History of Microbial Source Tracking Use in Texas

Terry Gentry

Department of Soil and Crop Sciences
Texas A&M University

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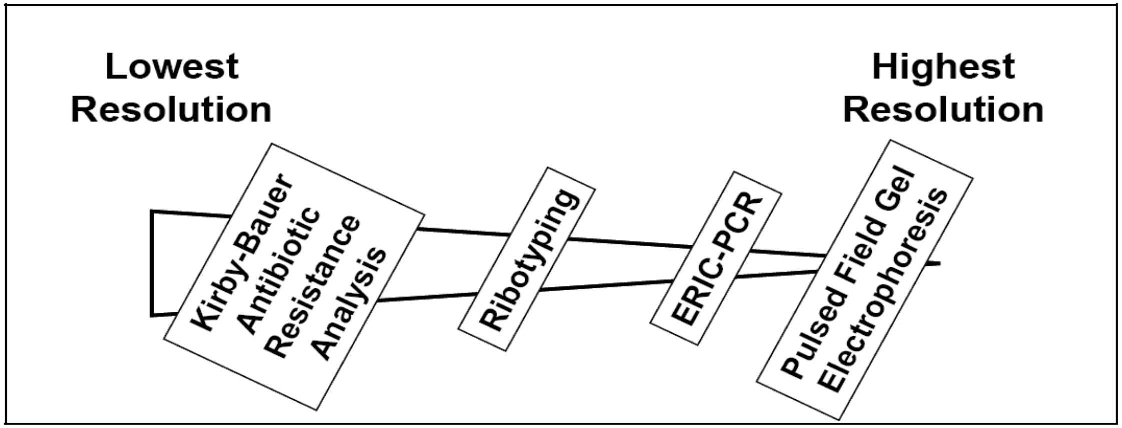
Texas A&M AgriLife Research

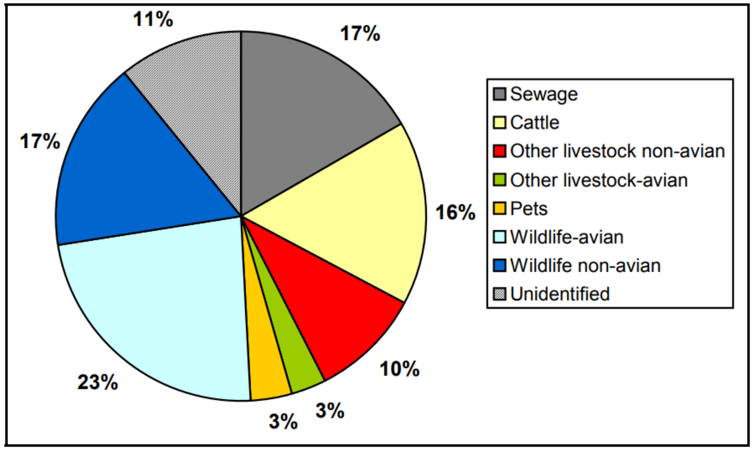
Texas Water Resources Institute
make every drop count

UTHealth | School of Public Health
The University of Texas
Health Science Center at Houston

Lake Waco/Belton Project

- Initiated Sep. 2002 with funding from TSSWCB
- 4-method composite performed better than individual methods
- 2-method composites appeared promising
  - ERIC-ARA = lower cost but more processing
  - ERIC-RP = higher cost but automated





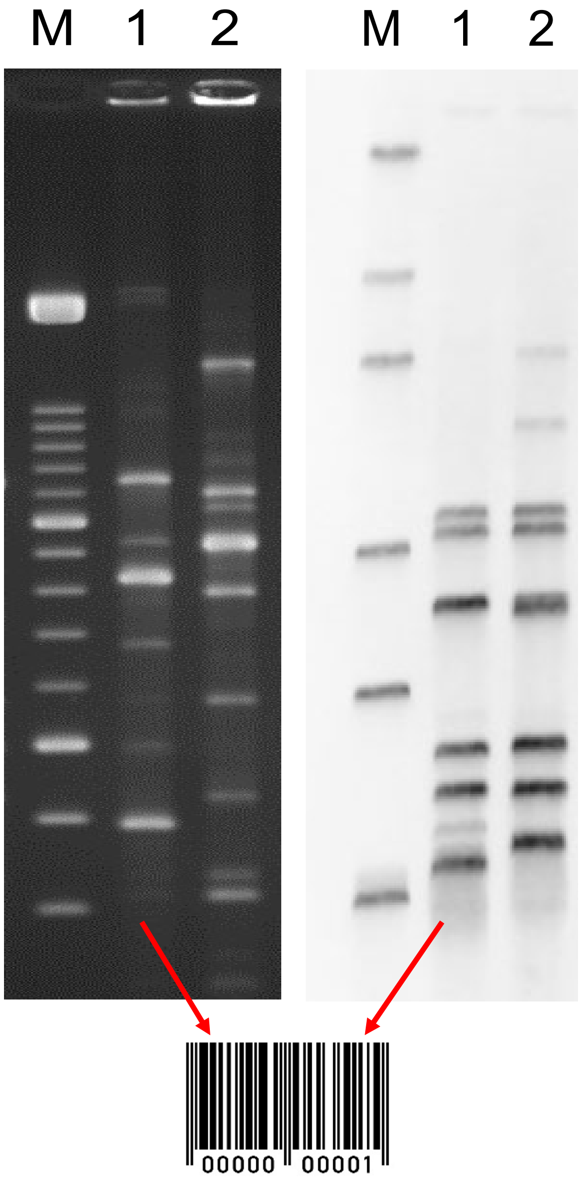
Bacteria TMDL Task Force

- Established in 2006 by the Texas Commission on Environmental Quality (TCEQ) and Texas State Soil and Water Conservation Board (TSSWCB)
- 7 members and 50 expert advisors
- Tasks included:
  - Evaluating a variety of models and bacteria source tracking (BST) methods available for developing TMDLs and I-Plans, and recommending under what conditions certain methods are more appropriate

Bacteria TMDL Task Force

- Methods evaluated include ERIC-PCR, Ribotyping, PFGE, KB-ARA, CSU and Bacteroidales PCR
- Recommended:
  - Library independent methods like Bacteriodales PCR for preliminary qualitative analyses (Tier 2)
  - Library-dependent methods if more quantitative data needed (Tier 3)
  - Confirmed ERIC-RP as a recommended method

Library-Dependent MST Methods



Methods:

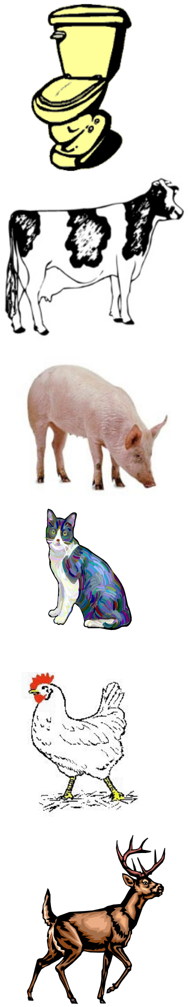
- DNA fingerprinting
  - Enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction (ERIC-PCR)
  - RiboPrinting® (RP)

Advantages/Disadvantages:

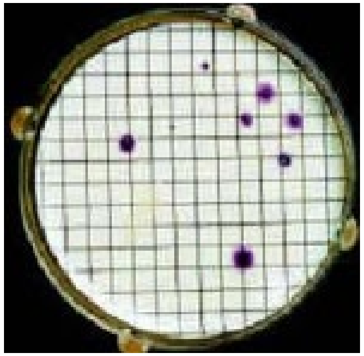
- More discriminating
- Allows ranking of sources
- Relatively expensive

Development of Texas *E. coli* BST Library

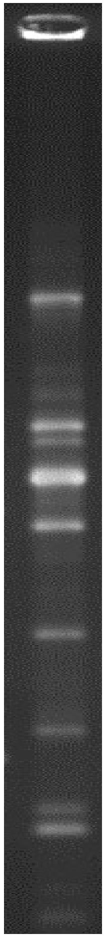
Sources



Isolate → *E. coli*



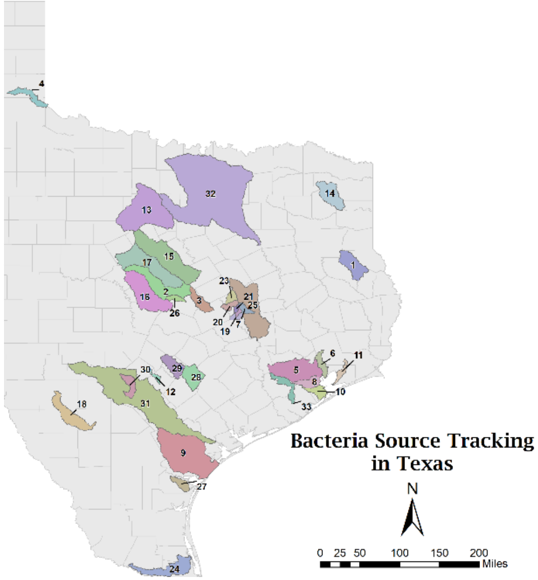
DNA → Fingerprint



Add to → Library

Texas *E. coli* BST Library (v. 03-20)

- **Contains 1,912 *E. coli* isolates from 1,653 different human and animal samples**
- **Developed by collecting over 4,000 domestic sewage, wildlife, livestock, and pet fecal samples and screening over 7,000 isolates for clones and host specificity**
- **Samples from >20 watersheds across Texas for MST including:**
    - **Plum Creek**
    - **San Antonio**
    - **Lake Granbury**
    - **Oyster Creek / Trinity River**
    - **Waco / Belton Lake**
    - **Little Brazos River Tributaries**
    - **Attoyac Bayou**



- **Additional isolates being added from ongoing and future MST projects in other areas of Texas**

Three-way v. Seven-way Split of Results

- Using the results
  - Is it from human sources?
  - Is it from livestock?
  - Is it from wildlife?
- Biology
  - Large variety of wildlife
  - Geographical and temporal differences
  - Cosmopolitan strains
- Statistics
  - Number of isolates collected
  - May only use three-way split for limited studies

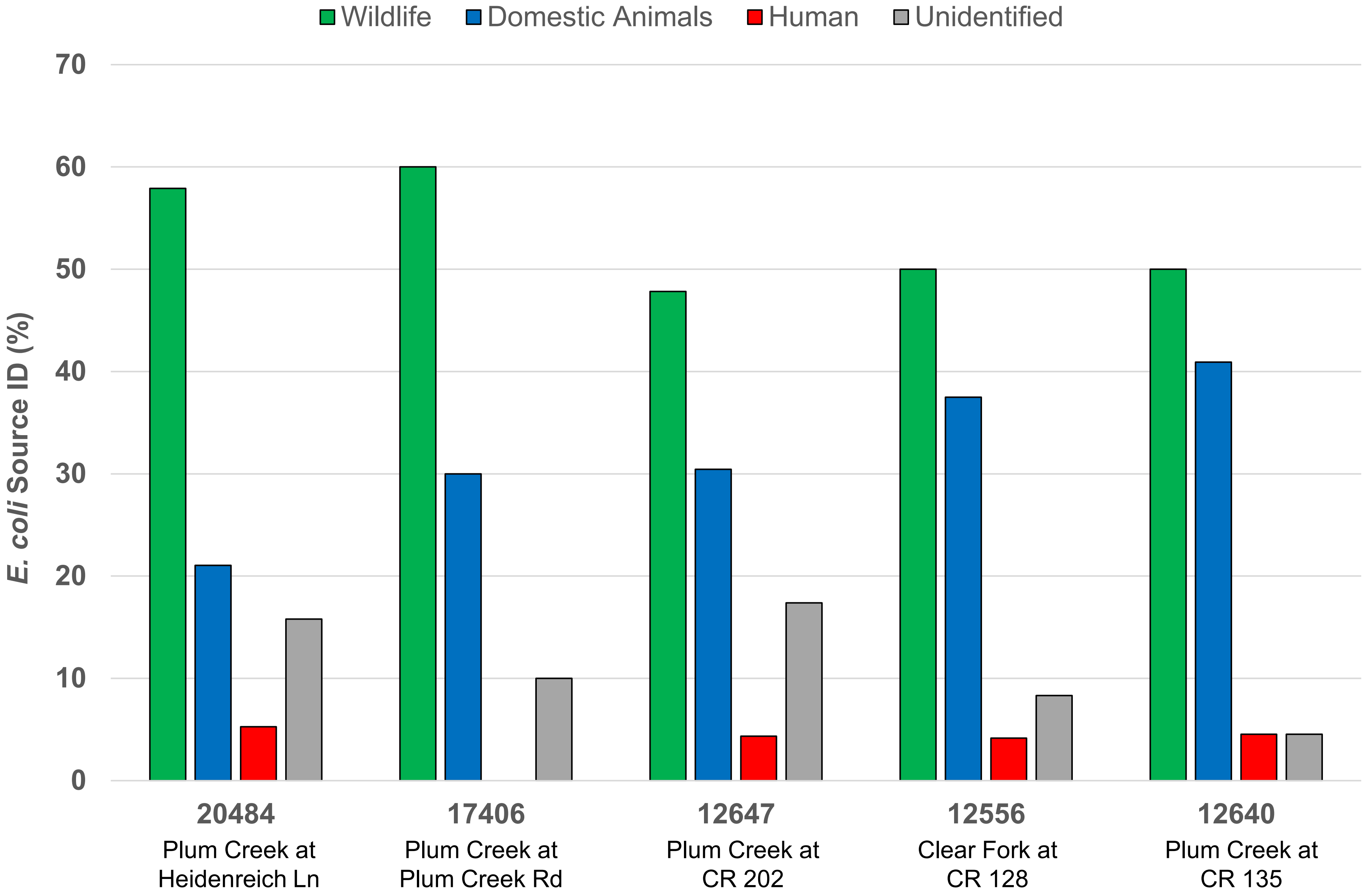
(1) Human
(2) Livestock & Pets
(3) Wildlife

vs.

Human (1)
Pets (2)
Cattle (3)
Other livestock, avian (4)
Other livestock, non-avian (5)
Wildlife, avian (6)
Wildlife, non-avian (7)

Plum Creek MST Results

5 Sampling Sites (3-Way Split)

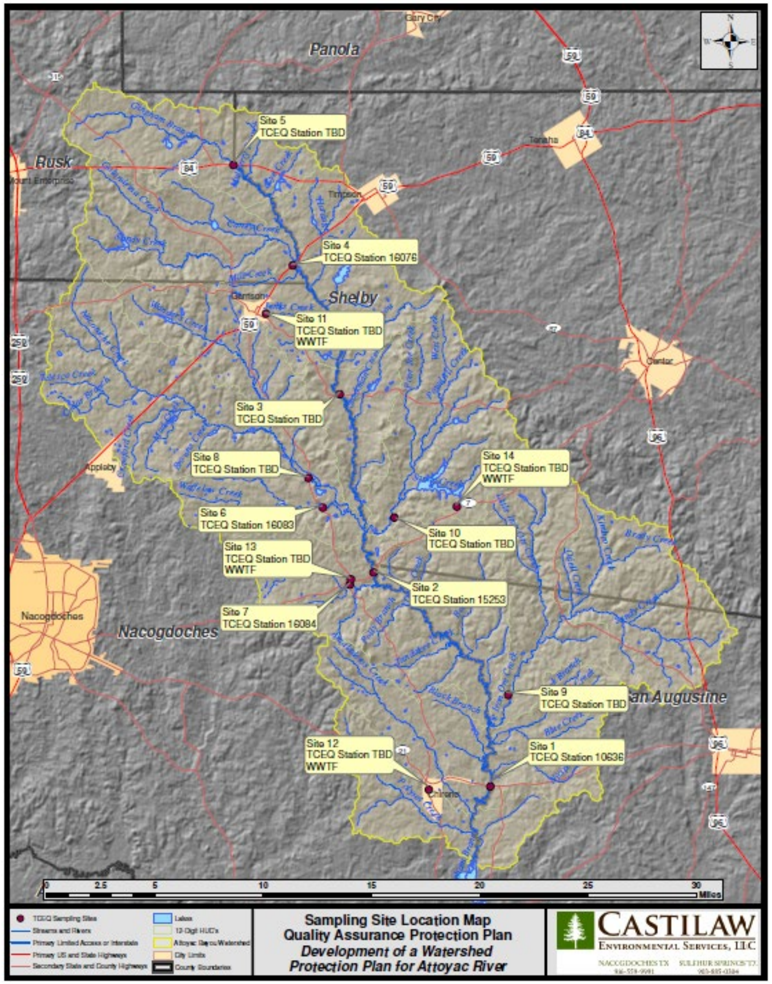


Library-Independent MST in Texas

- Several watersheds in Texas
  - Lake Granbury (UT)
  - Buck Creek (UT)
  - Little Brazos River Tributaries (TAMU)
  - Big Cypress (TAMU)
  - Attoyac Bayou (TAMU)
  - Leona River (TAMU)
  - Galveston (TAMU)
  - Beaumont (TAMU)
  - Nueces County (TAMU)
- Edge-of-field runoff (BMP evaluation)
  - Dairy manure (UT)
  - Grazing systems (TAMU)
- Oklahoma City (UT; waterborne disease outbreak)

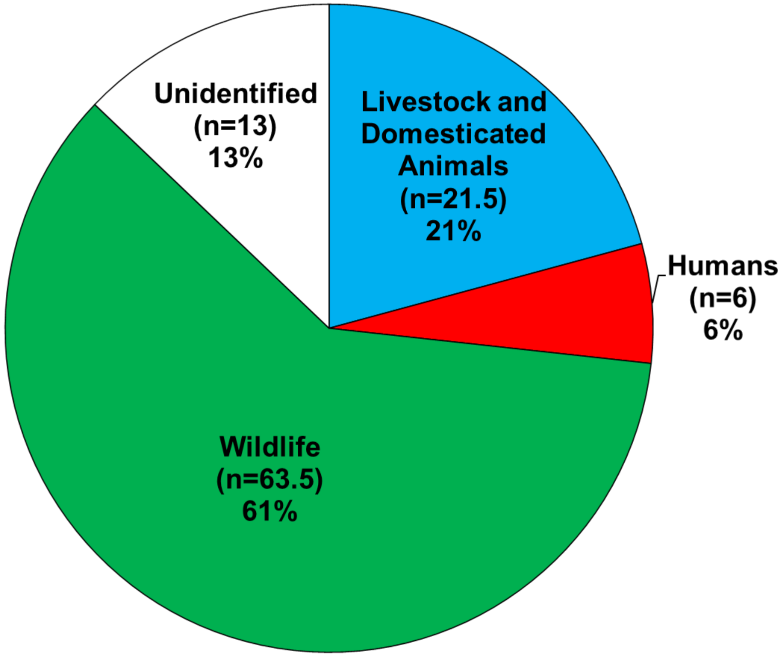
Attoyac Bayou MST

- **Limited library-dependent**
  - **Analyze *E. coli* from ~100 water samples from across the study area using both ERIC-PCR and RP fingerprinting**
- **Library-independent**
  - **Analyze ~250 water samples**
  - ***Bacteroidales* PCR (+/-)**
    - **Human (HF183)**
    - **Ruminant (CF128F)**
    - **Hog (PF163F)**
    - **Horse (Ho597F)**

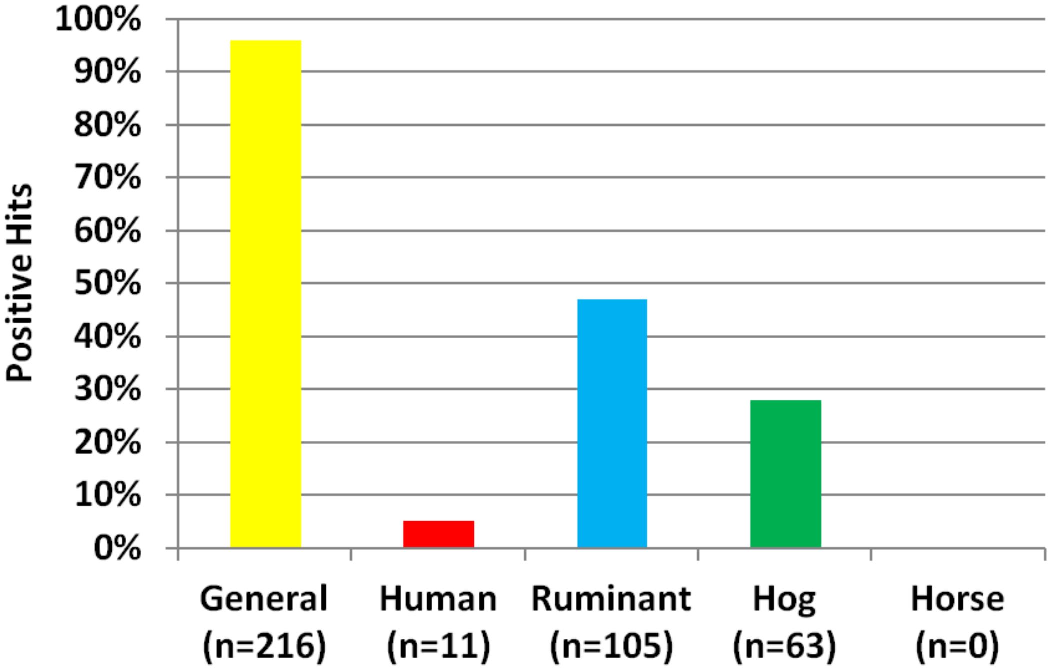


Attoyac Bayou *Bacteroidales* Base Flow Samples (n=225)

Library Dependent

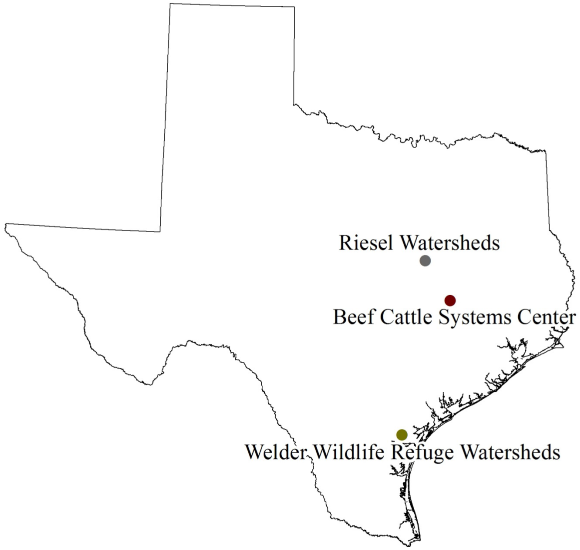


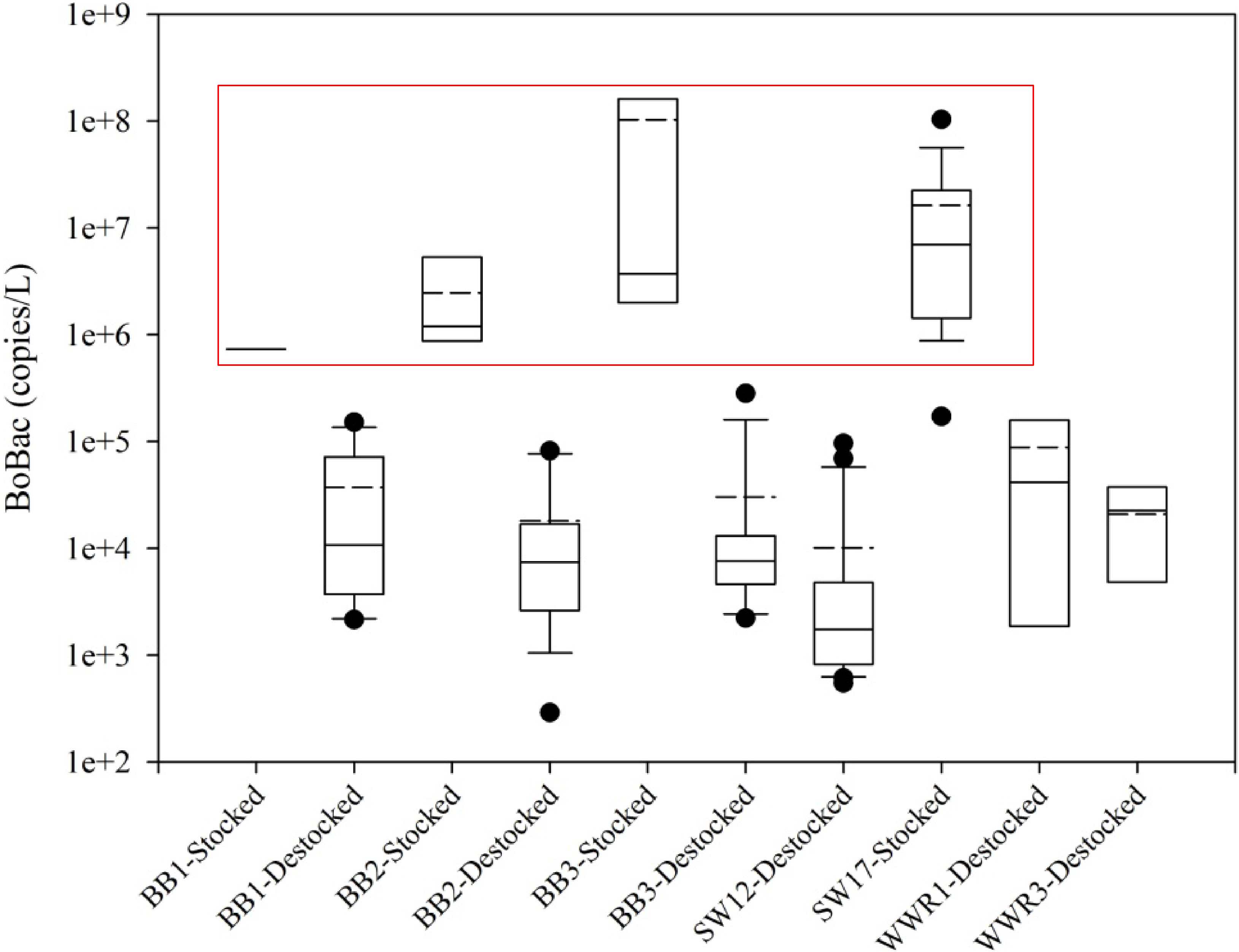
Library Independent

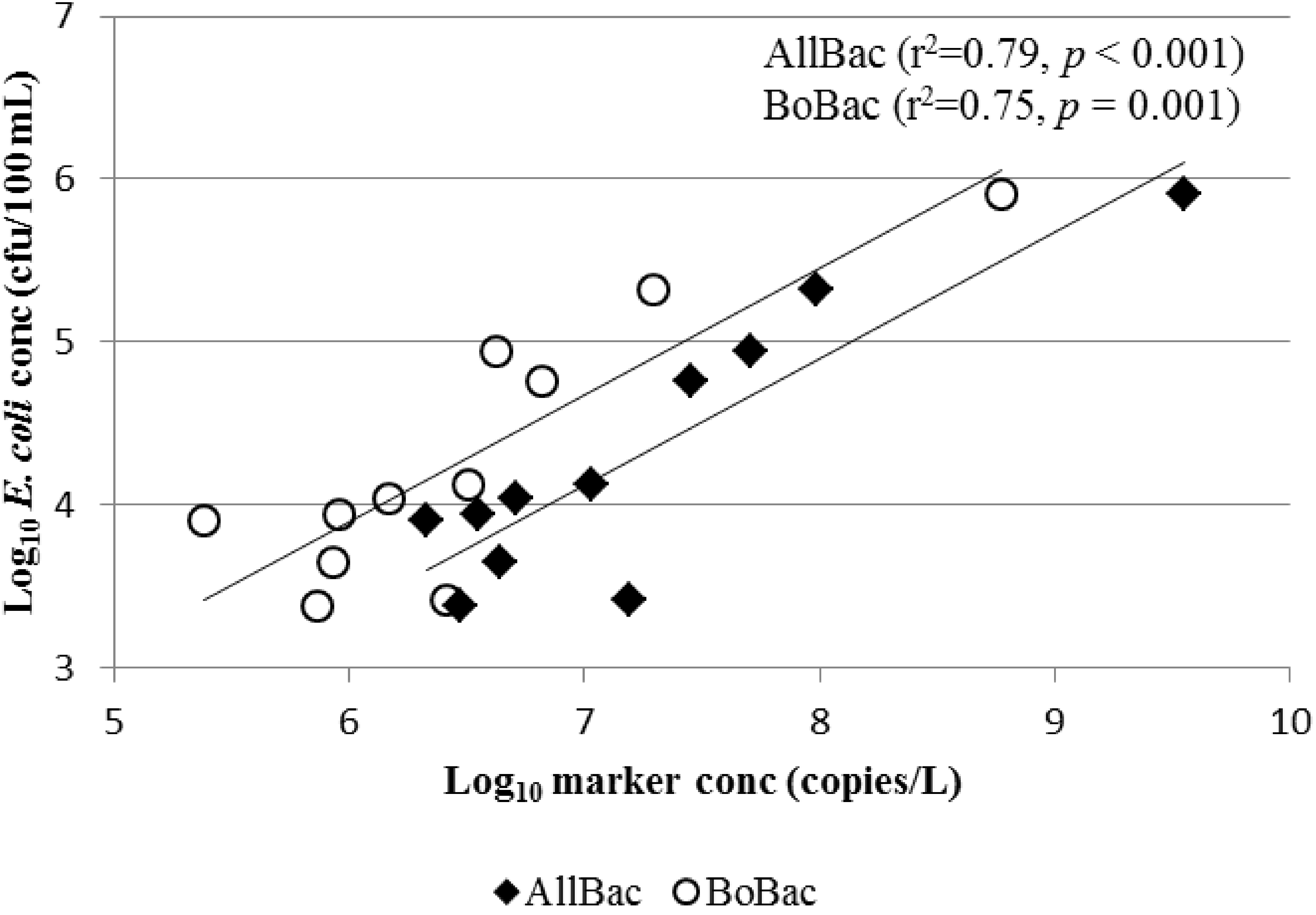


Grazing Management Evaluation

- **Runoff collected over three years**
- ***E. coli* - EPA Method 1603**
- ***Bacteroides* (Layton et al., 2006)**
  - **Total *Bacteroides* spp. (AllBac)**
  - **Bovine-associated *Bacteroides* spp. (BoBac)**



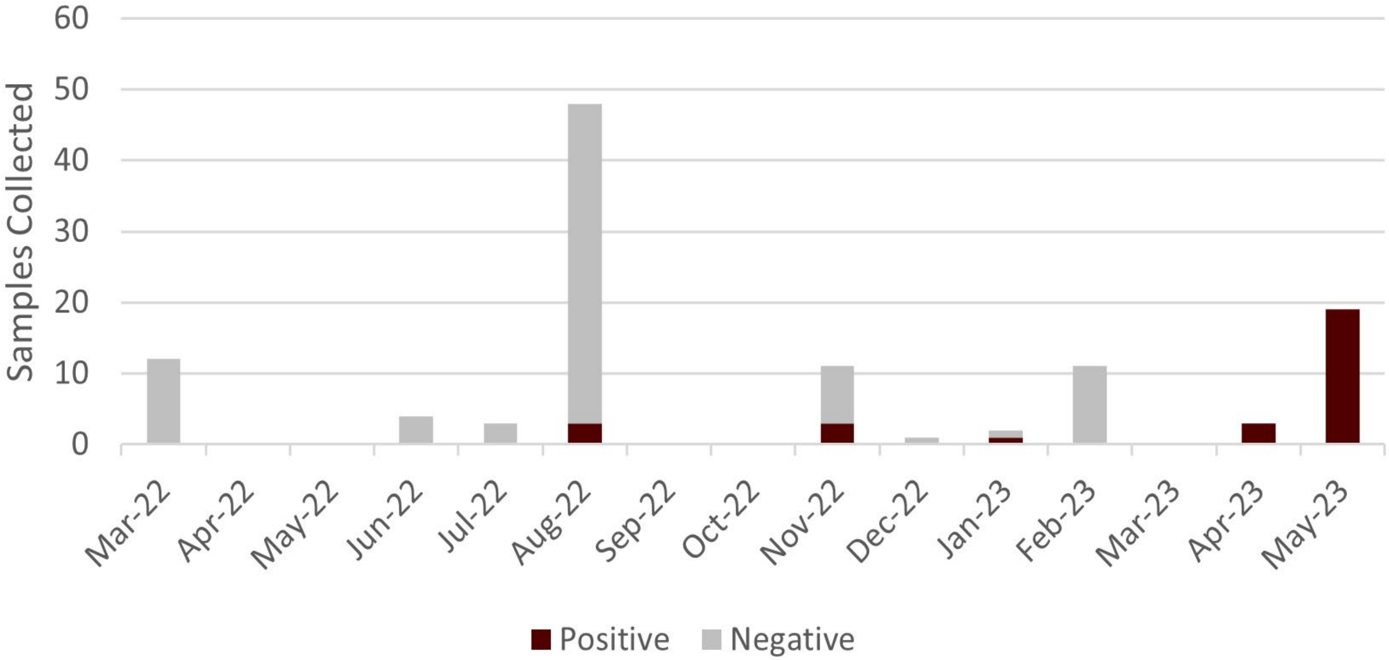

1e+9
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
BoBac (copies/L)
BB1-Stocked
BB1-Destocked
BB2-Stocked
BB2-Destocked
BB3-Stocked
BB3-Destocked
SW12-Destocked
SW17-Stocked
WWR1-Destocked
WWR3-Destocked


AllBac (r^2=0.79, $p < 0.001$)
BoBac (r^2=0.75, $p = 0.001$)
7
6
5
4
3
5
6
7
8
9
10
Log$_{10}$ *E. coli* conc (cfu/100 mL)
Log$_{10}$ marker conc (copies/L)
◆ AllBac
○ BoBac

Galveston MST

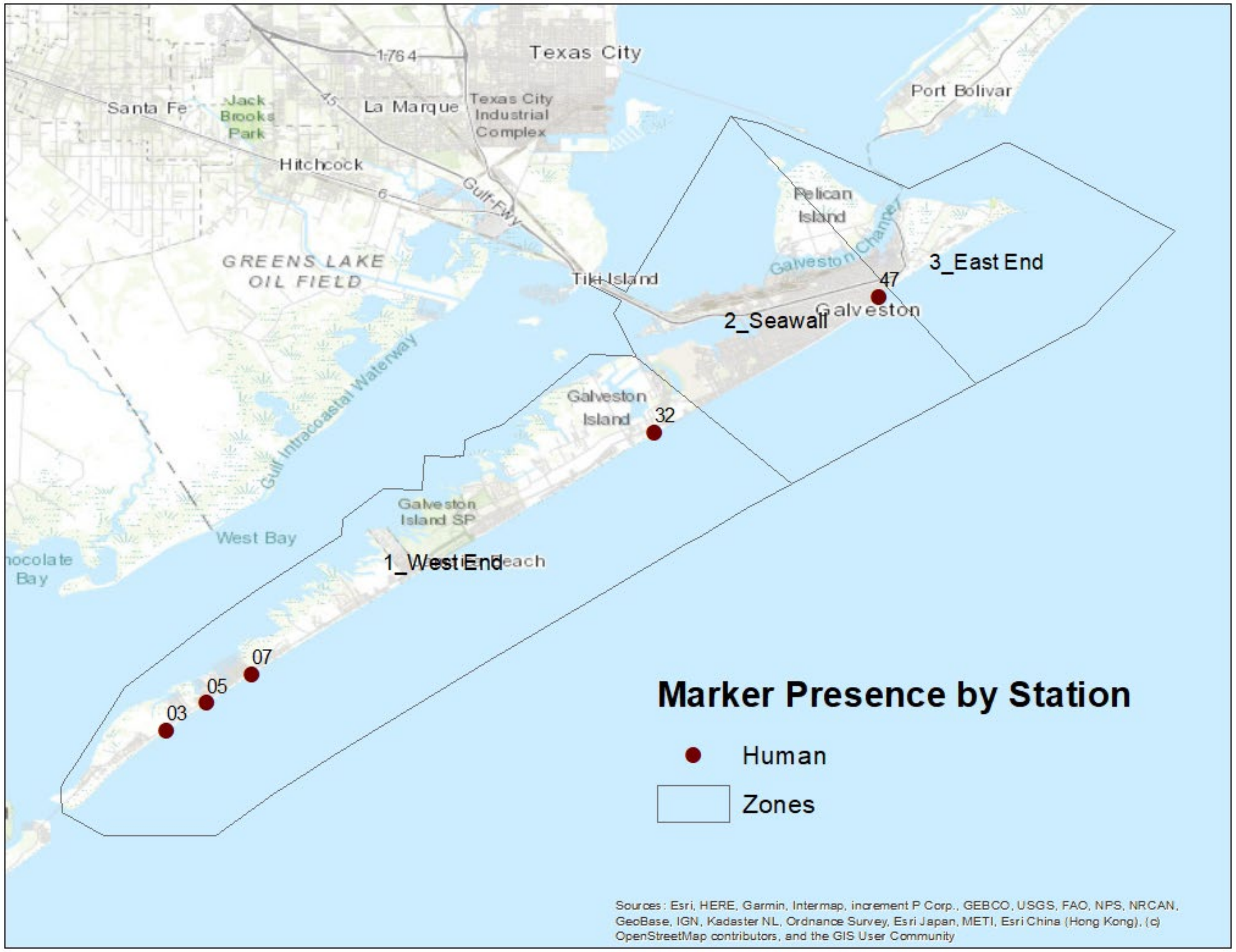
- TBW samples with enterococci levels >104 MPN/100 mL
- A total of 114 samples from 2022-2023
  - Quantitative PCR markers for specific sources
    - Human (HF183)
    - Dog (DogBact)
    - Gull (LeeSeaGull)
  - Microbiome-based BST (select 2022 samples)
    - Human (WWTF & Septic)
    - Dog & Coyote
    - Gull

Overall Marker Results



- ~25% of samples positive for at least one of the source markers
- Markers more prevalent in 2023 samples

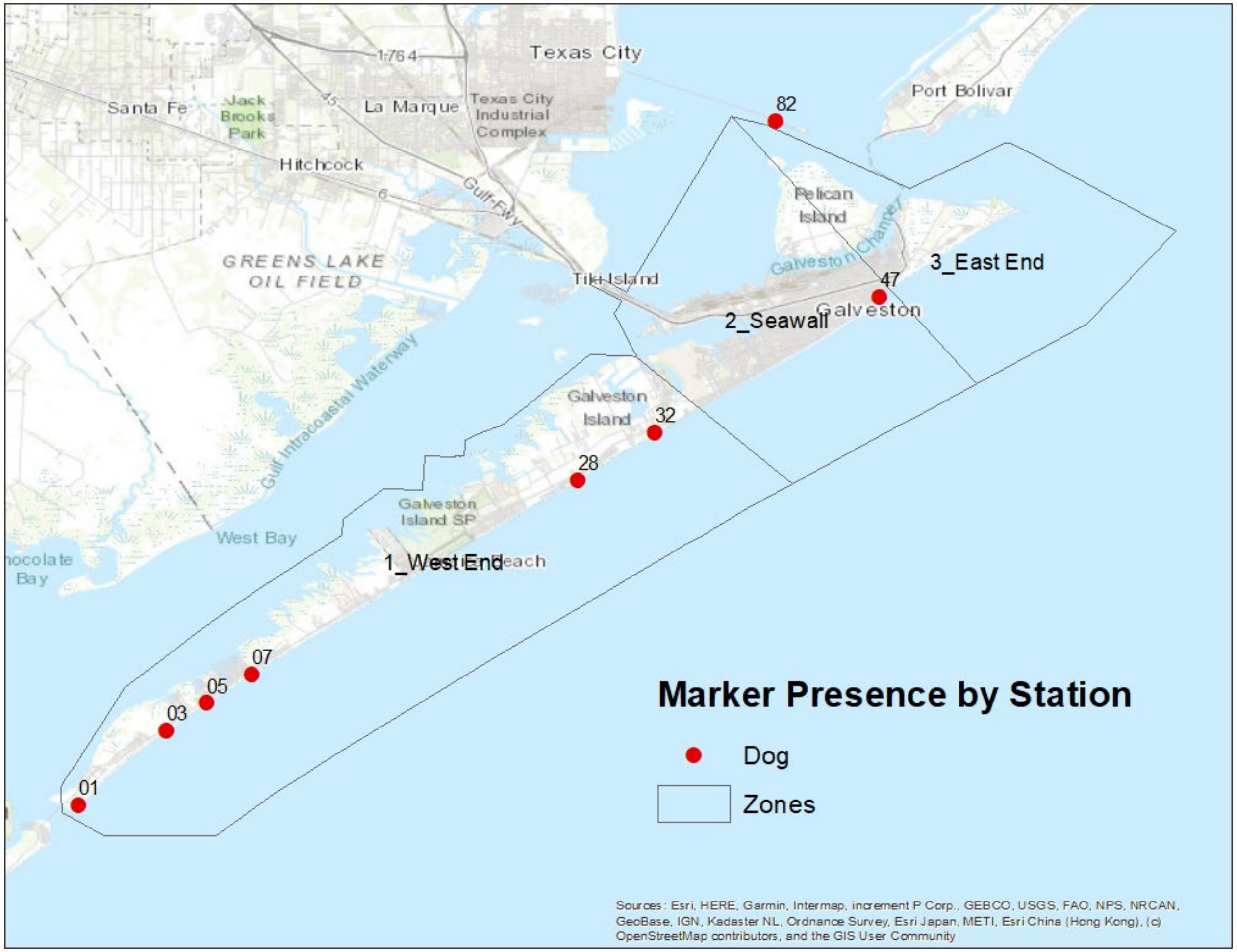
Human Marker



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

- **Detected in 4% of samples**
- **Highest level (1,138 copies/100 ml) in GAL032 sample from 5/23/2023**

Dog Marker



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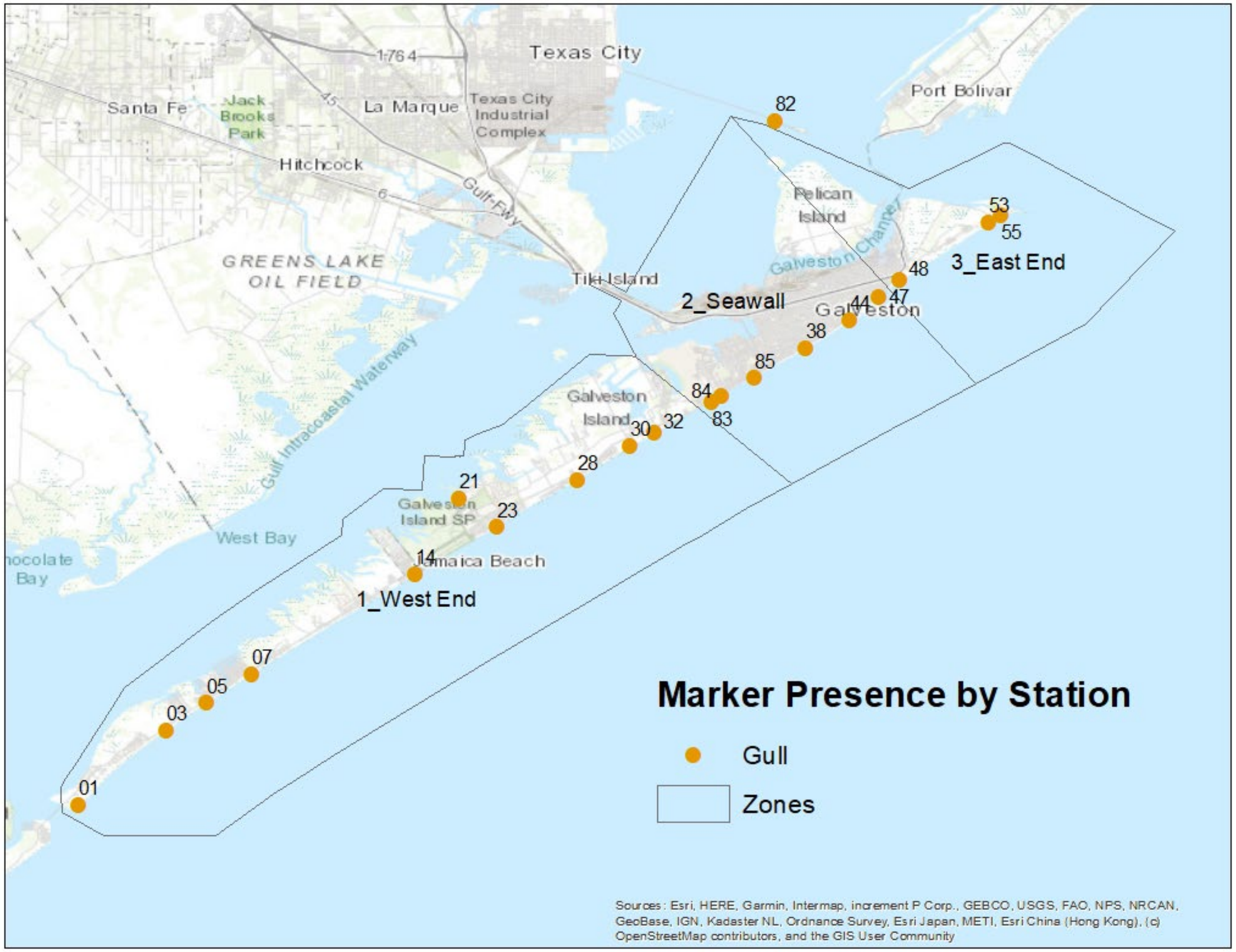
- Detected in 7% of samples
- Wider distribution but tended to overlap with human marker-positive sites

Gull Marker



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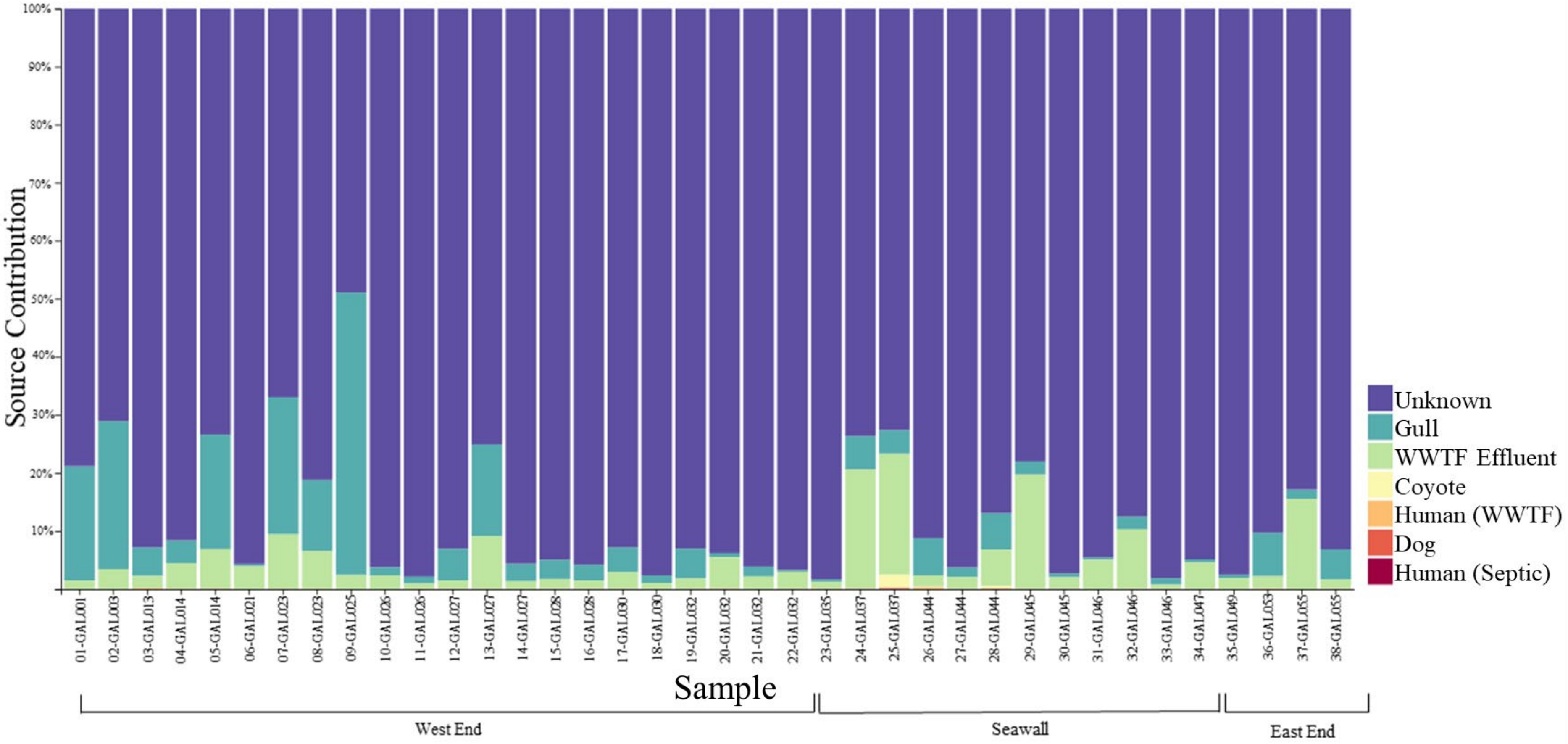
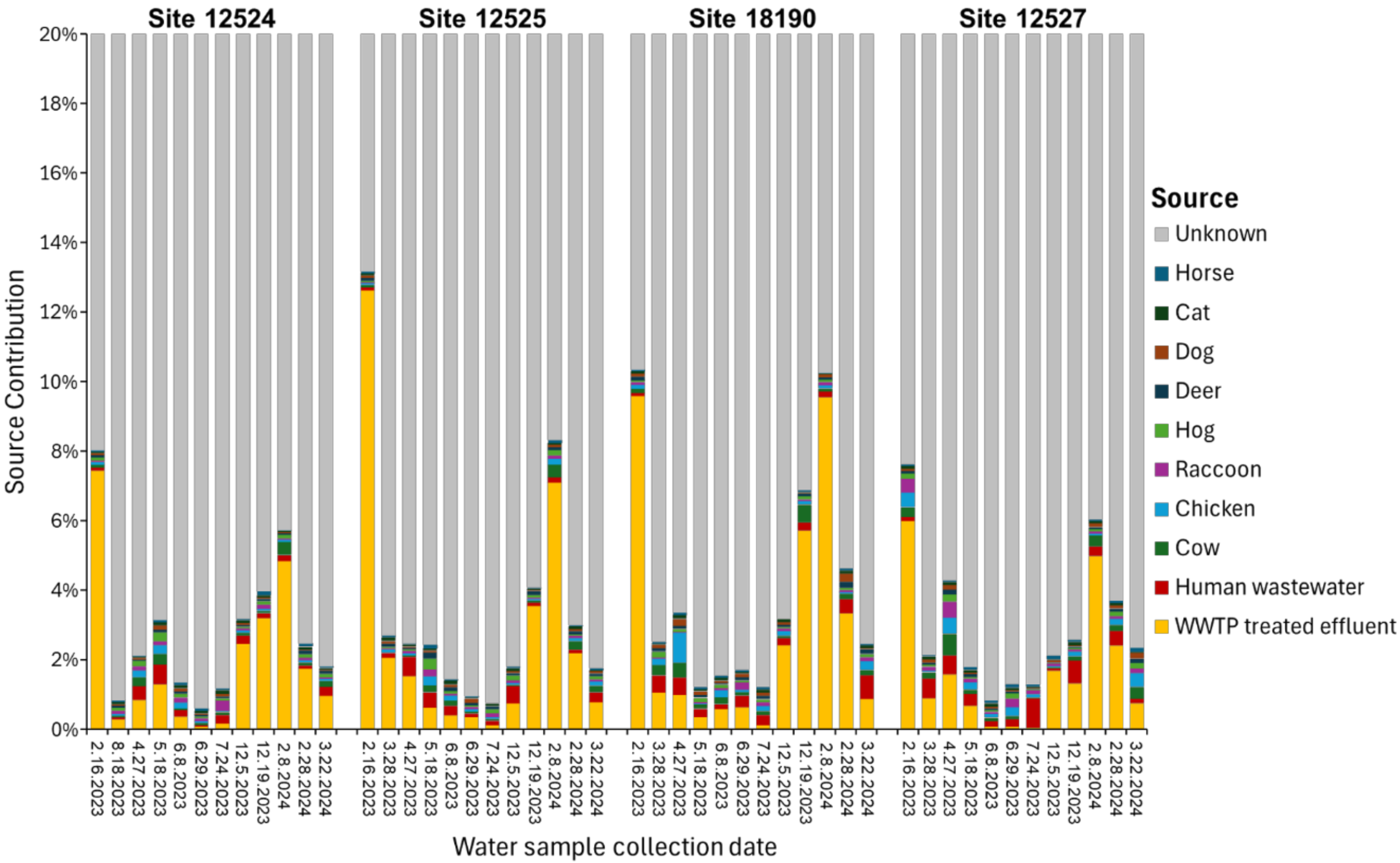
- **Detected in 24% of samples**
- **Widest distribution of tested markers**

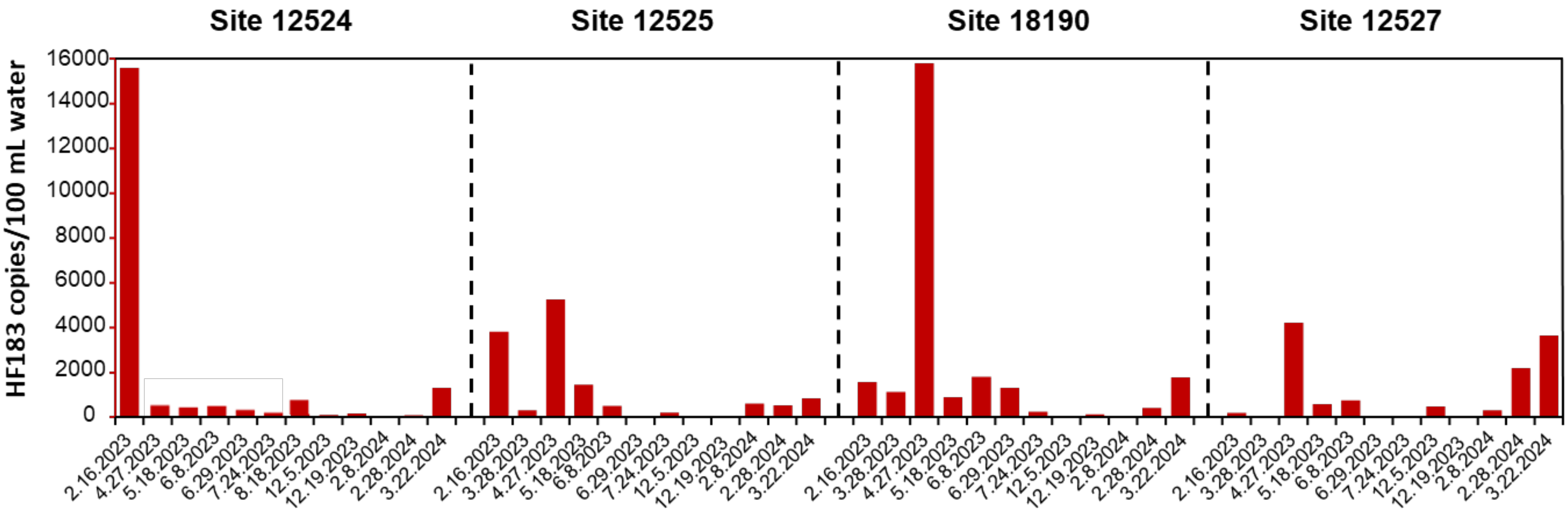
Microbiome BST (2022 samples)



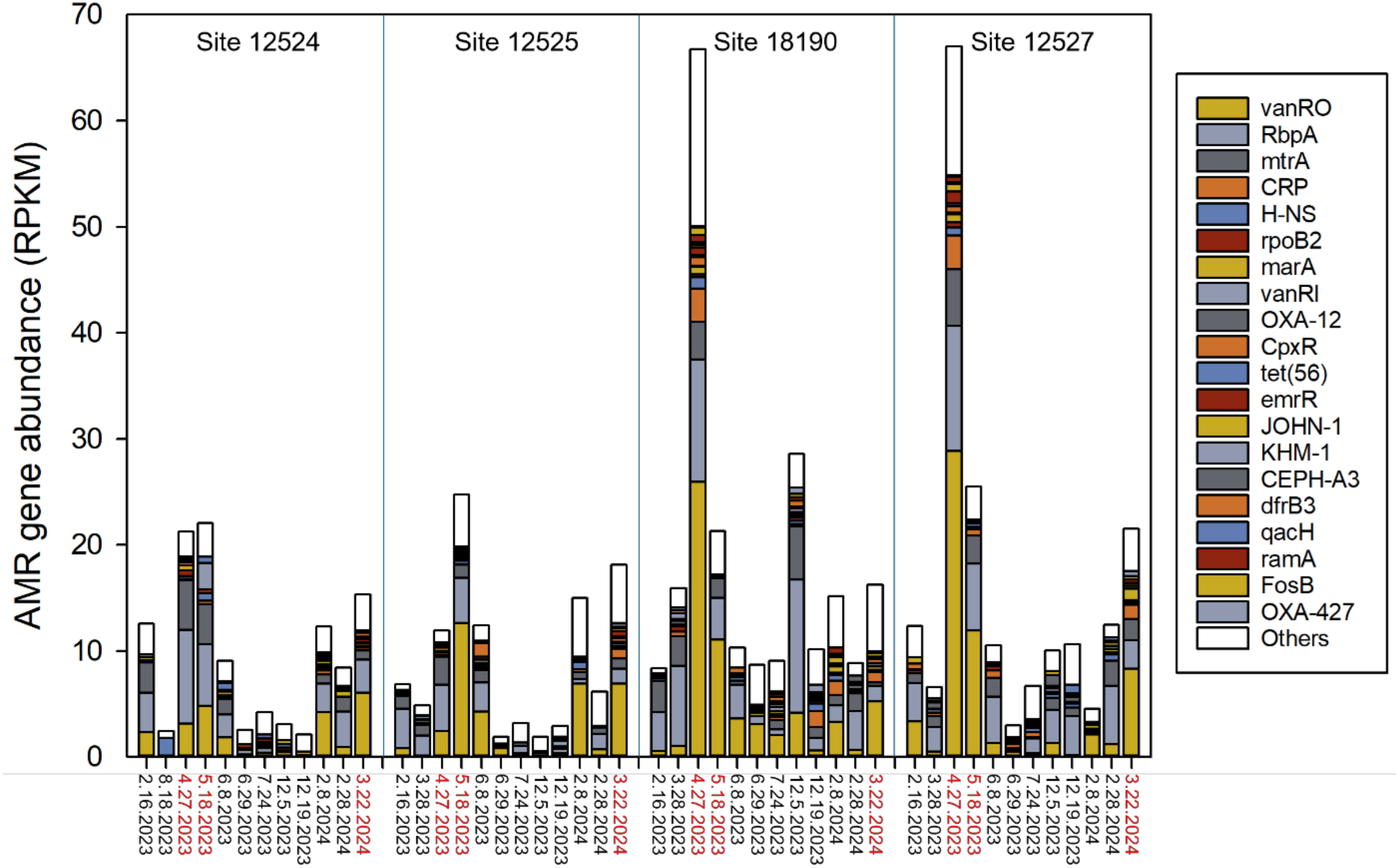
Lavaca River Watershed MST



Lavaca River Watershed MST



- **Human marker consistently detected at all four sites**
- **Precipitation significantly correlated with HF183**


AMR gene abundance (RPKM)
70
60
50
40
30
20
10
0
Site 12524
Site 12525
Site 18190
Site 12527
2.16.2023
8.18.2023
4.27.2023
5.18.2023
6.8.2023
6.29.2023
7.24.2023
12.5.2023
12.19.2023
2.8.2024
2.28.2024
3.22.2024
2.16.2023
3.28.2023
4.27.2023
5.18.2023
6.8.2023
6.29.2023
7.24.2023
12.5.2023
12.19.2023
2.8.2024
2.28.2024
3.22.2024
2.16.2023
3.28.2023
4.27.2023
5.18.2023
6.8.2023
6.29.2023
7.24.2023
12.5.2023
12.19.2023
2.8.2024
2.28.2024
3.22.2024
2.16.2023
3.28.2023
4.27.2023
5.18.2023
6.8.2023
6.29.2023
7.24.2023
12.5.2023
12.19.2023
2.8.2024
2.28.2024
3.22.2024
vanRO
RbpA
mtrA
CRP
H-NS
rpoB2
marA
vanRI
OXA-12
CpxR
tet(56)
emrR
JOHN-1
KHM-1
CEPH-A3
dfrB3
qacH
ramA
FosB
OXA-427
Others

Summary

- Continual evolution in Texas MST
- Other Texas MST researchers
- Important to reconcile with:
  - *E. coli,* enterococci enumeration data
  - Land use
  - Watershed source survey
  - Modeling
  - Stakeholder input
  - Common sense

Questions?

Terry Gentry
Texas A&M University
2474 TAMU
College Station, TX 77843
Phone: (979) 321-5918
Email: tjgentry@tamu.edu

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