North Central Texas Watershed Stakeholder Meeting

Staff Contact: Ryann Cline

North Central Texas Council of Governments
Environment & Development

www.nctcog.org/WaterResources

Welcome!

Use of Bacterial Source Tracking to Characterize Texas Watersheds

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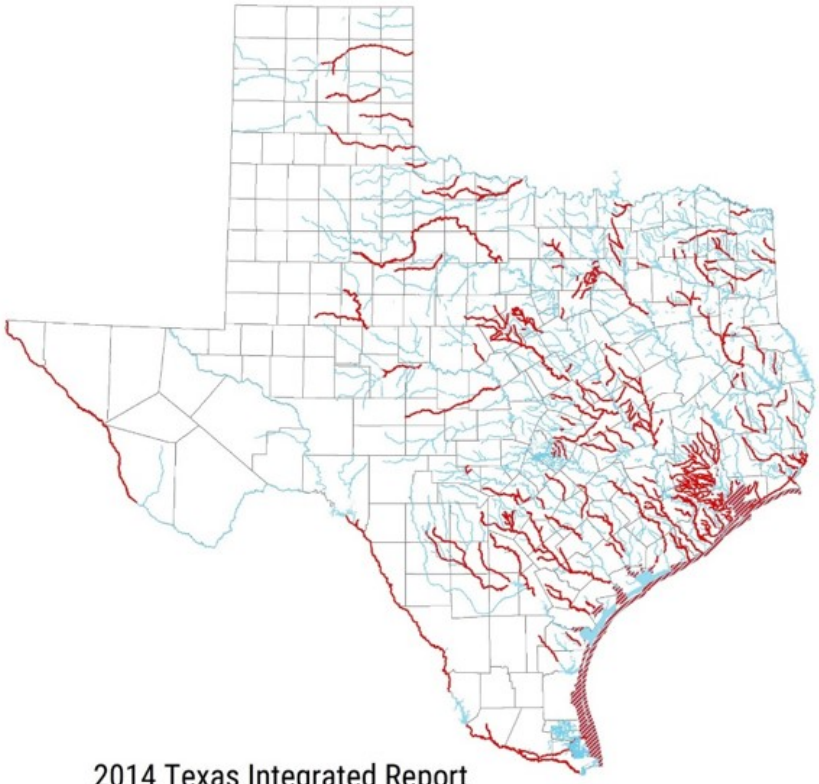
March 5, 2019

TEXAS A&M AGRILIFE RESEARCH

Where Did the Bacteria Come From?

- Potential sources
  - Humans
  - Domesticated animals
  - Wildlife
- Methods for determining sources
  - Source survey
  - Modeling
  - Bacterial source tracking



2014 Texas Integrated Report
Waterbodies with Bacteria Impairments

What is Bacterial Source Tracking?

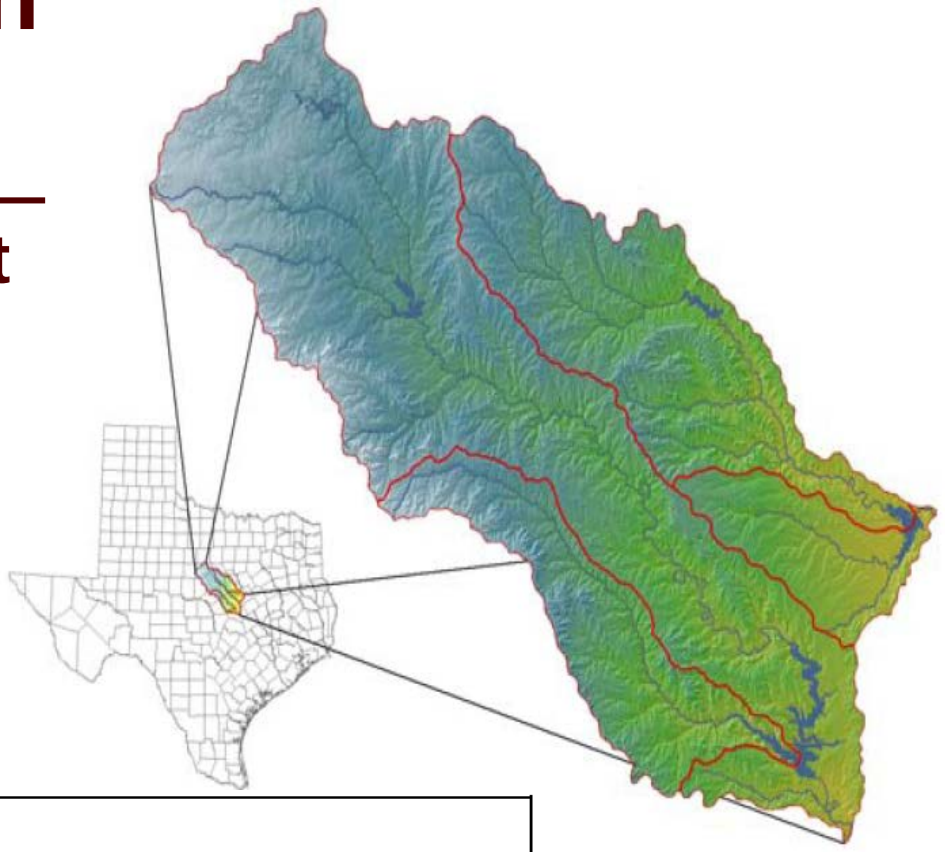
- Used to determine the sources of fecal contamination
- Based on uniqueness of bacteria from individual sources
- A variety of different methods are used
- Often works best as part of a “toolbox approach”

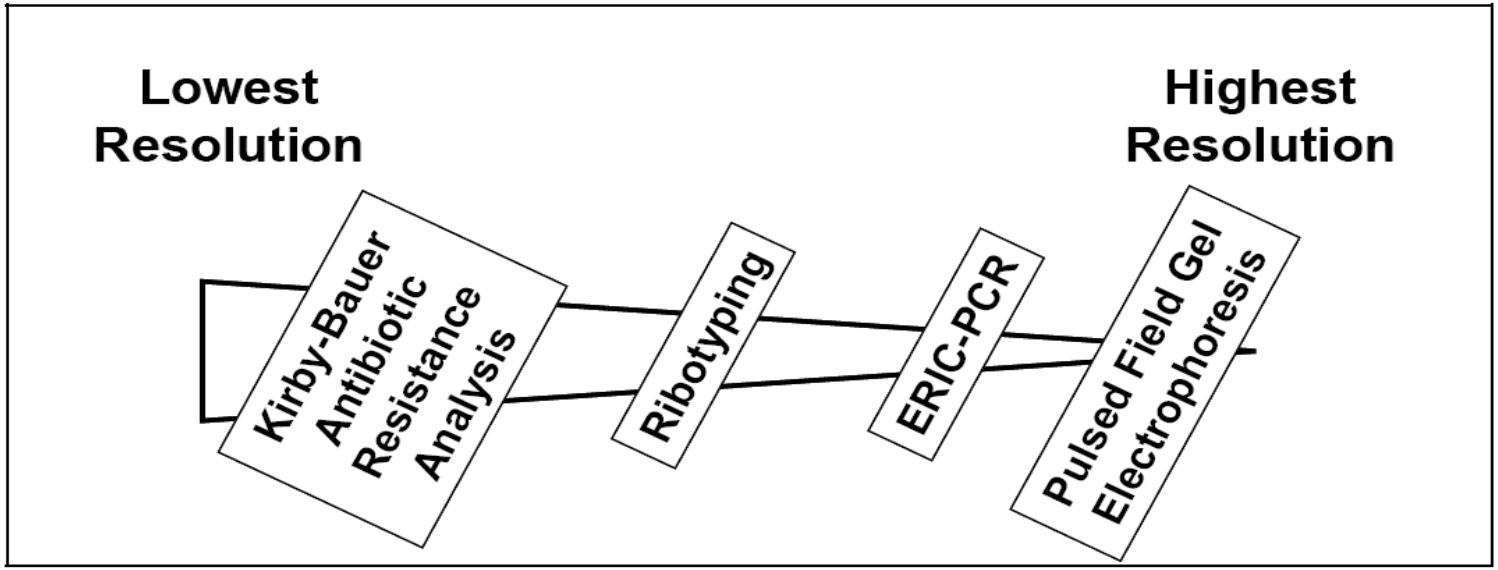
BST Target Organisms

- **Bacterial v. Microbial Source Tracking**
- **Different targets:**
  - ***E. coli***
  - ***Bacteroidales***
  - **Bacteriophage**
  - **Human viruses**
  - **Chemicals**

History of BST Use in Texas

- Lake Waco/Belton Project initiated Sep. 2002
- Funded by TSSWCB
- Evaluated utility & methods
- Completed Feb. 2006





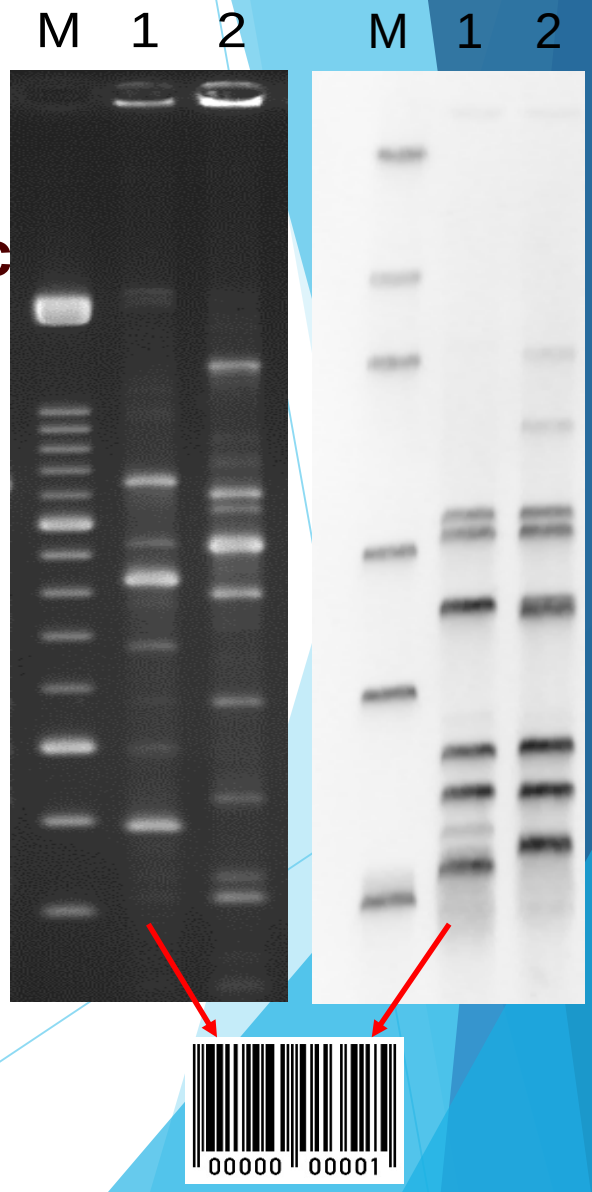
History of BST Use in Texas

- **Lake Waco/Belton Project Findings**
  - **4-method composite performed better than individual methods**
  - **2-method composites appeared promising**
    - **ERIC-ARA = lower cost but more sample & data processing**
    - **ERIC-RP = higher cost but automated**
- **TMDL Task Force Report – 2007**
  - **Confirmed ERIC-RP as recommended method**

Library-Dependent BST Methods

Methods:

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

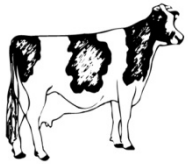


Advantages/Disadvantages:

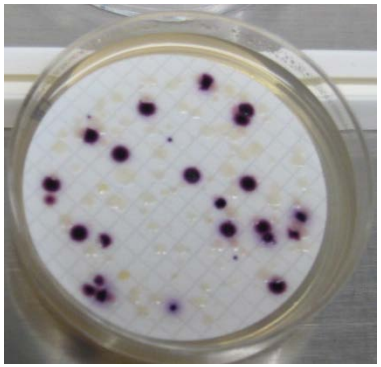
- More discriminating
- Allows ranking of sources
- Relatively expensive

Development of Texas *E. coli* BST Library

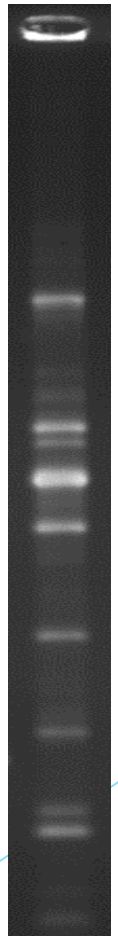
Sources



Isolate → ***E. coli***



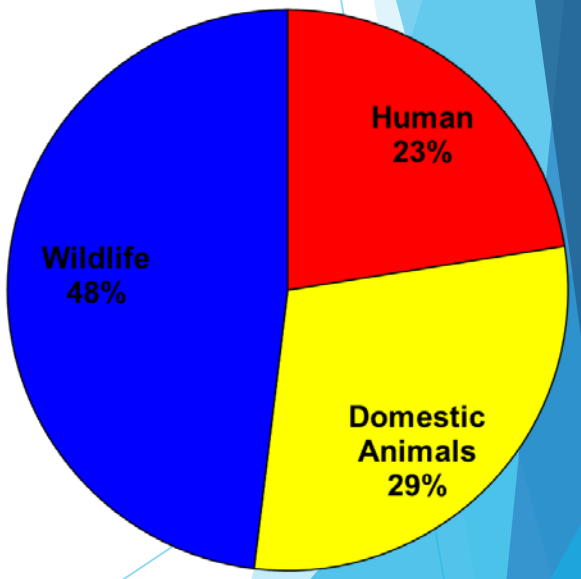
DNA → **Fingerprint**



Add to → **Library**

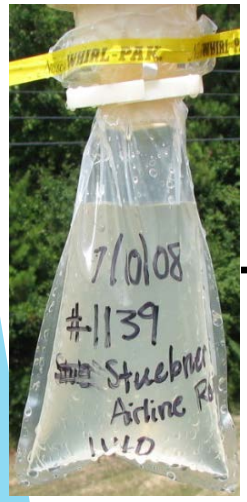
Texas *E. coli* BST Library (v. 12-17)

- Contains 1,853 *E. coli* isolates from 1,612 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 BST including:
  - Plum Creek
  - San Antonio
  - Lake Granbury
  - Oyster Creek / Trinity River
  - Waco / Belton Lake
  - Little Brazos River Tributaries
  - Attoyac Bayou



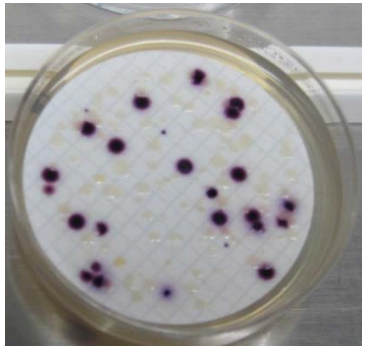
- Additional isolates being added from ongoing and future BST projects in other areas of Texas

Use of Texas *E. coli* BST Library for Identifying Water Isolates



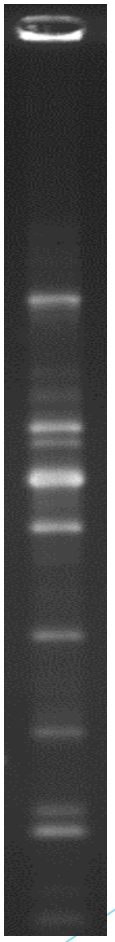
Isolate

E. coli



DNA

Fingerprint



Compare

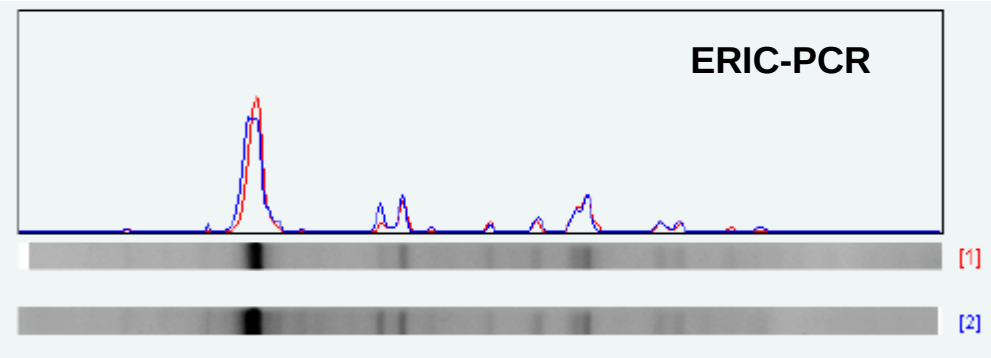
to Library

Source ID

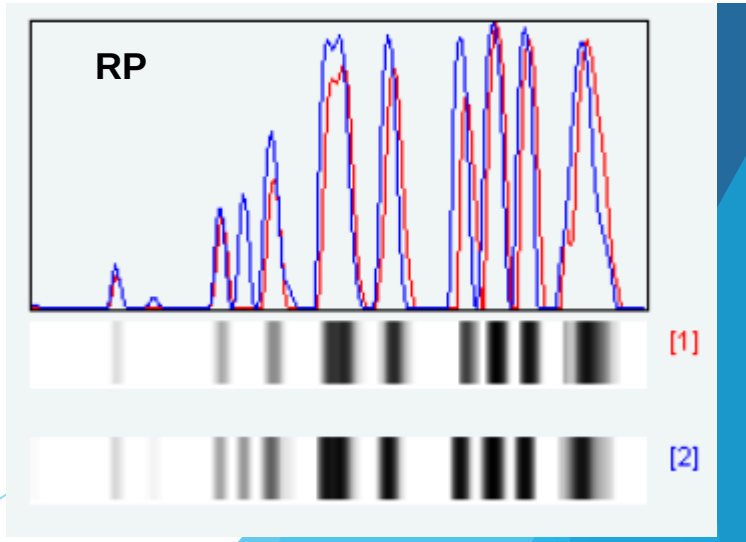
Comparison to Texas *E. coli* BST Library

- **Best match approach with 80% minimum similarity cutoff based on laboratory QC data**
  - **Water isolate must match library isolate ≥ 80% similarity or it is considered unidentified**
  - **Identification to single library isolate with highest similarity – max similarity approach**

Similarity: 96.94%

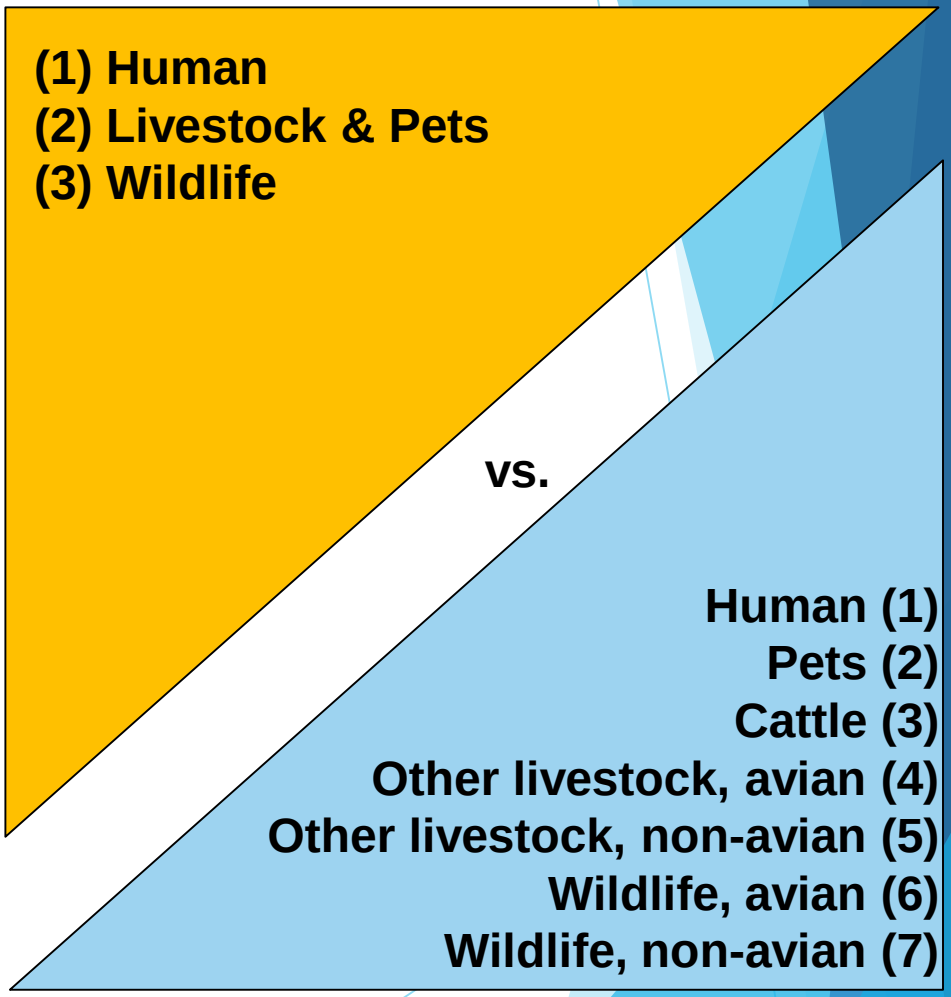


Similarity: 95.82%



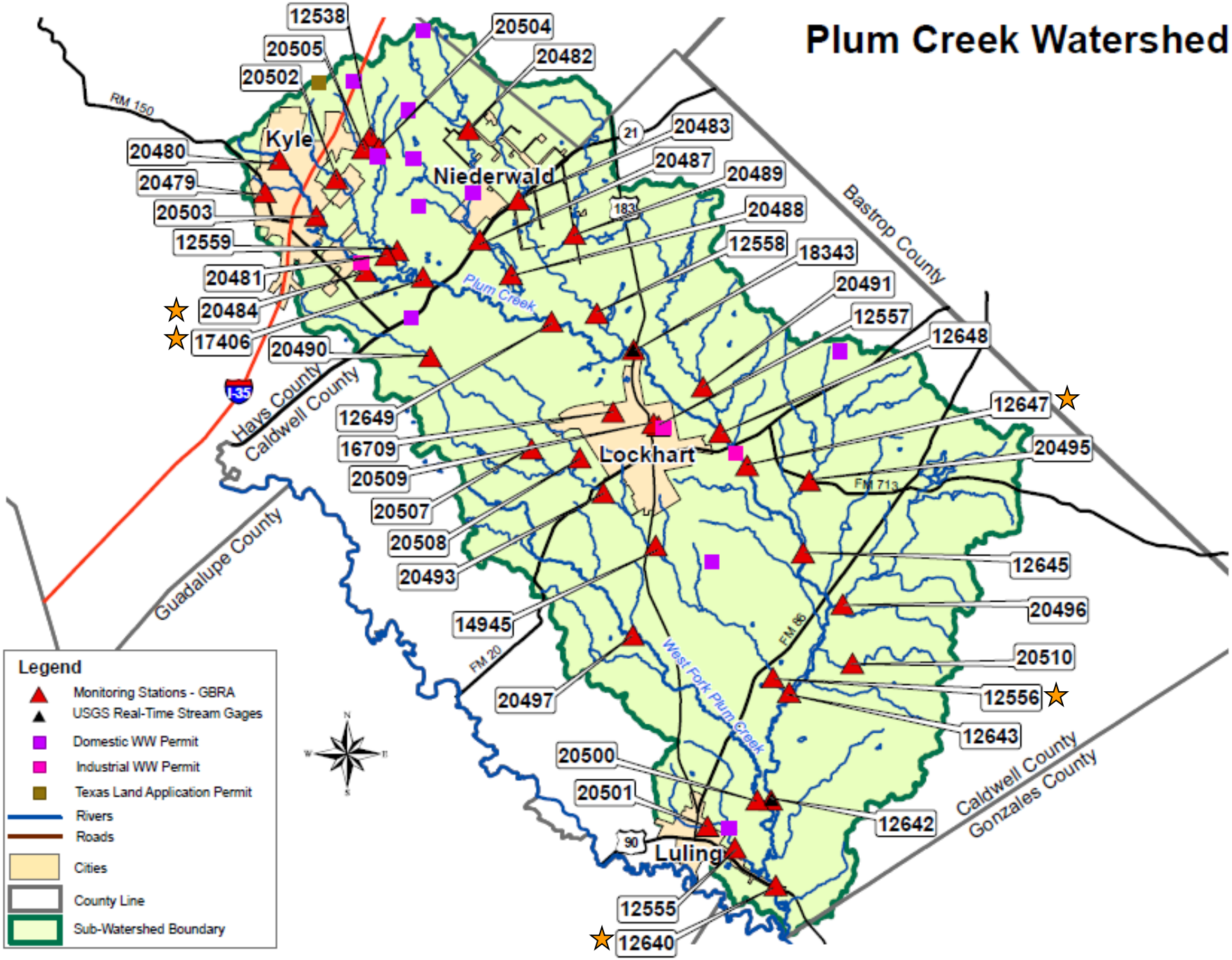
Three-way v. Seven-way Split of Results

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



Texas *E. coli* BST Library Composition & Rates of Correct Classification (RCC)

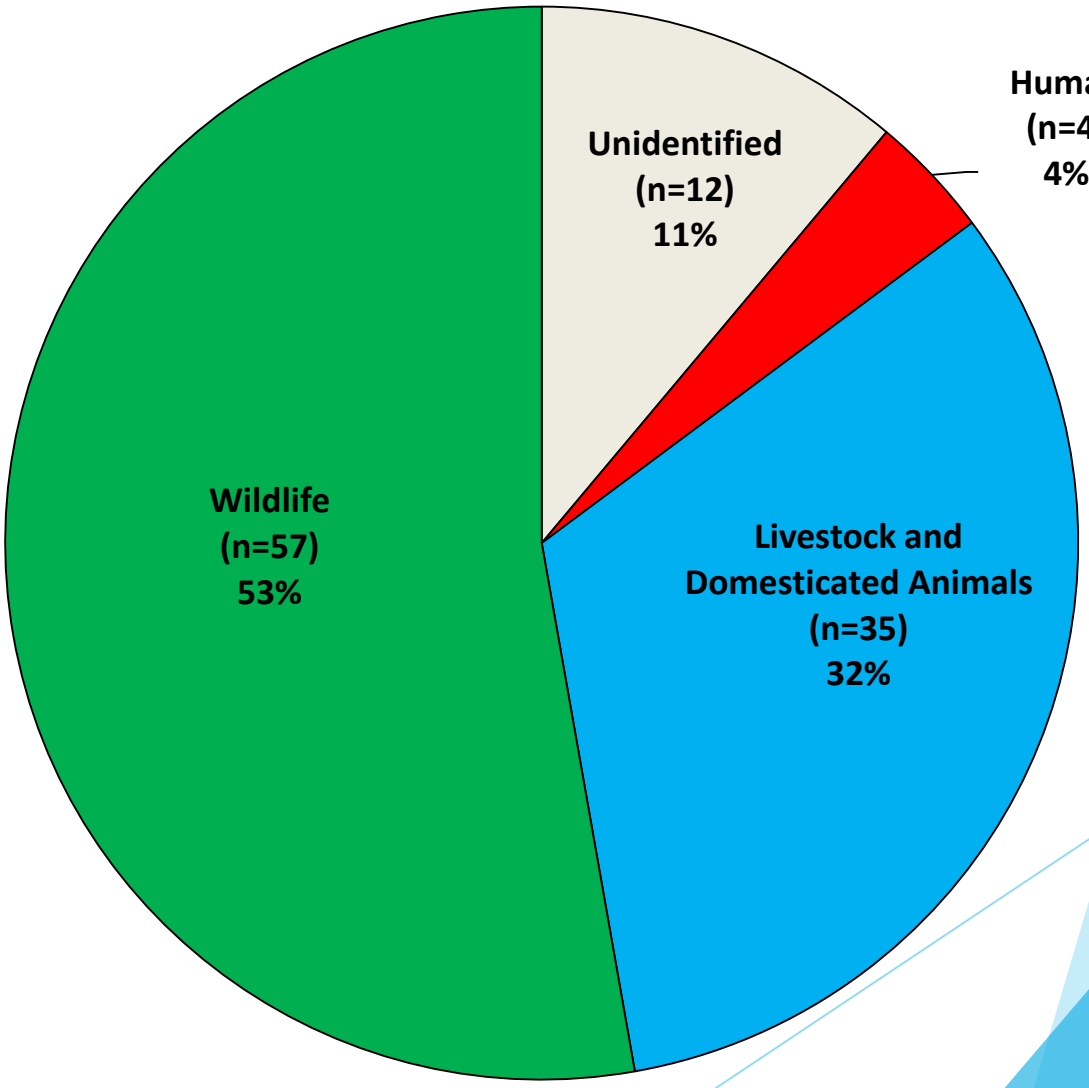
Source Class	Number of Isolates	Number of Samples	Library Composition and Expected Random Rate of Correct Classification	Calculated Rate of Correct Classification (RCC)	RCC to Random Ratio***	Left Unidentified (unique patterns)
HUMAN	**417**	**351**	**23%**	**100**	**4.3**	**22%**
DOMESTIC ANIMALS	**545**	**500**	**29%**	**100**	**3.4**	**19%**
Pets	83	74	4%	84	21.0	41%
Cattle	244	225	13%	94	7.2	11%
Avian Livestock	96	84	5%	89	17.8	27%
Other Non-Avian Livestock	122	117	7%	90	12.8	15%
WILDLIFE	**891**	**761**	**48%**	**100**	**2.1**	**16%**
Avian Wildlife	272	250	15%	79	5.3	18%
Non-Avian Wildlife	619	511	33%	91	2.8	15%
Overall	**1853**	**1612**		**ARCC** = 100% (3-way) 91% (7-way)**		**18%**


Plum Creek Watershed
12538
20504
20505
20482
20502
RM 150
Kyle
20480
20483
21
20487
20479
Niederwald
20489
20503
183
20488
12559
12558
18343
20481
Plum Creek
20491
20484
12557
17406
12648
20490
I-35
Hays County
Caldwell County
12649
12647
16709
Lockhart
20495
20509
FM 713
20507
20508
12645
20493
20496
14945
FM 20
FM 86
20510
20497
West Fork Plum Creek
12556
12643
20500
20501
12642
Caldwell County
Gonzales County
90
Luling
12555
12640
Guadalupe County
Bastrop County
Legend
Monitoring Stations - GBRA
USGS Real-Time Stream Gages
Domestic WW Permit
Industrial WW Permit
Texas Land Application Permit
Rivers
Roads
Cities
County Line
Sub-Watershed Boundary

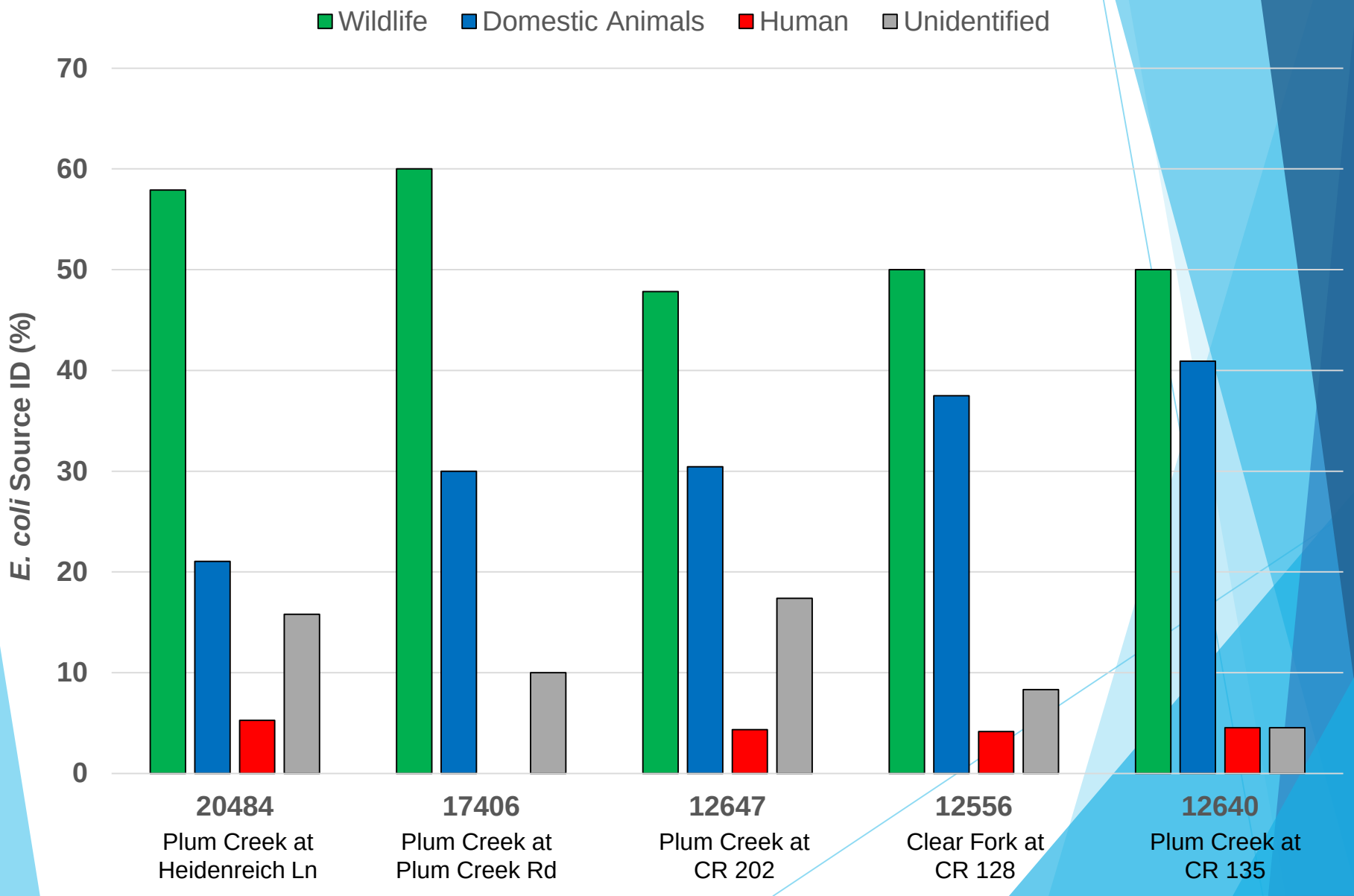
BST for Plum Creek

- **Addition of Known-Source *E. coli* Isolates**
  - **Isolated and DNA fingerprinted 76 *E. coli* from Plum Creek fecal/wastewater samples for addition to the Texas *E. coli* BST Library**
  - **Wastewater, poultry, cattle, wildlife, feral hogs, etc.**
- **Characterization of Water *E. coli* Isolates**
  - **Isolated *E. coli* from water samples collected monthly at five sites over one year (60 samples)**
  - **DNA fingerprinted 108 *E. coli* isolates and compared to Texas *E. coli* BST Library for source identification**

E. coli BST Results
3-Way Split



E. coli BST Results
5 Sampling Sites (3-Way Split)



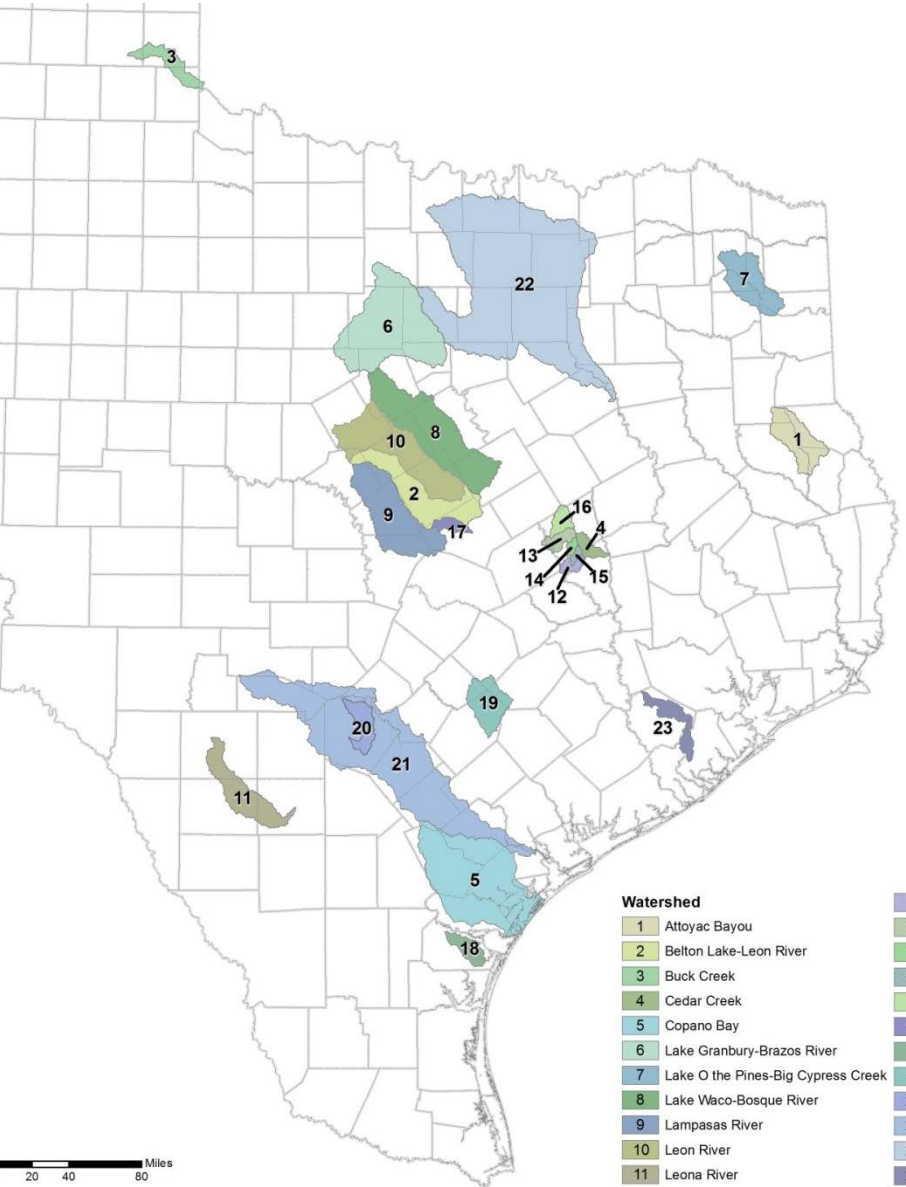
BST Summary for Plum Creek

- **Major *E. coli* sources in tested samples appear to be wildlife (feral hogs, small mammals, deer, birds) as well as domesticated animals (cattle)**
- **Domesticated animal contributions trended higher in samples from lower in the watershed**
- **Limited proportion of human *E. coli* isolates detected; primarily seen in samples collected below WWTF outfalls**

Use of BST Results

- **Reconcile with:**
  - ***E. coli* enumeration data**
  - **Land use**
  - **Watershed source survey**
  - **Modeling**
  - **Stakeholder input**
  - **Common sense**

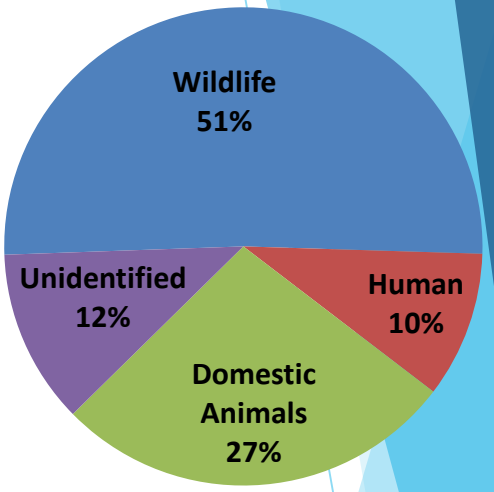
Texas BST Studies To Date



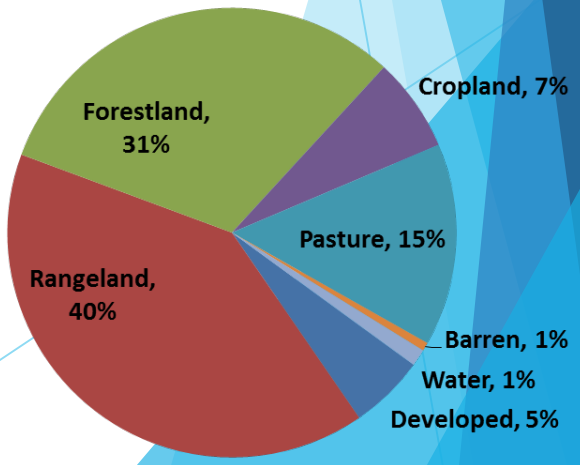
Watershed

1 Attoyac Bayou	12 Little Brazos Tributary-Campbells Creek
2 Belton Lake-Leon River	13 Little Brazos Tributary-Mud Creek
3 Buck Creek	14 Little Brazos Tributary-Pin Oak
4 Cedar Creek	15 Little Brazos Tributary-Spring Creek
5 Copano Bay	16 Little Brazos Tributary-Walnut Creek
6 Lake Granbury-Brazos River	17 Nolan Creek
7 Lake O the Pines-Big Cypress Creek	18 Oso Creek
8 Lake Waco-Bosque River	19 Peach Creek
9 Lampasas River	20 Salado Creek
10 Leon River	21 San Antonio River
11 Leona River	22 Trinity River
	23 Upper Oyster Creek

3-Way Split
(averages based on 11 watersheds)



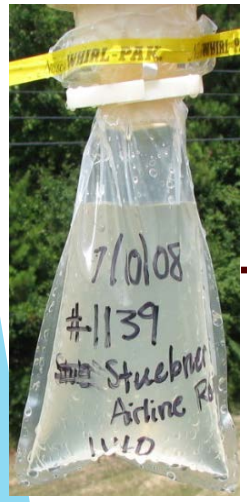
Typical Landuse in 11 BST Watersheds



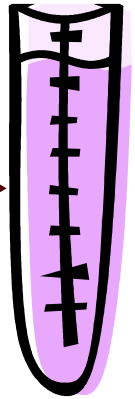
Library Independent BST

- Most common approach targets *Bacteroidales*
- *Bacteroidales* – human and animal fecal bacteria, more abundant than *E. coli*
- Markers available for
  - Ruminants (cattle, deer, elk, sheep, horses, llama)
  - Humans
  - Horses (needs optimization and validation)
  - Birds (needs optimization and validation)
  - Hogs (including feral hogs – in development)
- Highly (but not 100%) specific
- Limited markers for wildlife
- Relationship to *E. coli* and pathogens uncertain

Library Independent BST

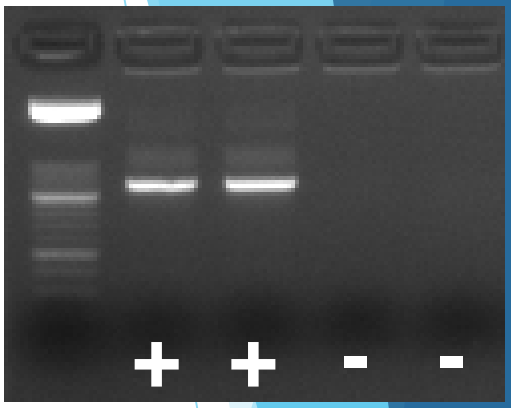


Extract DNA

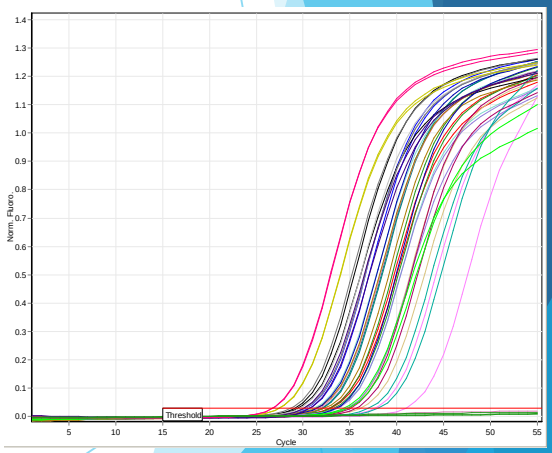


PCR amplify target sequence

Presence/ Absence



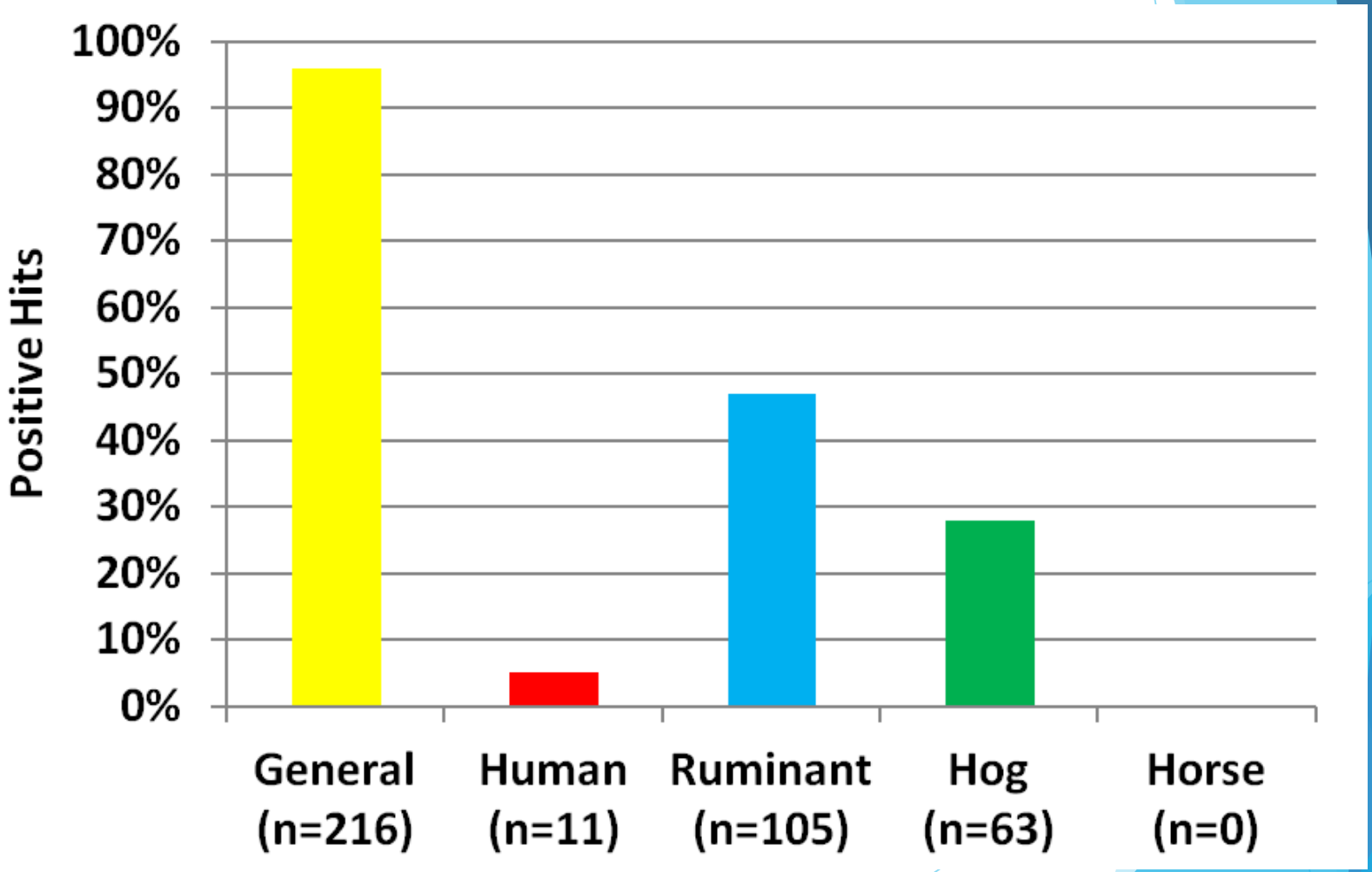
Quantitative



Advantages:

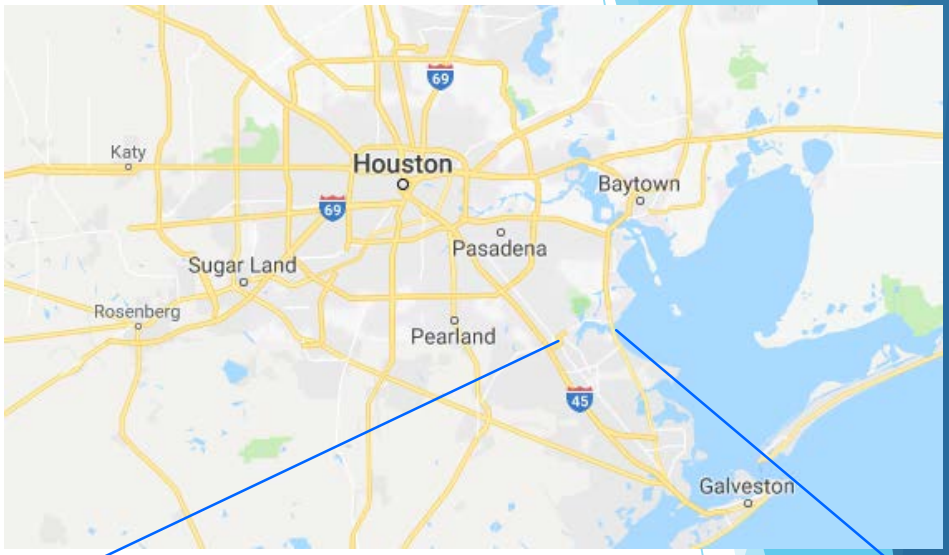
- Cost
- Time

Bacteroidales BST Results
Base Flow Samples (n=225)



Hurricane Harvey Flooding

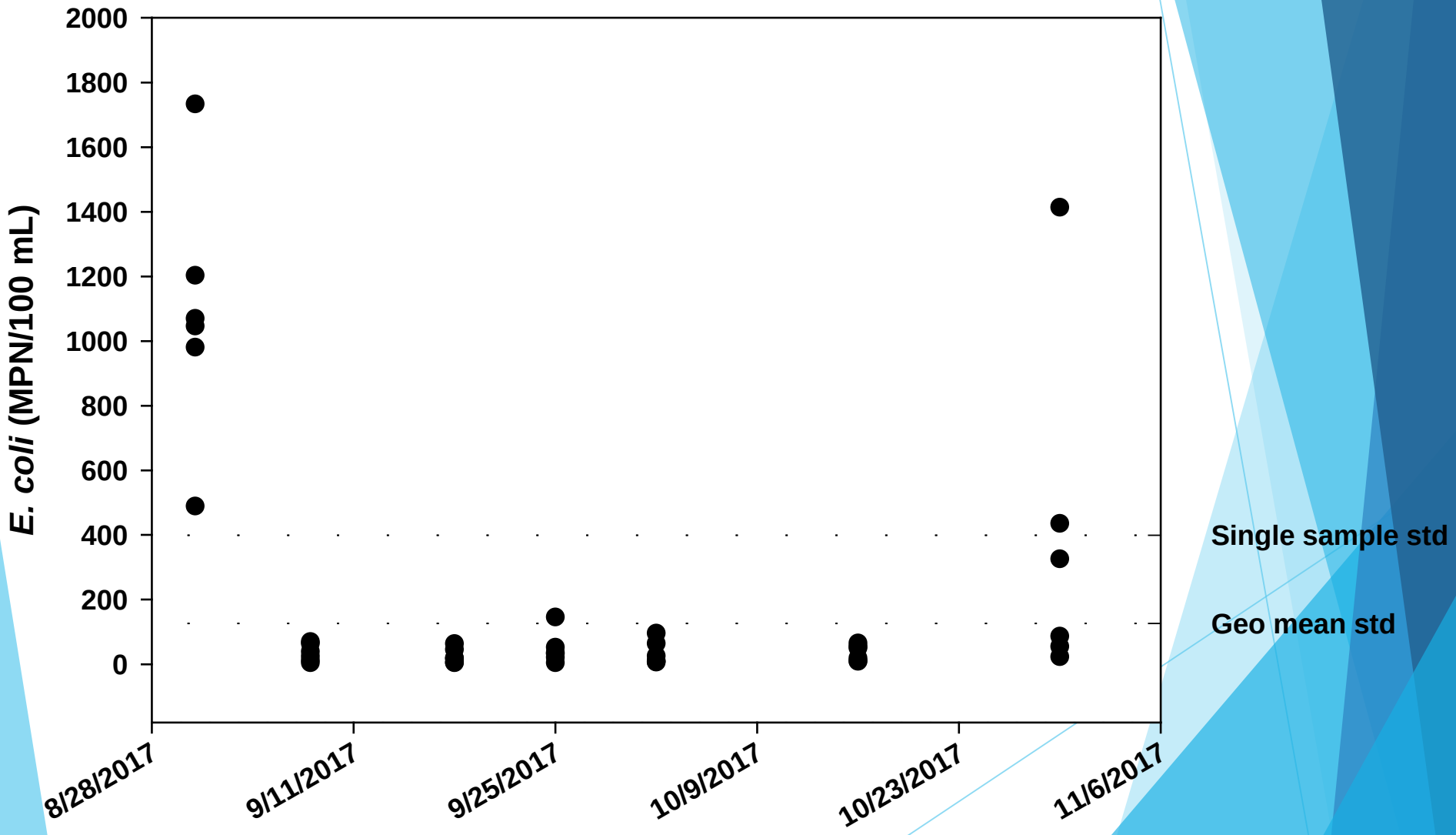
- Six locations in the southeastern Houston area around Clear Lake
- Surface water samples were collected as soon as sites were accessible following the hurricane and then every 1-2 weeks for ~2 months



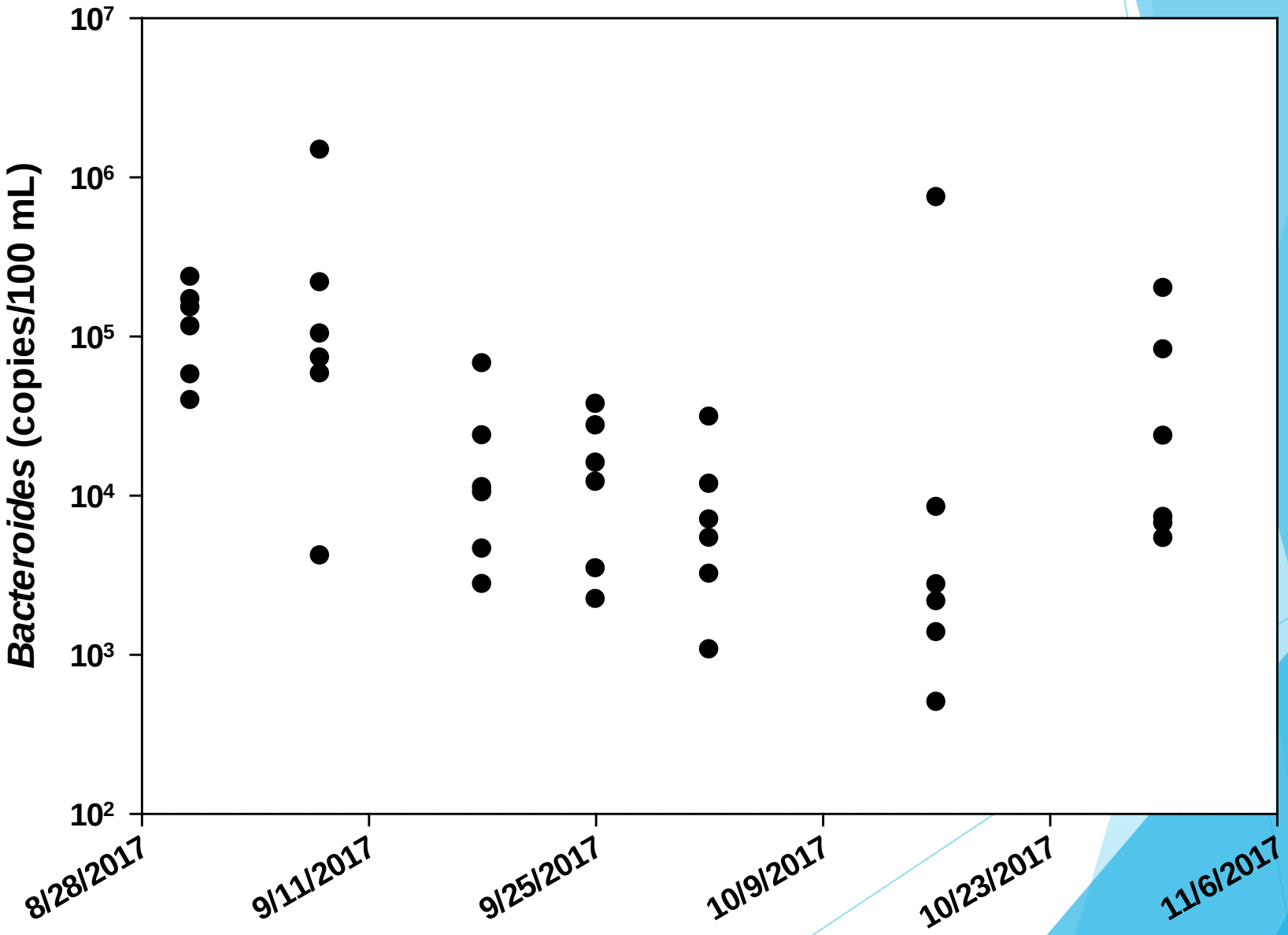
Analyses

- ***E. coli*** **using IDEXX Quanti-Tray/2000 with Colilert**
- **qPCR for total and human *Bacteroides***
  - **Passed through 0.2 µm filter**
  - **DNA extracted with Qiagen PowerWater kit**
  - **Primers GENBAC 32F/708R used for total *Bacteroides* (Bernhard and Field, 2000)**
  - **HumM2 primers used for human-specific *Bacteroides* (Shanks et al., 2009, 2010, 2016)**

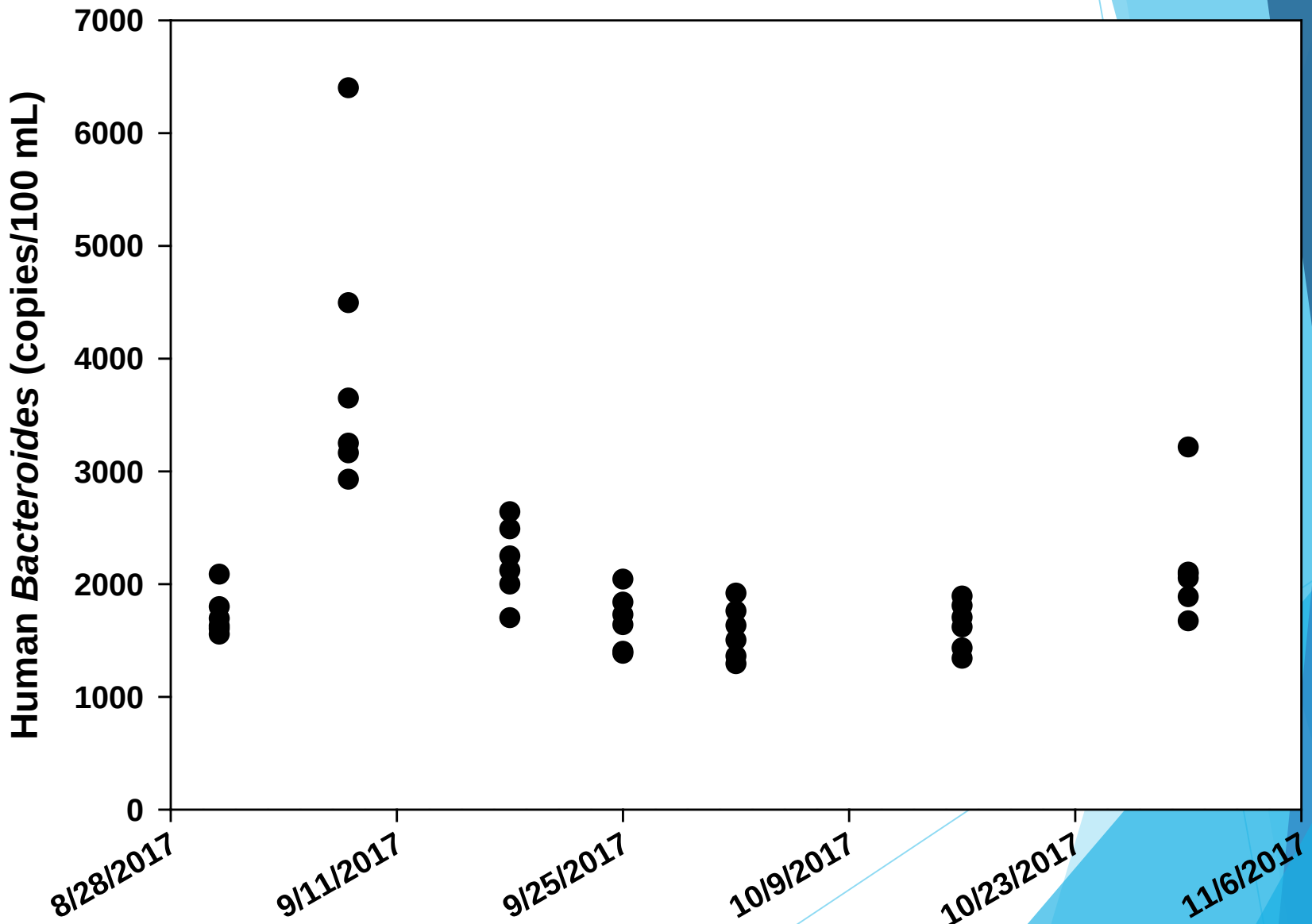
E. coli Levels



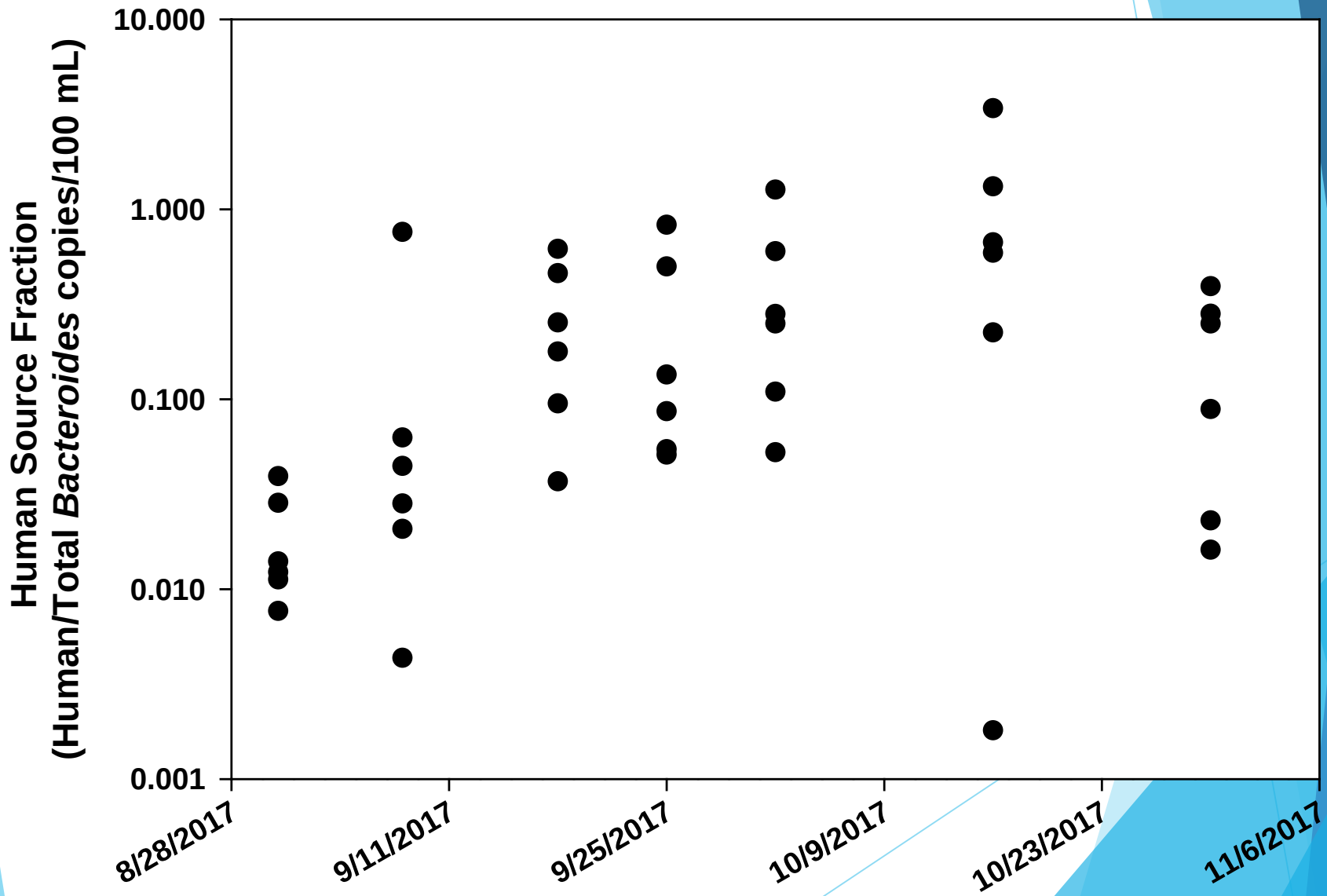
Total *Bacteroides* Levels



Human *Bacteroides* Levels



Human:Total *Bacteroides* Levels



Summary/Conclusions

- **Most fecal indicator and marker levels were elevated immediately after the hurricane but decreased within one week**
- **Low concentration of human *Bacteroides* detected at the first sampling date suggests non-human fecal sources were primarily responsible for contamination during the initial flooding**
- **The delayed (one week) spike in human *Bacteroides* marker abundance, and increased fraction over time, suggests the prevalence of human sources under normal conditions**

How to Start a BST Project?

- Government and commercial BST labs
- What is the goal of BST?
  - Characterize watershed or monitor specific sources?
  - How many potential sources?
    - All, most numerous…
    - One or a few (e.g., human)
  - What level of resolution is needed?
    - Individual species
    - Groups (e.g., humans, domesticated animals, and wildlife)
    - Presence/absence, relative ranking, or absolute number for various sources

Costs of a BST Project?

- **Current BST costs:**
  - **ERIC-RP = $250/isolate**
  - **Bacteroidales PCR**
    - **General + one specific marker = $250/sample**
    - **General + four specific markers = $325/sample**
- **Example watershed:**
  - **Three sites**
  - **Samples collected monthly for one year**
  - **ERIC-RP five isolates per sample**
  - **3 sites x 12 sampling events x 5 isolates/sample [180 total isolates] x $250/isolate = $45,000**
  - **Does not include sample collection, initial sample processing, and transport to lab**

Questions?

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TEXAS A&M AGRILIFE RESEARCH

5-minute Break

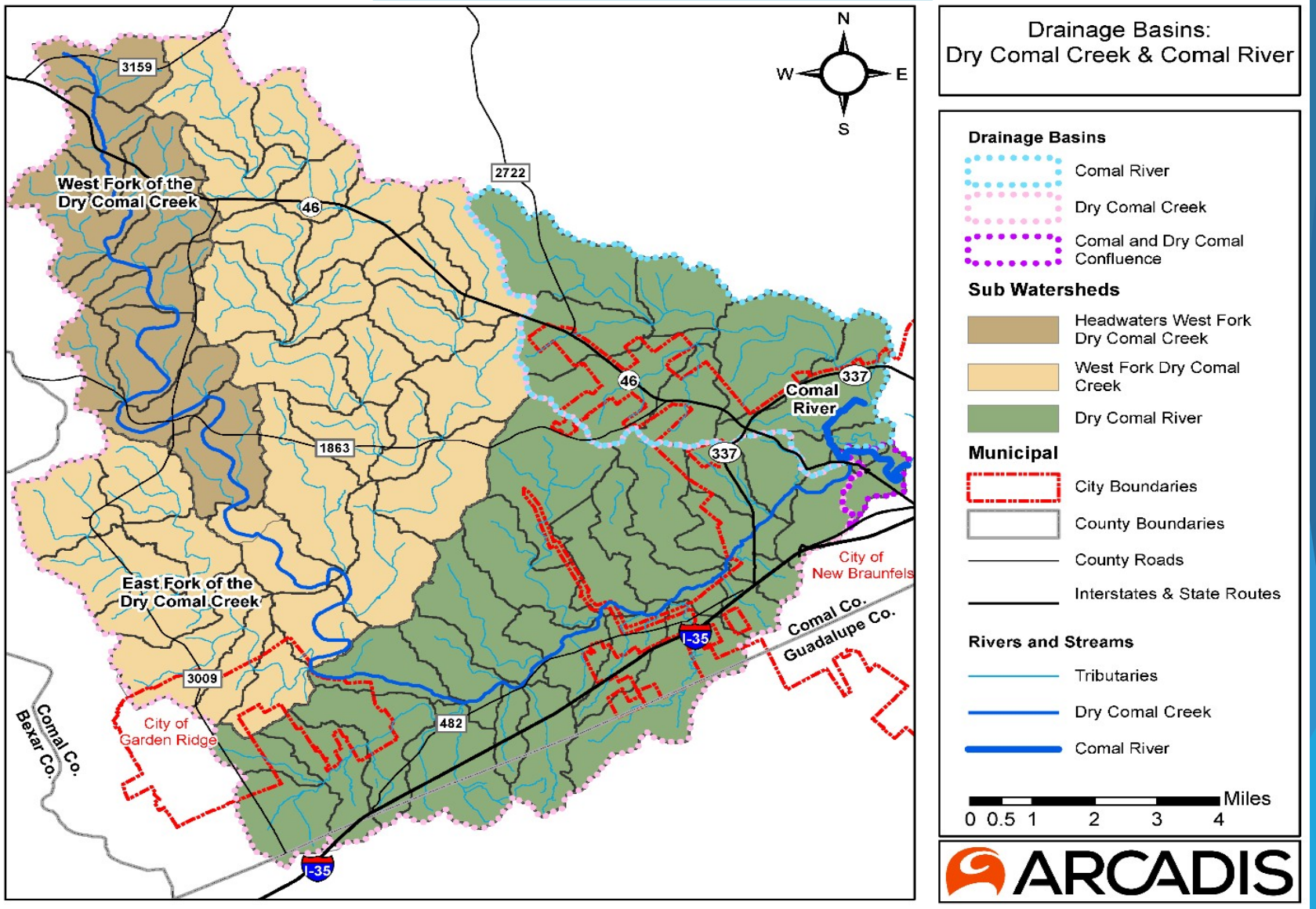
Dry Comal Creek & Comal River Watershed Protection Plan

Mark Enders
Watershed Program Manager
City of New Braunfels

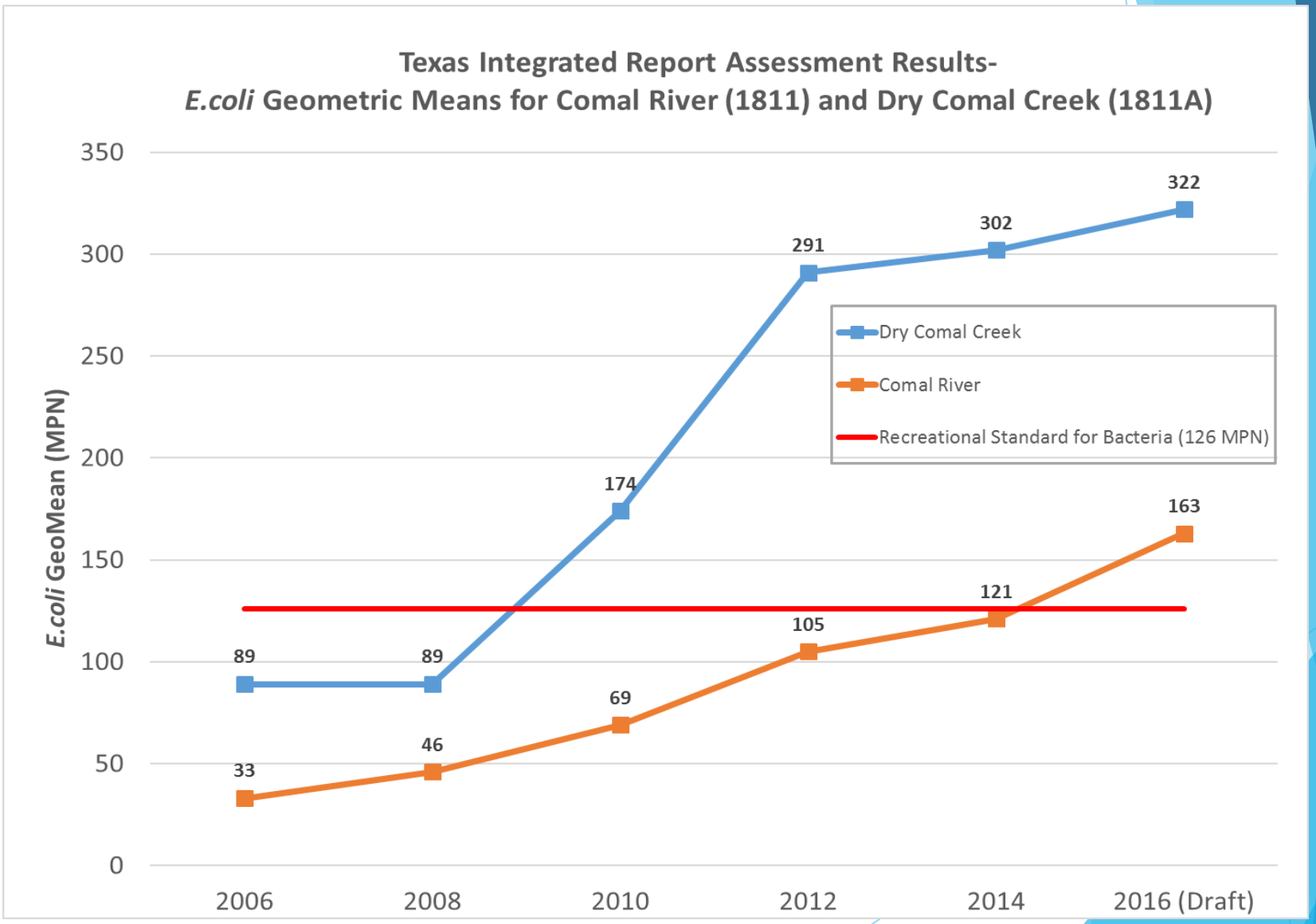
Phillip Quast
Watershed Coordinator
City of New Braunfels

Watershed

- Total Area: 83,160 acres
- Dry Comal Creek (Segment 1811A)
- Comal River (Segment 1811)



E. coli Concentrations

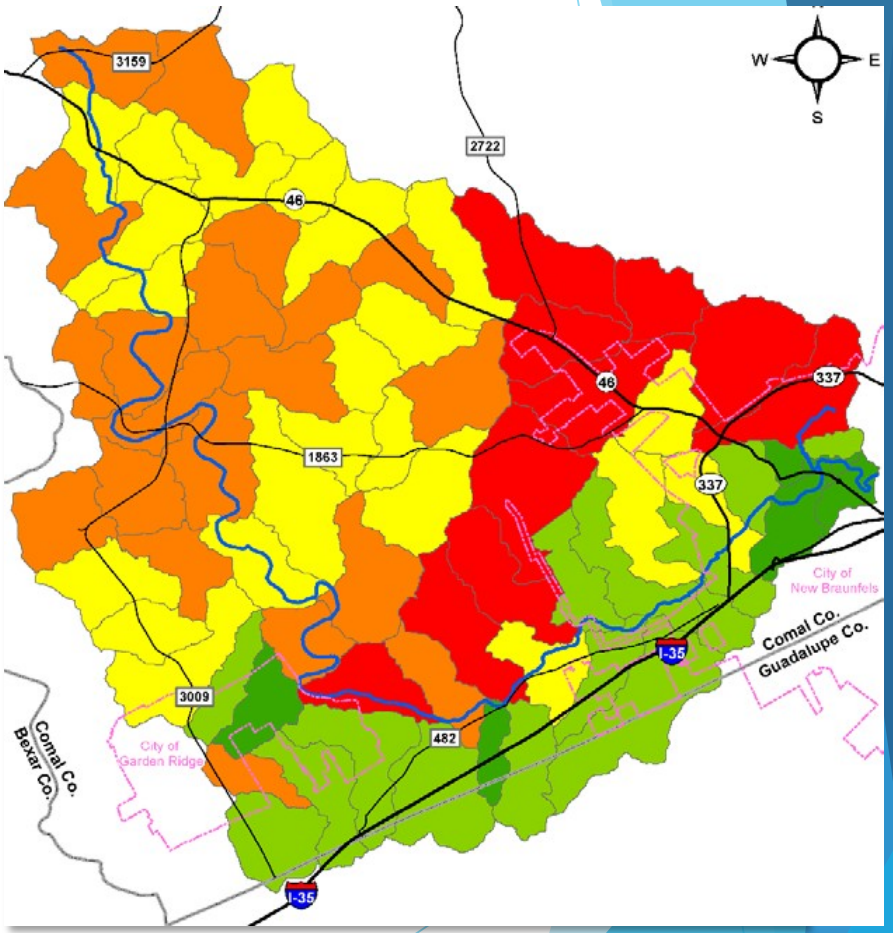


Watershed Protection Planning

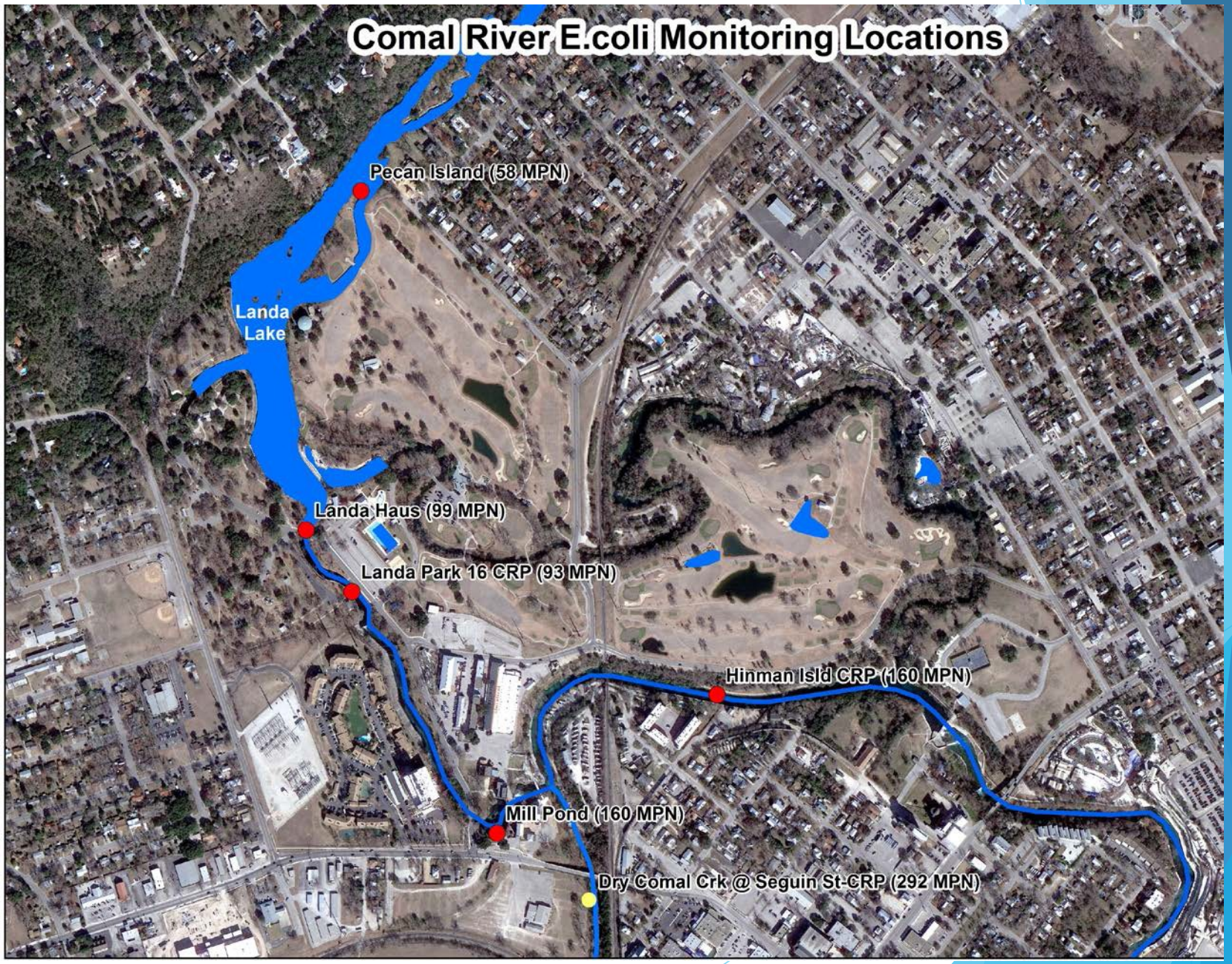
- City added additional *E.coli* monitoring stations, conducted BST
- Began development of the Dry Comal Creek and Comal River WPP in 2015
- City received grant funding through TCEQ
- City utilized a consultant (Arcadis) to assist with development of WPP

SELECT Used to Identify Source Locations

- SELECT = "Spatially Explicit Load Enrichment Calculation Tool"
- Highlights **location of *E. coli* sources** within the Watershed
- Sources examined:
  - Cattle
  - Other livestock
  - On-site sewage facilities
  - Deer
  - Feral hogs
  - Pets
  - Non-native avian wildlife
- **Didn't always reflect local knowledge!**



Comal River *E.coli* Monitoring

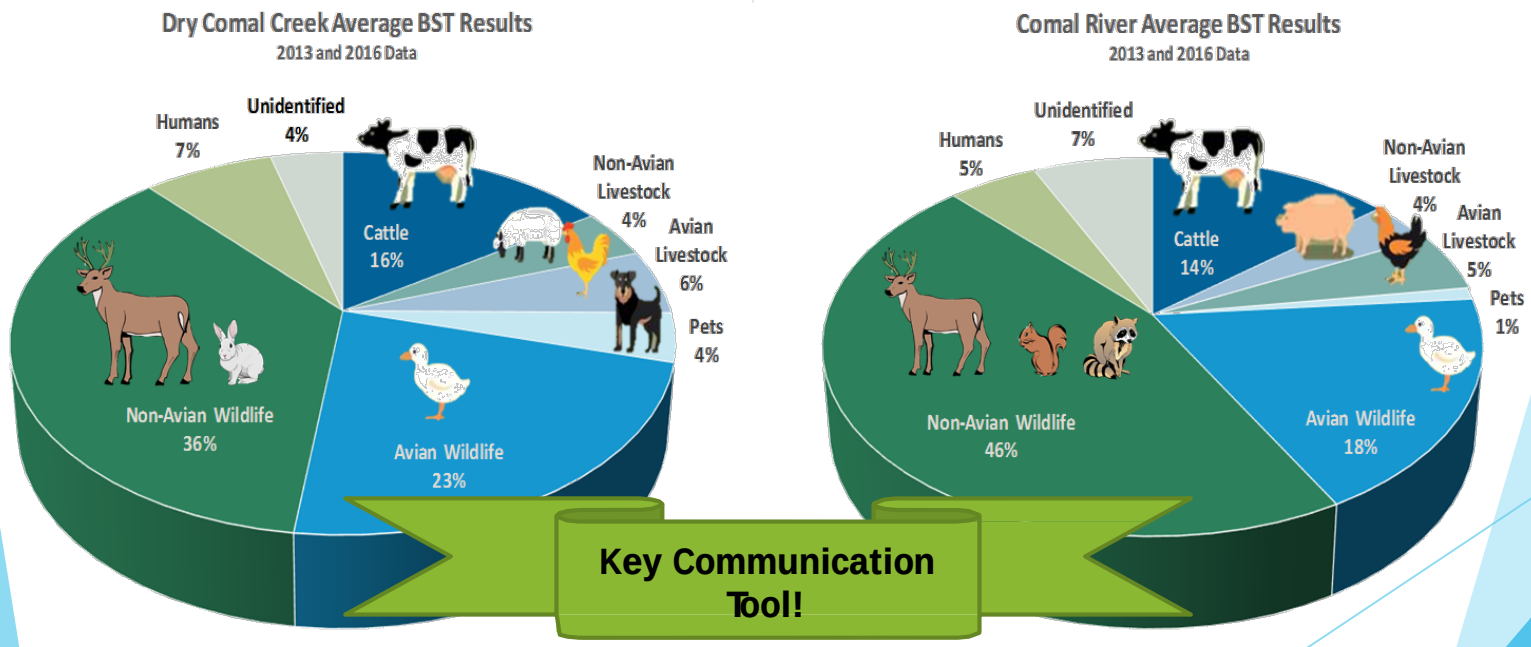


BST for Comal River & Dry Comal Creek

- **2013**
  - **2 sites**
  - **3 rounds of sampling (Sept – Oct)**
  - **≥ *25 E. coli* DNA fingerprinted per site**
- **2016**
  - **3 sites**
  - **3 rounds of sampling (Sept – Oct)**
  - **≥ 25 *E. coli* DNA fingerprinted per site**

BST Analysis Used to Prioritize Resources

- Bacteria source tracking highlights the **sources of *E. coli*** contributing to bacterial pollution
- Conducted in 2013 and 2016 (3 sampling events/year)
- Majority of *E. coli* bacteria contributed by wildlife (deer, non-native avian)



Stakeholder Engagement

- Includes local businesses, neighborhood associations, conservation groups, City departments, and technical advisors
- 4 Work Groups

Wildlife Management

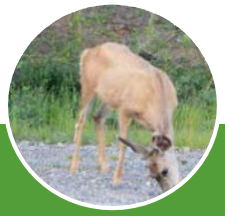
Livestock

Stormwater and Infrastructure

Outreach and Education

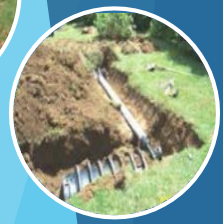
- Local knowledge used to inform locations of pollution not previously captured

Best Management Practices developed w/ **Stakeholder Input**







Overabundant Urban Deer

- Do-Not-Feed Wildlife Ordinance and Campaign within City Limits
- Deer Population Assessment
- Voluntary Do-Not-Feed Wildlife Campaign in Rural Neighborhoods
- Wildlife Management Workshops
- Active Management of Deer with
- City Council Approval

Non-Native Avian Wildlife

- Do-Not-Feed Wildlife Ordinance and Campaign within City Limits
- Non-Native Duck and Goose Population Assessment
- Discourage Non-Native Ducks and Geese from Congregating in the Park
- Wildlife Management Workshops
- Rapid Removal of Dead Animals
- Trap Non-Native Ducks and Geese
- Oil Coat Non-native Duck Eggs

Livestock

- WQMPs
- Livestock Outreach and Education

Stormwater

- Non-Structural Stormwater BMPs
- Outside of the City's MS4 Jurisdiction
- Stormwater Outreach and Education

Wastewater

- Wastewater Discharge Water Quality Assessment

Feral Hogs

- Feral Hog Workshops
- Bounty Program
- Trapping Intensity Assessment
- Feral Hog Website

Pet Waste

- Pet Owner Outreach and Education
- Pet Waste Stations
- Pet Code Enforcement
- Tailored Pet Solutions

OSSFs

- OSSF Education and Assistance
- Mandatory OSSF Inspection
- and Maintenance Program

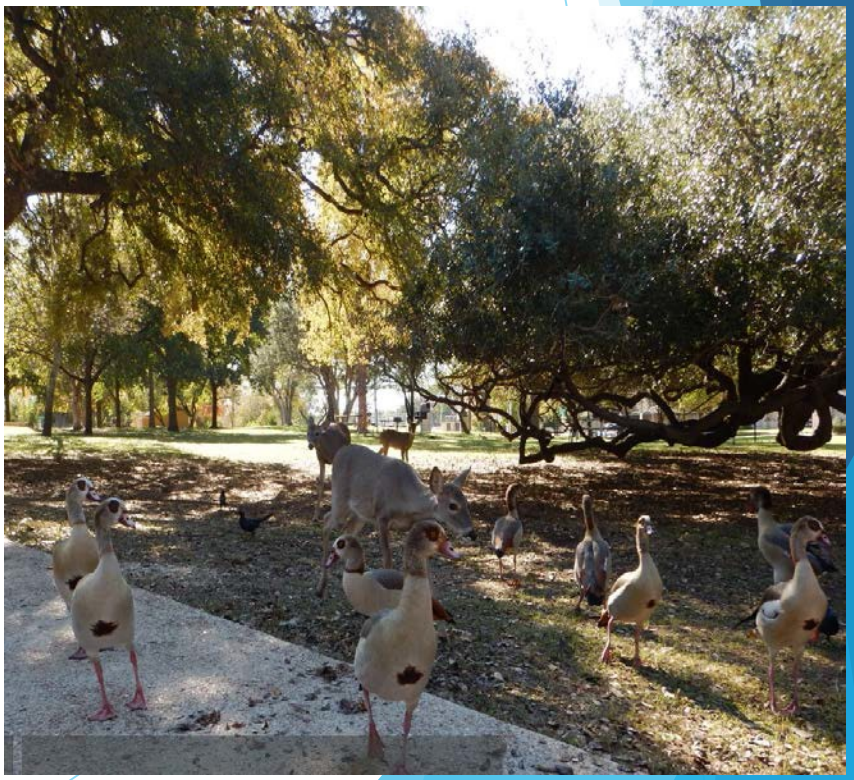
Dry Comal Creek & Comal River WPP

- WPP accepted by EPA in Sept 2018
- City of New Braunfels awarded WPP Implementation Grant: Sept 2018- Aug 2021

Negative Impacts of Overabundant Urban Wildlife

- High-density populations of both deer and waterfowl within City in portions of the watershed near waterways
- Negative Impacts include WQ degradation, deer/ auto collisions, wildlife health, etc.

Year	# of Deer Carcasses collected along roadways within City
2015	498
2016	644
2017	528
2018	632



Urban Wildlife Management

- Wildlife Feeding Ordinance passed in Sept 2018, becomes effective in March 2019
- Utilized demonstrated negative impacts as tool to inform City Council and residents
- Texas Parks and Wildlife Department Assistance
- Community education-workshops, surveys, etc

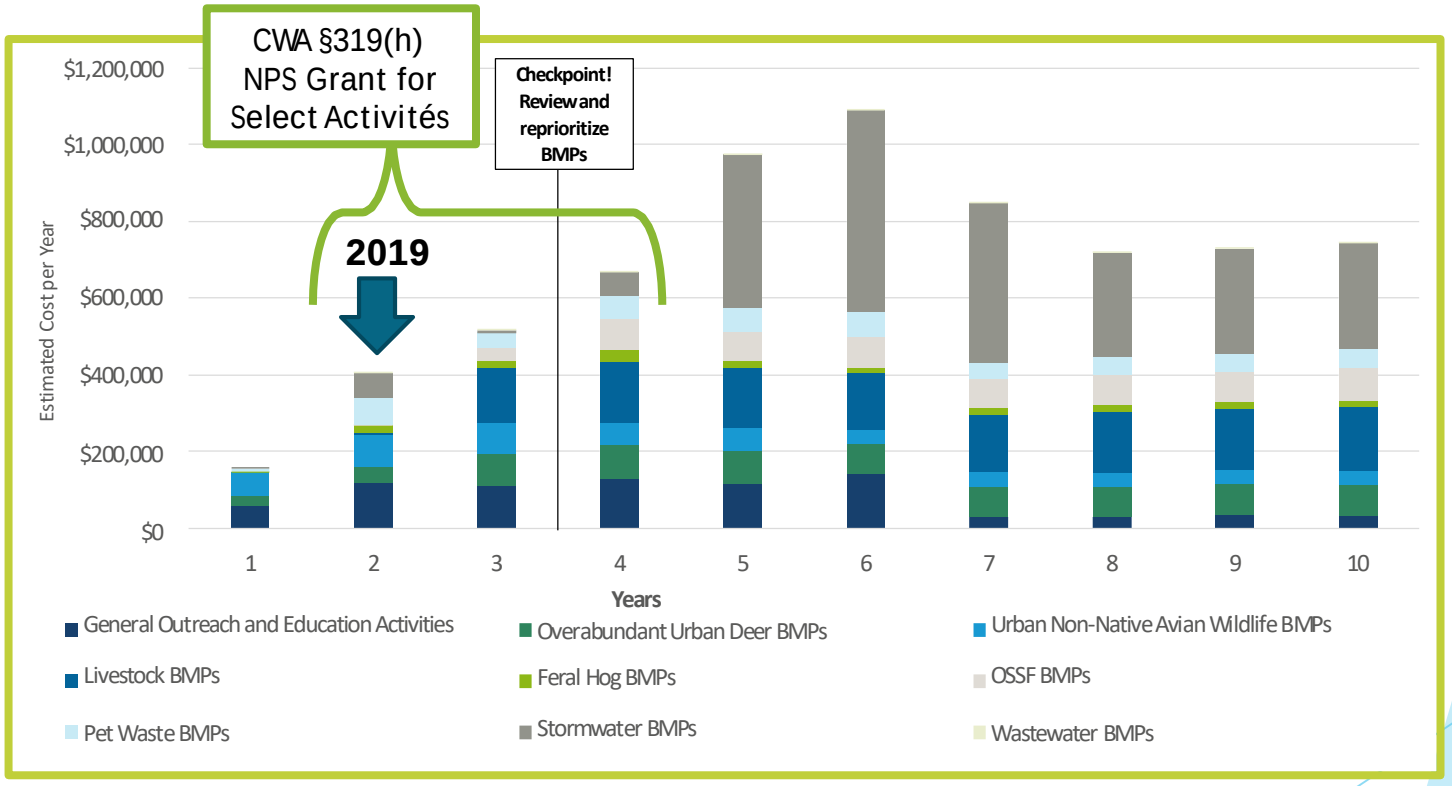
Urban Wildlife Management

- Active Management of Non-Native Waterfowl in Landa Park (includes trapping and oil-coating eggs)

Outreach & Education Critical!

- **Core Message:** Protect our springs, rivers, and watersheds by taking proactive steps to mitigate bacteria levels and enhance water quality.
- **Critical Activities:**
  - Social Media Campaign
  - News Campaign
  - Youth Activities
  - Local Community Events
  - Wildlife Feeding Campaign (including Do-Not-Feed Ordinance)
  - Wildlife Workshops

Implementation Schedule



Acknowledgements

- Guadalupe-Blanco River Authority
- Edwards Aquifer Authority
- Texas Commission on Environmental Quality
- Environmental Protection Agency
- Texas Parks and Wildlife Department
- Arcadis, Inc.
- **Local Stakeholders**

Questions?

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Roundtable Discussion

Thank you!

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