

Microbial Source Tracking: The Science

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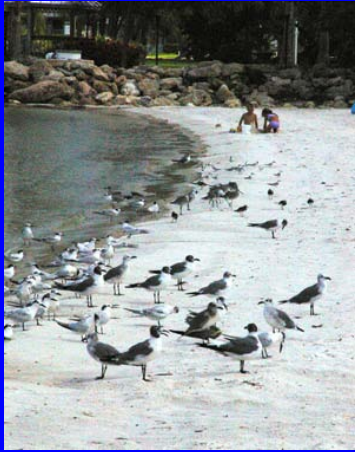
FDEP Tallahassee Meeting 06/10/08



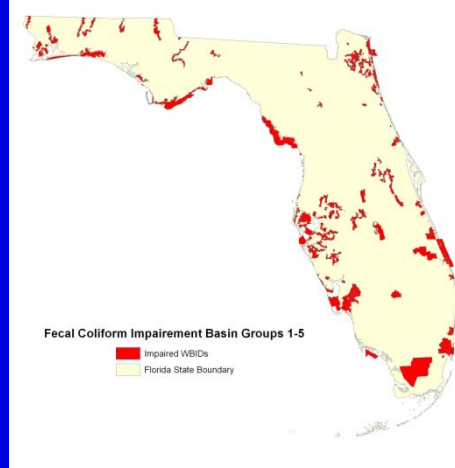
Part I: Some Background



The Central Question of Microbial Source Tracking (MST)



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Indicator Organisms (IOs) Are Generally Nonpathogenic!

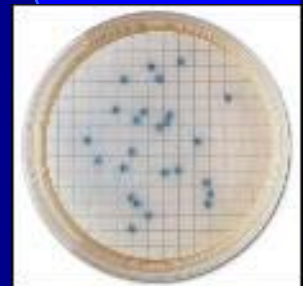
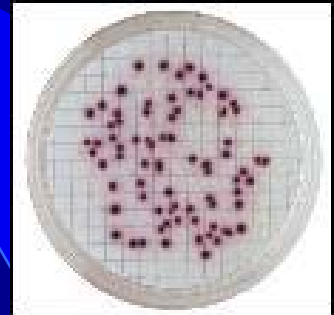
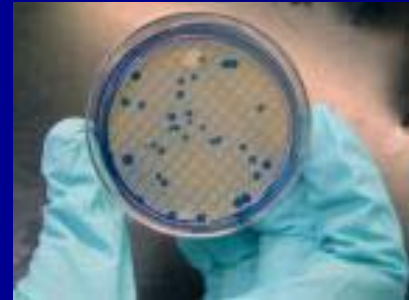


- Meant to act as a warning signal of fecal pollution and increased risk of associated pathogens in water



Current Recognized IOs for Recreational & Shellfishing Water Quality

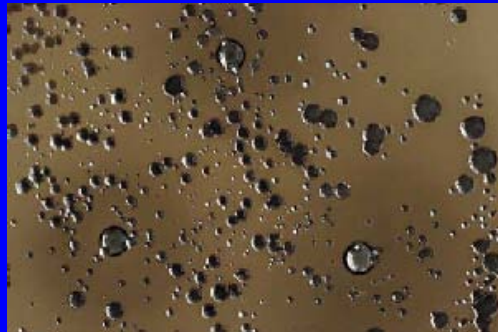
- Fecal coliforms – thermolerant coliforms of fecal origin (Florida & other states)
- *Escherichia coli* – correlated w/risk of gastroenteritis in freshwater (U.S. EPA)
- Enterococci –relatively salt-tolerant; correlated w/risk of gastroenteritis in freshwater & saltwater (U.S. EPA)



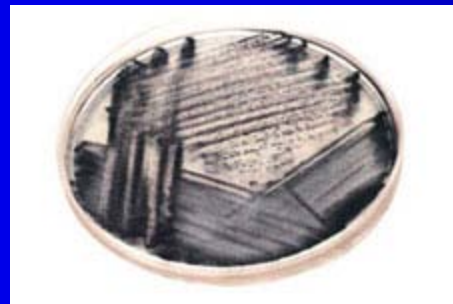


Water Quality Standards Must Protect Against Many Types of Pathogens

Bacterial pathogens

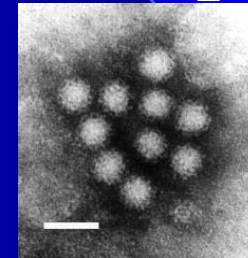


Salmonella



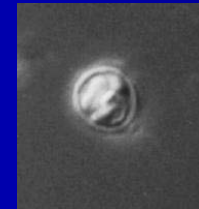
E. coli O157:H7

Viral pathogens



Norovirus

Protozoan pathogens



Cryptosporidium



Giardia

Basis for Continued Use of Indicator Bacteria to Protect Human Health

- Correlation of *E. coli* concentration with risk of gastroenteritis

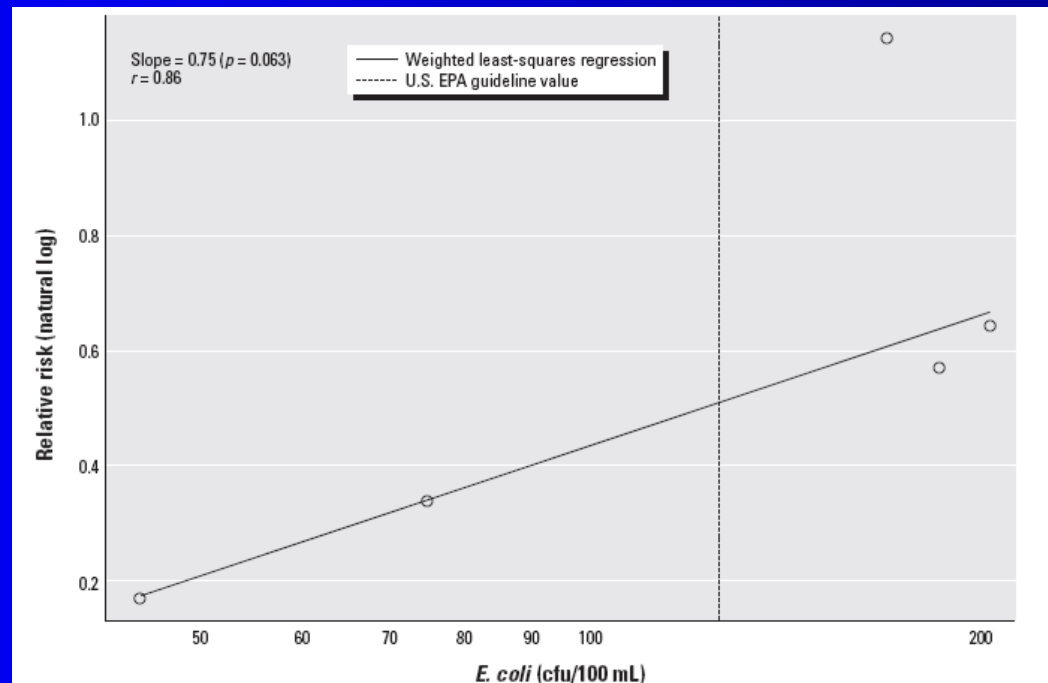


Figure 2. Scatterplot and weighted regression line (weighted by the inverse of the standard error of the natural log relative risk) of the natural log relative risk of GI illness from freshwater studies as a function of *E. coli* density.

Risk

- Epidemiology studies show increased risk of gastroenteritis from recreational water use of ~ 8 individuals/1,000 at these levels.
- Enterococci 61 CFU/100 ml freshwater & 104 CFU/100 ml marine water
- *E. coli* 235 CFU/100 ml
- ~equivalent to 400 CFU/100 ml fecal coliform



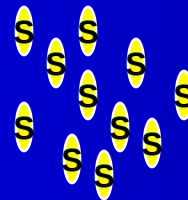
Why Do We Need to Know the Source?

- Sources vary from highest risk (human sewage)...
- ...to definite risk (cattle, poultry)
- ...to unknown risk (wildlife, pets, sediments, soils, stormwater)
- Can't remediate or implement w/o knowledge of dominant source(s)

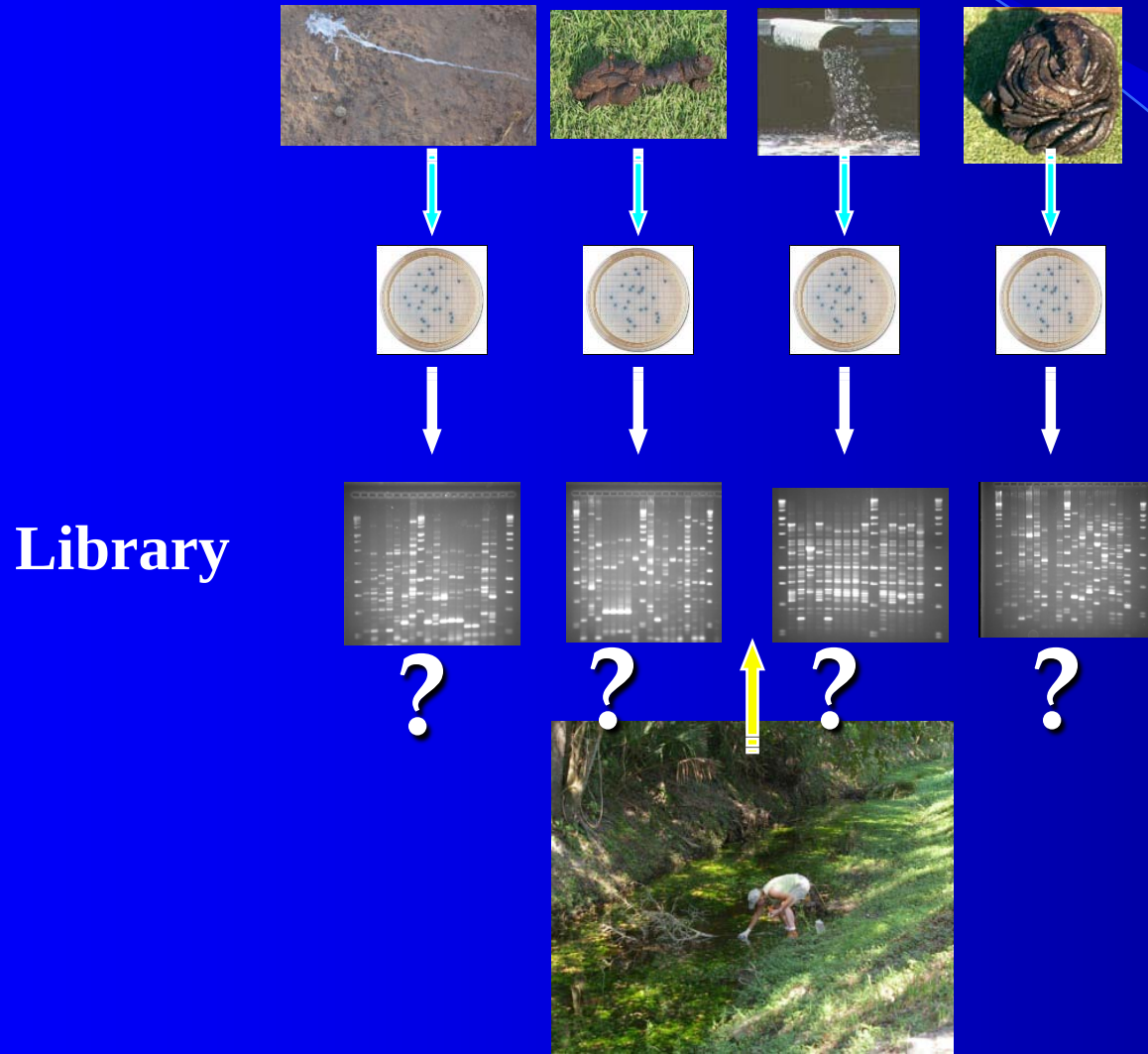


The Central Hypothesis of MST

- Certain microbial species or types are associated with the gastrointestinal tract of specific animals hosts.
- This association can be used to “track” the fecal microorganism back to its host.



Library-Dependent MST Approach



Library-Dependent Methods

Determine percentage of isolates assigned to each source category (proportional contribution of sources?)

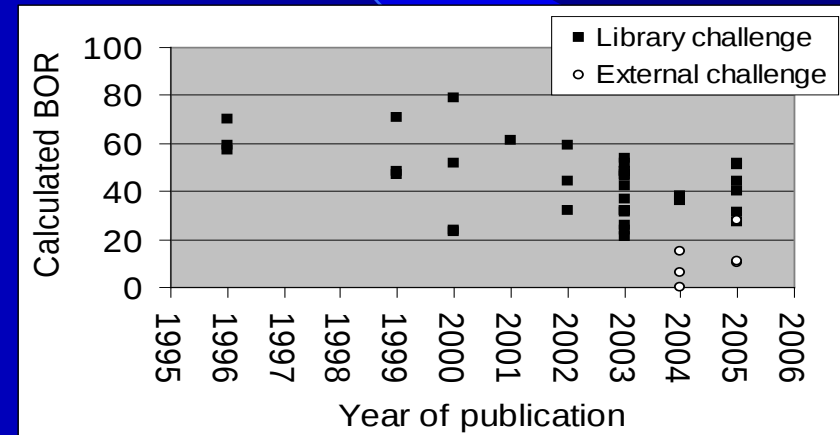
- **Representative sampling!**
 - Host species impacting watershed
 - Feces & sewage
 - Water
 - “Decclone” library at sample level

- **Geographic range**
- **Temporal variability**

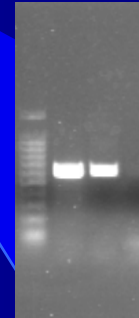
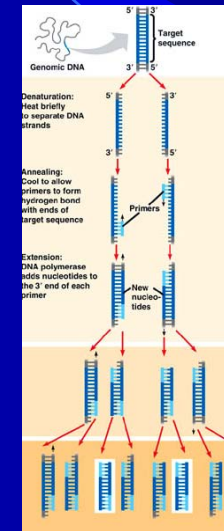
- **Sensitivity**
- **Specificity**



Use samples that are **NOT** represented in the library

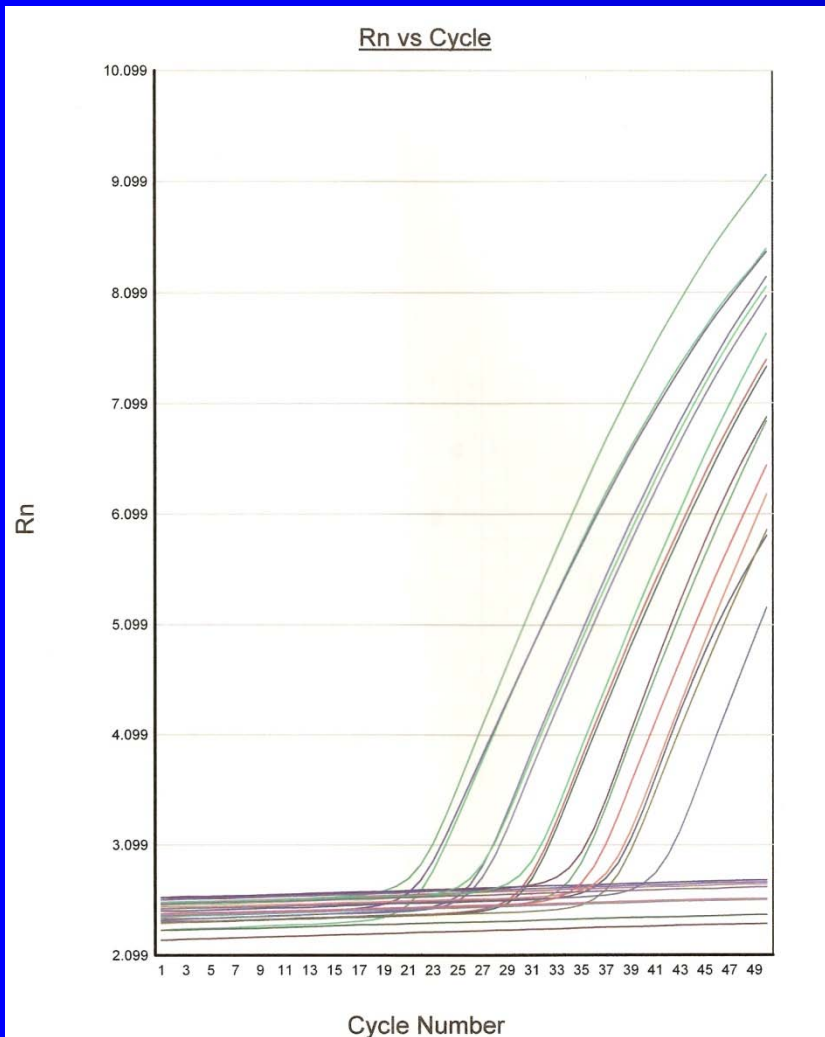


The Library-Independent MST Approach – Based on DNA “Markers”

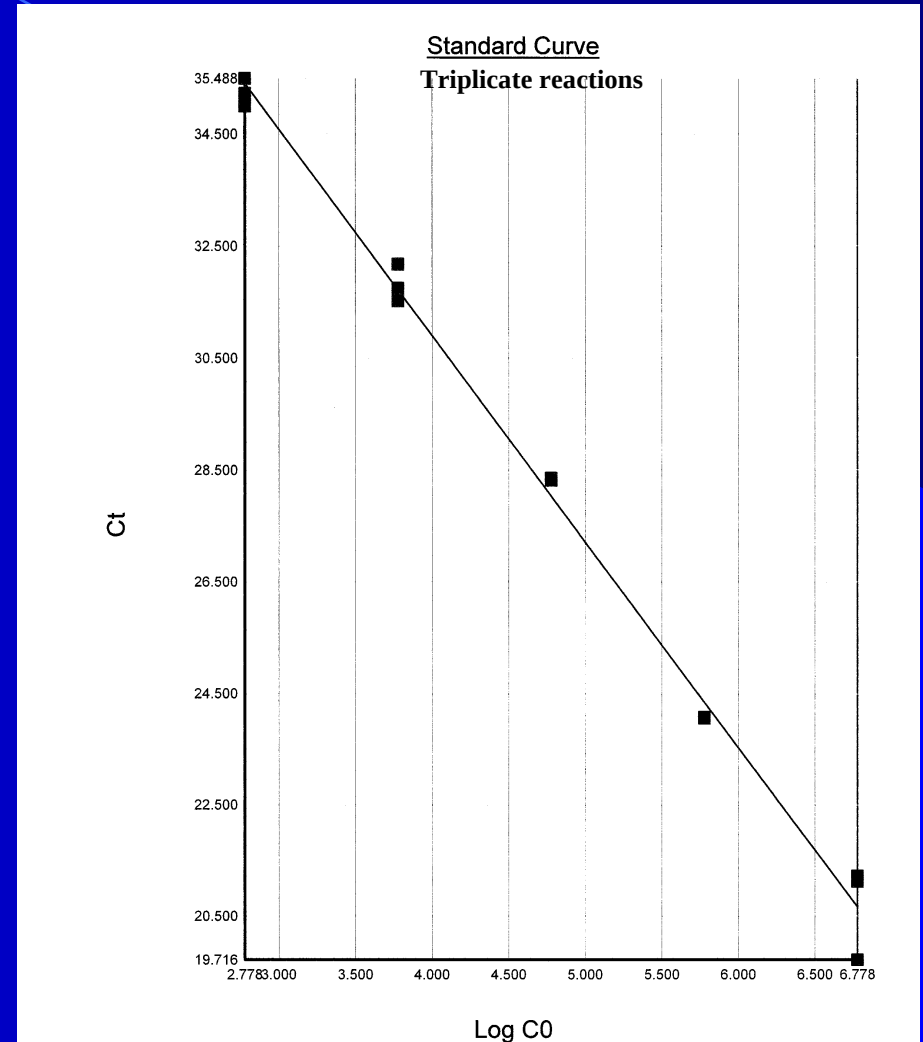


- Polymerase chain reaction targets a specific gene & copies it
- Forensic-type evidence for contamination source

Quantitative (Taqman) PCR for Human Polyomaviruses

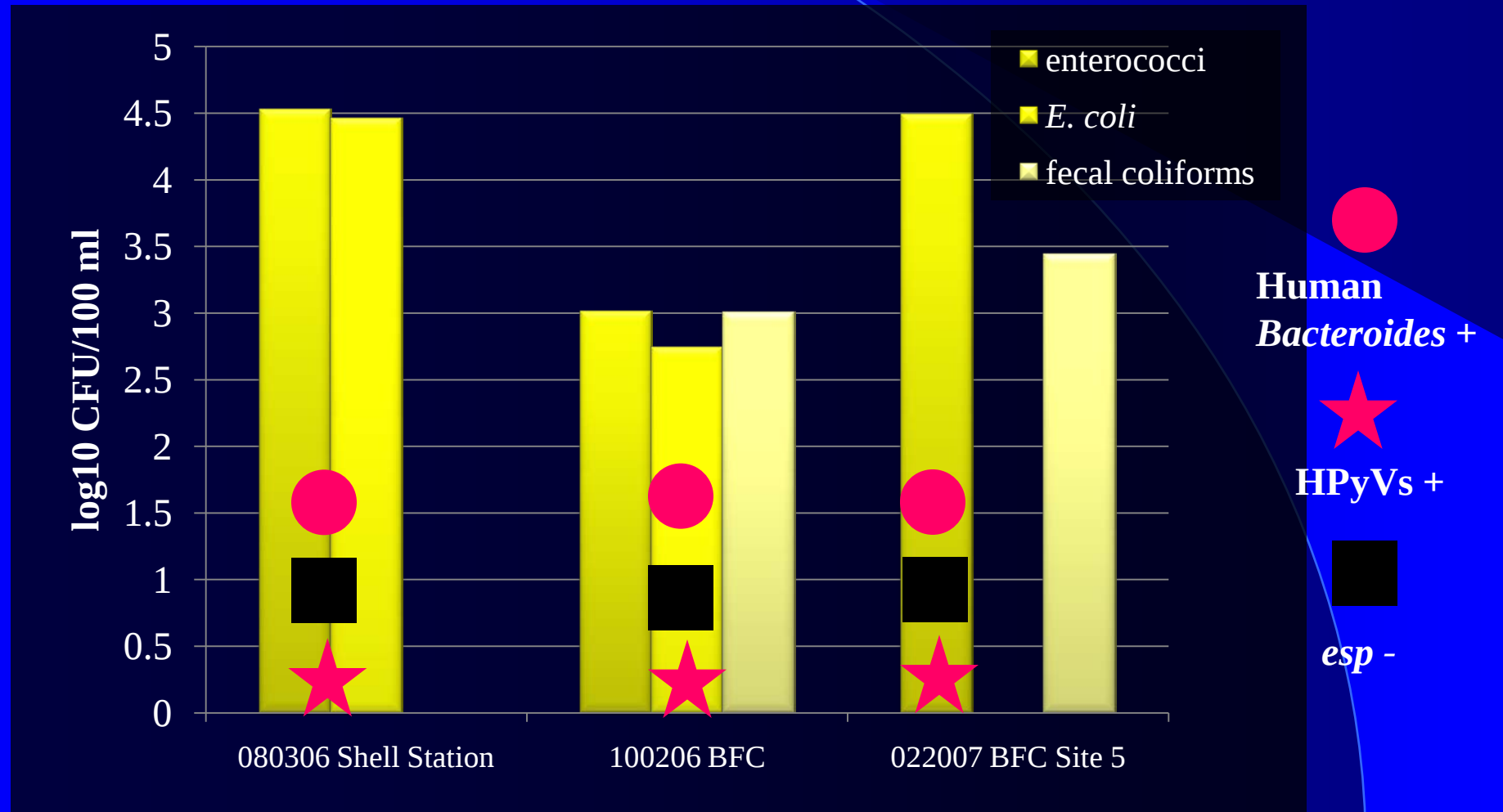


LOD ~10 viruses



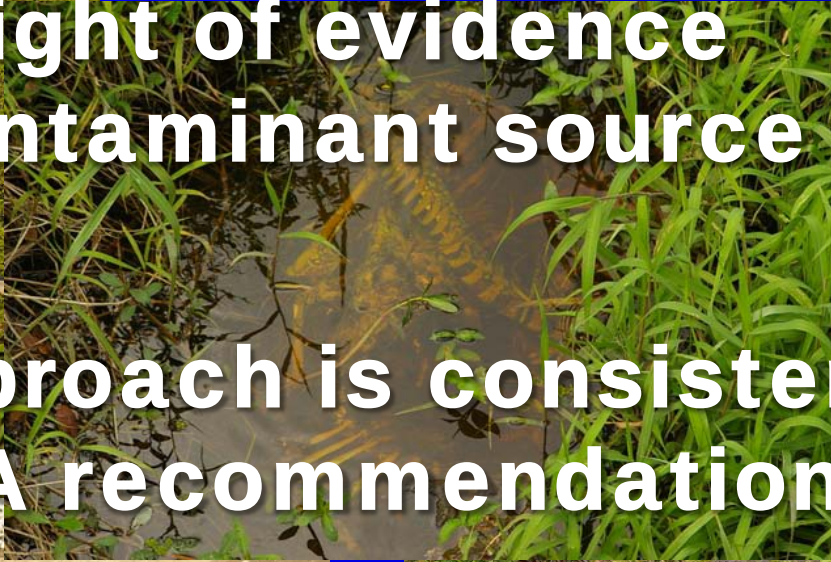
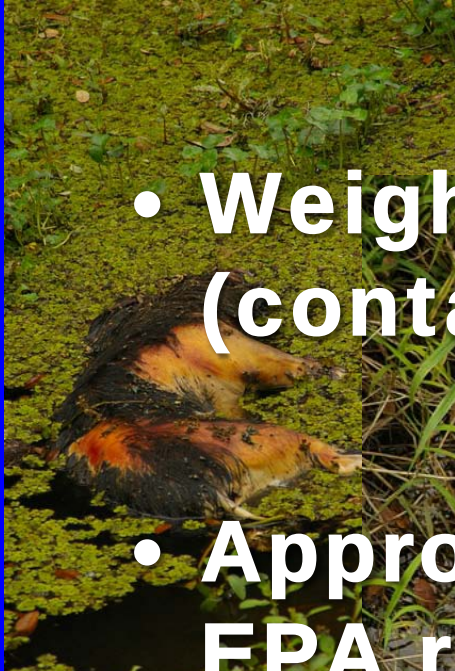
Concentration in sewage 10^3 - 10^4 /ml

Field Validation of Human *Bacteroides* Marker & HPyVs at Septic System Overflows



Part II: Merging Science with Application

- Weight of evidence (contaminant source survey)
- Approach is consistent with EPA recommendations
- Collaboration with stakeholders and buy-in



PCR

Alternative indicators

er

QPCR

Multiple markers

Weight of evidence – GIS, survey

Multiplex?

Microarray?

Alternative indicators

er

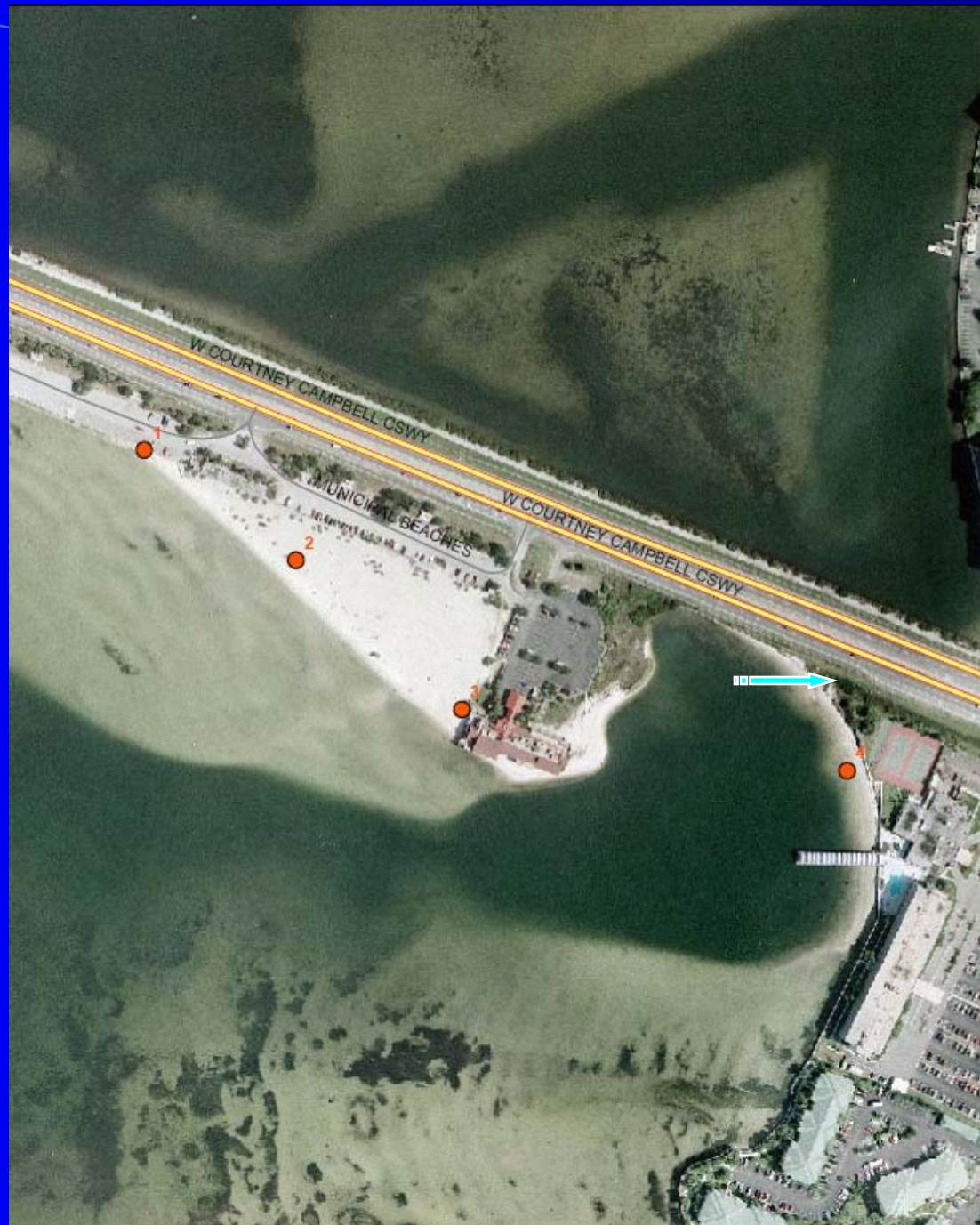


Walking the Walk

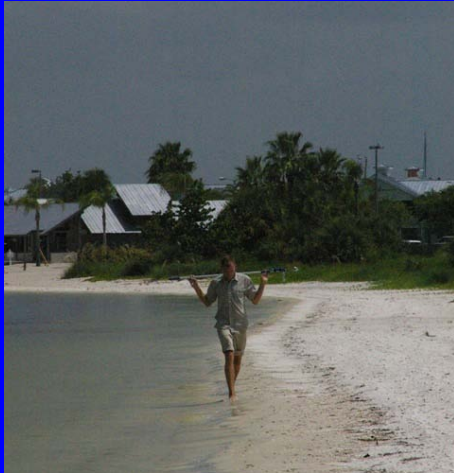
Knowledge of watershed and potential sources is crucial to successful MST application

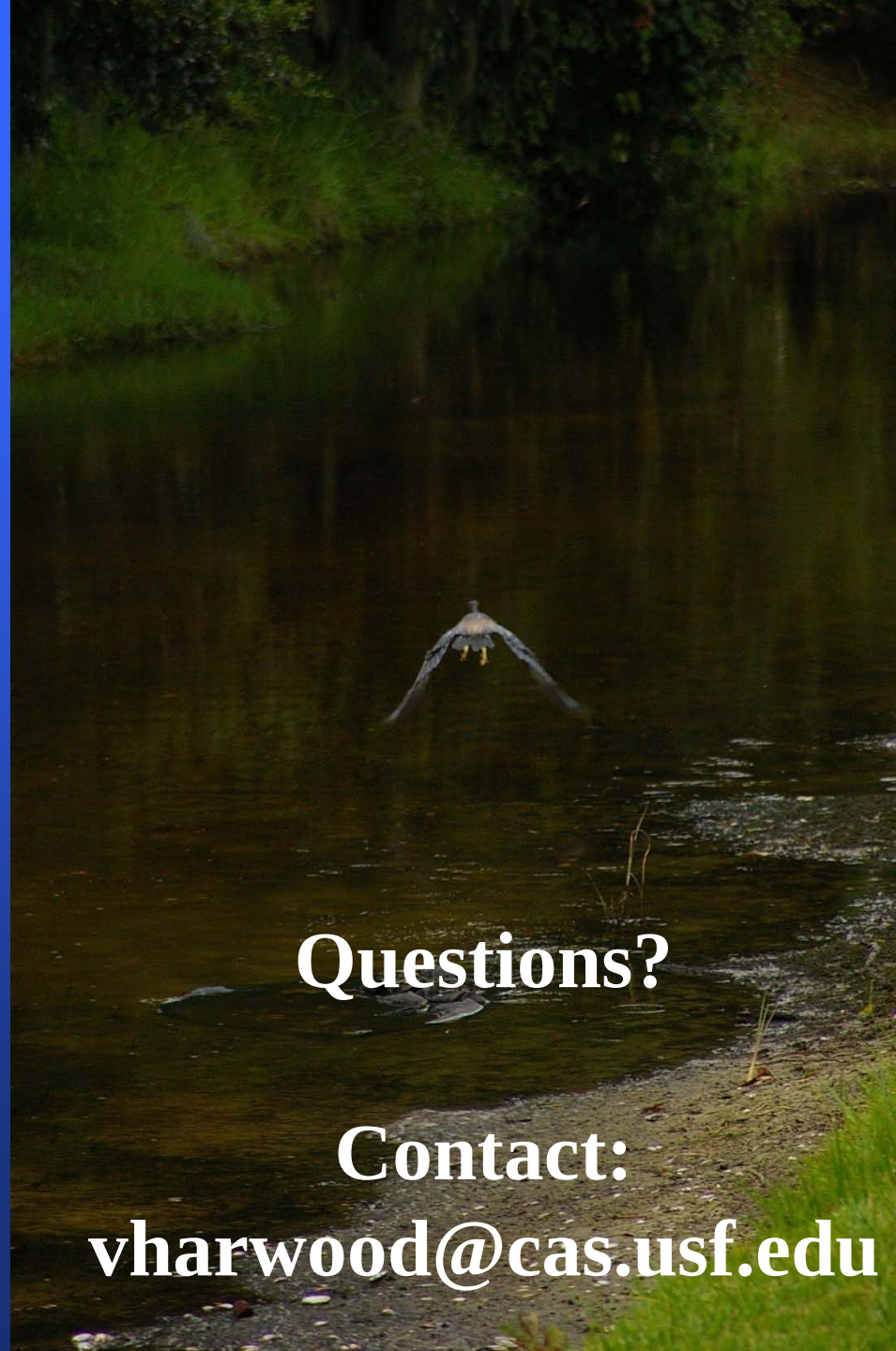
- **Maps – infrastructure, land use**
- **Partnerships – FDEP, DOH, DoACS; farmers; land-owners, residents**
- **Walk &/or boat the water body!**





Thanks to the Lab Crew!





Questions?

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