UNIVERSITY OF MINNESOTA

A High-Throughput DNA Sequencing-Based Approach for Determining Sources of Fecal Bacteria in the Lake Superior Watershed

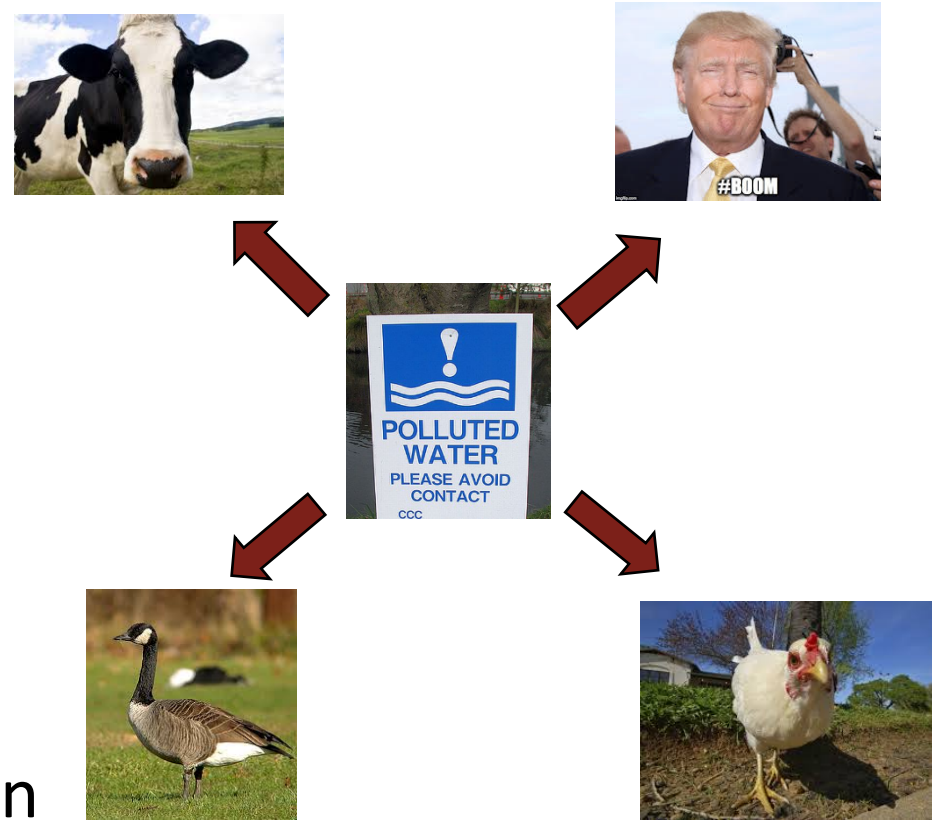
Clairessa Brown, Michael Sadowsky

everest
www.liren.info

Microbial Source Tracking (MST)

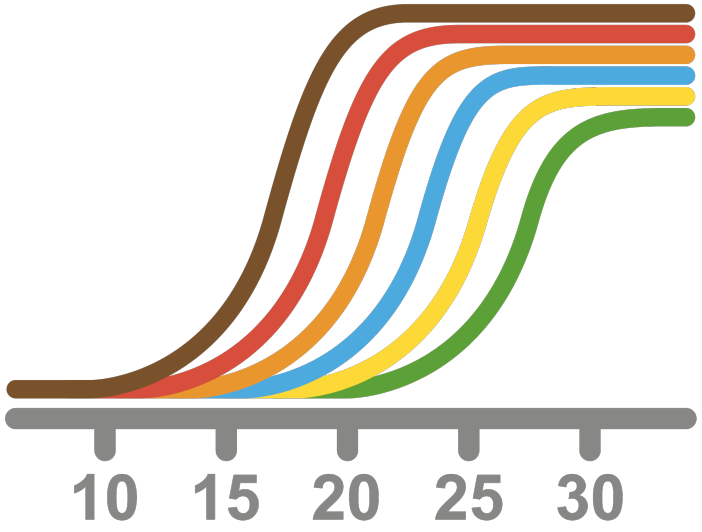
Assumptions:

1. Different animals have different gut microbiomes
2. Their fecal material harbors different microbial profiles
3. Identify sources based on differences in microbial profiles



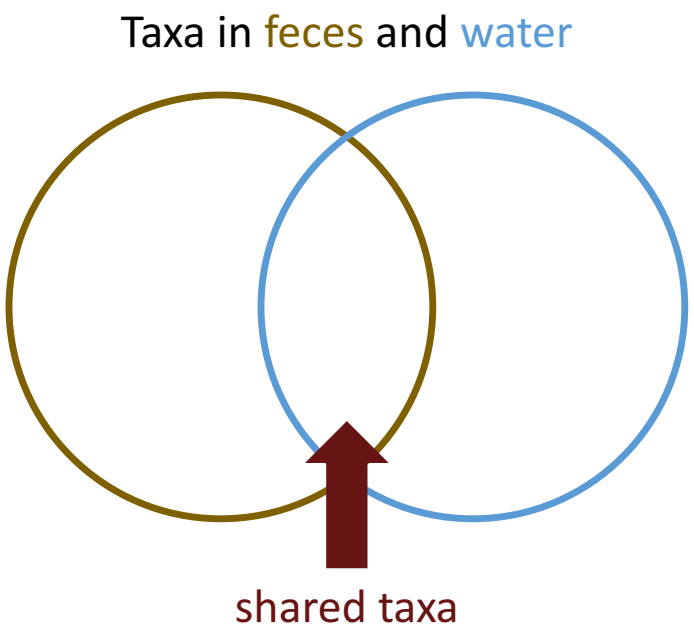
Quantitative-PCR (qPCR)

- Most widely used method
- Drawbacks:
  1. Issues with marker sensitivity
  2. Issues with marker specificity



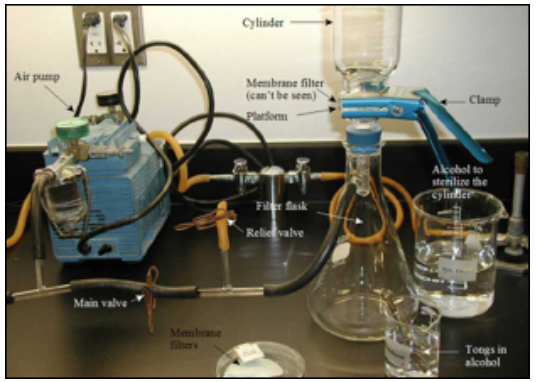
Community-Based MST

- Using 16S rRNA gene to get bacterial community profiles
- Then compare profiles from water and feces looking for shared taxa

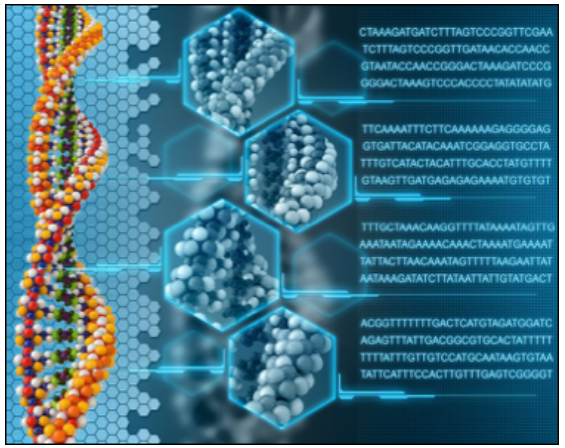


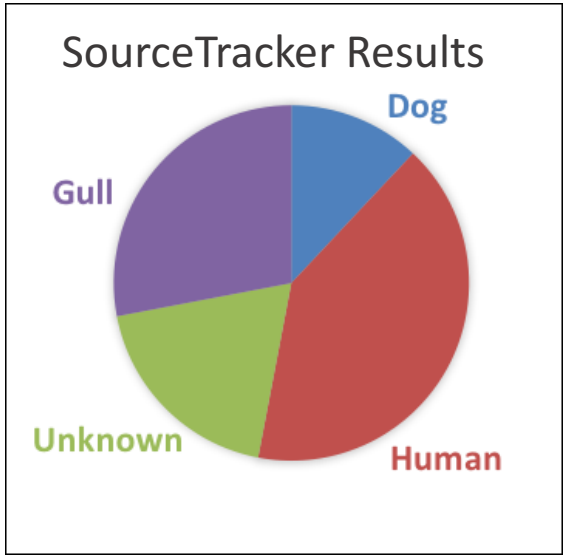
- Advantages:
  1. Get the complete bacterial profile of different sources
  2. Can screen for all potential sources simultaneously

Methods

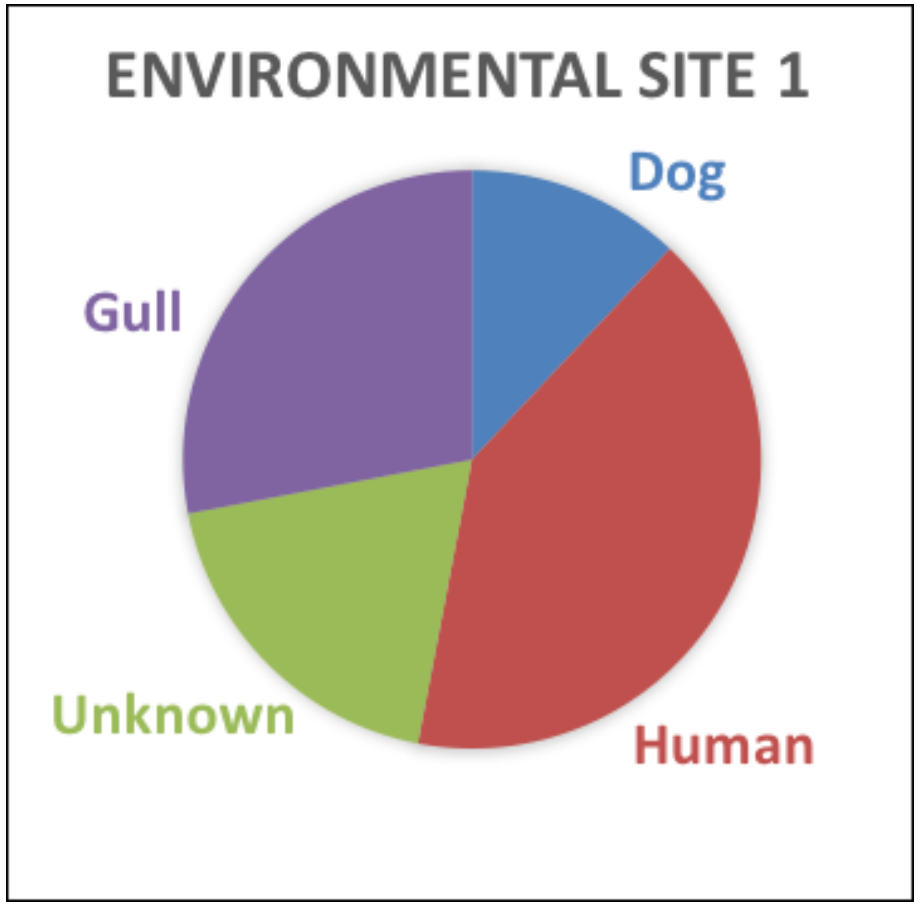

Cylinder
Air pump
Membrane filter (can't be seen)
Platform
Clamp
Alcohol to sterilize the cylinder
Filter flask
Relief valve
Main valve
Membrane filters
Tongs in alcohol

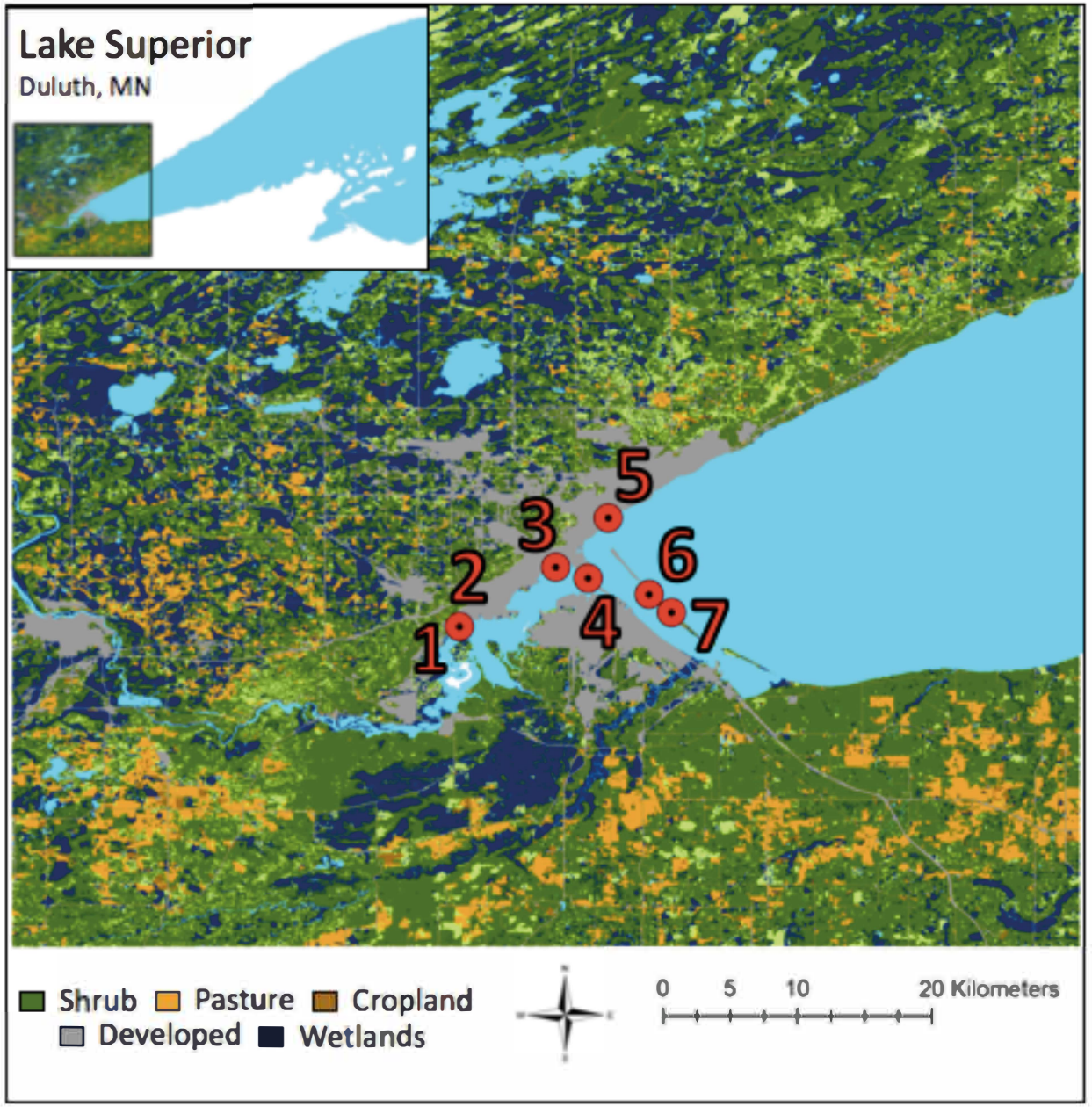
PowerSoil
DNA Kit




SourceTracker Results
Dog
Gull
Unknown
Human

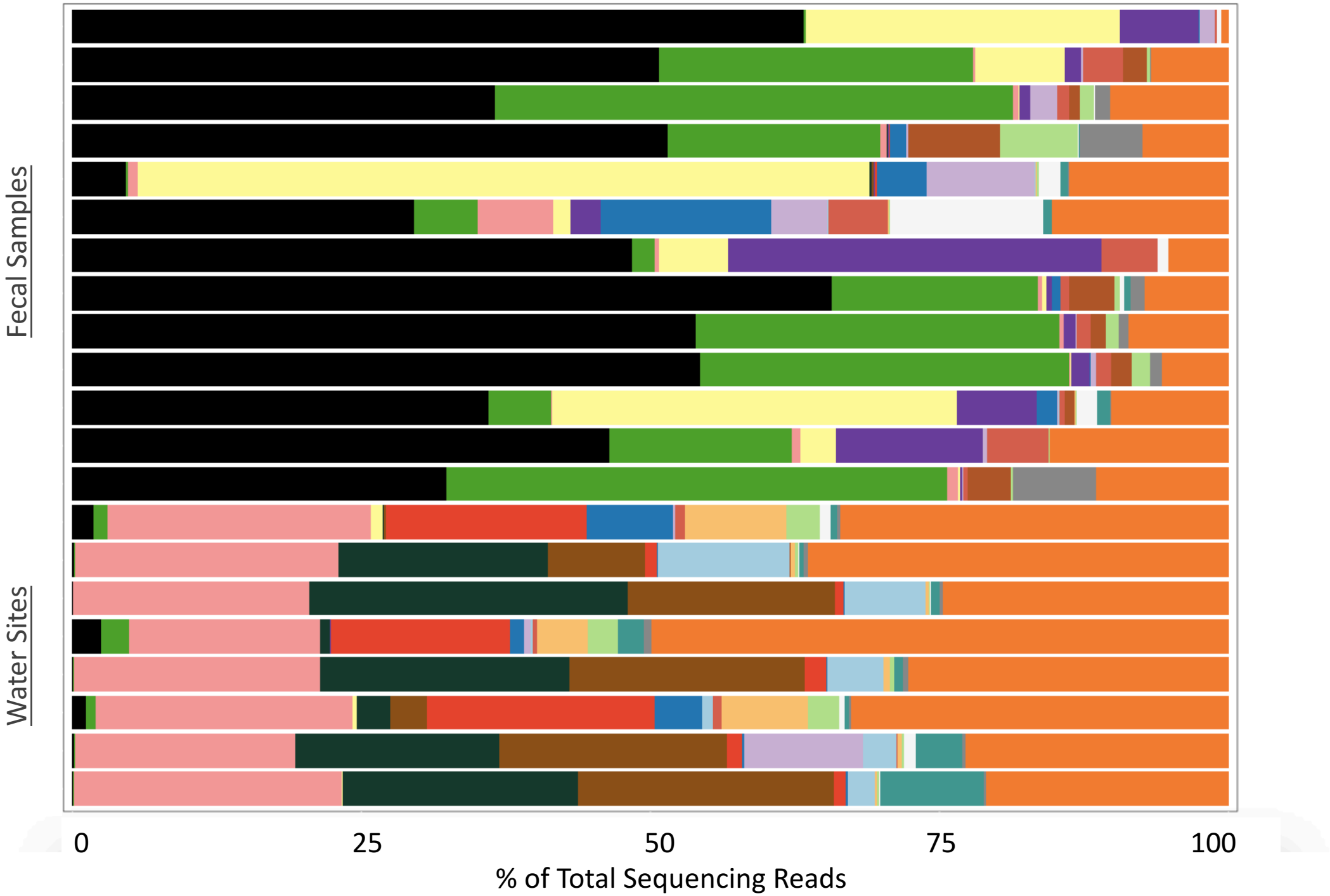
How does SourceTracker work?




Lake Superior
Duluth, MN
1
2
3
4
5
6
7
Shrub
Pasture
Cropland
Developed
Wetlands
0
5
10
20 Kilometers

Results

- The bacterial community structures between water and fecal samples are distinct
- The bacterial community structures between different animals are distinct

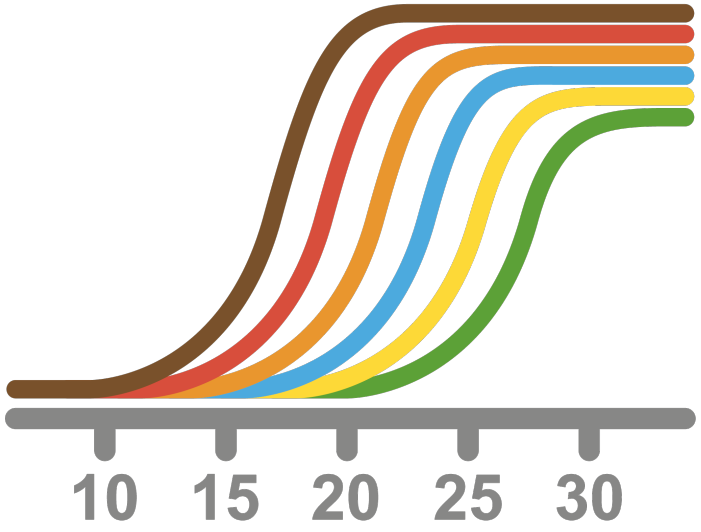

Fecal Samples
Water Sites
0
25
50
75
100
% of Total Sequencing Reads

- SourceTracker predicted that effluent from the waste water treatment plant was major source of fecal contamination
- Geese and gulls were the next most abundant

- SourceTracker used several different bacterial taxa to form unique markers for effluent, geese and gulls

Quantitative-PCR

- Used Human (HF183/BacR287), Gull (Gull-4), Geese (GFD) markers
- Do qPCR and SourceTracker agree on fecal pollution sources?



Conclusions

Community structures are…

- …different between fecal sources and water
- …different between animals species

SourceTracker predicted that…

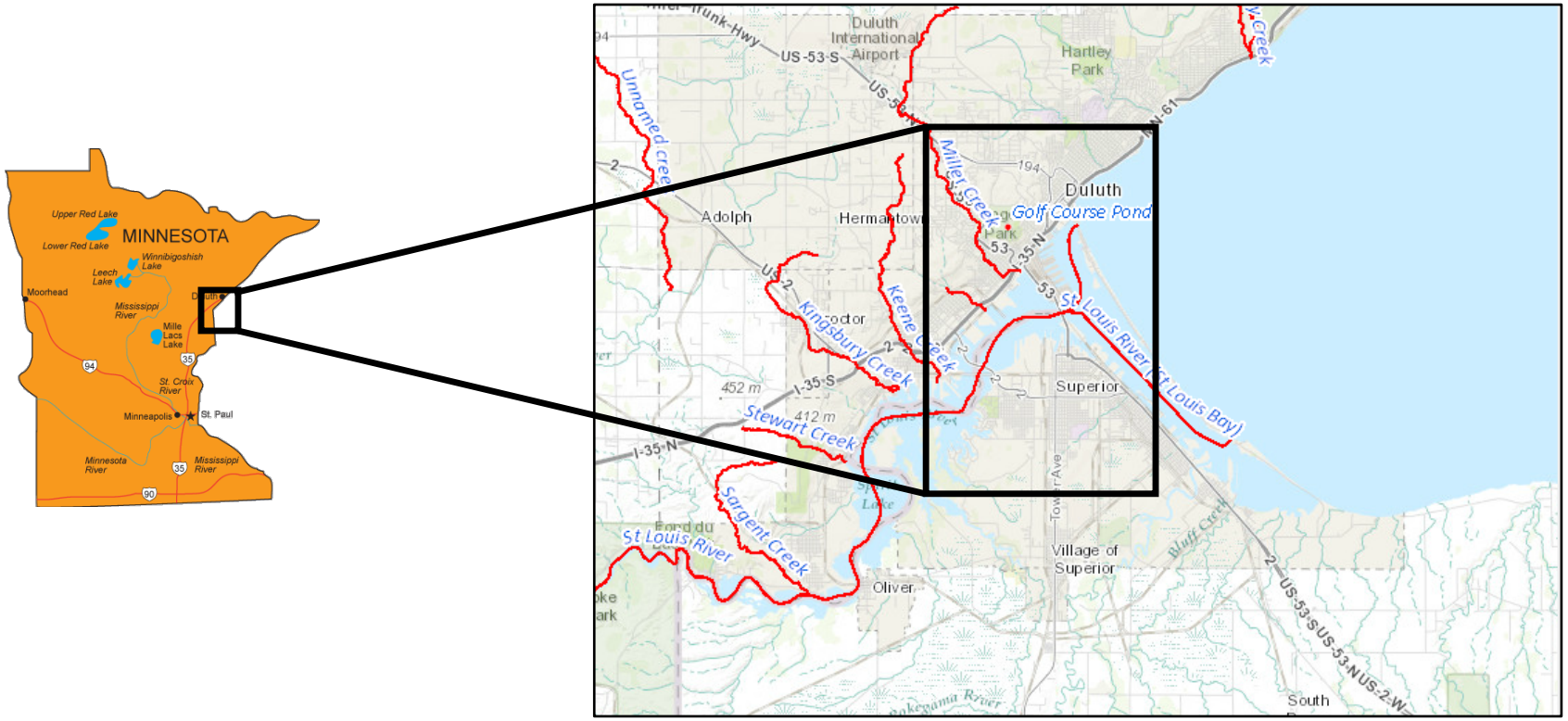
- …the major sources of contamination were from effluent, geese, and gulls

Quantitative-PCR

- SourceTracker and qPCR sometimes agree

Thankyou

- Dr. Michael Sadowsky
- Dr. Chan Lan Chun
  - University of Minnesota Duluth
- Sadowsky Lab
  - Dr. Chris Staley, Dr. Ping Wang
- Funding
  - NIH Biotechnology Training Grant, Sea Grant Minnesota
- Undergraduate Research Assistants
  - Eric Eisenberg
  - Sheue Torng Lee



U.S. E.P.A. (2012) National Water Quality Inventory Report (Section 303(d) of the Clean Water Act;