Macatawa Watershed Water Quality Research Project

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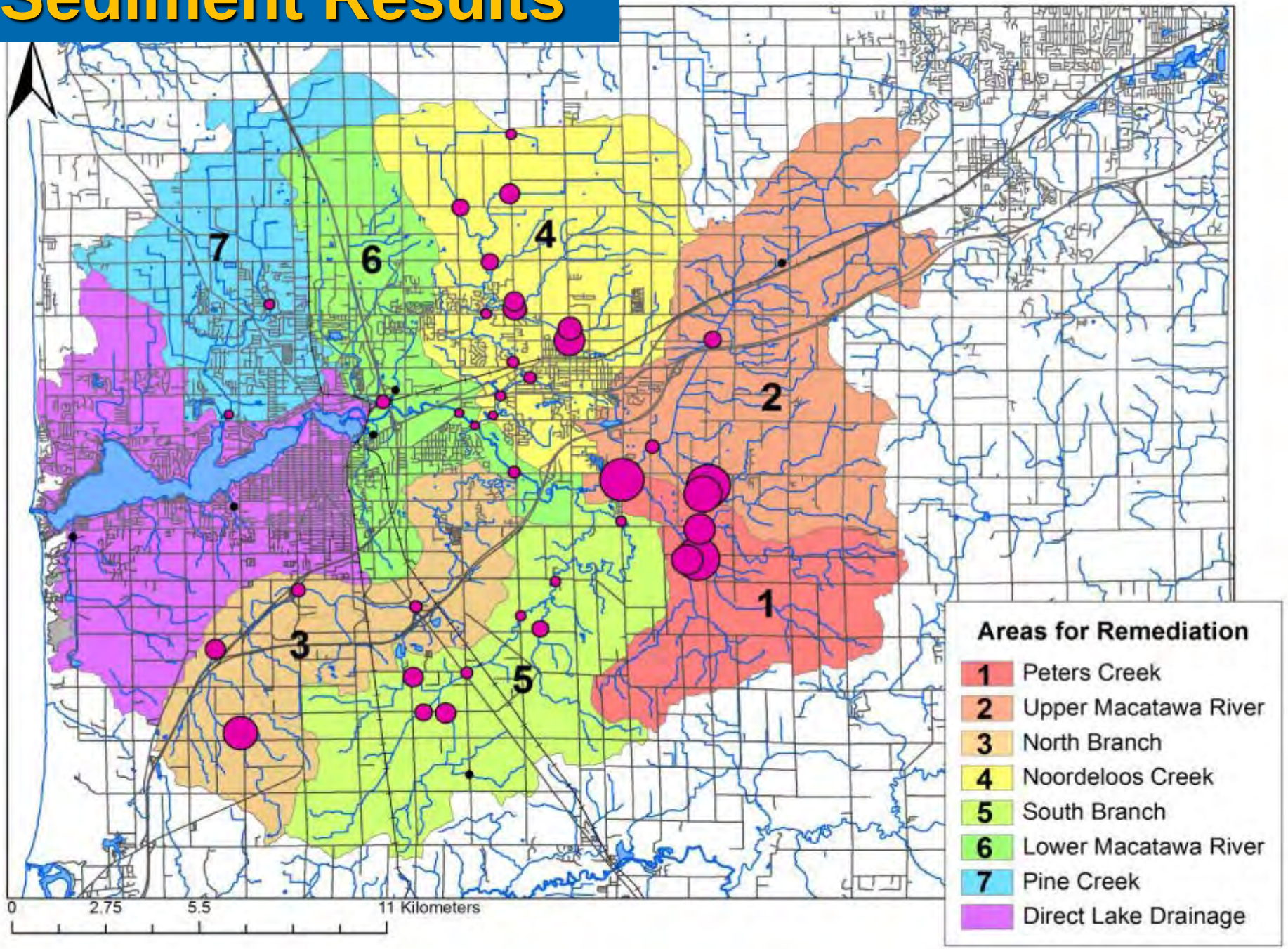
2011-2012

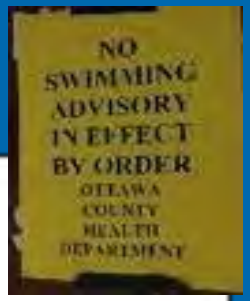
Lake Macatawa Watershed

Sediment Results



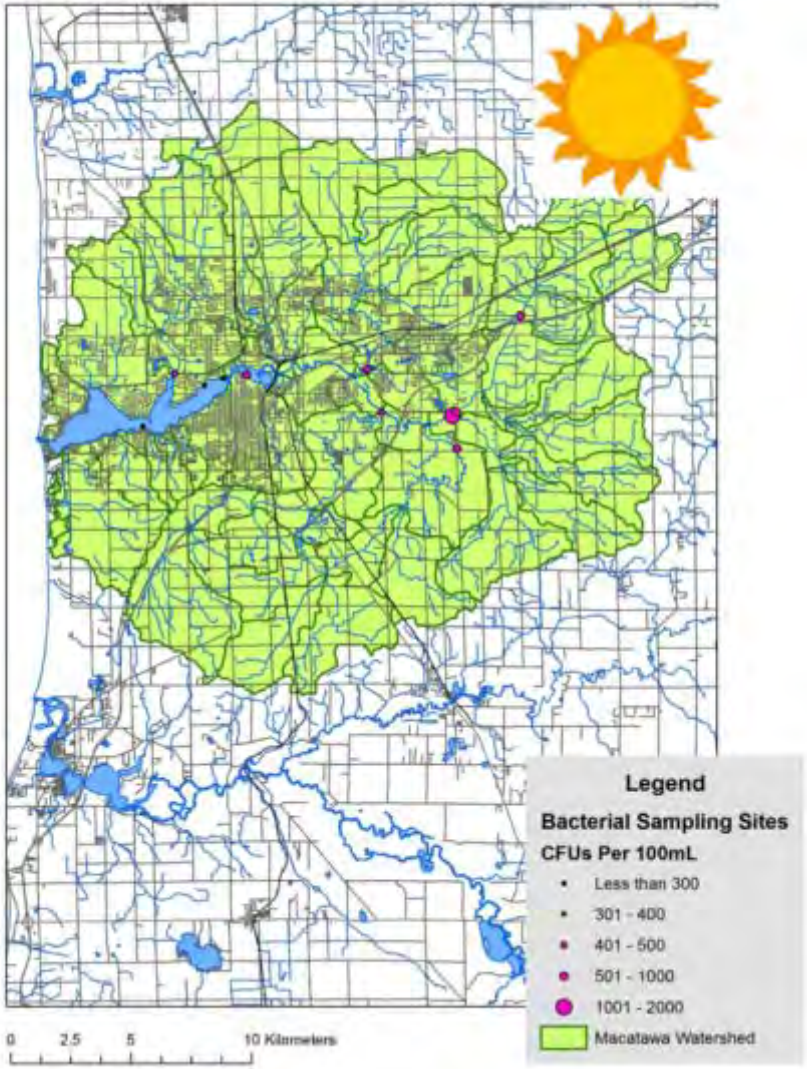
Coliform Bacteria

E. coli

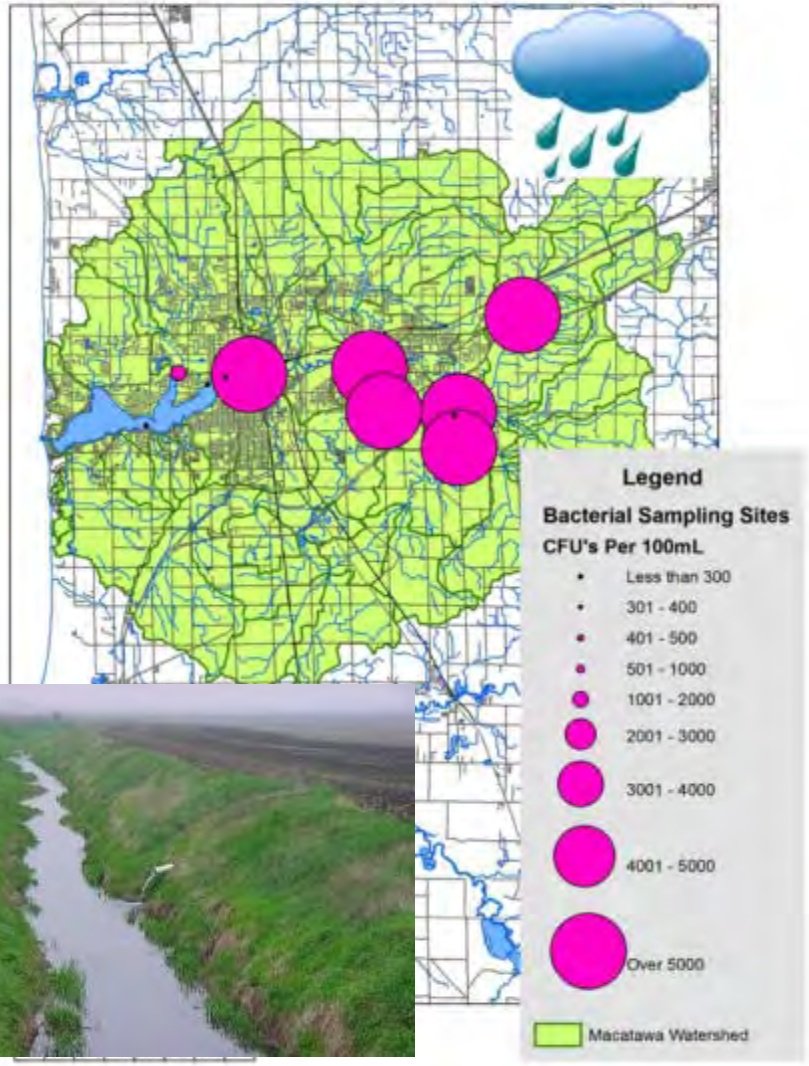


Initial Sampling Results

E. Coli Levels -- 6 July, 2011

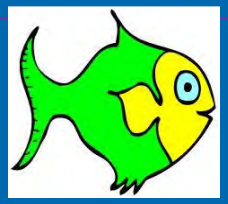


E. Coli Levels -- 12 July, 2011

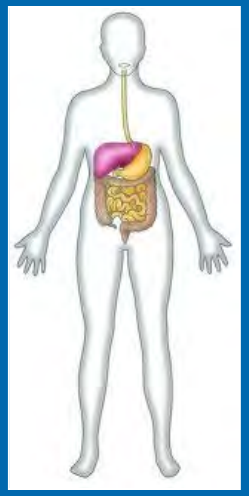


It’s a microbial zoo out there:

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



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



***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:

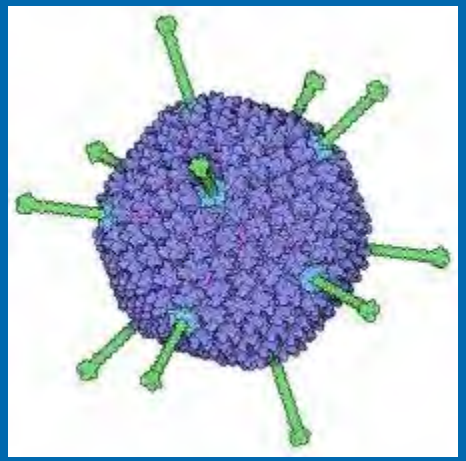
- ***Entamoeba***
- ***Cryptosporidium***
- ***Giardia***

Pathogenic bacteria like:

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

Viruses like:

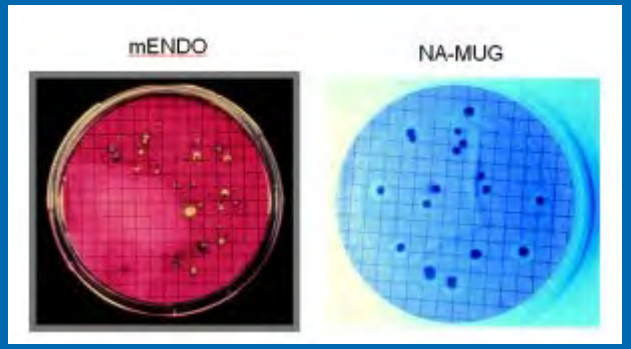
- **Adeno, parvo, corona**
- **Hepatitis A**
- **Polio**



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 can we predict risk more precisely?

Reminder of previous years' monitoring:

Widespread non-point source *E. coli* and *Enterococcus* associated with rain events – especially spring and early summer.

Limited "hits" to presumptive source hosts:

- Human in a storm drain along Lakewood Blvd.
- Human following lift station failure.
- Pig following manure spill.
- Cow (low levels) on occasion in vicinity of dairy farm.

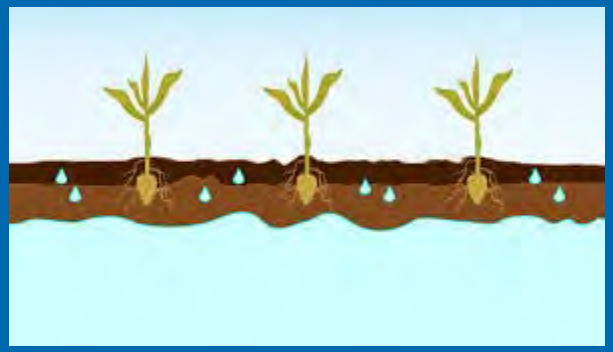
We found similar results in a study in South Haven area.

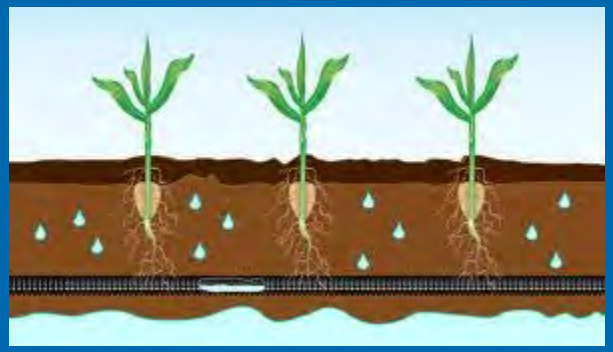
Keep pushing further upstream

Close-up analysis of possible agricultural run-off:

Local farm – about 100 acres corn
Manured – one field recently, one about six years ago
Drain tile installed over past ten years
- **6” corrugated plastic**
- **4-6’ below ground**
- **Empties into roadside county drain**

Possible mechanism to increase transit time of bacteria applied during manuring?





Monitoring points:

In soil around tile and sediment in tile (section of tile dug up for maintainance).

Core samples (two low/wet spots, two high spots) from field. Also compared against several non-agricultural core samples

Inside tiles – insert collection tray at outlets

Collection basin at tile outflow

Spring flow – high. Summer flow – low.

Agricultural site results (2012):

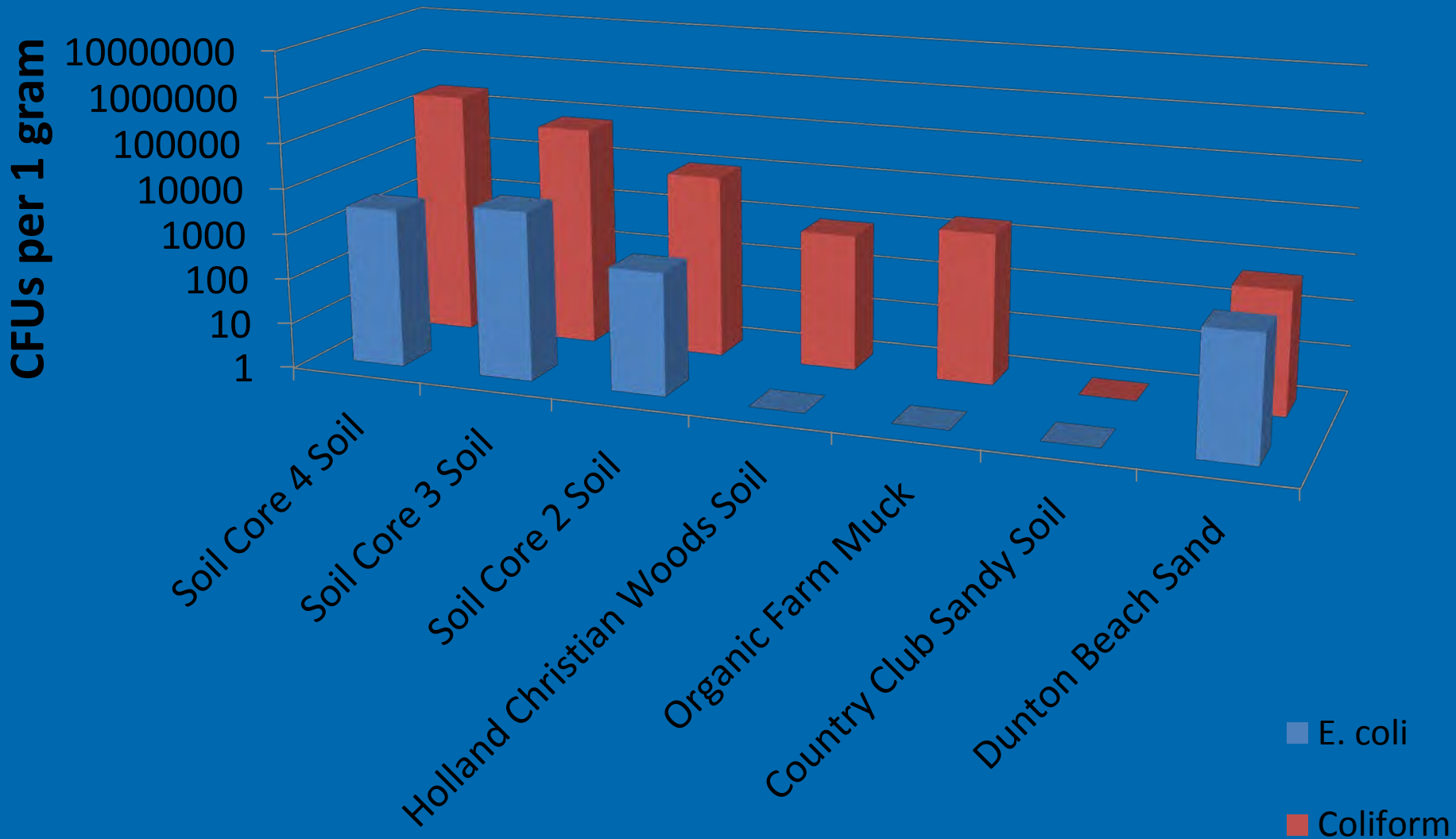
Sampling location \ Date:	3/2 (snow)	3/12 (thaw)	4/16 (rain)
Tile outflow (recent manure)	140/100	343/103	1300/4800
Tile Outflow (no recent manure)	53/40	250/247	720/10800
Surface drain	36/197	353/693	1800/9000

Counts are E coli/enterococci (mTEC and mEI), cfu/100 mL

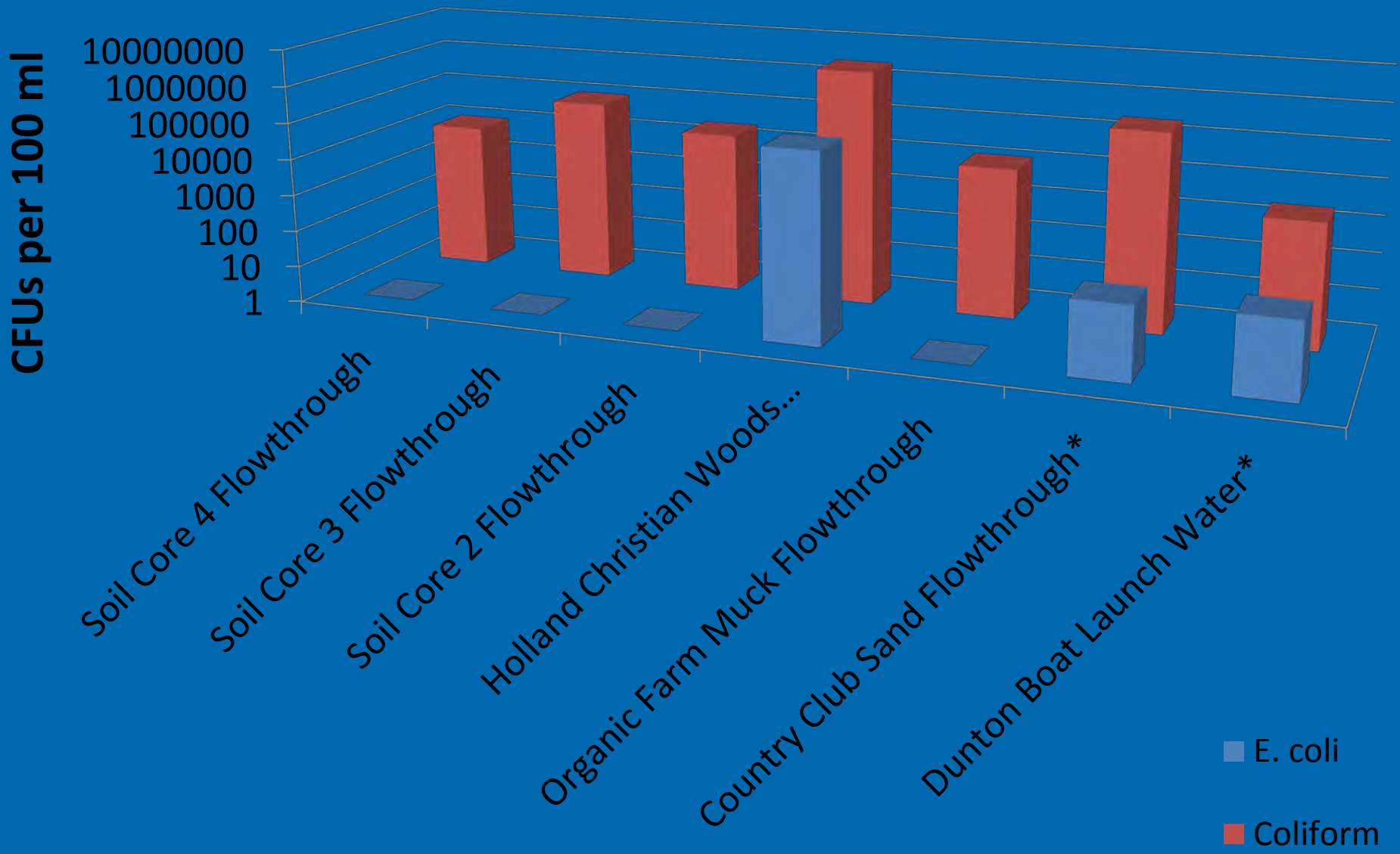
Bringing the field to the lab:
percolate sterile water through core sample and measure outflow

Complicated due to slow flow…

Idexx Results (Soil)



Idexx results (Soil Core Flow Through)



Bacteria levels in farm samples seem to be indigenous growth.

- Not increased in recently manured field
- Particularly high in early summer
- Found throughout soil core
- Again, tile may be conduit
- But what kind of bacteria are they? Are they a health hazard?

Monitoring through DNA Sequencing

Who are the *E. coli* that are being monitored in the watershed?

Are they environmentally adapted, endemic strains?

Are they associated with biofilms in tile drains?

What is their potential for virulence?

Monitoring through DNA Sequencing

Strategy:

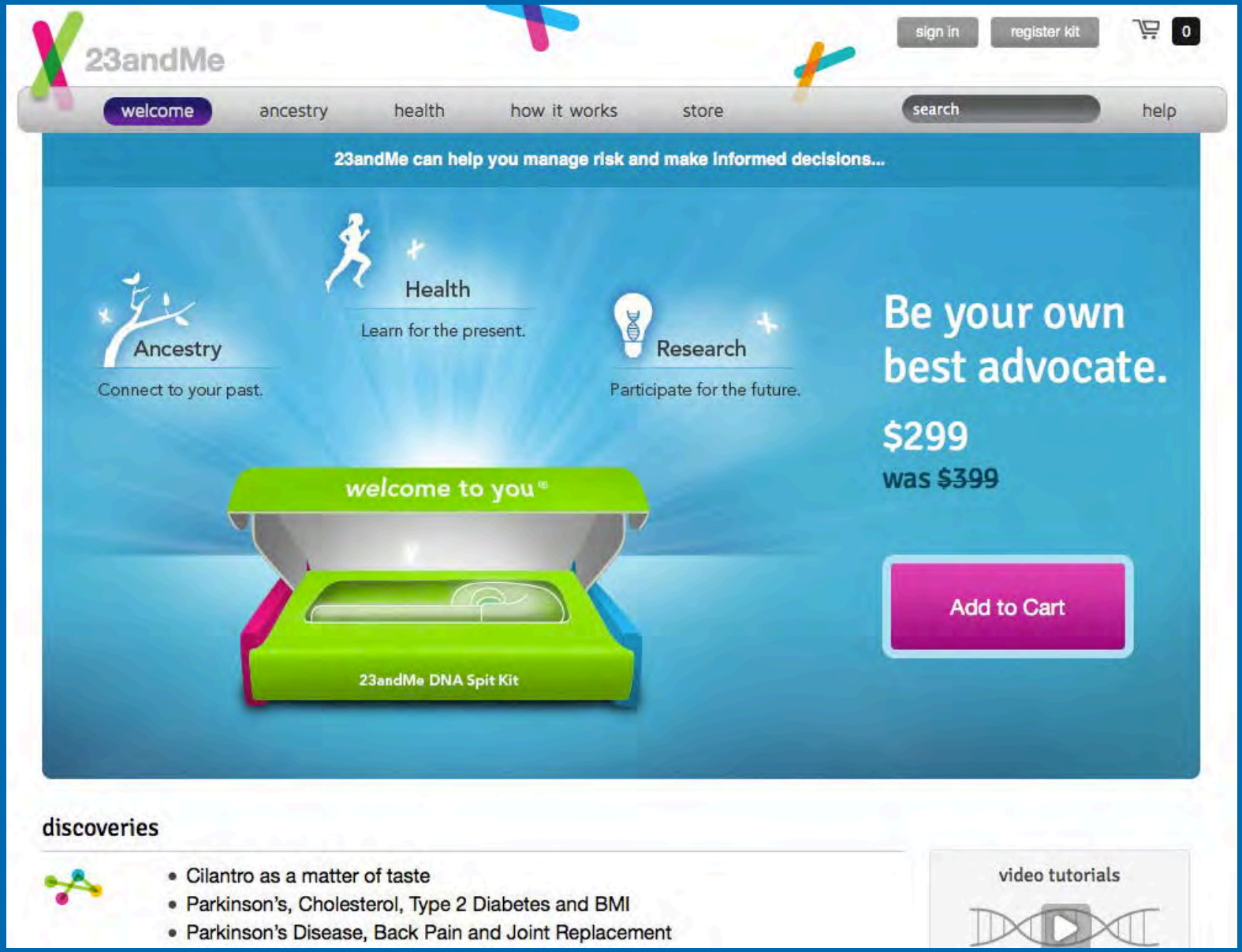
- Study site on local farm – drain box with multiple tile drains from surrounding fields
- Place small coupons of PVC or Black Corrugated Plastic in drains – allow biofilms to form over summer months

Monitoring through DNA Sequencing

Strategy:

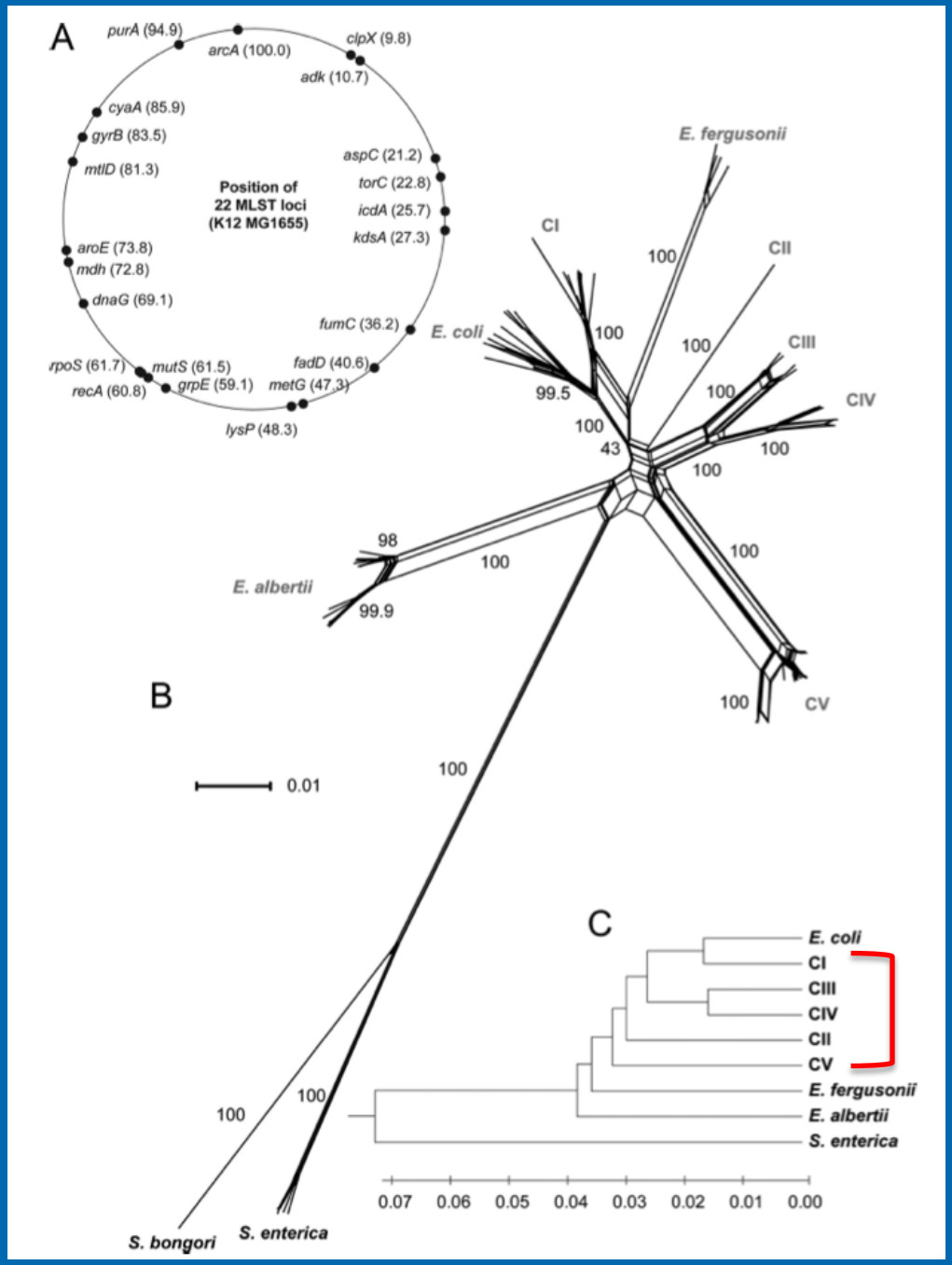
- Study site on local farm – drain box with multiple tile drains from surrounding fields
- Place small coupons of PVC or Black Corrugated Plastic in drains – allow biofilms to form over summer months
- Isolate *E. coli* and *Enterococcus* from water in drain box, efflux from drain tiles, biofilms on coupons, downstream samples
- Experiments to assess environmental adaptation
- Sequence full genome of each isolate

What can a genome sequence tell you?



Use the same principle for watershed isolates

- Different groups of *E. coli* – many clinical strains, some environmental strains
- What group are the watershed isolates in?
- What traits (genes) do they have known to be associated with causing disease (virulence factors)?
- What genes do they have that are different from pathogenic strains of *E. coli*?

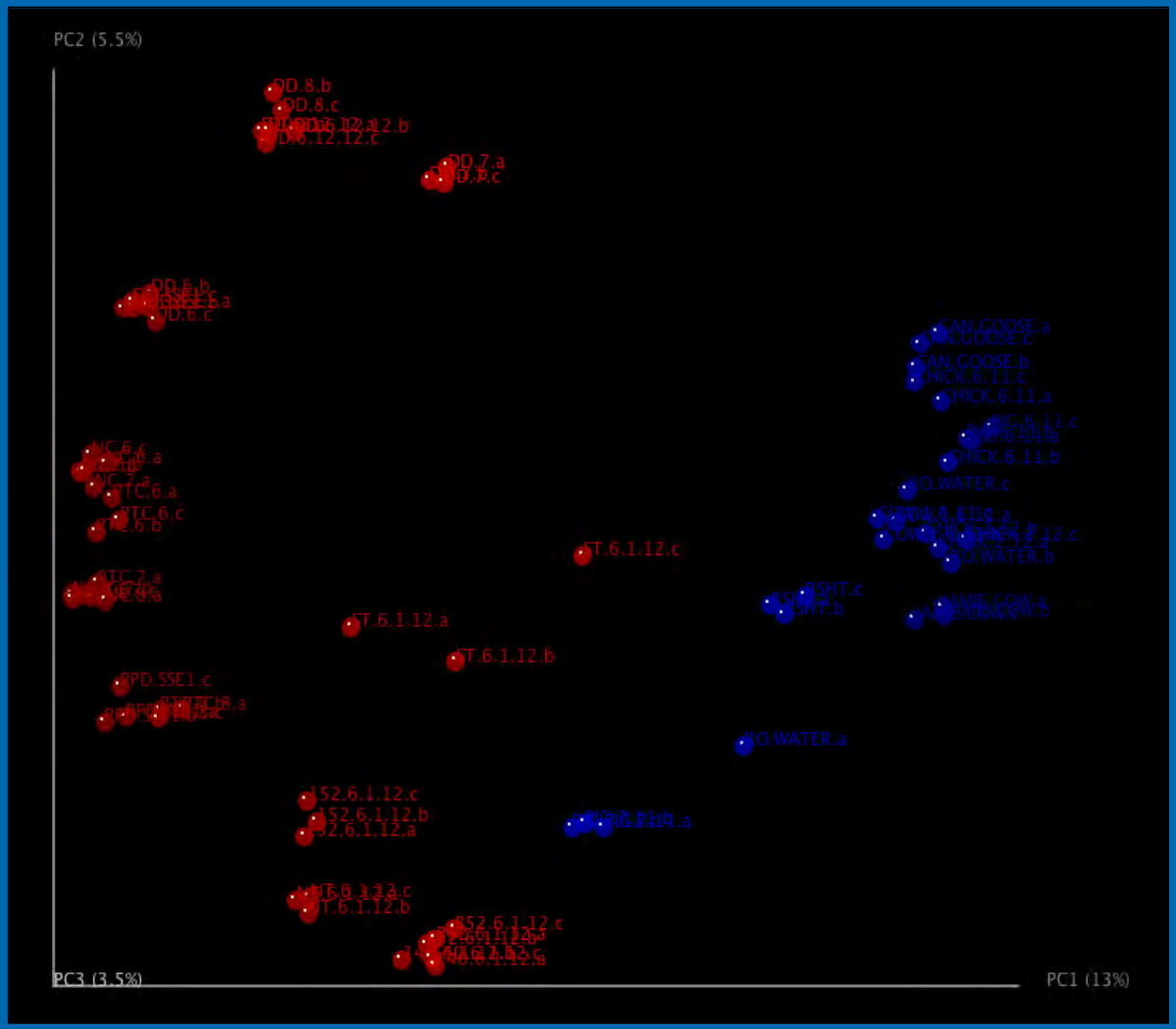

A
purA (94.9)
arcA (100.0)
clpX (9.8)
adk (10.7)
cyaA (85.9)
gyrB (83.5)
mtlD (81.3)
aroE (73.8)
mdh (72.8)
dnaG (69.1)
rpoS (61.7)
recA (60.8)
mutS (61.5)
grpE (59.1)
fumC (36.2)
fadD (40.6)
metG (47.3)
lysP (48.3)
aspC (21.2)
torC (22.8)
icdA (25.7)
kdsA (27.3)
Position of
22 MLST loci
(K12 MG1655)
B
E. fergusonii
E. coli
E. albertii
CI
CII
CIII
CIV
CV
100
99.5
43
98
99.9
0.01
S. bongori
S. enterica
C
E. coli
CI
CIII
CIV
CII
CV
E. fergusonii
E. albertii
S. enterica
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00

Monitoring the Entire Microbial Community – DNA Sequencing

- If *E. coli* are endemic and non-pathogenic, who should we be looking for?

- Use next generation DNA sequencing technology
- Identify ALL microbes that are in a sample
- Compare samples and look for groups of microbes that distinguish
- Potentially use for monitoring “health state” of the watershed

Monitoring the Entire Microbial Community – DNA Sequencing



Acknowledgements

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HOPE COLLEGE

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