North Central Texas Watershed Stakeholder Meeting

Staff Contact: Ryann Cline
Welcome!
Use of Bacterial Source Tracking to Characterize Texas Watersheds

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Where Did the Bacteria Come From?

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

1. Isolate *E. coli*
2. DNA Fingerprint
3. 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

1. Isolate *E. coli*
2. DNA Fingerprint
3. Compare to Library
4. 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
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

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

Human (1)
Pets (2)
Cattle (3)
Other livestock, avian (4)
Other livestock, non-avian (5)
Wildlife, avian (6)
Wildlife, non-avian (7)
**Texas *E. coli* BST Library Composition & Rates of Correct Classification (RCC)**

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

*ARCC** = 100% (3-way), 91% (7-way)***
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

- Wildlife (n=57) 53%
- Livestock and Domesticated Animals (n=35) 32%
- Human (n=4) 4%
- Unidentified (n=12) 11%
E. coli BST Results
5 Sampling Sites (3-Way Split)

Wildlife
Domestic Animals
Human
Unidentified

<table>
<thead>
<tr>
<th>Source ID</th>
<th>20484</th>
<th>17406</th>
<th>12647</th>
<th>12556</th>
<th>12640</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Plum Creek at Heidenreich Ln</td>
<td>Plum Creek at Plum Creek Rd</td>
<td>Plum Creek at CR 202</td>
<td>Clear Fork at CR 128</td>
<td>Plum Creek at CR 135</td>
</tr>
</tbody>
</table>

E. coli Source ID (%)
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

3-Way Split
(averages based on 11 watersheds)

- Wildlife: 51%
- Unidentified: 12%
- Human: 10%
- Domestic Animals: 27%

Typical Landuse in 11 BST Watersheds

- Rangeland: 40%
- Forestland: 31%
- Pasture: 15%
- Cropland: 7%
- Barren: 1%
- Water: 1%
- Developed: 5%
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

Advantages:
- Cost
- Time
**Bacteroidales BST Results**

**Base Flow Samples** (n=225)

![Bar graph showing positive hits by category: General (n=216), Human (n=11), Ruminant (n=105), Hog (n=63), Horse (n=0).]
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

E. coli (MPN/100 mL)


Single sample std
Geo mean std
Total *Bacteroides* Levels

<table>
<thead>
<tr>
<th>Date</th>
<th>Bacteroides (copies/100 mL)</th>
</tr>
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<tbody>
<tr>
<td>8/28/2017</td>
<td>10^2</td>
</tr>
<tr>
<td>9/11/2017</td>
<td>10^3</td>
</tr>
<tr>
<td>9/25/2017</td>
<td>10^5</td>
</tr>
<tr>
<td>10/9/2017</td>
<td>10^4</td>
</tr>
<tr>
<td>10/23/2017</td>
<td>10^6</td>
</tr>
<tr>
<td>11/6/2017</td>
<td>10^7</td>
</tr>
</tbody>
</table>
Human *Bacteroides* Levels

![Graph showing human *Bacteroides* levels from 8/28/2017 to 11/6/2017. The x-axis represents dates from 8/28/2017 to 11/6/2017, and the y-axis represents *Bacteroides* levels in copies per 100 mL. The graph displays scattered data points indicating varying levels over the specified period.](image-url)
Human: Total *Bacteroides* Levels

![Graph showing human to total Bacteroides levels over time](image-url)
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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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)
$E. \text{coli}$ Concentrations

Texas Integrated Report Assessment Results -
$E. \text{coli}$ Geometric Means for Comal River (1811) and Dry Comal Creek (1811A)

- **Dry Comal Creek**
- **Comal River**
- **Recreational Standard for Bacteria (126 MPN)**

<table>
<thead>
<tr>
<th>Year</th>
<th>Dry Comal Creek</th>
<th>Comal River</th>
<th>Recreational Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>89</td>
<td>33</td>
<td>126 MPN</td>
</tr>
<tr>
<td>2008</td>
<td>89</td>
<td>46</td>
<td>126 MPN</td>
</tr>
<tr>
<td>2010</td>
<td>174</td>
<td>69</td>
<td>126 MPN</td>
</tr>
<tr>
<td>2012</td>
<td>291</td>
<td>105</td>
<td>126 MPN</td>
</tr>
<tr>
<td>2014</td>
<td>302</td>
<td>121</td>
<td>126 MPN</td>
</tr>
<tr>
<td>2016</td>
<td>322</td>
<td>163</td>
<td>126 MPN</td>
</tr>
</tbody>
</table>
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

Comal River E.coli Monitoring Locations

- Pecan Island (58 MPN)
- Landa Haus (99 MPN)
- Landa Park 16 CRP (93 MPN)
- Hinman Blvd CRP (160 MPN)
- Mill Pond (160 MPN)
- Dry Comal Crk @ Seguin St CRP (292 MPN)
BST for Comal River & Dry Comal Creek

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

- **2016**
  - 3 sites
  - 3 rounds of sampling (Sept – Oct)
  - \( \geq 25 \) 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
- Local knowledge used to inform locations of pollution not previously captured
## 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.

<table>
<thead>
<tr>
<th>Year</th>
<th># of Deer Carcasses collected along roadways within City</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>498</td>
</tr>
<tr>
<td>2016</td>
<td>644</td>
</tr>
<tr>
<td>2017</td>
<td>528</td>
</tr>
<tr>
<td>2018</td>
<td>632</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Years</th>
<th>Estimated Cost per Year</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>$800,000</td>
</tr>
<tr>
<td>2</td>
<td>$600,000</td>
</tr>
<tr>
<td>3</td>
<td>$400,000</td>
</tr>
<tr>
<td>4</td>
<td>$200,000</td>
</tr>
<tr>
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<tr>
<td>6</td>
<td>$0</td>
</tr>
<tr>
<td>7</td>
<td>$0</td>
</tr>
<tr>
<td>8</td>
<td>$0</td>
</tr>
<tr>
<td>9</td>
<td>$0</td>
</tr>
<tr>
<td>10</td>
<td>$0</td>
</tr>
</tbody>
</table>

**CWA §319(h) NPS Grant for Select Activities**

**Checkpoint!**
- Review and reprioritize BMPs

**Years**
- General Outreach and Education Activities
- Overabundant Urban Deer BMPs
- Livestock BMPs
- Feral Hog BMPs
- Pet Waste BMPs
- Stormwater BMPs
- Urban Non-Native Avian Wildlife BMPs
- OSSF BMPs
- Wastewater BMPs
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?

- Mark Enders
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Roundtable Discussion
Thank you!
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