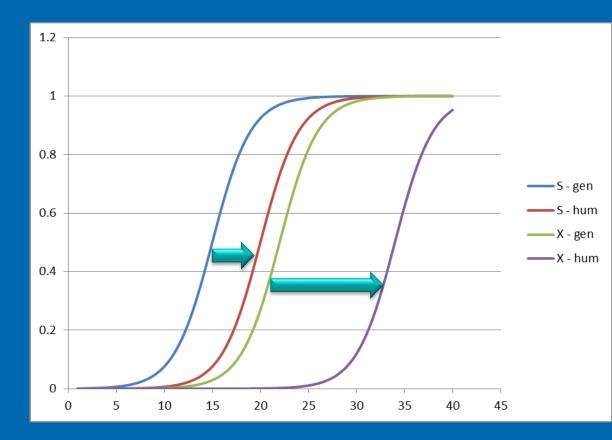
- present in pig feces;
- in drainage surrounding a local hog farm;
- in days following pig manure spill immediately downstream
- Completely absent in routine samples

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 2⁸ = 258 times more original target DNA than sample B

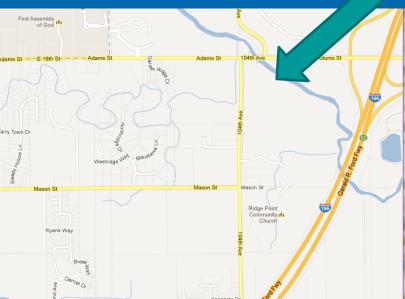


Cow-specific probes:

- Conventional PCR
- qPCR (bacteroides and nonbacteroides)
- 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

Felch St

Vriesland

Cemeter

Felch St

196

Gerald R. Ford Fwy

- None detected!

Felch St

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

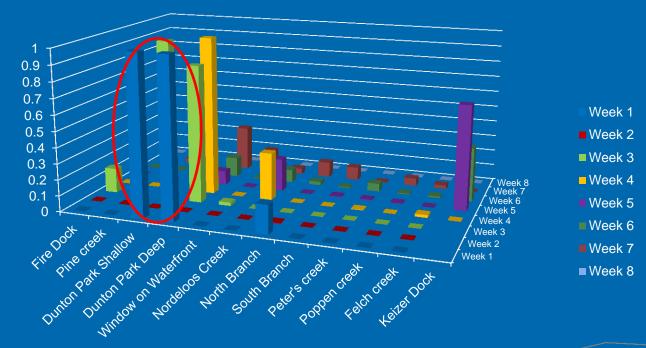
Remember: Less is more!!

(and ND means none detected) Even better, we can turn that into a ratio of human compared to total; let's call that:

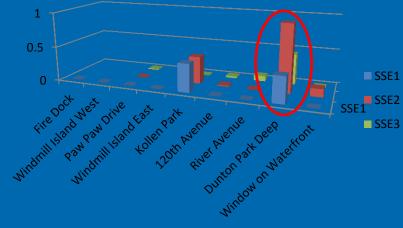
By comparing the ratio in our environmental sample to that in sewage (presumably entirely human in origin), we can calculate the fraction of bacteroides present in a given sample attributable to human sewage:

	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 \rightarrow



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 \rightarrow molecular source tracking

Pinpoint: Dunton 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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