BST as part of an integrated approach towards understanding adoption and efficacy of BMPs in an agriculture dominated watershed
Acknowledgements

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• Previous BST projects
  – Funding Organizations: TDEC, EPA, TVA, USGS, USDA, Blount County Soil Conservation, City of Gatlinburg, KUB, UT
  – Watersheds and Watershed groups: Stock Creek, Hiwasse River, Little River, West Prong of the Little Pigeon River, Pond Creek, Duck River
Outline

• Overview of water quality issues in Tennessee/southeastern US
• The use of *Bacteroidales* quantitative PCR assays for BST
• Integration of source identification assays in the Oostanaula Watershed
• Identification of bacteria in fecal sources using 16S rRNA amplicon pyrosequencing
Tennessee has >60,000 miles of streams and rivers and a relatively low per capita income.
64% of assessed streams do not meet water quality standards
What are the pollutant sources?

- Agriculture: 42%
- Hydrologic Modification: 20%
- Municipal: 18%
- Other: 15%
- Construction: 5%
Purported Ag Pollutant Sources

- Grazing in Riparian Zone: 64%
- Non-irrigated Crop Production: 31%
- Animal Feeding Operations: 3%
- Other: 2%
Grazing in the riparian zone

Fixing these watershed problems requires the implementation of voluntary BMPS
BST as a method to assess pollution sources at a farm scale and guide BMP implementation

- Rapid, cost effective *Bacteroidales* assays
  - Identify sources (cattle, human)
  - Estimate concentration of fecal material in water
    - Calculate fecal loading in streams based on stream discharge and concentrations
    - Map locations and types of fecal inputs
    - Model transport and predict load reductions
    - Verify effectiveness of the BMP
Direct PCR from water sample (no DNA extraction). The number of genes can be determined over a wide range of concentrations.

log-linear relationship between concentration and # of cycles to detect fluorescence
Relationship of *E. coli* concentrations and sources determined by BST

- **All**
  - >126 CFU/100ml (81%)
    - 35% Bovine (*E. coli* GM = 1384)
    - 22% Human (*E. coli* GM = 664)
    - 12% Bovine & Human (*E. coli* GM = 2365)
    - 31% Neither (*E. coli* GM = 3122)
  - <126 CFU/100ml (19%)
    - 84% No Source Marker
Relationship of *E. coli* to *Bacteroidales* assays

**E. coli by source vs no Source**

Mosaic Plot

**E. coli by different sources**

Mosaic Plot

Significant  $P < 0.001$
Correlations of molecular assays to colilert *E. coli*

**Bacteroidales to colilert *E. coli***

**colilert *E. coli* to m*E.coli***

![Graph showing correlations](image)

- AllBAc ($r^2 = 0.22$)
- HuBac ($r^2 = 0.01$)
- BoBac ($r^2 = 0.10$)

![Graph showing correlations](image)

- $r^2 = 0.68$
Differential decay of *E. coli* and *Bacteroidales* in cow patties (summer)

**E. coli**

![Graph showing the decay of E. coli with the slope and R² values](image1)

**Bacteroidales**

![Graph showing the decay of Bacteroidales with the slope and R² values](image2)

Markers measured in 8 cow patties for up to 28 days
Comparison of bacterial markers by season

Best correlation between *E. coli* and Bacteroidales may occur in the winter.
Application of BST assays to watersheds

• What area of the watershed has the most/least pathogen impairment?
  – Can I use this information to help determine where I should spend my time/money on remediation?
  – Have the levels or locations of pathogen impairment changed over time?

• We do **NOT** attempt to address:
  – If a sample is negative is the water safe?
  – Can *Bacteroidales* assays be used as a daily monitoring tool?
Oostanaula Watershed

• 303d list of impaired stream
• *E. coli*, Phosphorus, Siltation
  – 54.2 to 72.2% in *E. coli* load reduction
• Landuse
  – Upper – Pasture/Row Crop/AFO
  – Mid – Urban/Residential
  – Lower – Pasture/Forest
Preliminary data: *E. coli* and *Bacteroidales* in Hiwasse Watershed

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Main Channel Length</th>
<th>Correlation between <em>E. coli</em> and <em>Bacteroides</em></th>
<th>Host animal Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chatata/Little Chatata</td>
<td>8.5 miles</td>
<td>0.65</td>
<td>0.1-10% Bovine 1-25% Human</td>
</tr>
<tr>
<td>Oostanaula</td>
<td>50 miles</td>
<td>0.98</td>
<td>22%-92% Bovine 0.5%-7.0% Human</td>
</tr>
</tbody>
</table>

6 to 8 sites in each sub watershed sampled on one date
Sampling

- 10 sites
- USGS gage at site 1
- Sampled 28 times over 18 months
- 260 samples
  - Colilert
  - Turbidity
  - Quantitative PCR
    - Total *Bacteroidales*
    - Bovine *Bacteroidales*
Are *E. coli* concentrations correlated to Bacteroidales concentrations?

• Across all samples *E. coli* is equally correlated to both total *Bacteroidales* and bovine associated *Bacteroidales*.
• However, turbidity is not well correlated with *Bacteroidales*. 
Which site has highest concentrations of *E. coli* and Bacteroidales?

<table>
<thead>
<tr>
<th>Site #</th>
<th>E. coli (MPN)</th>
<th>ABAC mg/L</th>
<th>BoBac mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>40</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1500</td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2000</td>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>2500</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>120</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Site 9 (upstream site) has the highest concentrations of *E. coli*, total *Bacteroidales* and bovine associated *Bacteroidales*
- *E. coli*, total *Bacteroidales* and bovine associated *Bacteroidales* drop at sites around the Athens city limit.
Is bacterial concentration related to hydrology?
Oostanaula Creek near Sanford, TN
Flow Duration Curve

USGS Gage: 03565500

Flow Duration Interval (%)

Flow (cfs)

176 cfs
71 cfs
44 cfs
23 cfs

High Flows
Moist Conditions
Mid-range Flows
Dry Conditions
Low Flows

USGS Flow Data

57 square miles
Discharge vs Site vs Marker

**E. coli**

**Bovine Bacteroidales**
Discharge vs Site vs Marker

Total Bacteroidales

Turbidity

Athens

Stream

Discharge
Summary

- The majority of fecal contamination (*Bacteroidales* and *E. coli*) arises at site 9
  - Highest concentrations and potential longest distance of travel is associated with high flows (e.g. runoff during storms)
  - Compare empirical model with calibrated ArcSwat model
What types of BMPs lead to improved water quality and which ones will producers adopt?

- Economic perspective
- Enhance capability to model effect of BMP adoption on water quality (e.g., *E. coli*, Bacteriodales)
- Supplement biophysical model
  - Farm demographic, operator attributes
  - Livestock management practices: beef cattle dominate over dairy cattle
Types of BMPs

- Rotational grazing
- Waterers
Pasture improvement
Stream Crossing
BMP economic survey

- Surveyed producers in 6 watersheds in McMinn County
- Tax Parcel records used to generate survey list for properties zoned for agriculture
- Estimate supply response of BMPs
  - Waterers, stream crossings, rotational grazing, pasture improvement (modeled after EQIP programs)
  - Hold operator, farm business, and environmental data constant
  - Vary incentives, management practices (e.g., stocking density)
- Final Survey (February, 2010)
  - 437 responses (30% response rate)
  - 143 beef cattle operations (34% of responses)
  - 13,962 pasture acres represented
## BMP adoption patterns

<table>
<thead>
<tr>
<th>Practice</th>
<th>Adopters (% of total cattle operations)</th>
<th>Total Units of practice adopted across 5 watersheds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream crossing -4</td>
<td>23 (16%)</td>
<td>1,302 ft$^2$</td>
</tr>
<tr>
<td>Rotational grazing -2</td>
<td>62 (42%)</td>
<td>3,456 ac</td>
</tr>
<tr>
<td>Pasture improvement -1</td>
<td>100 (68%)</td>
<td>4,694 ac</td>
</tr>
<tr>
<td>Waterer -3</td>
<td>61 (41%)</td>
<td>171 troughs</td>
</tr>
</tbody>
</table>
Summary

• Pasture improvement was the preferred BMP by producer
  – Positive benefit for producer-More grass = More beef
  – Positive benefit for watershed-Less runoff = Less pathogens, nutrients, sediment
• Stream crossing improvement was the least preferred BMP by producers
  – Positive benefit for watershed, producers less convinced of benefit
• The willingness of adoption of the improvement was not dependent on the cost
• BMP implementation is focusing on the upper end of the watershed and emphasizes both pasture improvement and stream crossing improvements
• BST and water quality monitoring will be performed after BMPs have been become established to determine efficacy of the BMPs
  – Hypothesize that establishment of BMPs will lead to acceptable *E. coli* concentrations and low Bacteroidales concentrations
Questions?
Can beef and dairy manure be differentiated by monitoring multiple fecal bacteria or by total community analysis?

• Different diets/husbandry may result in different gut and fecal bacteria

• Multiple new technologies to identify bacteria in complex systems:
  – Phylochip
  – 16S rRNA amplicon pyrosequencing
  – PCR arrays
Differentiation of fecal sources using 16S rRNA amplicon pyrosequencing

- Microbial community analyses using high throughput sequencing (1000’s of sequences per library)
- 14 beef cow patties collected in fall, winter, spring and summer (8 fresh and 6 aged 72 hrs)
- 8 dairy cows patties, a dairy lagoon and a dairy floor
- Data being analyzed and compared to microbial communities from sewage
16S amplicon pyrosequencing

DNA extractions

16S PCR amplification using 27f and 1492r

PCR Amplification using Barcoded Primers

Clean-up

Emulsion PCR

Mixed 10 samples/region

454 Sequencing

Data Analysis

Clean-up
Data analysis requires bioinformatics

- Ribosomal Database Project (RDP)
  - SSU rRNA collection contains 1400 Bacteria
  - Sequences arranged in phylogenetic order and aligned form
  - Each sequence is annotated with the sequence data origin and organismal source

- MG-Rast
  - Web based program with easy to visualize graphics
  - Comparisons of the user’s data and previously sequenced Prokaryotes and Eukaryotes can be made
  - Analysis is based on phylogenetic annotations only
  - Difficult to find statistical measurements

- Mothur
  - Command line
  - Analysis can be done based on OUT’s or phylogeny
  - More difficult to do comparisons with other sample sets

- Qiime
  - Command line/Cloud version
Characterization of bacteria based on phylogenetic identification
Uses of Phylogeny

• Identify dominant groups and potentially host specific strains within the groups
  – Bacteroidales
  – Clostridium

• Identify potential pathogens
  – *Streptococcus parauberis*, causes mastitis in dairy cattle, found in the dairy lagoon, floor and 1 dairy cows
  – *Campylobacter jejuni* subsp. *Jejuni* and *Campylobacter* sp. 86/06 found in 1 dairy cow.
  – *E. coli* found in dairy lagoon and 4 dairy cows
Principal Component Analysis

Possible approach for differentiating fecal sources of fecal based on complete community analysis

- Sewage samples grouped separately from cattle manure samples
  - More samples are needed to determine whether dairy will group separately from beef
Summary: Uses of *Bacteroides* assay for BST

- Allows prioritization of remediation and implementation of BMPs in subwatersheds
- Provides before and after data for confirmation of reduction in pathogens for TMDL implementation
- Future use of multiple bacterial groups may allow better resolution of sources of fecal contamination