Palmer River: Collaborative Sampling and Fecal Source Tracking with the PhyloChip

NORTHEAST AQUATIC BIOLOGISTS CONFERENCE

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Summary

• Watershed Description and Context
• Timeline and Sampling
• Farmer Engagement and Best Management Practices
• PhyloChip Technology and Work in the Palmer
Palmer River Watershed
“Where’re you steering to? You’ve run us into the eel-grass!”

“That’s so! I wasn’t looking for it so soon. We can’t be far from the channel,” . . . and presently we were in a sort of lane of clear water, on either side of which the eel-grass dotted the surface as far as we could see.
Palmer River
Palmer River Timeline

1990s
- RI Shellfish Area Permanently Closes
- RIDEM TMDL Sampling

2000s
- RIDEM Bacteria TMDL
- MADEP Bacteria TMDL
- RIDEM Issues Permits with Nitrogen Limits for Warren WWTF and Blount Seafood

2010s
- RIDEM, MADEP, and EPA Joint Sampling Project
- MADEP Bacteria Source Tracking
- NRCS Designates Watershed for Targeted Funds
- Significant Investment in Agriculture BMPs
RIDEM, MADEP, and EPA Joint Sampling Project
RIDEM, MADEP, and EPA Joint Sampling Project Sampling Stations

Canoe Trip

2016-2019 April-November Monthly Sampling
MassDEP Bacterial Source Tracking Toolbox

Meter: pH, temp, salinity, conductivity, TSS

IDEXX

E. coli and Enterococcus Bacteria Analyses

Spectrophotometer
MassDEP Bacterial Source Tracking Toolbox

- Ammonia Test Strips
- Optical Brightener Pads (Fluorescence)
- Detergents (Surfactants) Chemetrics Kit
- WES-Human Marker Tests and Limited Additional Biological and Chemical Testing
MassDEP
Bacterial Source Tracking Success
MassDEP Bacterial Source Tracking Success
BMP Project Partnership
Engagement with Farmers

• Ongoing dialogue to build trust and awareness of opportunities to implement conservation practices.

• Assisting with grant applications, engineering, financing, contracting, inspecting and completing paperwork related to successful conservation practice implementation.

• Showcasing successful implementations to build interest and confidence with other area landowners regarding installation of conservation practices.
Dairy
Cattle
Other BMP Projects
Rocky Run at Davis Street
Rocky Run at Davis Street 2000
2018 – Owners have decided that the parcel is too small for animals.
RIDEM Continues Bacteria Monitoring in the Estuarine Palmer River
Fecal Source Tracking: DNA Microarray Analysis in the Palmer River Watershed
Fecal Indicator Bacteria

• Over 10,000 impaired waterbodies
  • Human health risk, beach closures, economic impacts

• Fecal indicator bacteria (FIB) are a proxy
  • False positives

• Human waste a better indicator of potential disease

• Direct measurement pathogens challenging
  • Cost
  • Hard to culture
  • Takes time
PhyloChip Technology

- Based on probing the 16S rRNA gene
  - Present in all bacteria and archaea
  - Highly conserved
  - Nine hypervariable regions
- Doesn’t rely on culturing
  - Most bacteria aren’t culturable
- Rapid, high throughput
PhyloChip Technology

• Based on probing the 16S rRNA gene
  • Present in all bacteria and archaea
  • Highly conserved
  • Nine hypervariable regions
• Doesn’t rely on culturing
  • Most bacteria aren’t culturable
• Rapid, high throughput
PhyloChip Technology

- DNA extraction
- PCR amplification of 16S rRNA gene
- 1.2 Million probes per chip
- 59,000 individual microbial taxa
- Relative fluorescence measured
- Algorithm determines source based on gut microbiome “reference libraries”

Taken from: Dubinsky et al. 2012
Palmer River Project

- Samples collected 2017-2019
- Analysis is costly
- How do we select a sub-set of samples that is representative of:
  - Space
  - Time
  - Contamination source
- 50 samples analyzed
- EPA contracted with Horsley-Whitten and FB Environmental for analysis
Sample Selection Considerations
<table>
<thead>
<tr>
<th>Site ID</th>
<th>PhyloChip® Strong Source</th>
<th>PhyloChip® Marginal Source</th>
<th>MST-DNA Results</th>
<th>Other Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR01</td>
<td>Bird</td>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR02</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR03</td>
<td>Human</td>
<td>Bird, Cow</td>
<td>Cow, pig isolates from ribotyping study</td>
<td></td>
</tr>
<tr>
<td>PM31</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM30</td>
<td>Human</td>
<td>Bird, Cow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM44</td>
<td>Human, Bird</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR23</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR22</td>
<td>Human, Bird, Cow</td>
<td></td>
<td>Cow, pig, horse, human, deer, rabbit, dog isolates from ribotyping study</td>
<td>Historic septic system failure at RR02 (upstream); remediated by 2015</td>
</tr>
<tr>
<td>TC07</td>
<td>Human, Cow</td>
<td>Bird, Pig, Dog, Horse</td>
<td>Cow, pig isolates from ribotyping study</td>
<td>Waterfowl identified in 2004 MA TMDL</td>
</tr>
<tr>
<td>TC08</td>
<td>Human, Bird</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM29</td>
<td>NA</td>
<td>NA</td>
<td>Weak human Bacteroidetes marker</td>
<td>Major geese congregation</td>
</tr>
<tr>
<td>PM43</td>
<td>Bird</td>
<td>Human</td>
<td></td>
<td>Major geese congregation</td>
</tr>
</tbody>
</table>

Fecal Sources Detected
Strong Signals of Fecal Source Types
Table 3. Taxonomic richness of pathogenic bacteria in the Palmer River. Values represent the number of detected OTUs summed across all samples for each site. Shading indicates the following: no shading (< 10 OTUs), light yellow (10-50 OTUs), yellow (51-100 OTUs), orange (101-150 OTUs), red (151-300 OTUs), and dark red (>300 OTUs). Taxa (rows) are ordered from greatest to least total counts for all sites. Sites (columns) are ordered from upstream to downstream.

<table>
<thead>
<tr>
<th>[Class] Genus Species</th>
<th>CR01</th>
<th>CR03</th>
<th>PM30</th>
<th>PM44</th>
<th>RR22</th>
<th>TC07</th>
<th>TC08</th>
<th>PM43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacilli Staphylococcus spp.</td>
<td>68</td>
<td>265</td>
<td>104</td>
<td>87</td>
<td>260</td>
<td>178</td>
<td>189</td>
<td>82</td>
</tr>
<tr>
<td>Bacilli Streptococcus spp.</td>
<td>50</td>
<td>125</td>
<td>83</td>
<td>42</td>
<td>126</td>
<td>106</td>
<td>150</td>
<td>175</td>
</tr>
<tr>
<td>Gammaproteobacteria Serratia marcescens</td>
<td>20</td>
<td>46</td>
<td>43</td>
<td>29</td>
<td>51</td>
<td>43</td>
<td>43</td>
<td>32</td>
</tr>
<tr>
<td>Gammaproteobacteria Proteus mirabilis</td>
<td>9</td>
<td>12</td>
<td>22</td>
<td>17</td>
<td>16</td>
<td>20</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>Gammaproteobacteria Salmonella enterica</td>
<td>11</td>
<td>10</td>
<td>20</td>
<td>12</td>
<td>13</td>
<td>16</td>
<td>17</td>
<td>31</td>
</tr>
<tr>
<td>Epsilonproteobacteria Helicobacter spp.</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Epsilonproteobacteria Campylobacter subantarcticus</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gammaproteobacteria Legionella pneumophila</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gammaproteobacteria Vibrio cholerae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Pathogenic Bacteria Detected
False Positives and Negatives

Table 6. Summary of samples (n, %) by source type (human, bird, dog, horse, pig, and cow) for *E. coli* and enterococci for the classifications of false negative, false positive, true negative, and true positive (refer to text and Figure 5 for determination criteria).

<table>
<thead>
<tr>
<th></th>
<th>Number of Samples by Source Type</th>
<th>Portion of Samples by Source Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human</td>
<td>Bird</td>
</tr>
<tr>
<td><em>E. coli</em> (235 MPN/100mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>false negative</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>false positive</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>true negative</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>true positive</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td><em>Enterococci</em> (104 MPN/100mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>false negative</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>false positive</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>true negative</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>true positive</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>
Potential For Use in Other Watersheds

- Summarize source types by site and compare to expected sources
- Determine the dominant OTUs (Observed Taxonomic Units) for each source type and how community composition and structure change in relation to space, time or water quality
- Track water quality improvements over time
- Identify factors that help predict presence of source types or best capture possible source types
Citations and for More Information


• https://ipo.lbl.gov/lbnl2229/


• Hazen et. al. (2010). Deep-Sea Oil Plume Enriches Indigenous Oil-Degrading Bacteria. Science, 330 (6001), 204-208. DOI: 10.1126/science.1195979

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QUESTIONS?

THANK YOU!