

# Bacterial counts and metabolic activity from water samples along the Buffalo National River

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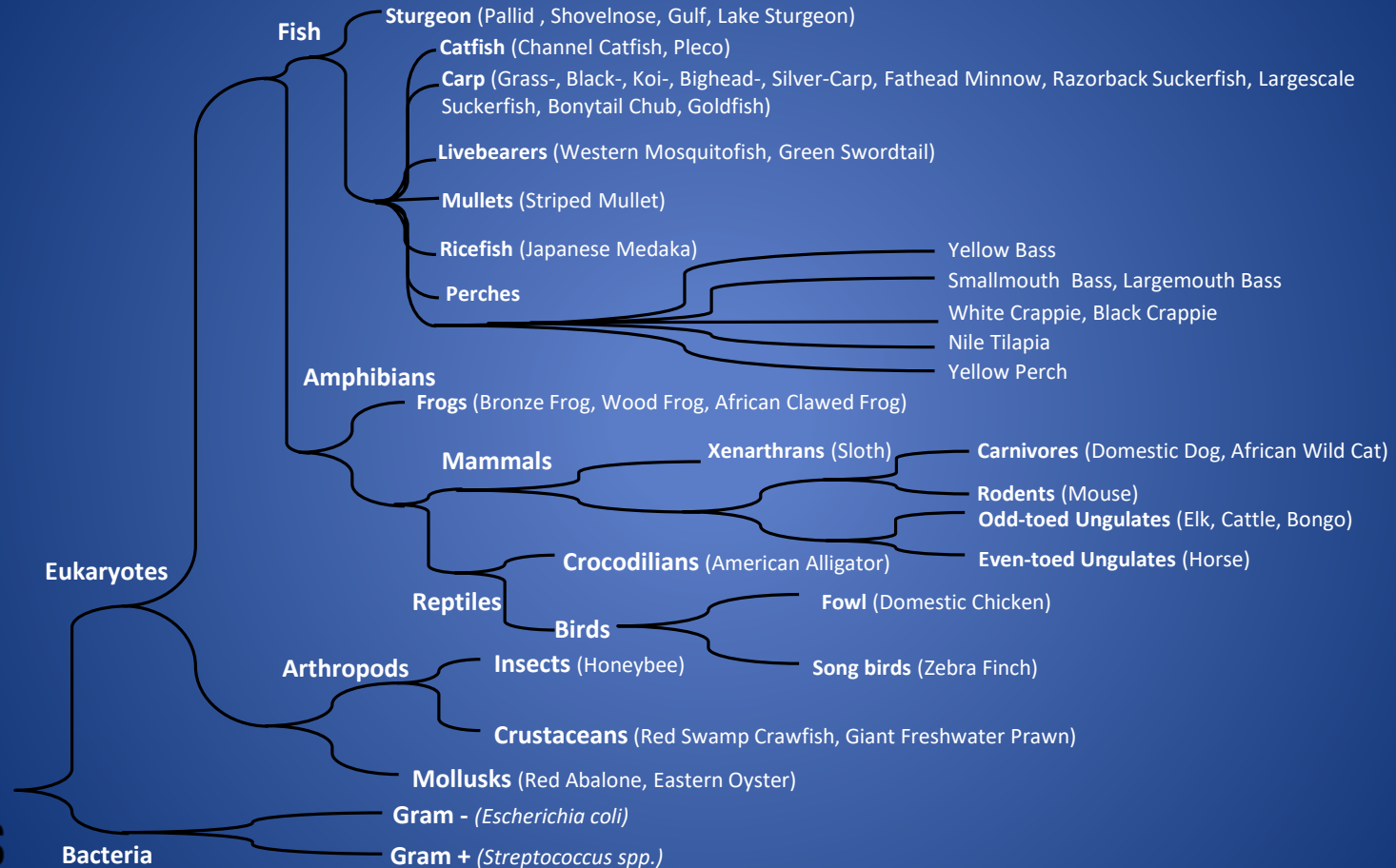
-with Billy Justus, Lucas Driver, Shawn Hodges, and Ashley Rodman

# Today's Objectives

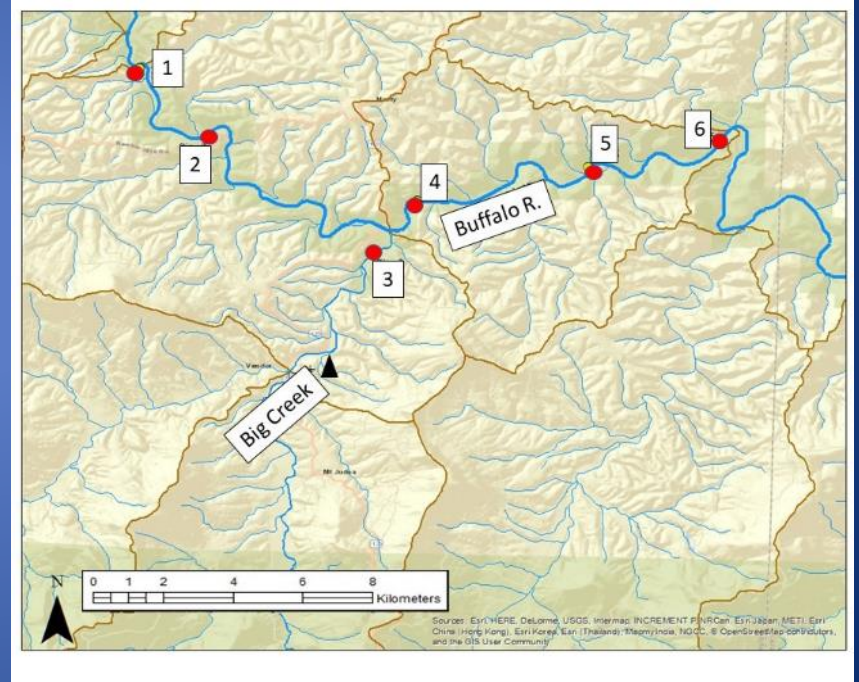
- Present hypothesis and results
- Show how hypotheses are tested
- Introduce lab capabilities
- Consider ideas



# Phylogenetic tree displaying species studied by flow cytometry in this laboratory



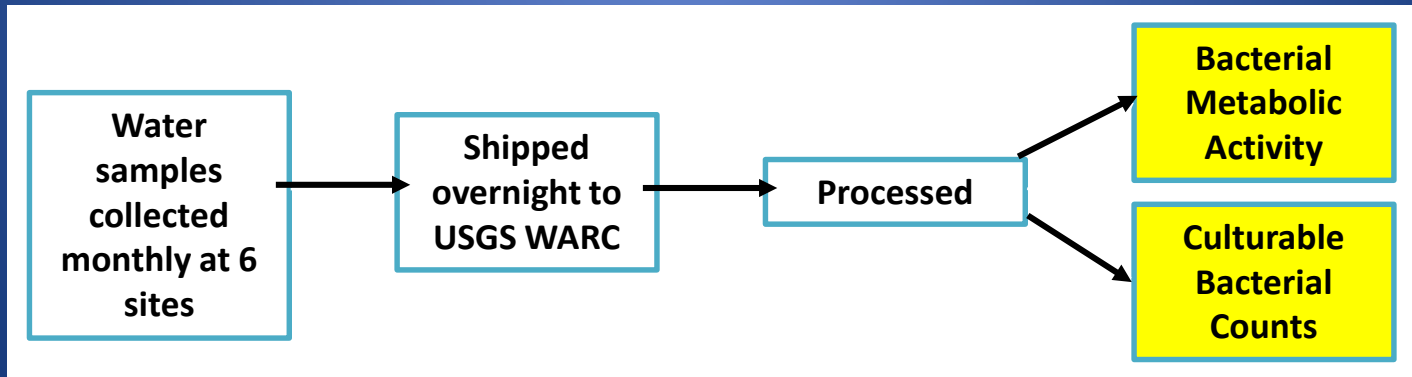
# Hypothesis: Potential differences in water quality along the Buffalo National River: determined via overall bacterial counts and metabolic activity



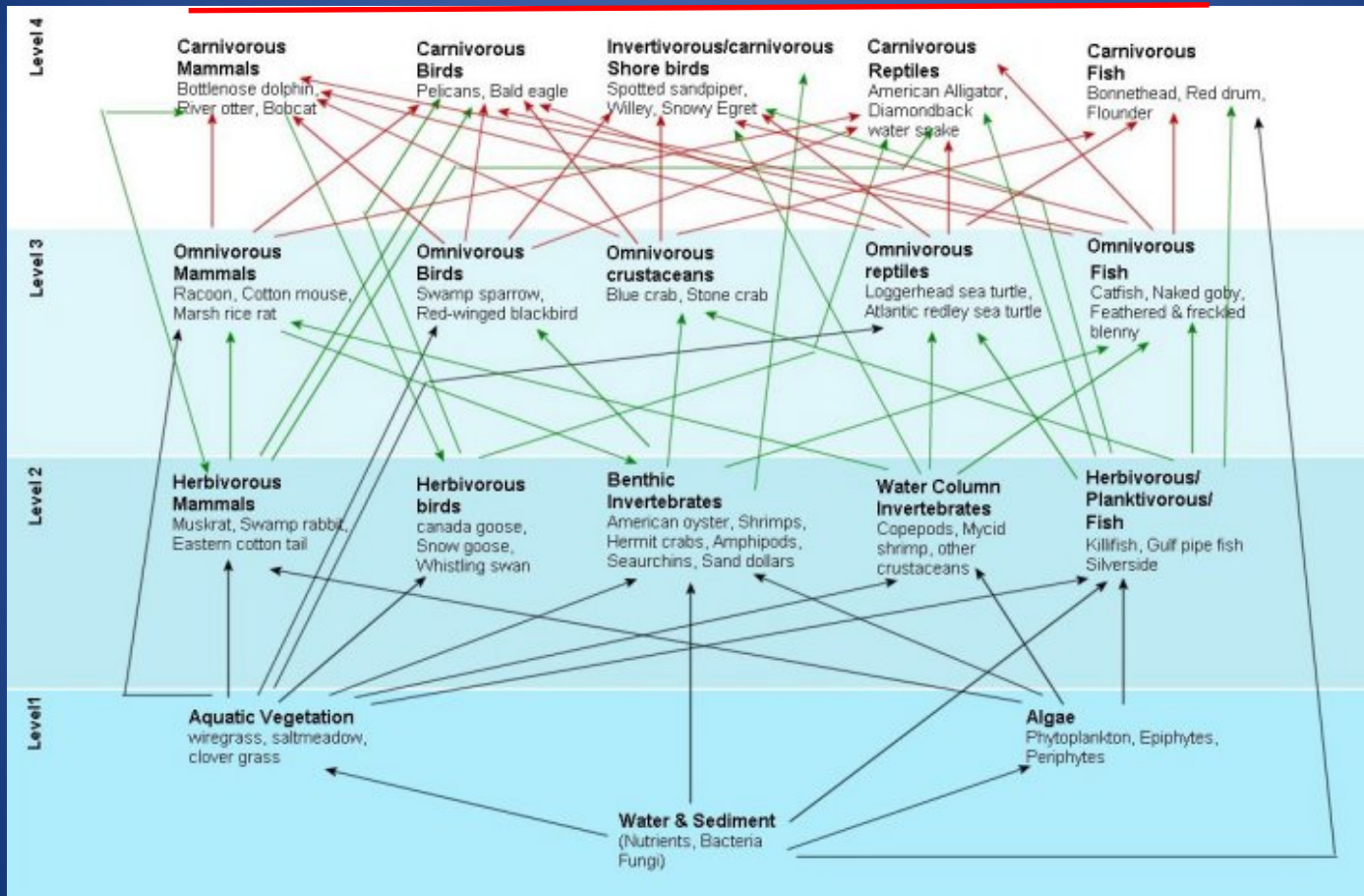
Red dots are the water sampling sites.

## Proposal Concepts-Experimental Design

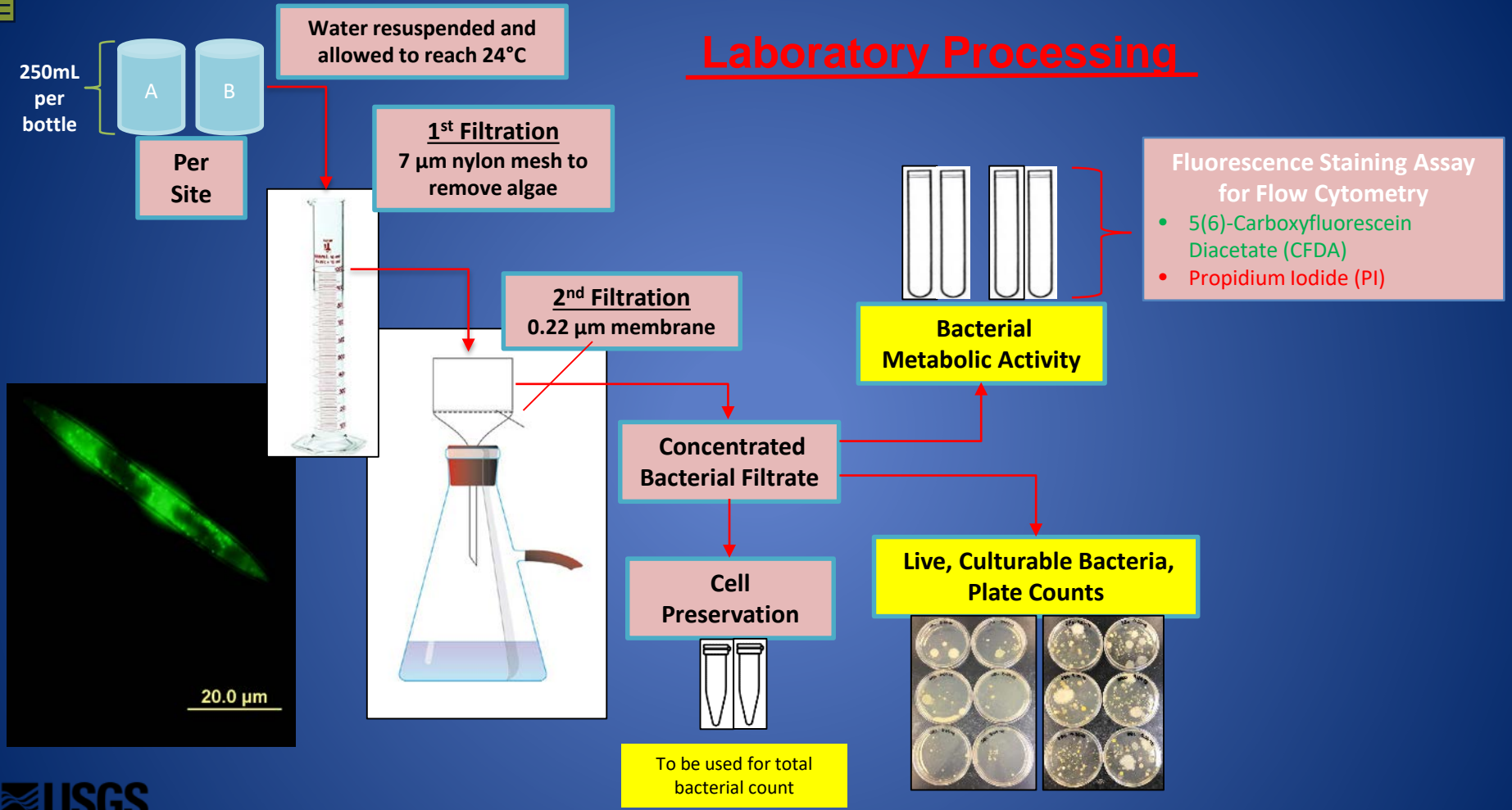
- Basic water quality parameters: turbidity, DO, pH, temp., conductivity etc. (USGS database - NWIS)
- Nutrient compounds: N species ( $\text{NH}_4$ ,  $\text{NO}_2$ ,  $\text{NO}_3$ ), P species (Ortho P, etc.)
- Hydrology: use of gages and hydrographs, flow regime, mass balance of water, precipitation
- Periphyton, Chl A quarterly
- Total coliforms and *Esherichia coli*, fecal indicators with IDEXX system
- **Heterotrophic bacteria**

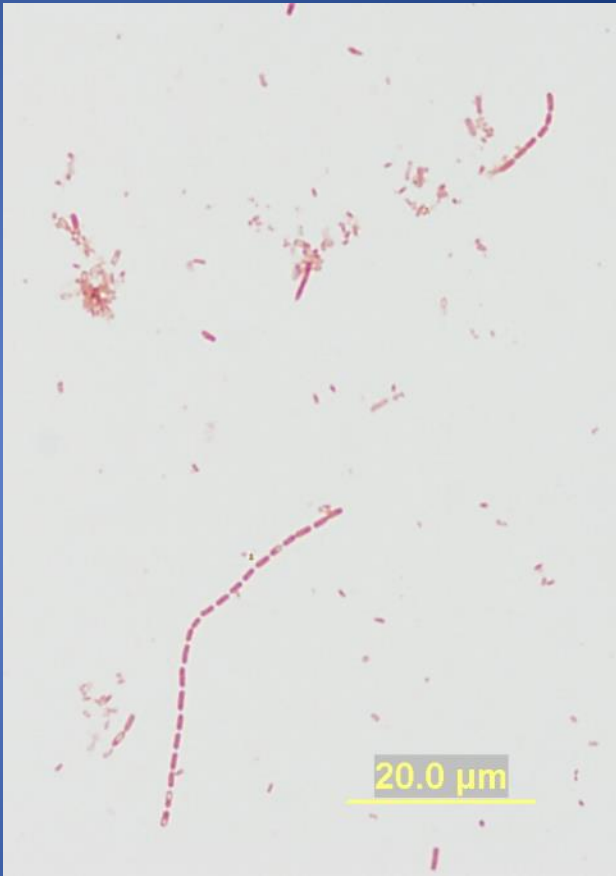
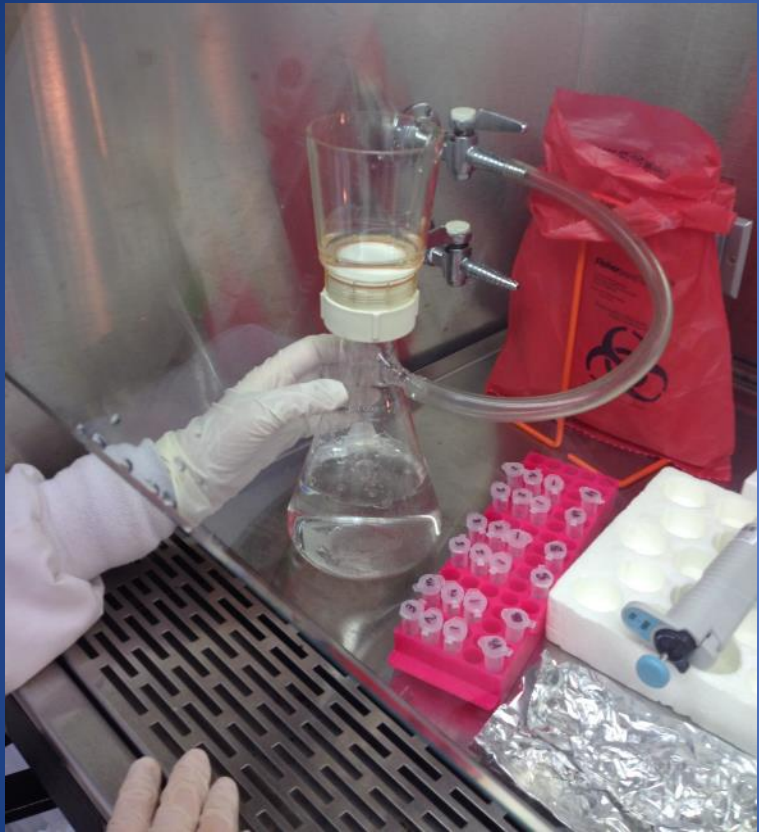


# Aquatic Food Web

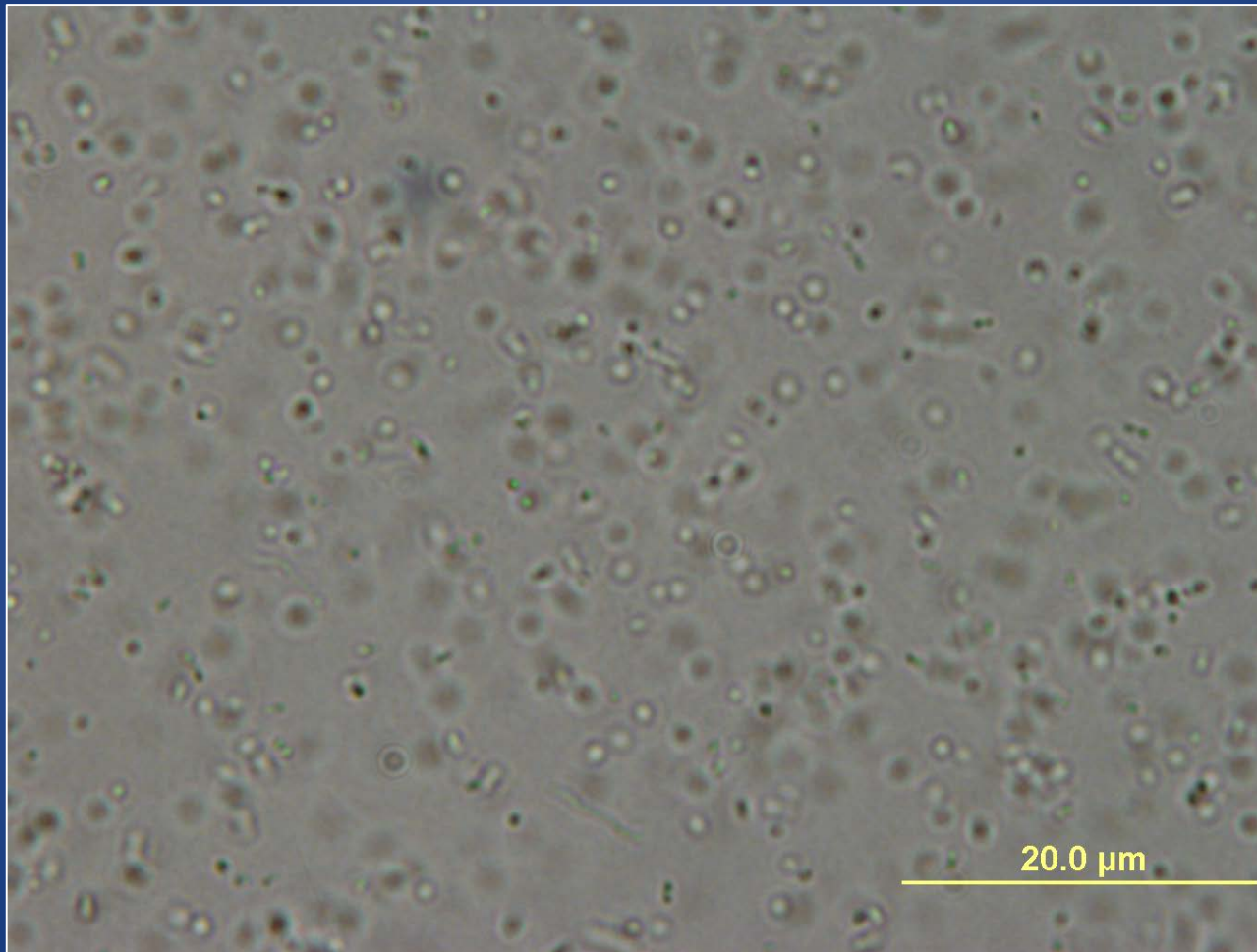


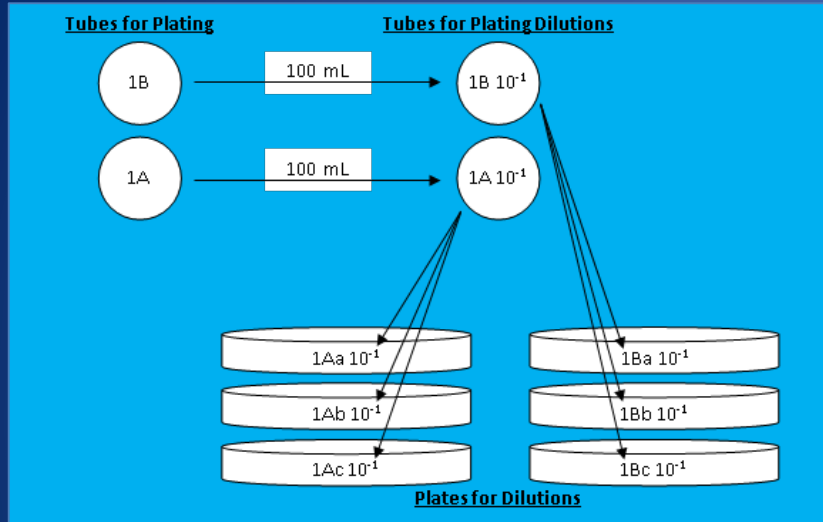
# Laboratory Processing





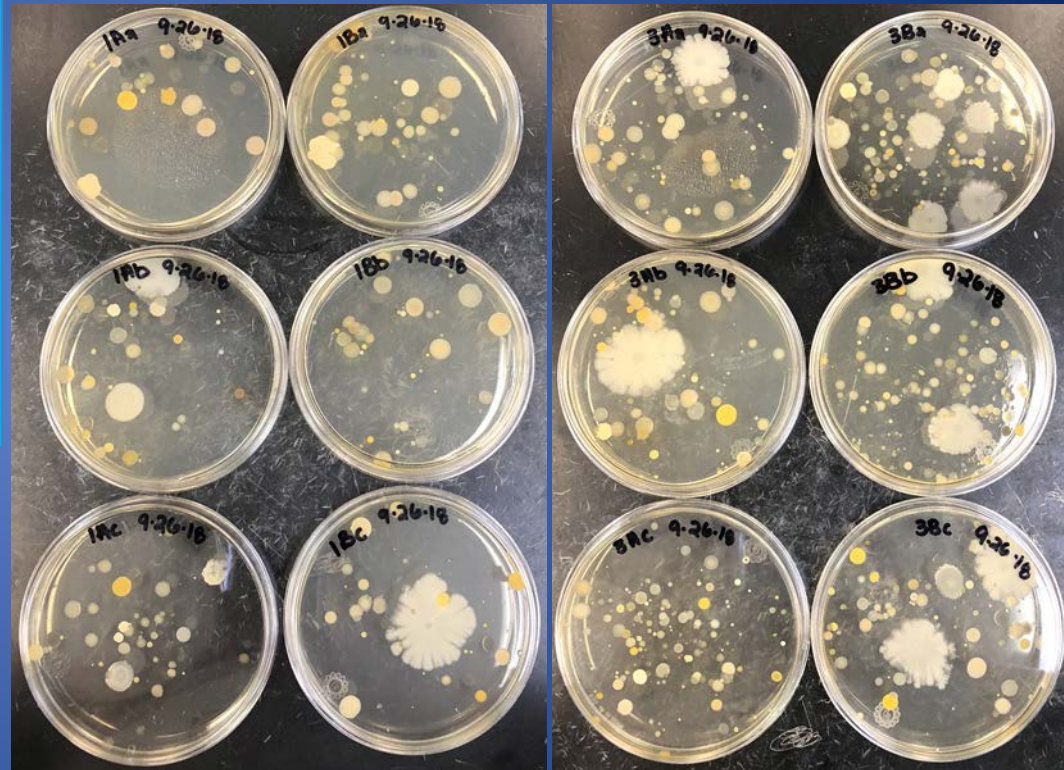






Site 1

Site 3



## **On the Results of Cultural bacterial colonies per site analyzed by ANOVA:**

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**Heterotrophic plate count (HPC) measures a range of Gram positive and Gram negative bacteria that are naturally present in the environment.**

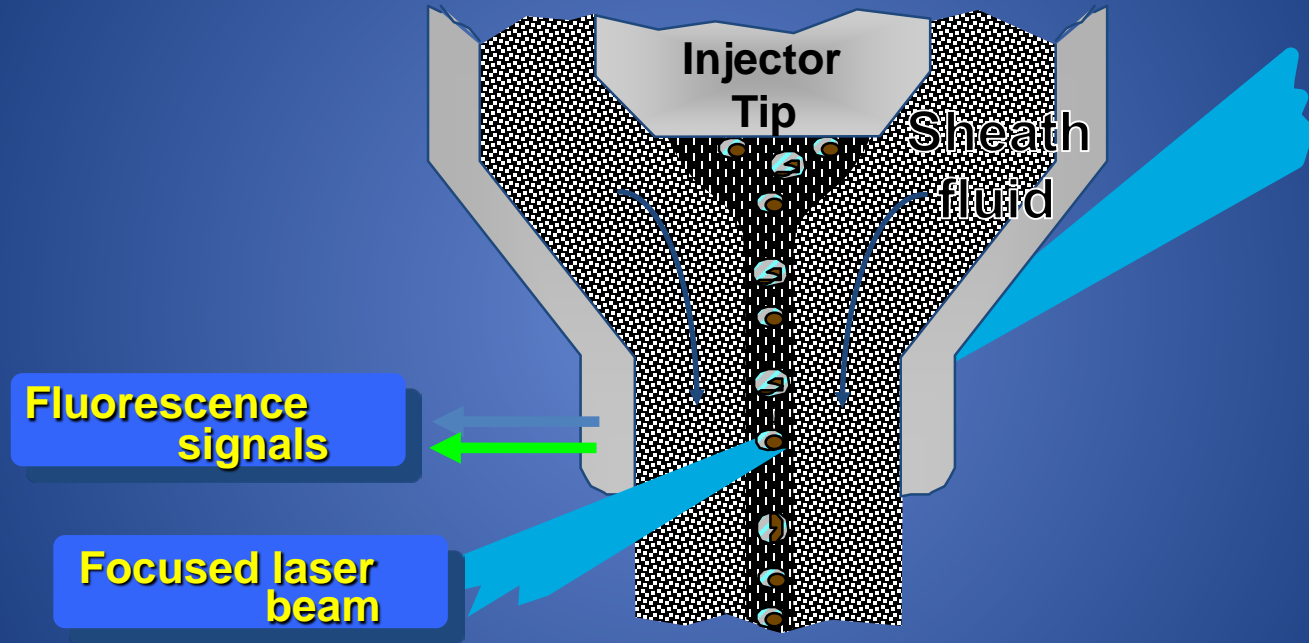
**March 2018 – flooding event in AR made sampling unsafe so no samples were received.**

**October 2018 – unable to process samples for sites 4, 5, and 6 due to delayed receipt of shipment. 48 hours too late for processing.**

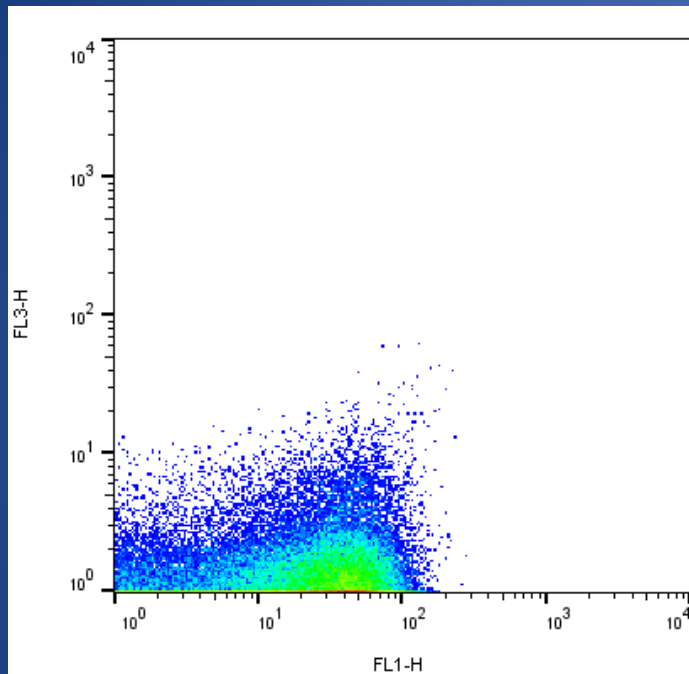
**11 out of 15 months showed significant differences in bacterial counts among sites sampled (73%)**

**Site 3 showed the highest bacterial counts for 5 of those 11 months (45%)**

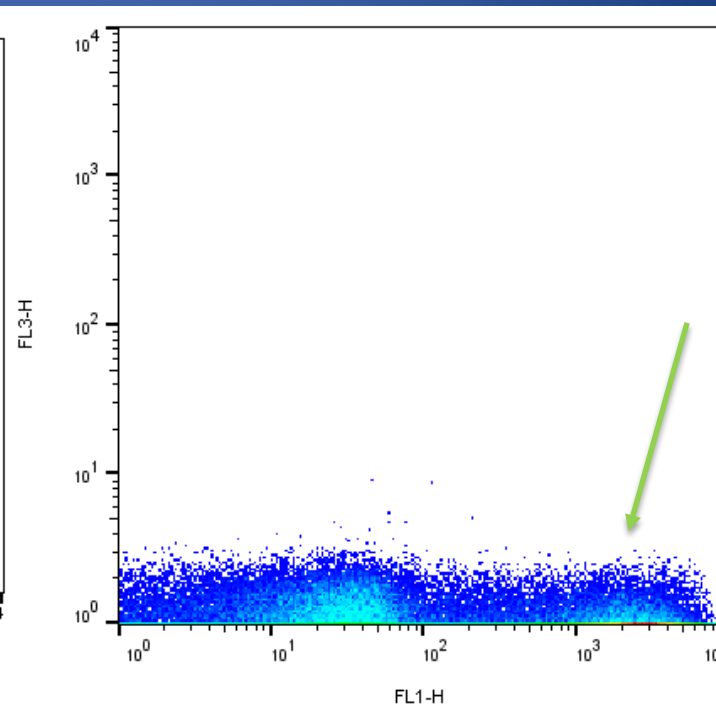
# Flow Cytometry



# Staining Controls Using 5(6)-Carboxyfluorescein Diacetate (CFDA)

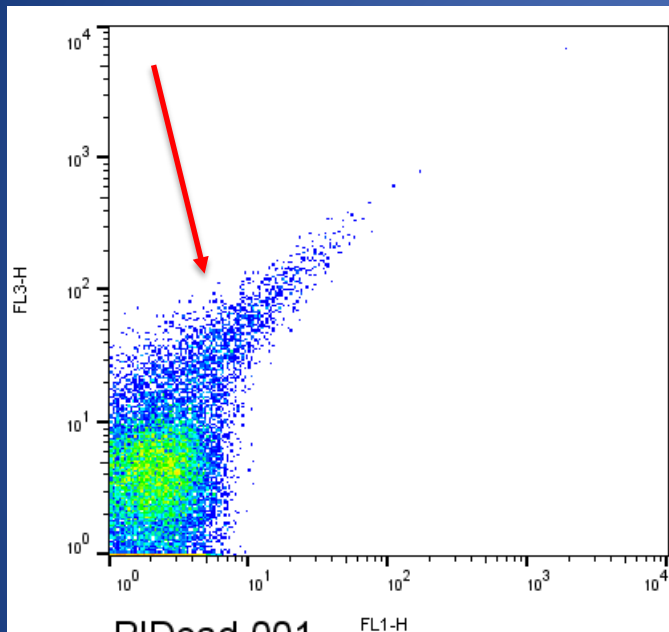


CFDA Dead.001  
Ungated  
47081



CFDA Live.001  
Ungated  
94291

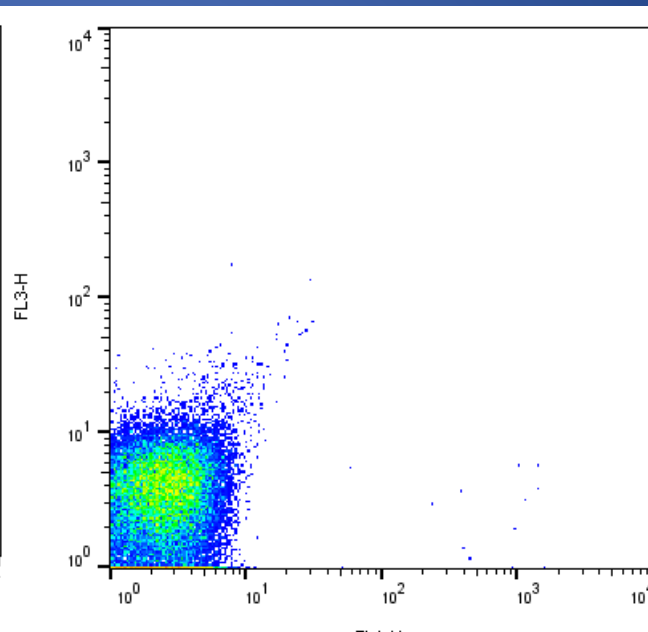
# Staining Controls Using Propidium Iodide (PI)



PIDead.001

Ungated

22078

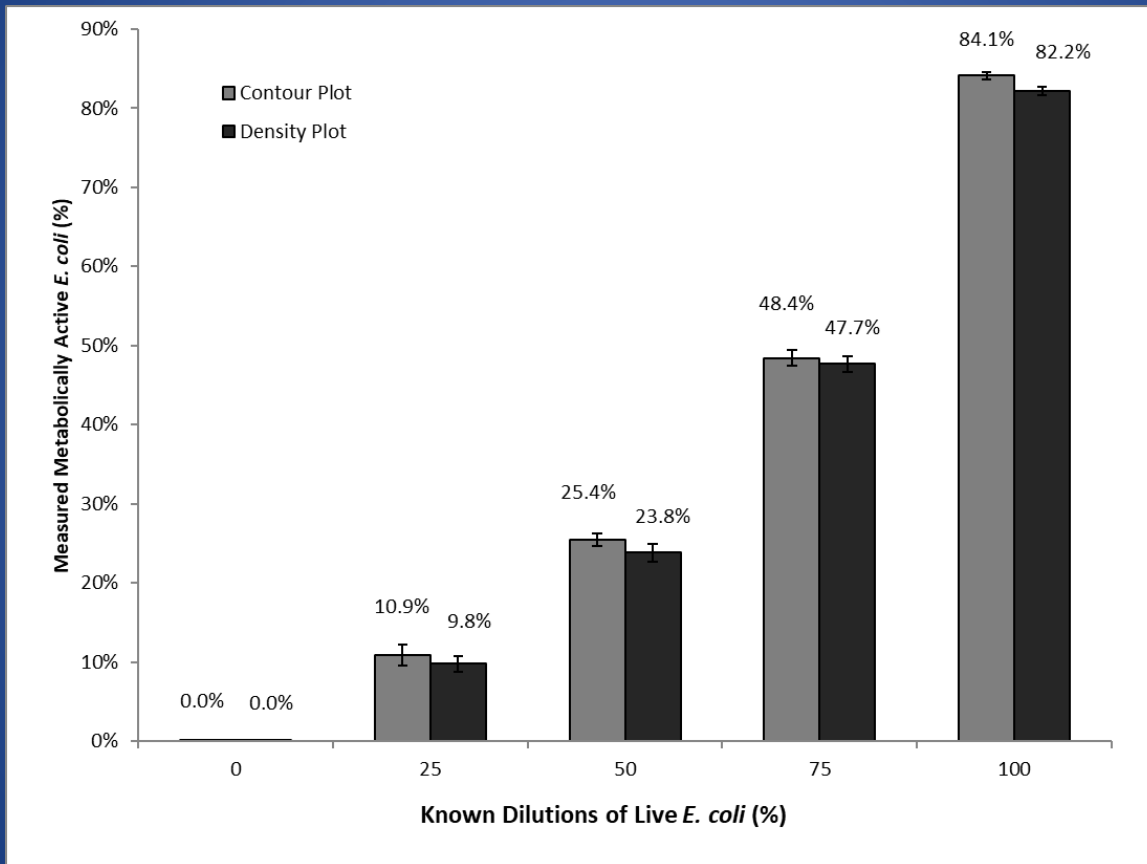


PILive.001

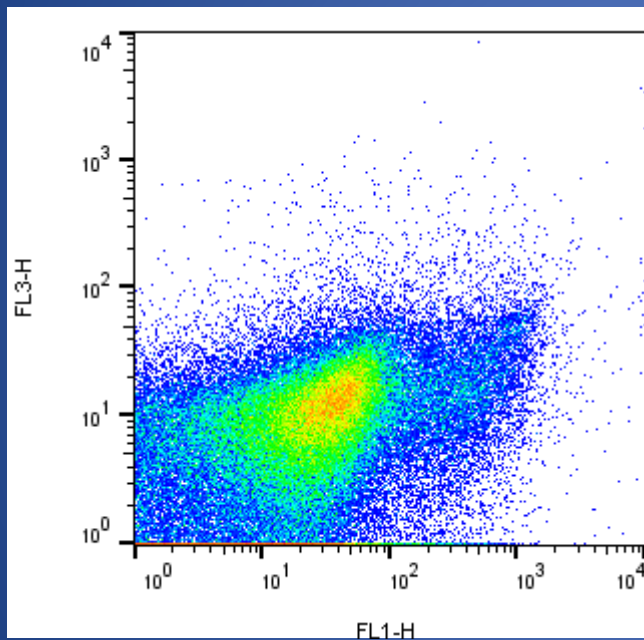
Ungated

32138

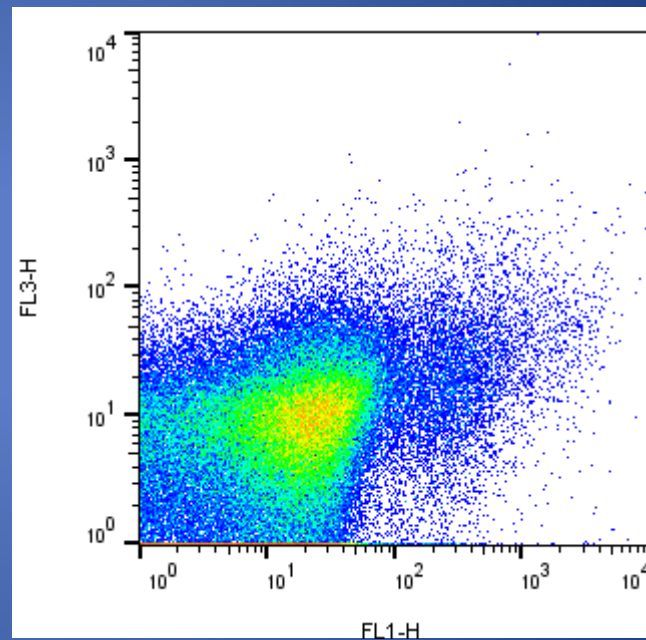
# Method Validated: *E. coli* esterase analyzed by two flow cytometric software



# Typical Cytograms, Month 6, Oct 2017



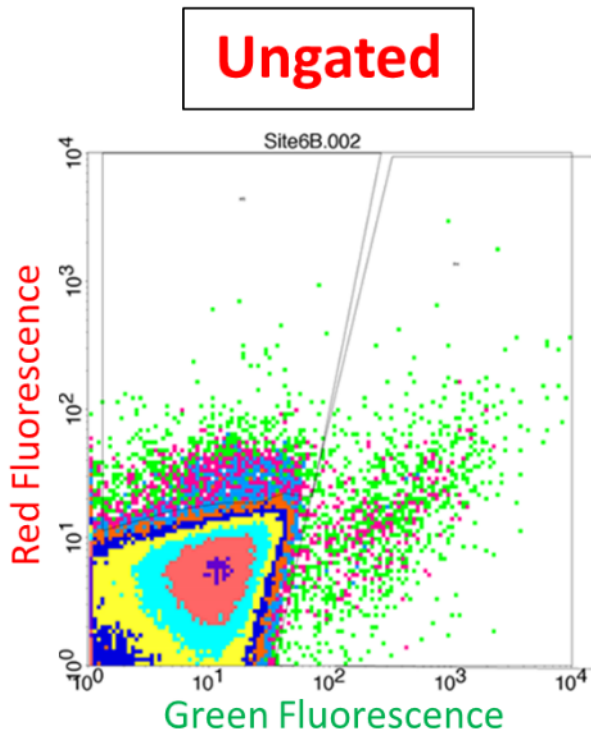
Site 1



Site 3

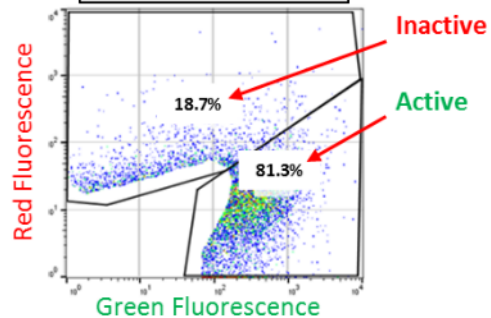


# Sample Flow Cytograms

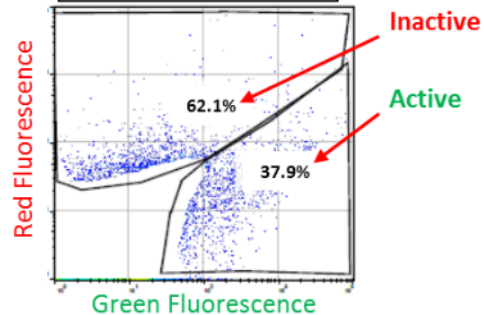


**Gated**

Oct '17 - Site 2



Feb '18 - Site 3



## Bacterial metabolic activity (n = 4) by site analyzed by ANOVA

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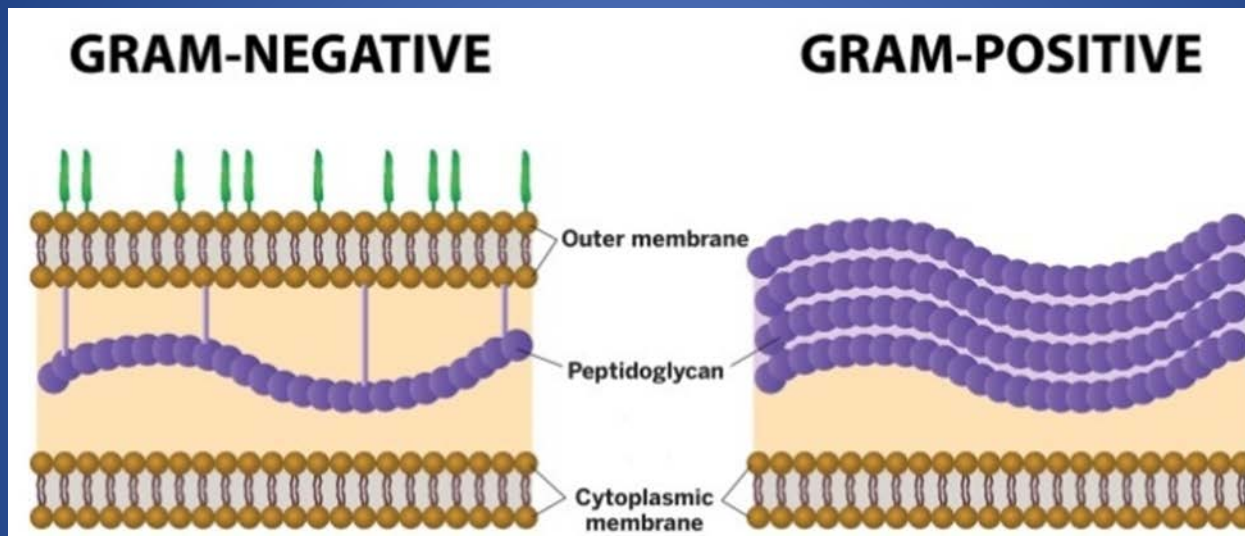
We investigated if there was a statistical difference in metabolic activity among sites for 17 months.

16 out of 17 months showed significant differences in metabolic activity among sites sampled (94%)

Site 3 showed the lowest metabolic activity for 12 of those 17 months (71%)

There were 4 months out of the 15 months analyzed that showed site 3 as having the lowest metabolic activity as well as the highest bacterial counts. Perhaps the total cell counts will yield insight into this result.

# Bacterial Cell Wall Types



# Environmental Fungi





# DNA sequencing: Environmental Metagenomics: Univ. of NH – Dr. B. Brown

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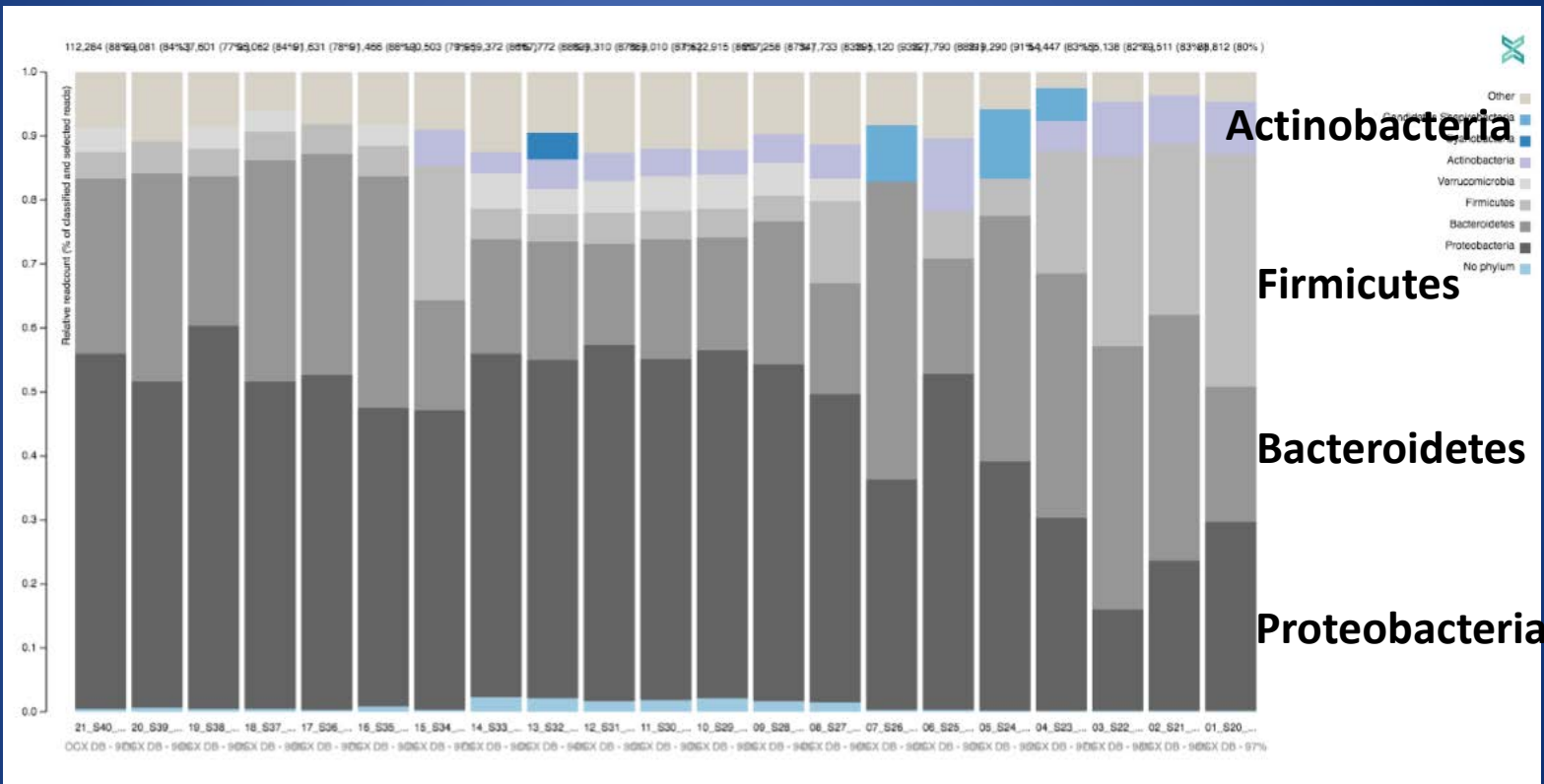
21 BNR water samples: PCR amplified 16S ribosomal subunit V4.

Looks at large database of sequences from known microbes, sees the taxonomic composition of that water sample.

The relative proportions gives a rough estimate of the abundance of the taxa identified in a sample.

The data also can be used to predict functions of the microbial consortia.

# NPS Buffalo National River - Phylum



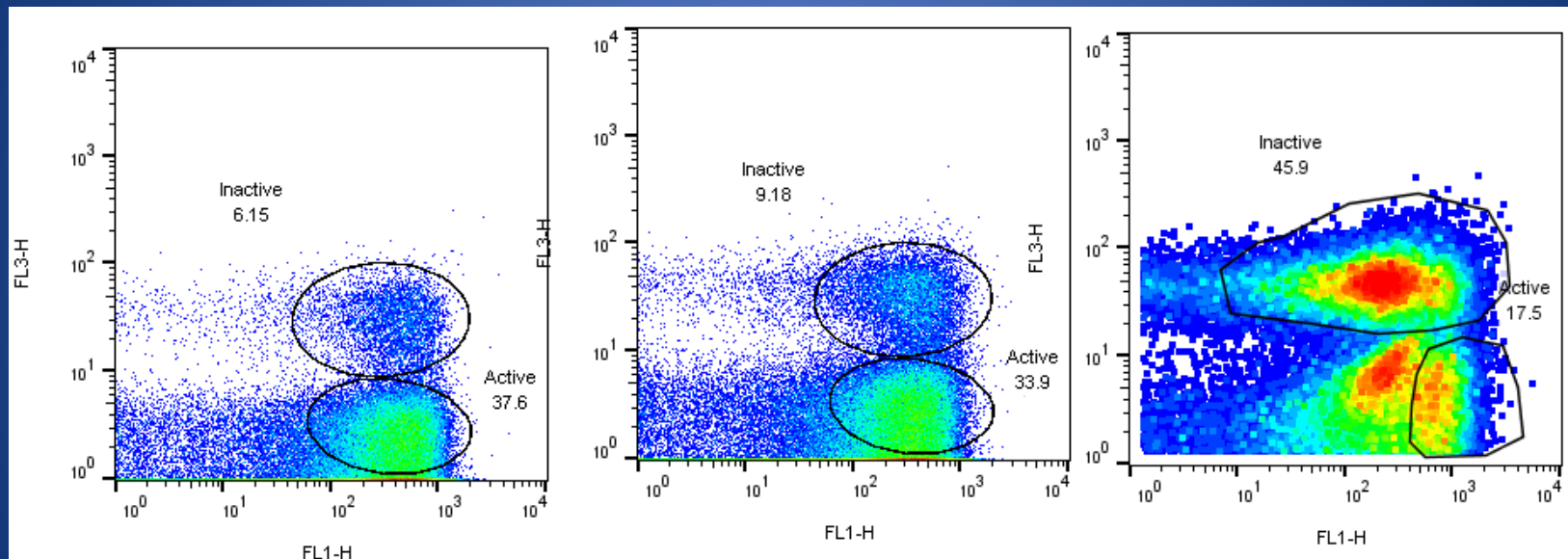
# Pharmaceutically Active Compounds

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17 $\alpha$ ethinylestradiol (EE2):	25, 5, 1 ng/L
Trenbalone	80, 40, 10 ng/L
Atrazine	10, 3, 1 $\mu$ g/L
Penicillin	1500, 750, 375 $\mu$ g/L
Pen/Streptomycin	100 units-100 $\mu$ g/mL; 50-50; 25-25
Tylosin	1000, 500, 100 $\mu$ g/L
Tetracycline	1500, 750, 375 $\mu$ g/L

**Hypothesis: Is metabolic activity influenced by simulated confined animal feeding operation (CAFO) mixtures?**

# Penicillin



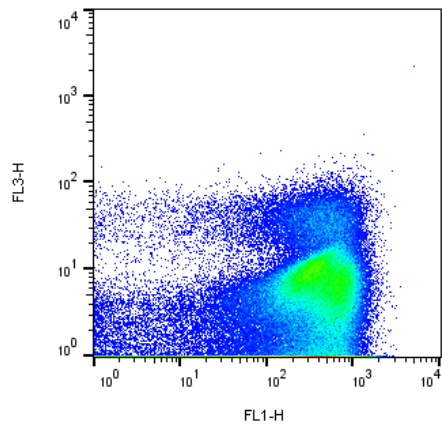
Control

Low

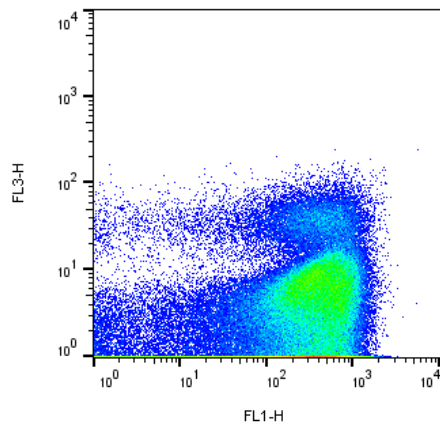
High



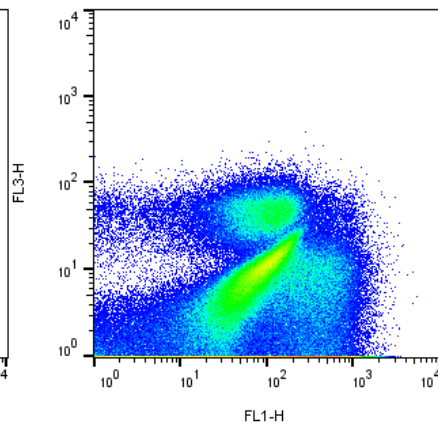
# Atrazine



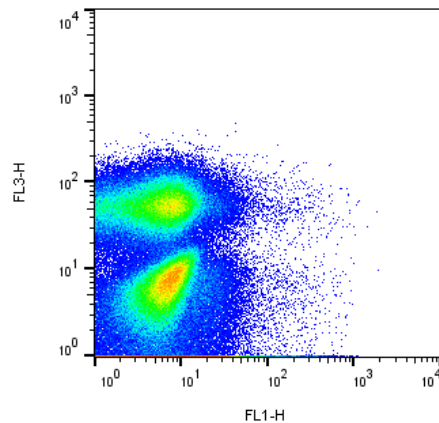
**Control**



**Low**

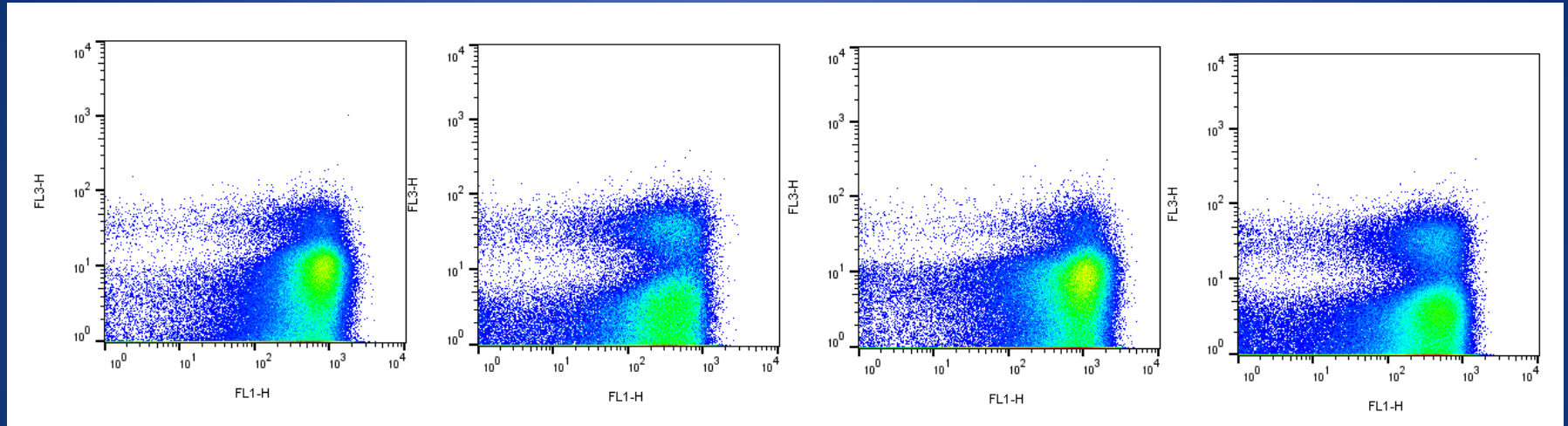


**Med**



**High**

# Trenbolone



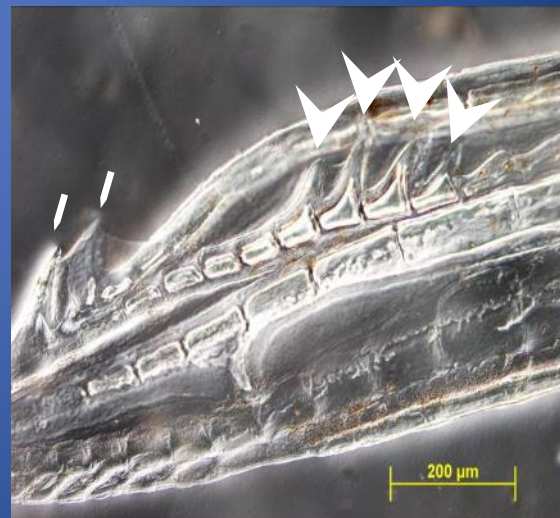
**Control**

**Low**

**Med**

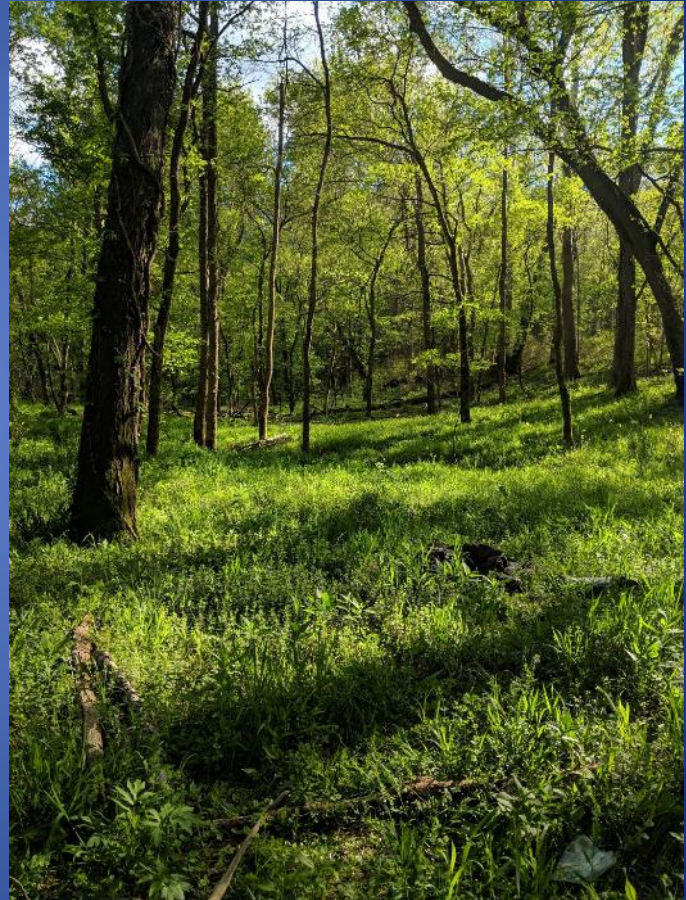
**High**

Western Mosquitofish, *Gambusia affinis*  
Biomarker study w/ simulated CAFO runoff



## Results

- Although higher bacterial metabolic activity was hypothesized to occur at Big Creek due to likely introduction of organic nutrients into BNR from a swine farm, Big Creek showed the lowest metabolic activity for 71% of the months analyzed.
- Overall bacterial metabolic activity at all sites was lower in the colder, winter months.
- Highest live bacterial counts were associated with local rain events.



# Conclusions



- The results presented in this study are part of a 19-month project and will be combined with other environmental water-quality data and fecal bacterial indicators.
- Nutrients/organics and bacteria may originate from other distant sources in the Big Creek watershed.
- Results point to broader implications for river ecosystem services.



## USGS-NPS Natural Resources Preservation Project (NRPP)

### USGS Ecosystems Mission Area

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