# Tracking and Investigating Microbial Sources (TIMS)

Brett Goodman, P.E.
Gainesville Regional Utilities
Orange Creek Basin Working Group
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# Overview

- Background
- Purpose
- MST Scope
- Results
- Conclusions

# Gainesville Creeks

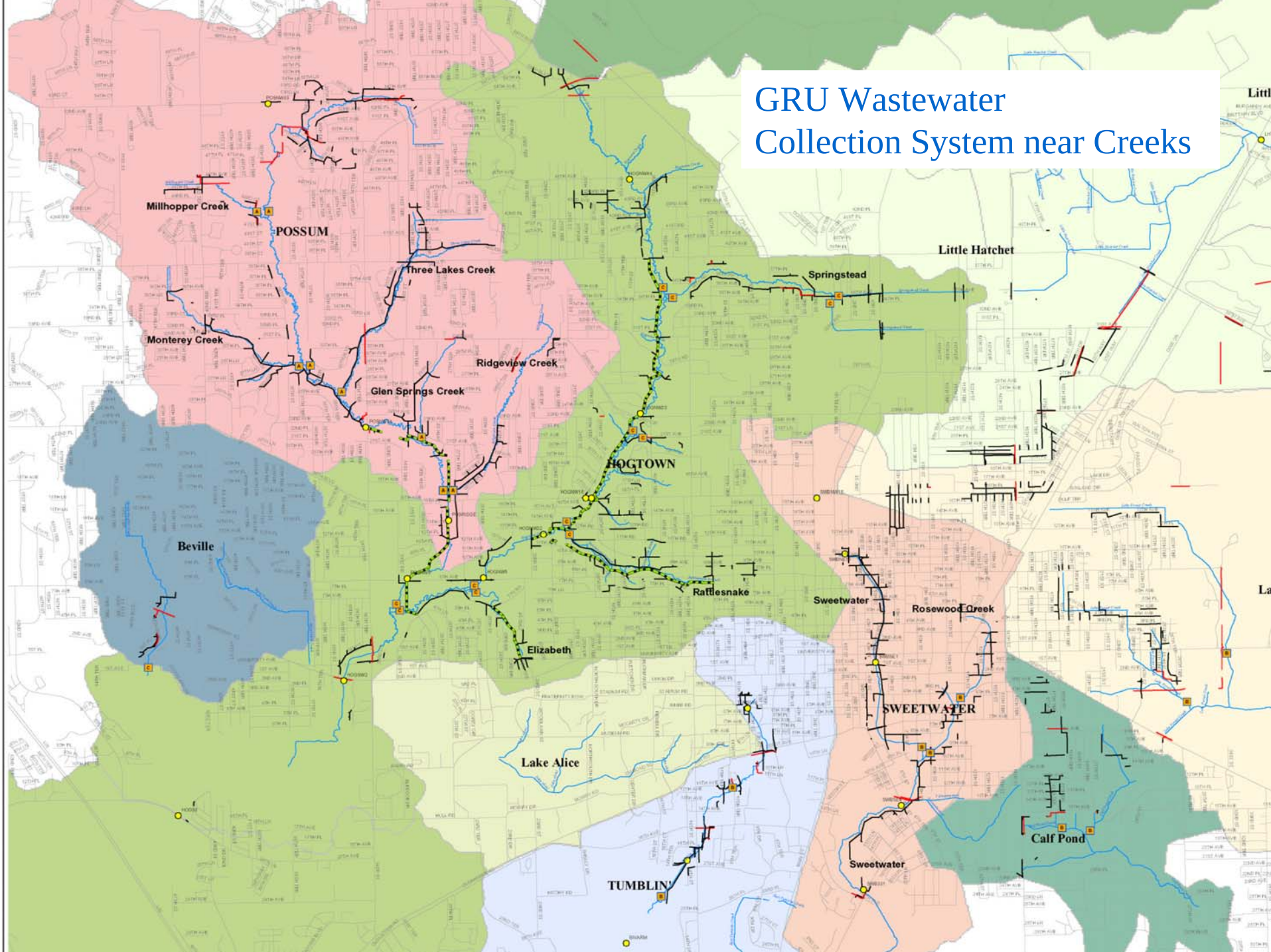
- **3 primary creek systems in Gainesville are listed as impaired by elevated levels of fecal coliform bacteria. FDEP has develop TMDLs for each system:**
  - **Hogtown Creek: 51% Reduction**
  - **Sweetwater Branch Creek: 70% Reduction**
  - **Tumblin' Creek: 74% Reduction**
- **So what happens next given that fecal coliforms can come from multiple sources and conventional methods can't identify source?...**

# Eating the Elephant…
## One Bite at a Time

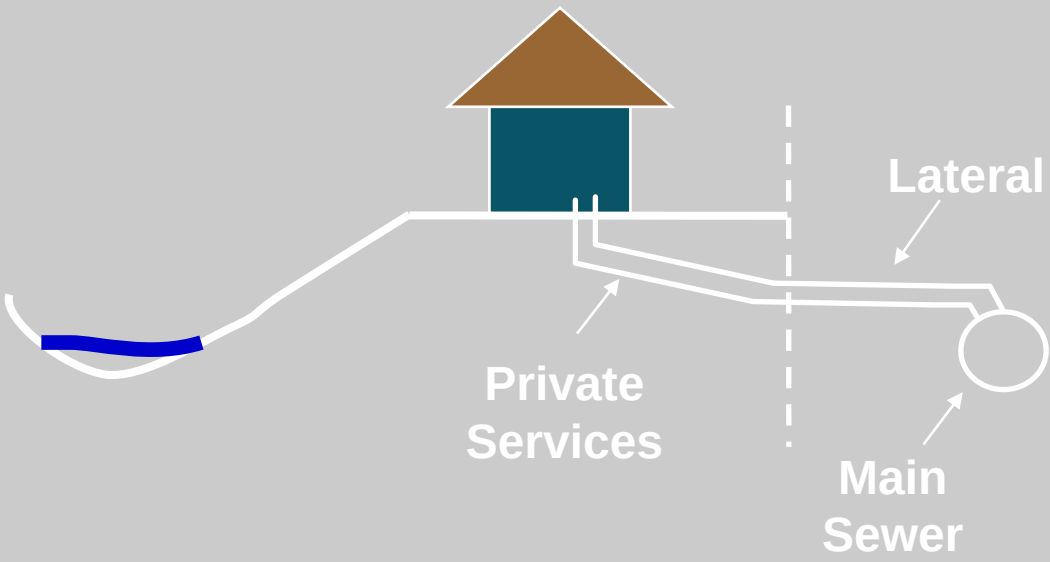
- **Solving bacteria water quality problems will be the ultimate application of the "watershed" approach**
- **Need to break this problem in to manageable pieces to identify sources, establish responsibilities, and implement the most cost effective solutions**

# Possible Sources

- **Exfiltration and releases from wastewater collection systems (public and private)**
- **Malfunctioning septic systems**
- **Urban campsites near creeks**
- **Domestic animals**
- **Wildlife**
- **Re-growth in conducive environment**

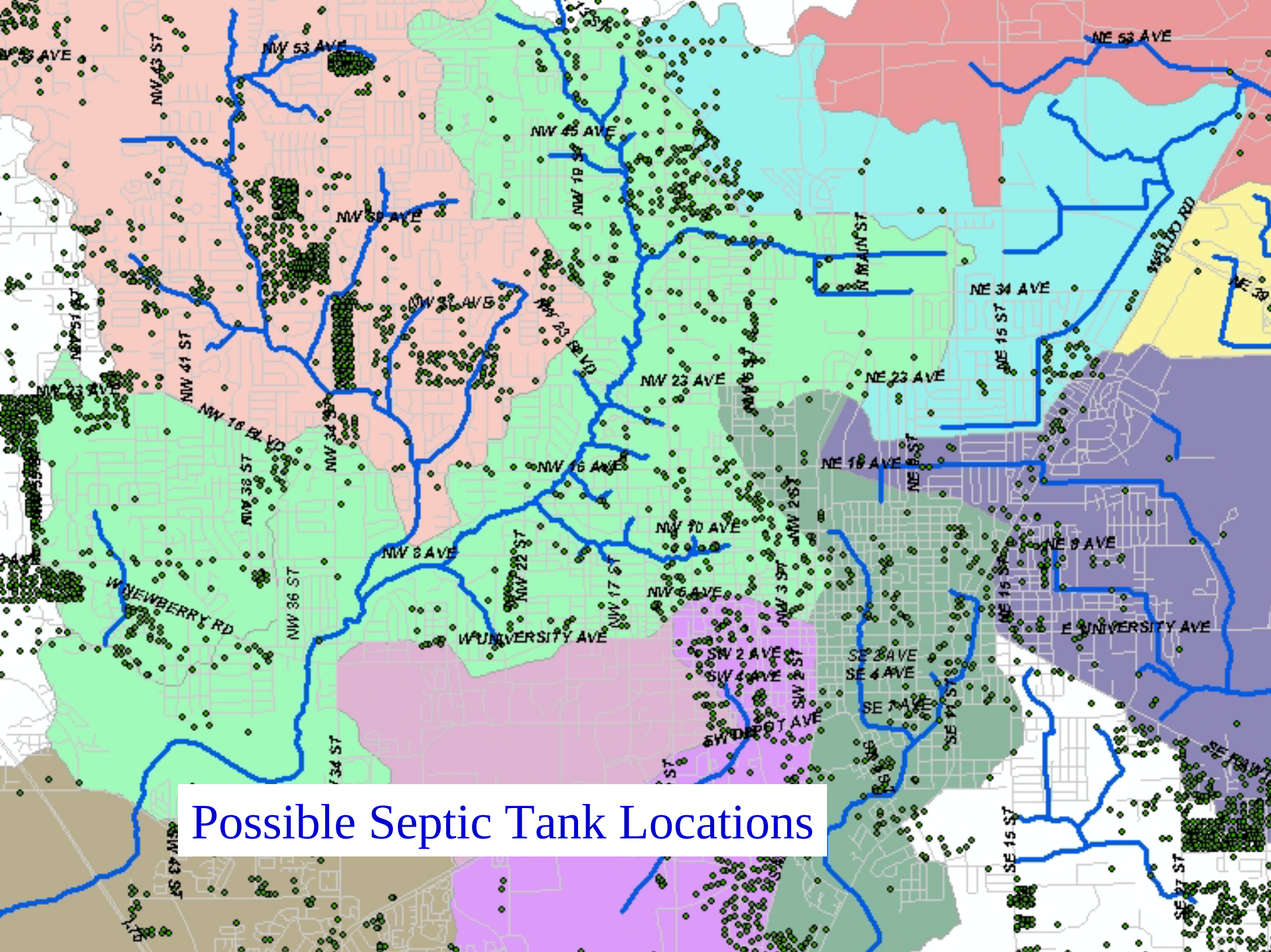

GRU Wastewater
Collection System near Creeks
Millhopper Creek
POSSUM
Three Lakes Creek
Monterey Creek
Ridgeview Creek
Glen Springs Creek
Little Hatchet
Springstead
HOGTOWN
Beville
Rattlesnake
Sweetwater
Rosewood Creek
Elizabeth
SWEETWATER
Lake Alice
Calf Pond
Sweetwater
TUMBLIN

# Private Systems




Private Collection System

GRU Collection System




# Possible Septic Tank Locations

# Urban Campsites

# Animals

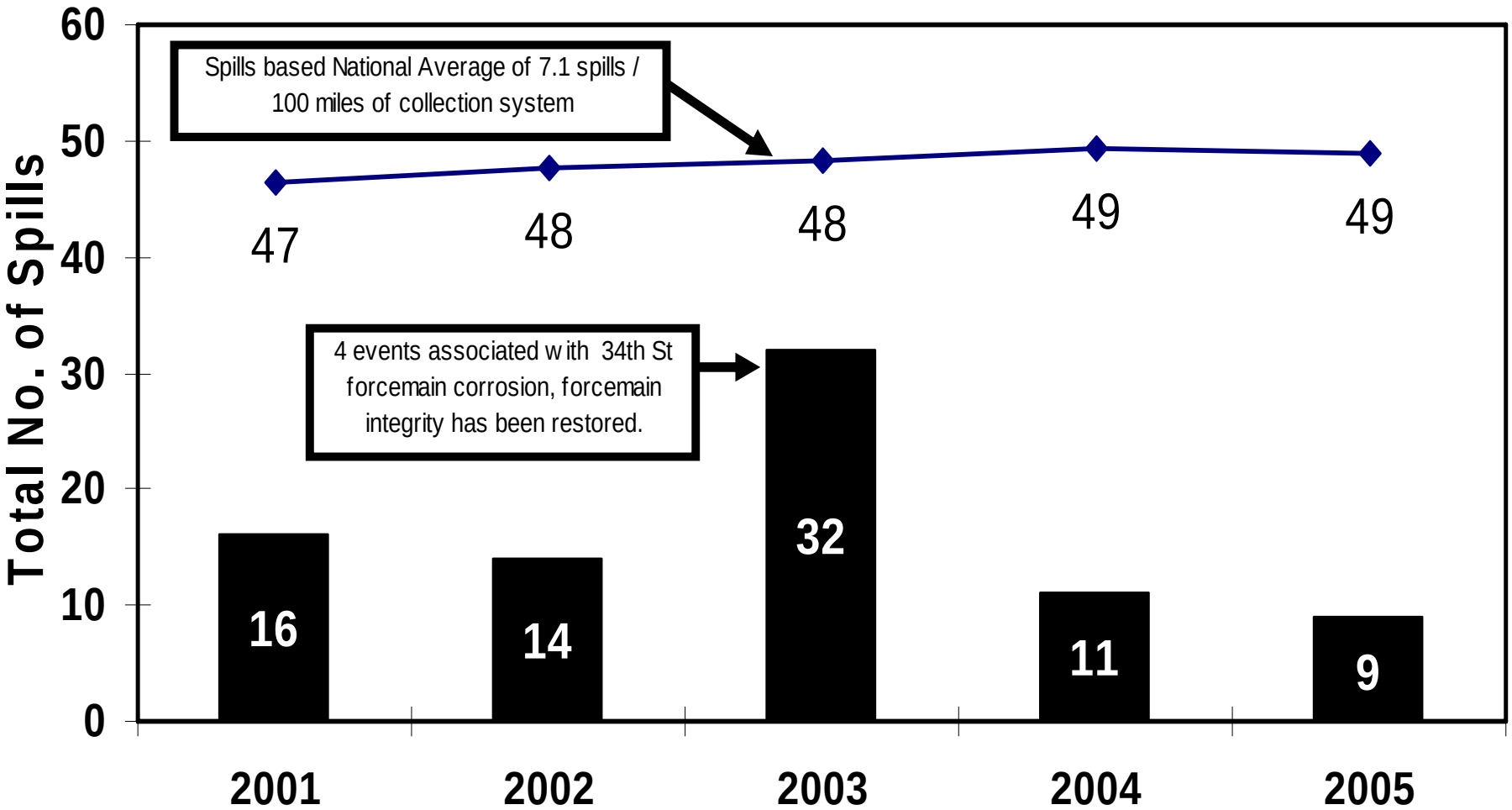
# Environmental Conditions

# GRU's Role in Watershed

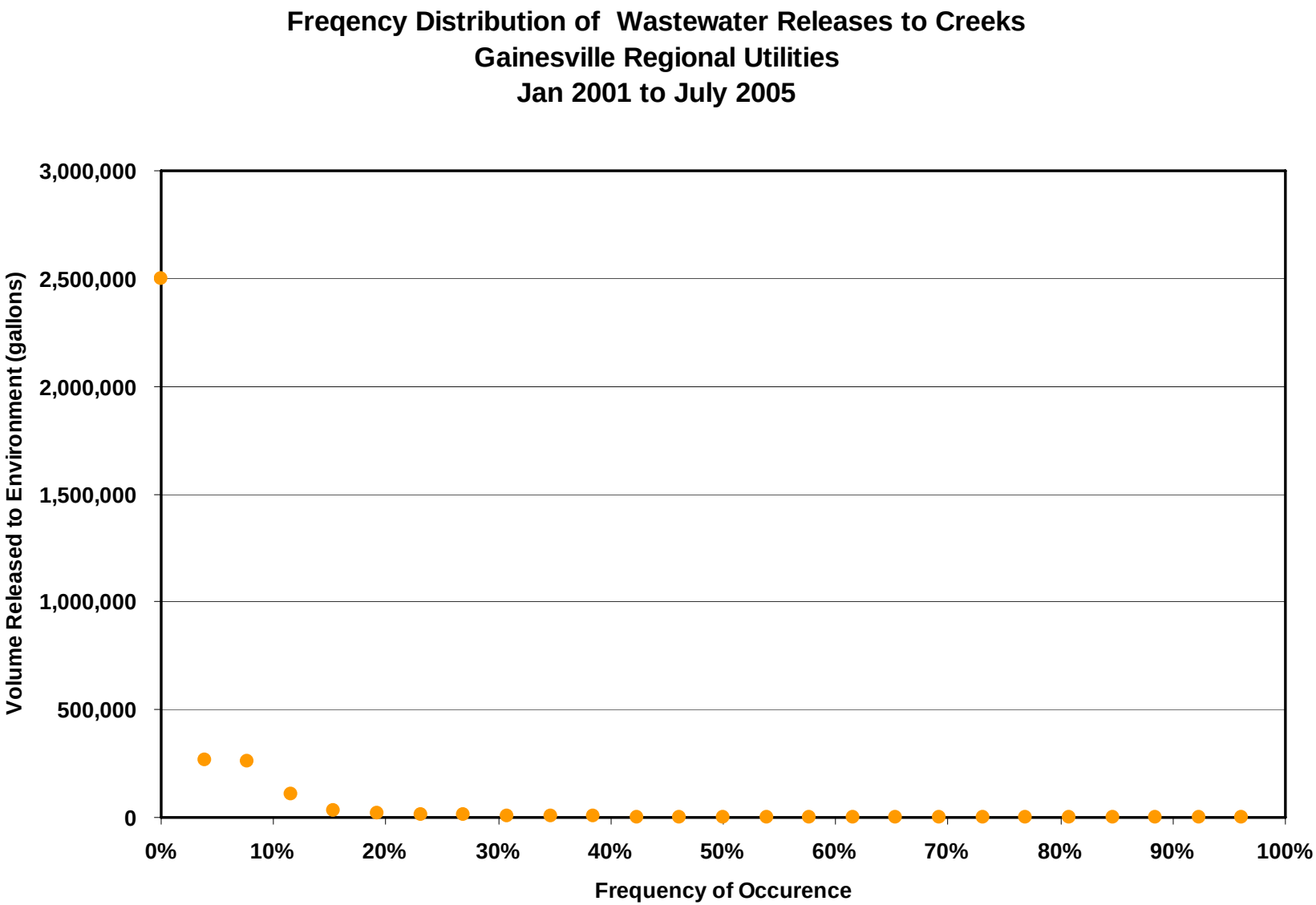
- **Operate and maintain the municipal wastewater system**
- **Be accountable to our customers**
- **Protect public health and safety**

**Keep it in the pipe!**

# GRU Wastewater System Performance vs National Averages




Source: "Effective Practices for Sanitary Sewer and Collection System Operations and Maintenance", Water Environment Research Federation. (June 2003).


Freqency Distribution of Wastewater Releases to Creeks
Gainesville Regional Utilities
Jan 2001 to July 2005
Volume Released to Environment (gallons)
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Frequency of Occurence

# Impact from Spills

- **Total loading from typical release**
  - **TSS = 2 lbs**
  - **TKN = 0.3 lbs**
  - **Fecal coliform levels back within 1 day**

# Purpose of GRU Study

- Leverage in-kind project with FDEP
- Put our wastewater collection system management programs to ultimate test
- Identity areas to focus capital improvement programs
- Help community solve creek bacteria problems using best available science

# Factors in Selecting Approach

- **Phenotypic vs. Genotypic MST**
- **Reliance on reference libraries**
- **Ability to answer multiple questions**
- **Flexibility to change course or make modifications during study**

# Study Scope

- **3 Phase Approach:**
  - **Phase 1: Synoptic Sampling – series of "snapshots" in time of the urban creek system using conventional tests (total coliform, fecal coliform, and enterococcus)**
  - **Phase 2: Detailed Bacterial Source Tracking (BST) Pilot Testing**
  - **Phase 3: Focused BST Sampling Program**
  - **Cost: $300,000**

# MST Questions

- **Is human marker present?**
- **Is human marker persistent?**
  - **Test for human genetic markers (Enteric Virus, Enterococcus, Polyoma Virus, Bacteroides)**
- **What is contribution from each source?**
  - **Isolate e.coli and use genetic fingerprint (ribotyping)**

# Human Markers

- Specific to Humans (no organism sharing)
- Highly Sensitive
- Presence / Absence
- Persistence
- Initially look at enteric viruses and enterococci

# Human Markers (PCR)

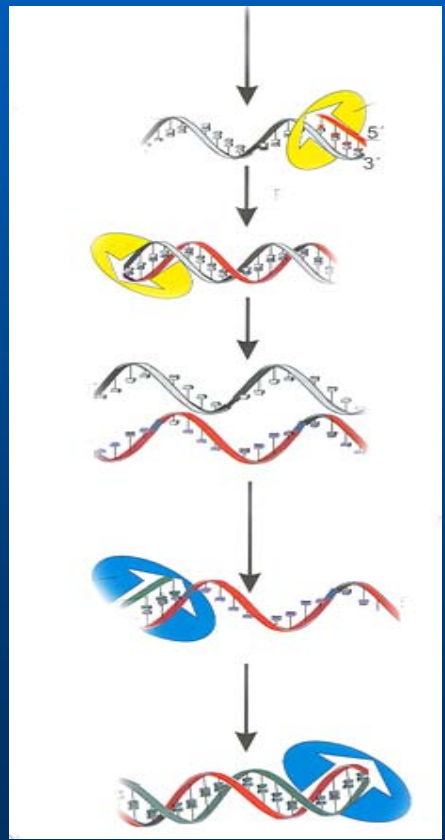
Add primer to isolate gene sequence of interest

Primer finds matching DNA sequence and elongates sequences.

Raise temperature to split elongated sequence DNA

Continue matching, elongation cycles until product is amplified

DNA from the sample

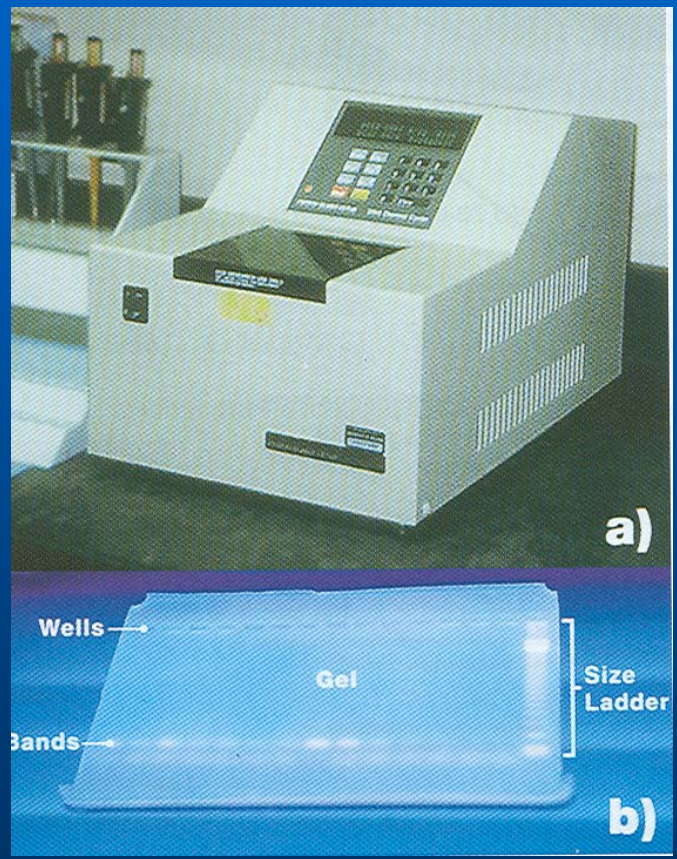





From Dr. Teplitski and Environmental Microbiology, Maier et al

# Human Markers

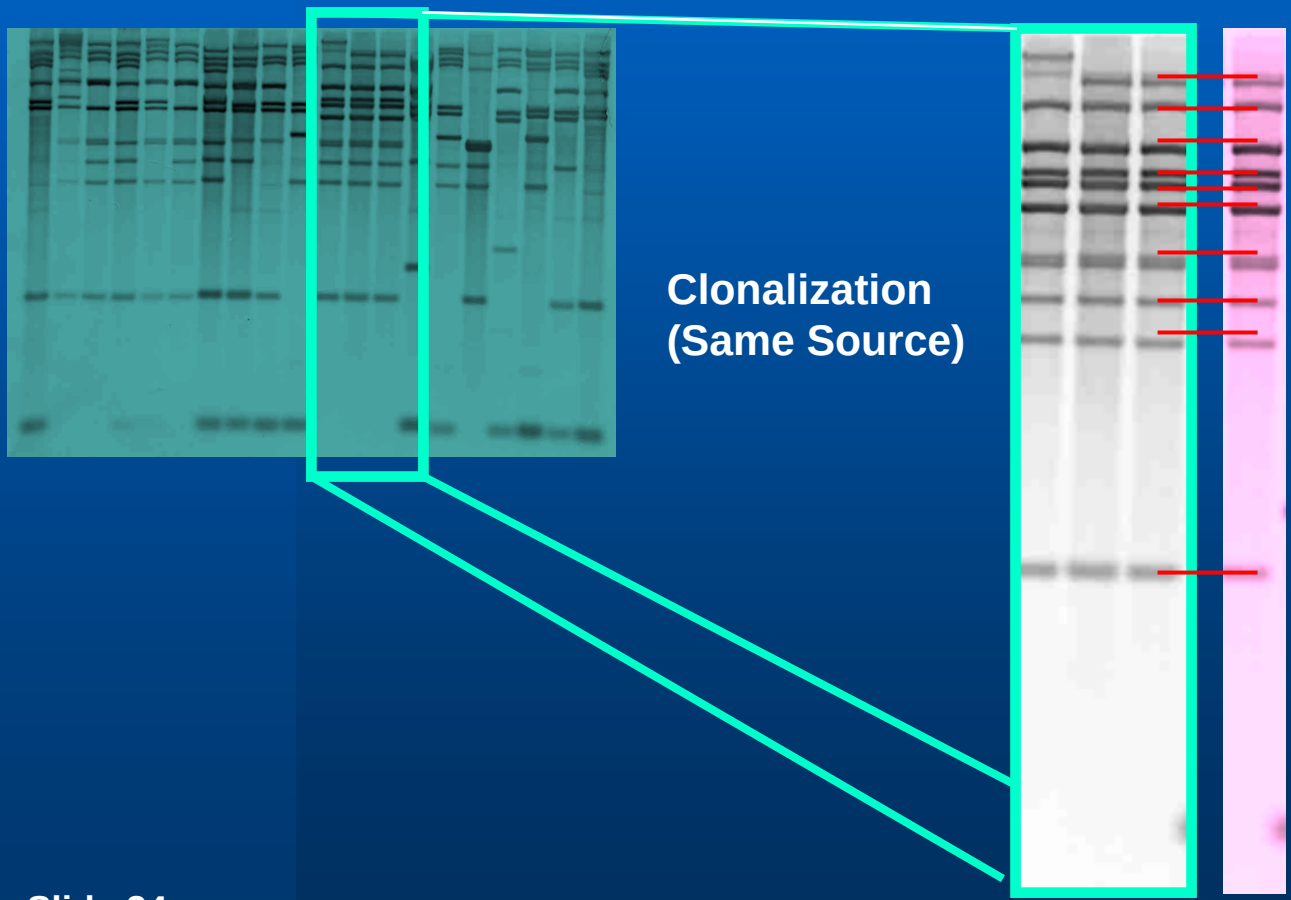
- Polymerase Chain Reaction (PCR)
- Detecting presence by multiplying DNA of marker
- If marker present in sample, PCR will multiply it $2^{35}$ times making detection very sensitive



# Genetic Fingerprinting (Ribotyping)

- Involves analyzing isolates of e. coli
- Provides snapshot of the sample's composition
- Compares isolates to library database for various species
- Computer matching is confirmed with human interpretation
- Analytes are not forced into categories – creates indeterminate category for shared bacteria and isolates not in database

# Genetic Fingerprinting



**Clonalization (Same Source)**

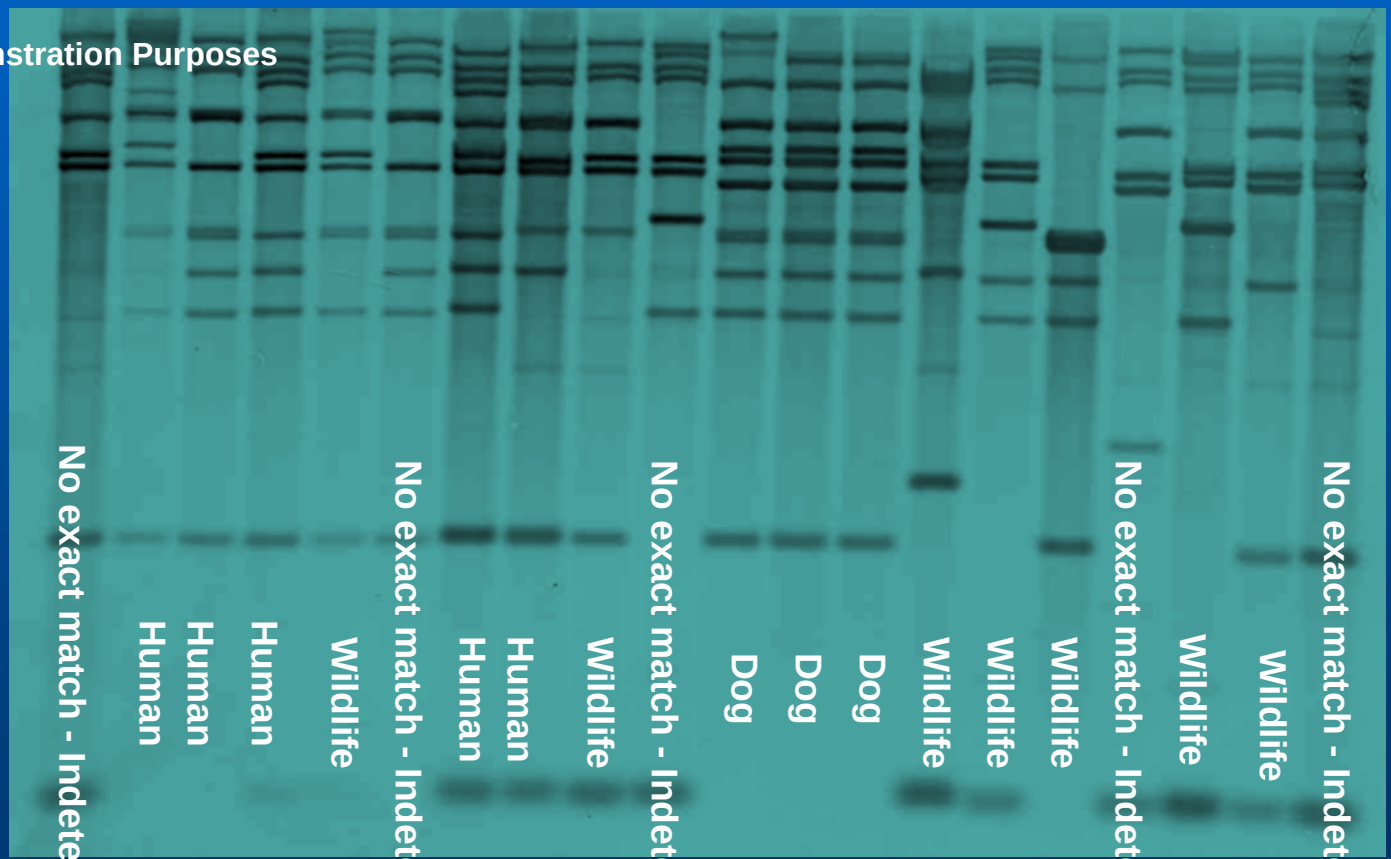
Database Archive Matching



**Source: Biological Consulting Services**

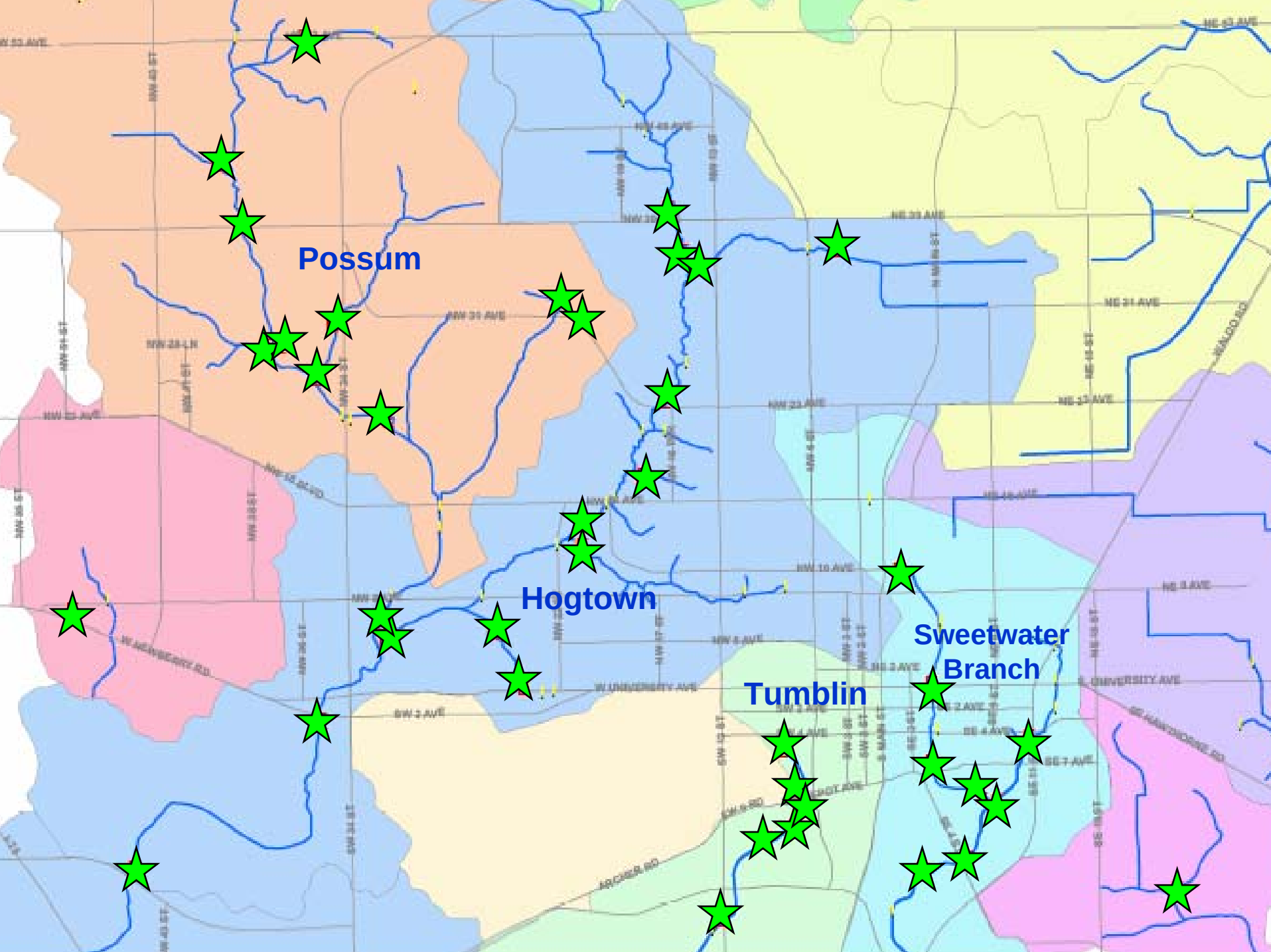
# Genetic Fingerprinting

For Demonstration Purposes



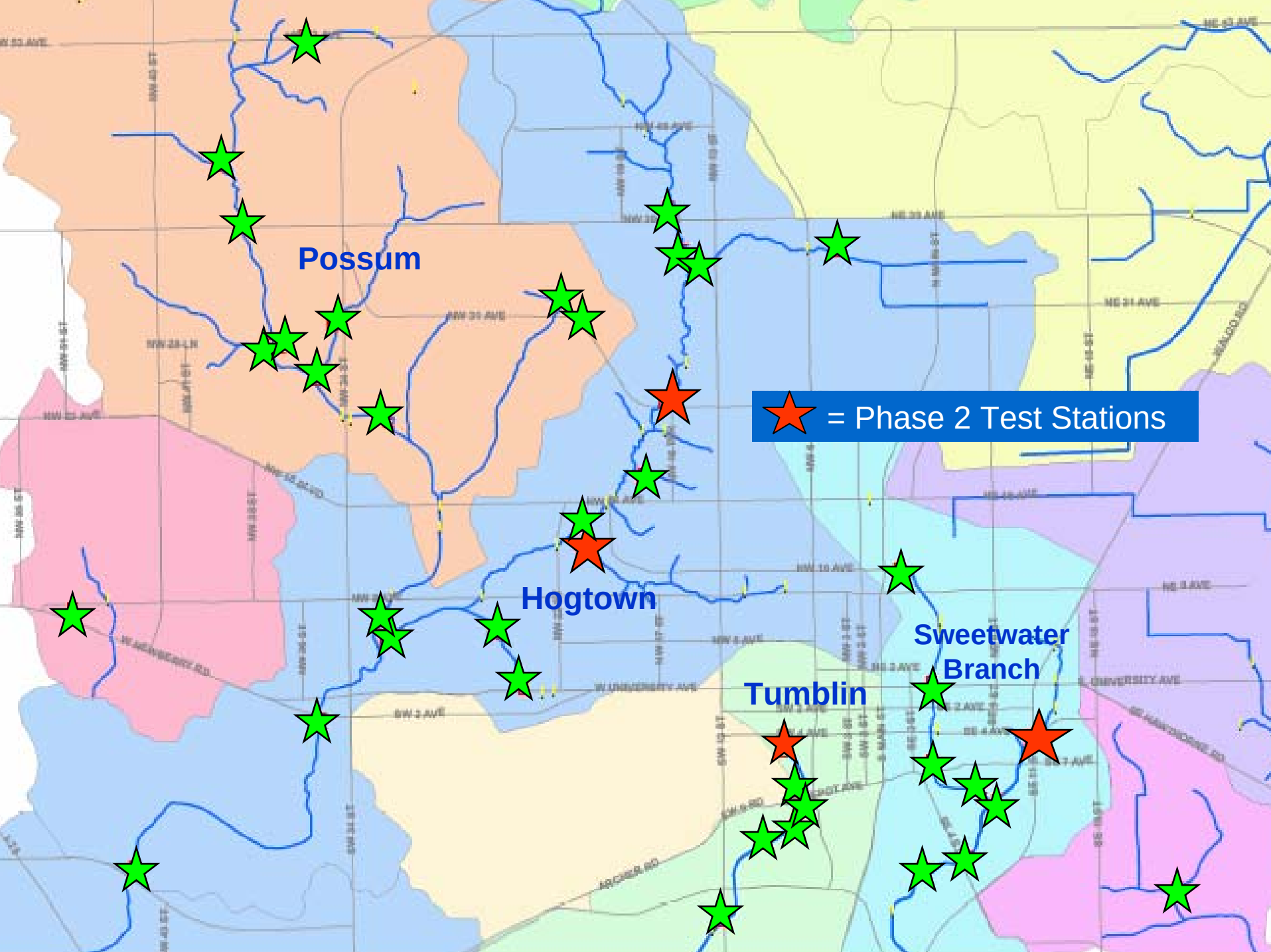
# Status

- **Phase 1: 6 of 6 sampling events completed (3 dry weather, 3 wet weather at 43 locations)**
- **Phase 2: Pilot Testing conducted at 3 sampling locations to test best methodologies for achieving study goals - Developed a hybrid approach**
- **Phase 3: 3 of 4 events complete. Stations coordinated with local DOH septic tank evaluation and County ambient monitoring program**


Possum
Hogtown
Tumblin
Sweetwater
Branch

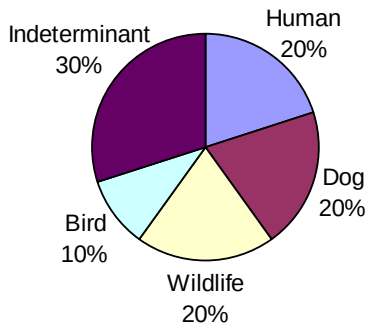
# Phase 1 Results

- Bacteria levels high everywhere most of the time under wet or dry conditions
- Fecal coliform to Enterococcus ratios not consistent with wastewater ratios
- Creeks are impacted from urban setting
- Fecal numbers and past history used to select 3 Phase 2 pilot stations


Possum
Hogtown
Tumblin
Sweetwater Branch
= Phase 2 Test Stations

# Phase 2 Results

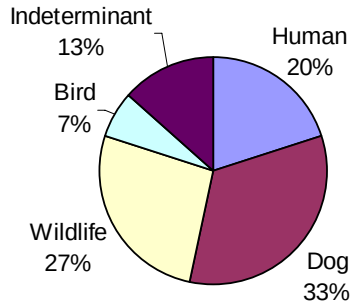
**S-7 Sweetwater branch**




Enteric Virus DNA Marker = Positive

Enterococcus DNA Marker = Positive

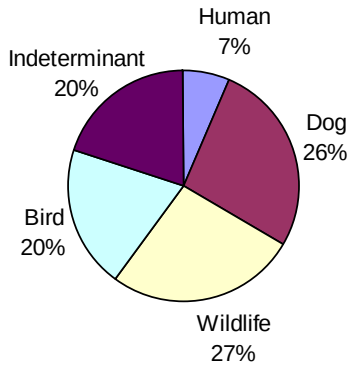
**S11 Hogtown Creek**




Enteric Virus DNA Marker = Negative

Enterococcus DNA Marker = Positive (Faint)

**S37 Hogtown Creek**




Enteric Virus DNA Marker = Negative

Enterococcus DNA Marker = Negative

# Phase 2 Results

- Decided on multiple method approach (ribotyping and presence/absence for human markers)
- Added additional human markers (polyoma virus, bacteroides) to address persistence
- Selected Phase 3 sites based on high fecal counts and input from others

# Phase 3 Results (through 3 events)

- Still interpreting data and completing analysis
- Locations with high fecal counts w/o human marker presence
- Indeterminants in ribotyping show limitations of methods, but indicate that majority likely from non-human sources
- Human markers are present in the urban creeks
- Contribution from each human source still not distinguishable, but results can be used to target assessments on based local conditions

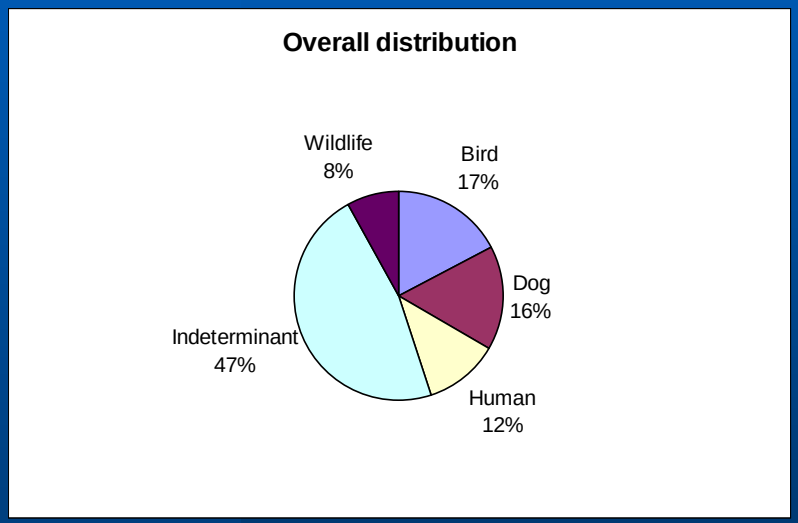
# Conventional vs. MST Example

## Possum Creek Examples

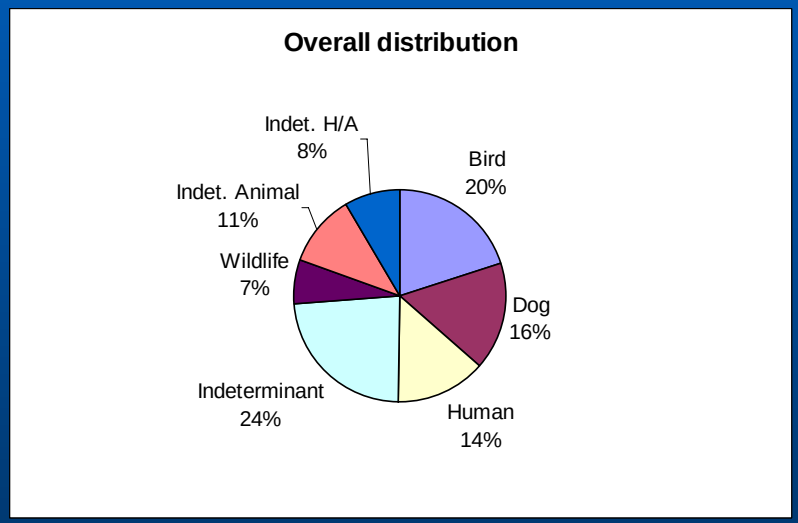
| Date | Station | Fecal Coliiforms MPN/100ml | Enterococci CFU/100ml | Human Virus Marker | Enterococcus Marker | Bacteriodes Marker |
|---|---|---|---|---|---|---|
| 6/21/2005 | S13P | 543 | 1400 | NEGATIVE | NEGATIVE | NEGATIVE |
| 10/3/2005 | S13P | 1100 | 1000 | NEGATIVE | NEGATIVE | NEGATIVE |
| 6/7/2005 | S23 | 130 | 2000 | NEGATIVE | NEGATIVE | NEGATIVE |
| 10/3/2005 | S107 | >2400 | 800 | NEGATIVE | NEGATIVE | NEGATIVE |
| 6/21/2005 | S41 | 1600 | 2000 | NEGATIVE | NEGATIVE | NEGATIVE |

# Ribotyping Results

## After Second Event



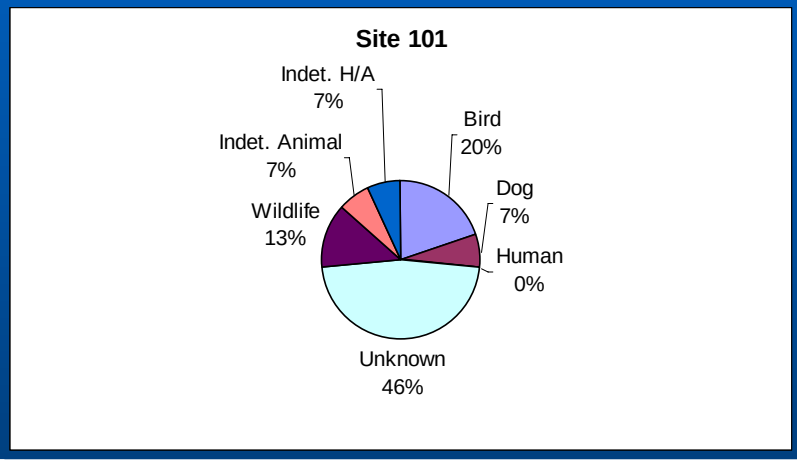

## After Third Event




Libraries have limits: database population and organism sharing.
From 2nd to 3rd event, tried to get a better understanding of indeterminants.

# Ribotyping vs Markers

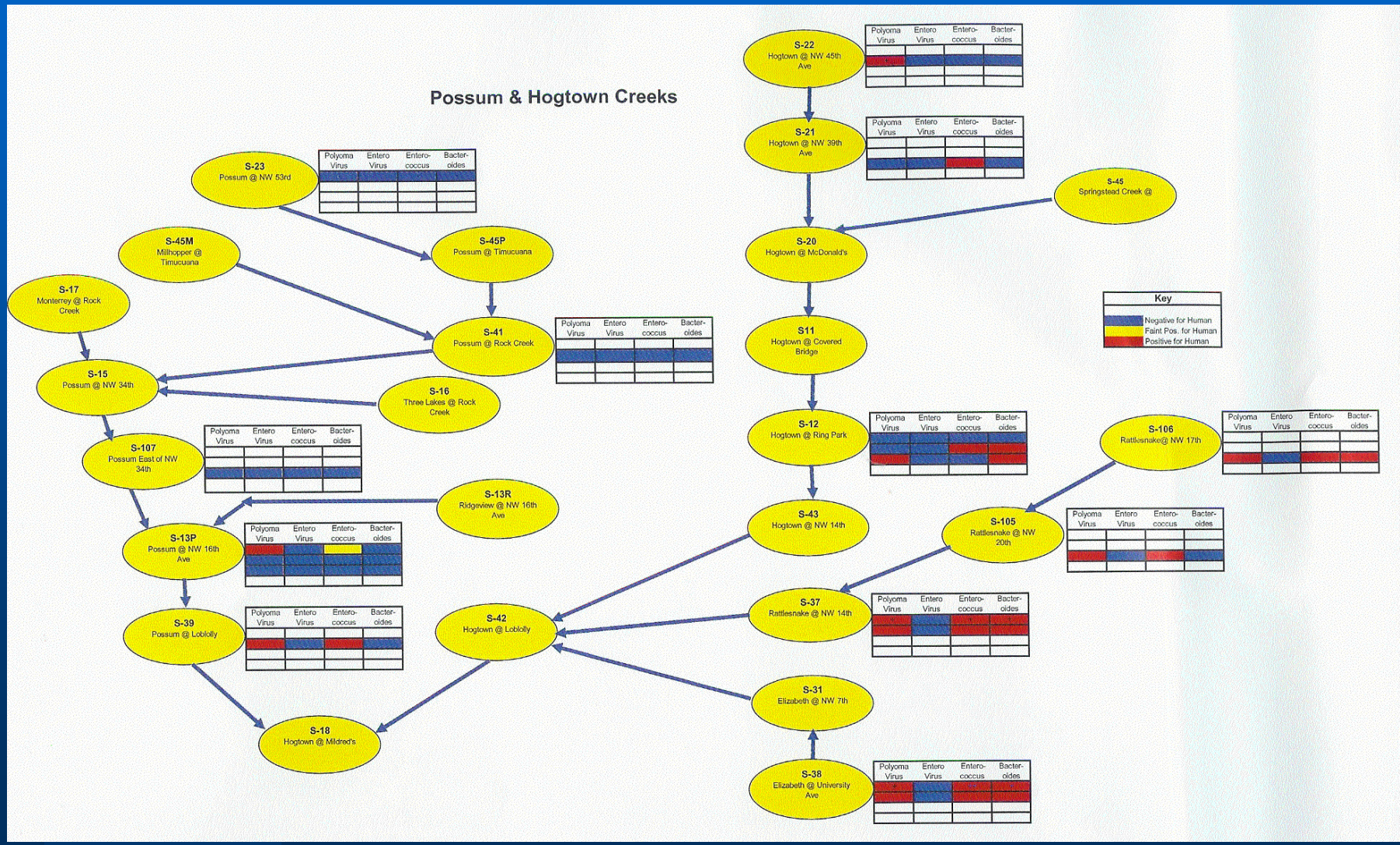
## Stormwater Outfall to Tumblin Creek



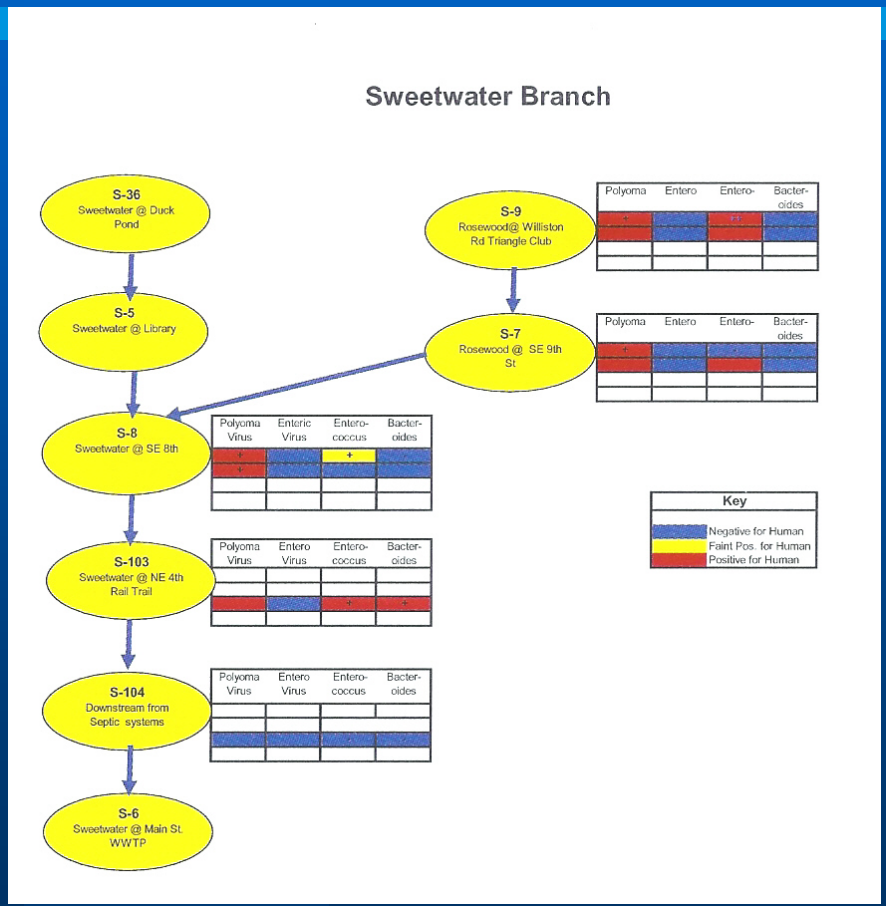

| Polyoma\ Virus | Entero Virus | Enterococcus | Bacteroides |
|---|---|---|---|
| + | | + | + |

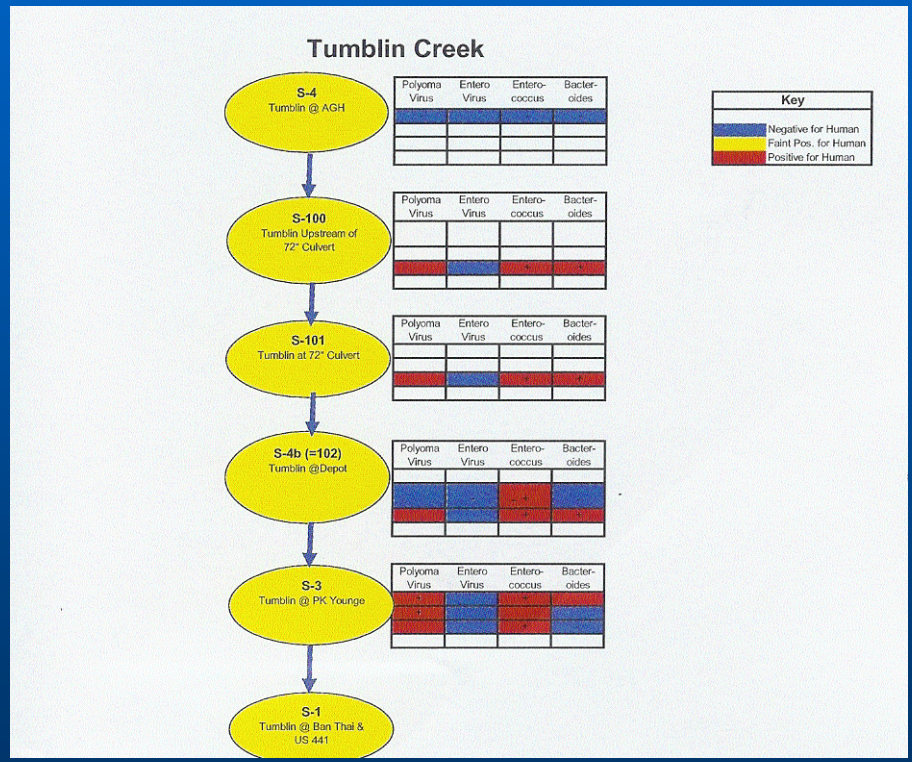
Fecal Coliforms > 2400 cfu / 100 ml

# Human Marker Results



# Human Marker Results



# Targeted Sample Sites

## Sites Downstream of Septic Tank Sites

**S107**

| Event | Polyoma Virus | Enteric Virus | Entero-coccus | Bacteroides |
|---|---|---|---|---|
| Dry 1 | | | | |
| Dry 2 | | | | |
| Wet 1 | | | | |

**S104***

| Event | Polyoma Virus | Enteric Virus | Entero-coccus | Bacteroides |
|---|---|---|---|---|
| Dry 1 | | | | |
| Dry 2 | | | | |
| Wet 1 | | | | |

* Phase 2 Pilot Test showing human markers

**S7**

| Event | Polyoma Virus | Enteric Virus | Entero-coccus | Bacteroides |
|---|---|---|---|---|
| Dry 1 | | | | |
| Dry 2 | | | | |
| Wet 1 | | | | |

# Targeted Sample Sites

## Sites Downstream of Stormwater Outfalls

**S101**

| Event | Polyoma Virus | Enteric Virus | Entero-coccus | Bacteroides |
|---|---|---|---|---|
| Dry 1 | | | | |
| Dry 2 | | | | |
| Wet 1 | | | | |

**S106**

| Event | Polyoma Virus | Enteric Virus | Entero-coccus | Bacteroides |
|---|---|---|---|---|
| Dry 1 | | | | |
| Dry 2 | | | | |
| Wet 1 | | | | |

# Targeted Sample Sites

## Sites Downstream of Urban Campsites

**S100**

| Event | Polyoma Virus | Enteric Virus | Entero-coccus | Bacteroides |
|---|---|---|---|---|
| Dry 1 | | | | |
| Dry 2 | | | | |
| Wet 1 | | | | |

**S102**

| Event | Polyoma Virus | Enteric Virus | Entero-coccus | Bacteroides |
|---|---|---|---|---|
| Dry 1 | | | | |
| Dry 2 | | | | |
| Wet 1 | | | | |

**S103**

| Event | Polyoma Virus | Enteric Virus | Entero-coccus | Bacteroides |
|---|---|---|---|---|
| Dry 1 | | | | |
| Dry 2 | | | | |
| Wet 1 | | | | |

# Urban Campsites

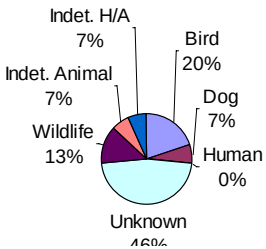
## Sweetwater Branch

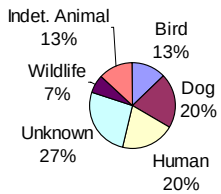
## Tumblin Creek

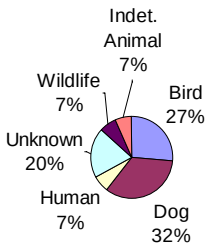
# Tumblin Creek

| Polyoma Virus | Entero Virus | Entero-coccus | Bacter-oides |
|---|---|---|---|
| | | | |

Phase 2 Event Negative for Human Markers







= MST Sample Sites

Urban Campsite Area

Stormwater Outfall

Private Collection Systems

Septic System with Significant Potential

GRU Collection System (Typ)

Draft

# Sweetwater Branch

= MST Sample Sites

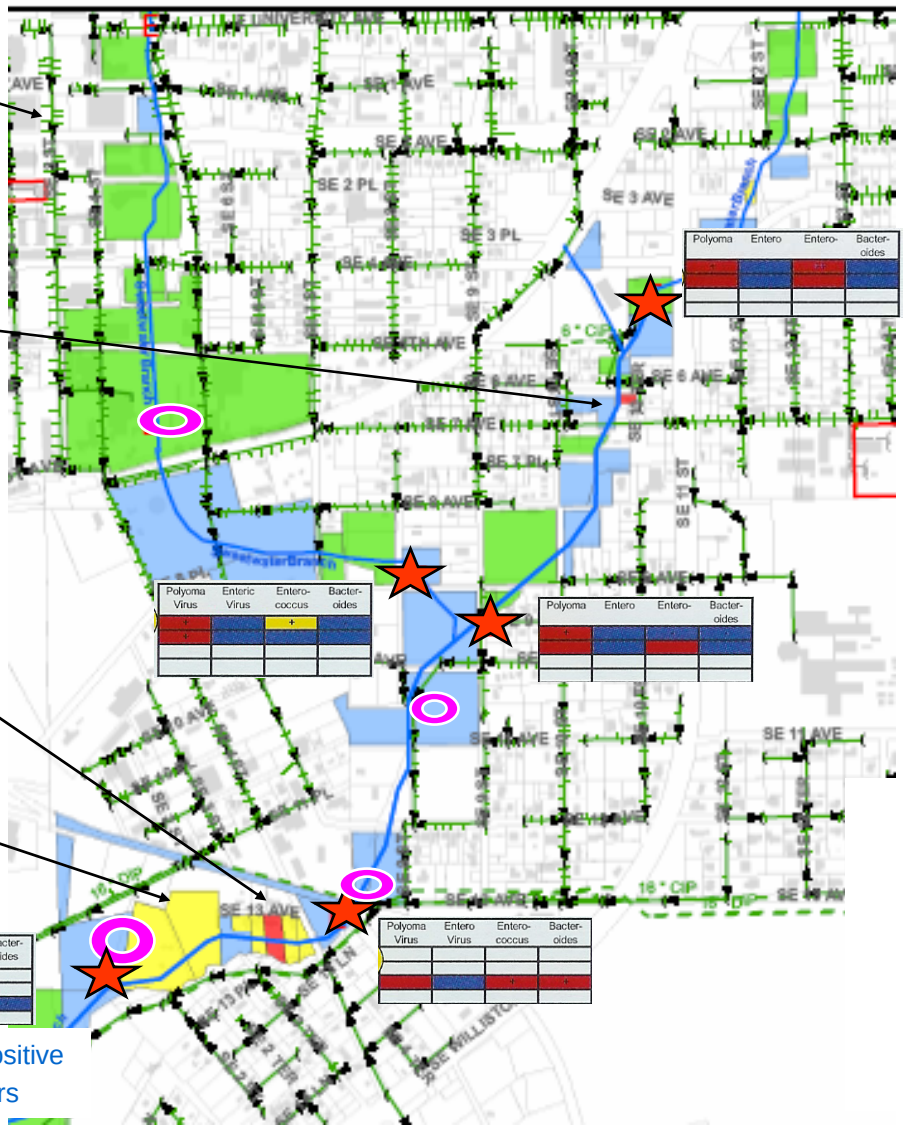
= Urban Campsite Area

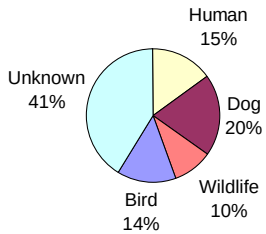
GRU Collection System (Typ)

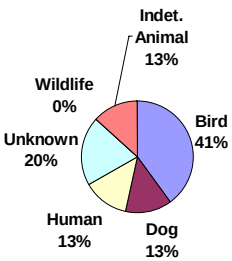
Septic System with Confirmed Impact

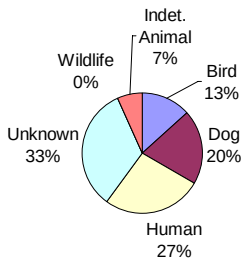
Septic System with Confirmed Impact (Red)

Septic Systems with Significant Potential (Yellow)




Phase 2 Event Positive for Human Markers

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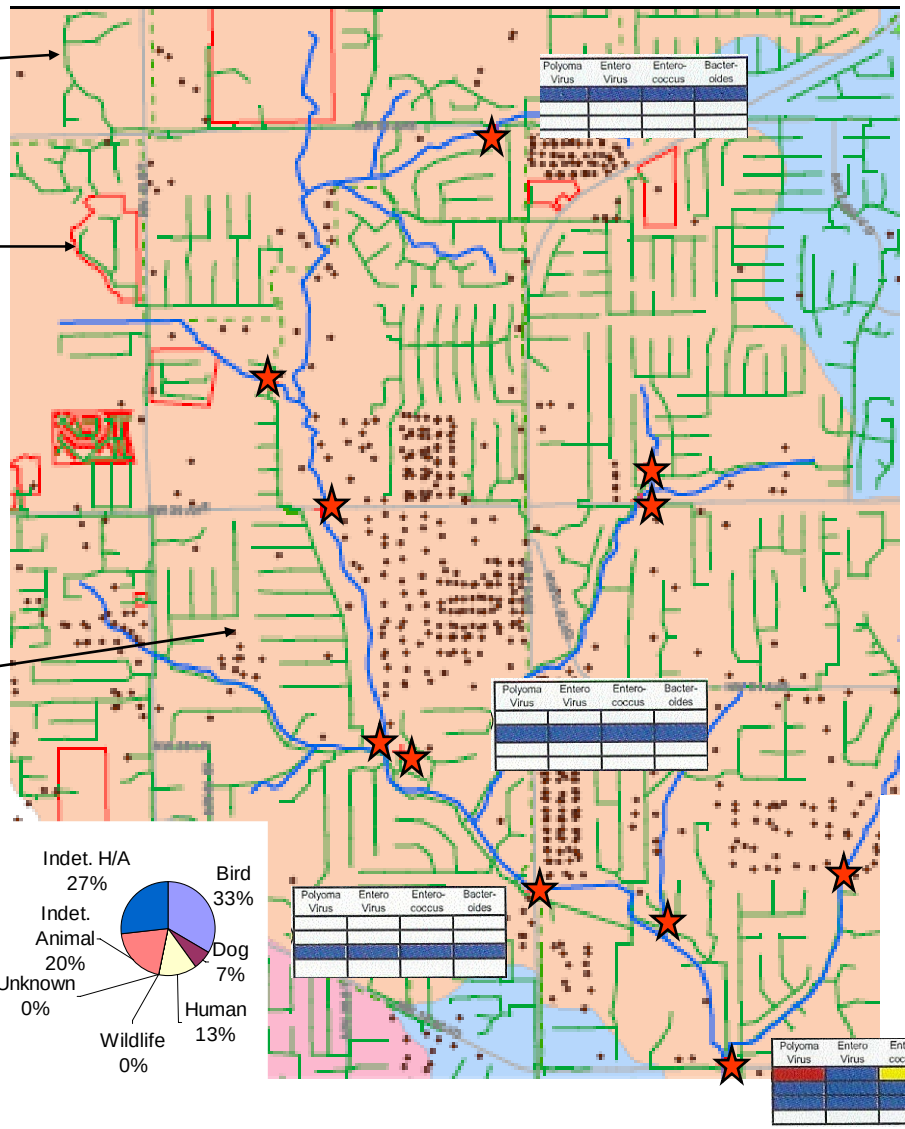
# Possum Creek

= MST Sample Sites

GRU Collection System (Typ)

Private Collection Systems

Unverified Septic Tanks

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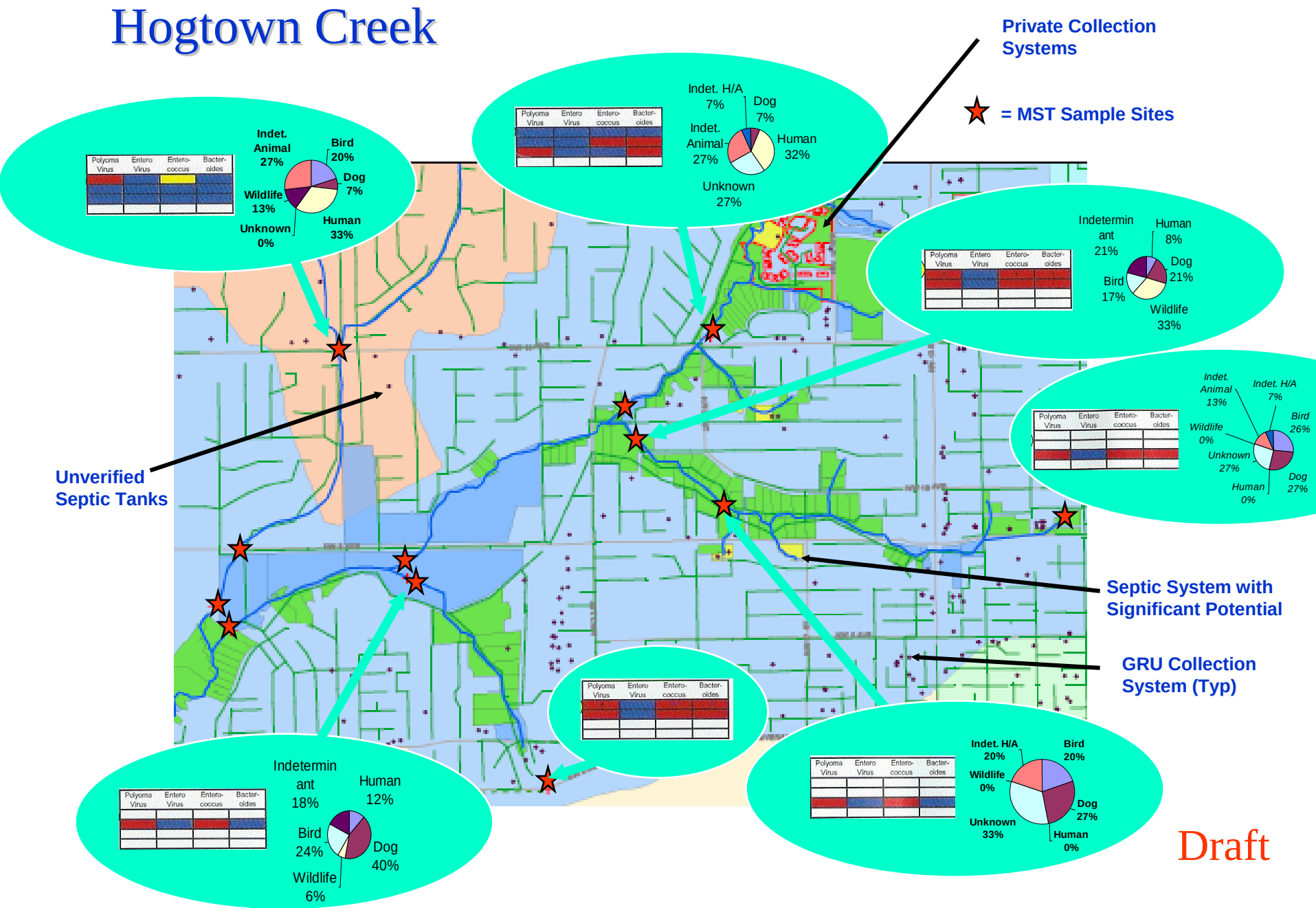
# Hogtown Creek




Draft

# Conclusion

- **Advanced methods make solutions more available, but they don't answer all the questions**
- **Results can be used to target assessments based on local conditions**
- **Each stakeholder will have to do their part**
- **Animal contribution of bacteria will likely need to be addressed to achieve water quality standards based on fecal coliforms**

# Acknowledgements


- **BST WORK**
  - **Bill Dunn and Martha Klein, CH2M Hill (Gainesville)**
  - **Troy Scott, Biological Consulting Services of North Florida (Gainesville)**