UPDATE:
Understanding Nutrient Impacts and Sources at the Watershed Scale to Enhance Environmental Stewardship

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Proposal Objectives

• Analyze archived water samples for bovine, swine, and poultry source-specific DNA markers
• Identify associations between nutrients, MST markers, land use, land characteristics, agricultural practices, and climate
• Create maps identifying locations to focus mitigation strategies
Relationship to Corn Growers goals

• “support non-proprietary, common good research to optimize economic returns and environmental stewardship for corn production”

• By linking microbial source tracking (MST) with nutrient loading, we will be able to identify which agricultural practices that degrade water quality
Sampling approach

Baseflow (October 2010)
Snow melt (March 2011)
Early summer rain (June 2011)

64 River systems

84% Lower Peninsula drainage area
Approach – Sample analysis

*Escherichia coli (E. coli)*

- General indicator of fecal contamination
- Linked to gastrointestinal illness through epidemiological studies *(DuFour et al. 1982; Wade et al. 2006, 2008, 2010)*

- USEPA recreational freshwater criterion: 2.5 log CFU/100 ml

- IDEXX Colilert® Quanti-Tray 2000®
Approach for MST Analysis

- droplet digital PCR (ddPCR)
  - Absolute quantification
  - High accuracy and precision
  - No standard curve
- Microbial Source Tracker (MST)
M2 Bovine Marker

• Bovine M2 Marker
• Specific to *Bacteriodales* bacteria in cows
• 100% Sensitivity
• >90% Specificity
• Tested by 5 separate Labs
Porcine Marker

- Pig2Bac
- Specific to *Bacteriodales* bacteria in pigs
- 100% Sensitivity
- 99% Specificity
Approach: Statistical Methods

• Basic stats: Correlation (Spearman Rank), Regression

• Classification And Regression Tree (CART)
  – Automated trial-and-error algorithm to classify data according to the influence of a potentially large number of independent variables (land use, nutrients, soils)
  – Sequentially splits dependent variable (bovine marker) into groups
  – Split variable and value selected that produces the least variance within groups
  – A tree is formed as subgroups are then split hierarchically
  – Final tree is pruned to include only significant splits
Updated analyses
  Baseflow, 
  Snowmelt and
  Summer Rain

• New variables in CART analyses
  • % Cropland Data Layers (CDL from National Ag, Stat Services) provides crop types
  • National Land Cover Data (NLCD from USGS)
  • Annual mean concentration of N applied to the land from all animal manure
  • Ag fertilizer
### NLCD classifications

<table>
<thead>
<tr>
<th>NLCD Land Cover Classification Legend</th>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 Open Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Perennial Ice/ Snow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 Developed, Open Space</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 Developed, Low Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23 Developed, Medium Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Developed, High Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 Barren Land (Rock/Sand/Clay)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41 Deciduous Forest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42 Evergreen Forest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43 Mixed Forest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51 Dwarf Scrub*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52 Shrub/Scrub</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71 Grassland/Herbaceous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 Sedge/Herbaceous*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>73 Lichens*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>74 Moss*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>81 Pasture/Hay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82 Cultivated Crops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 Woody Wetlands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95 Emergent Herbaceous Wetlands</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Alaska only
# Results of the M2P Bovine Marker

<table>
<thead>
<tr>
<th></th>
<th>Baseflow (Fall)</th>
<th>Snow Melt (Spring)</th>
<th>Summer Rain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percent Positive</strong></td>
<td>45% (28/63)</td>
<td>62% (38/61)</td>
<td>75% (47/63)</td>
</tr>
<tr>
<td><strong>Average Concentrations (CE/100ml)</strong></td>
<td>35.7</td>
<td>21.2</td>
<td>31.2</td>
</tr>
<tr>
<td><strong>Range of Concentrations (CE/100ml)</strong></td>
<td>5.4 – 224</td>
<td>1.6-807</td>
<td>1.6 – 748.8</td>
</tr>
</tbody>
</table>
Baseflow - Bovine

Bovine MST marker
Baseflow
Non-detect
Detectable levels

Baseflow
Non-detect
Lowest 25%
Middle 50%
Highest 25%
Baseflow – Bovine (PRE 58%)

(PRE: Proportional Reduction Error, Percentage of Variance Explained)

NLCD Woody Wetlands (27%)
- NOX (25%)
- TDN (22%)

NLCD Open Water (11%)
- TDN (7%)

NLCD Deciduous Forest (10%)
- NLCD Low Intensity Urban (7%)

Soil Hydraulic Conductivity (10%)
- No competitors

N=15
- Bovine 1.6

N=7
- Bovine 1.4

N=14
- Bovine 0.28

N=19
- Bovine 0.14

N=9
- Bovine 1.3

* Bovine reported in log10. Detection limit = 0.11
Snowmelt - Bovine

Bovine MST marker

Snowmelt
- Non-detect
- Detectable levels

Lowest 25%
Middle 50%
Highest 25%

Bovine MST marker

Snowmelt
- No sample
- Non-detect
- Lowest 25%
- Middle 50%
- Highest 25%
Snowmelt – Bovine (PRE 44%)

(PRE: Proportional Reduction Error, Percentage of Variance Explained)

**NLCD Barren (27%)**
Dissolved Oxygen (25%); Shed area (22%)

**Dam density (6%)**
No competitors

**Riparian Water (7%)**
Shed area (6%); CDL Other Tree Crops (5%)

N=8
Bovine 1.5

N=12
Bovine 0.77

N=7
Bovine 0.82

N=34
Bovine 0.28

* Bovine reported in log10. Detection limit = 0.11
Summer Rain – Bovine (PRE 63%)

Riparian Septics (31%)
- CDL Soybeans (29%)
- NLCD Grassland/Herbaceous (28%)
- Septics (26%)

Prior Rain, 3-days (14%)
- No Competitors

Soil Hydraulic Conductivity (5%)
- WWTP & Septics (3.5%
- SRP (2%)

Chlorophyll a (13%)
- Prior Rain, 4-days (9%)

Prior Rain, 4-days (9%)
- No Competitors

Chlorophyll a (13%)
- Prior Rain, 4-days (9%)

Soil Hydraulic Conductivity (5%)
- WWTP & Septics (3.5%)
- SRP (2%)

* Bovine reported in log10. Detection limit = 0.11
Baseflow – TDN (PRE 58%)

(PRE: Proportional Reduction Error, Percentage of Variance Explained)

CDL Other Crops (45%)
No Competitors

CDL Low Intensity Urban (10%)
NLCD Low Intensity Urban (10%); Riparian Barren (7.5%); Impervious (5%)

N=42
TDN 827

N=8
TDN 3794

N=14
TDN 1132
Baseflow – NOX (PRE 52%)

(PRE: Proportional Reduction Error, Percentage of Variance Explained)

CDL Other Crops (43%)
No Competitors

MSU estimated N from fertilizer (9%)
NLCD Cultivated Crops (8.5%); Riparian Ag (8.5%); Riparian Barren (8%)

N=8
NOX 3123

N=37
NOX 240

N=19
NOX 1109
Baseflow – TP (PRE 42%)

Riparian Septics (36%)
Septics (34%); CDL Grassland/Pasture (32%); NLCD Developed Open Space (31%)

NLCD Shrub/Scrub (6%)
No Competitors

N=40
TP 18

N=17
TP 47

N=7
TP 127

(PRE: Proportional Reduction Error, Percentage of Variance Explained)
Baseflow CART Results top variables explaining the dependent variables.

<table>
<thead>
<tr>
<th>Bovine marker</th>
<th>TDN</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Woody wetlands</td>
<td>• Other crops</td>
<td>• Total septic tanks in 60 m buffer (riparian zones)</td>
</tr>
<tr>
<td>• NOX &amp; TDN (inverse relationship)</td>
<td>• Low intensity urban</td>
<td>• Less shrubs (inverse relationship)</td>
</tr>
<tr>
<td>• Less open water (inverse relationship)</td>
<td>• Impervious surfaces</td>
<td></td>
</tr>
<tr>
<td>• Grassland/pasture</td>
<td>• Other crops</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• N from fertilizer</td>
<td></td>
</tr>
</tbody>
</table>
Snow melt CART Results top variables explaining the dependent variables.

**Bovine marker**
- Less barren soils (inverse relationship)
- Lower DO (inverse relationship)
- Smaller watersheds
- More dams
- Less riparian areas (inverse relationship)

**TDN**
- N-fertilizer with AG
- Potassium
- Corn

**NOX**
- Corn
- Magnesium
- Cultivated crops

**TP**
- Winter wheat
- Specific conductance
- Less Calcium (inverse relationship)
## Summer Rain CART Results top variables explaining the dependent variables

<table>
<thead>
<tr>
<th>Bovine marker</th>
<th>TDN</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy and grass lands</td>
<td>Total precipitation in the prior 4 days</td>
<td>Less Open land, grasses (inverse relationship)</td>
</tr>
<tr>
<td>Less Septic tanks</td>
<td></td>
<td>Corn</td>
</tr>
<tr>
<td>Less Chlorophyll a (inverse relationship)</td>
<td></td>
<td>Total precipitation in the prior 8 days in small watersheds</td>
</tr>
<tr>
<td>Total precipitation in the prior 3 days</td>
<td>Less barren land (inverse relationship)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry beans</td>
<td></td>
</tr>
<tr>
<td>NOX</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impact*</td>
<td>River Name</td>
<td>Watershed Area (km²)</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>HIGH</td>
<td>Little Pigeon Creek</td>
<td>14</td>
</tr>
<tr>
<td>HIGH</td>
<td>Belangers Creek</td>
<td>25</td>
</tr>
<tr>
<td>HIGH</td>
<td>Monroe Creek</td>
<td>27</td>
</tr>
<tr>
<td>HIGH</td>
<td>Little Trout River</td>
<td>28</td>
</tr>
<tr>
<td>HIGH</td>
<td>Silver Creek</td>
<td>41</td>
</tr>
<tr>
<td>HIGH</td>
<td>Harrington Drain</td>
<td>53</td>
</tr>
<tr>
<td>HIGH</td>
<td>Trout River</td>
<td>82</td>
</tr>
<tr>
<td>HIGH</td>
<td>Sandy Creek</td>
<td>82</td>
</tr>
<tr>
<td>HIGH</td>
<td>Carp River</td>
<td>119</td>
</tr>
<tr>
<td>HIGH</td>
<td>Rush Creek</td>
<td>152</td>
</tr>
<tr>
<td>HIGH</td>
<td>Boyne River</td>
<td>199</td>
</tr>
<tr>
<td>HIGH</td>
<td>Lincoln River</td>
<td>215</td>
</tr>
<tr>
<td>HIGH</td>
<td>Tawas River</td>
<td>403</td>
</tr>
<tr>
<td>HIGH</td>
<td>Black River</td>
<td>1250</td>
</tr>
<tr>
<td>HIGH</td>
<td>Cass River</td>
<td>2174</td>
</tr>
<tr>
<td>HIGH</td>
<td>Au Sable</td>
<td>5287</td>
</tr>
<tr>
<td>HIGH</td>
<td>Tiltabawasee River</td>
<td>6211</td>
</tr>
<tr>
<td>Impact*</td>
<td>River Name</td>
<td>Watershed Area (km²)</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Low</td>
<td>Macatawa River</td>
<td>292</td>
</tr>
<tr>
<td>Low</td>
<td>North Branch Black River</td>
<td>398</td>
</tr>
<tr>
<td>Low</td>
<td>Pine River</td>
<td>440</td>
</tr>
<tr>
<td>Low</td>
<td>Big Sable River</td>
<td>476</td>
</tr>
<tr>
<td>Low</td>
<td>Boardman</td>
<td>716</td>
</tr>
<tr>
<td>Low</td>
<td>Rifle</td>
<td>858</td>
</tr>
<tr>
<td>Low</td>
<td>White River</td>
<td>1049</td>
</tr>
<tr>
<td>Low</td>
<td>Elk-Torch</td>
<td>1308</td>
</tr>
<tr>
<td>Low</td>
<td>Pere Marquette</td>
<td>1790</td>
</tr>
<tr>
<td>Low</td>
<td>Cheboygan</td>
<td>2317</td>
</tr>
<tr>
<td>Low</td>
<td>Manistee</td>
<td>3559</td>
</tr>
<tr>
<td>Low</td>
<td>Muskegon</td>
<td>6418</td>
</tr>
<tr>
<td>Low</td>
<td>St. Joseph</td>
<td>11061</td>
</tr>
<tr>
<td>Low</td>
<td>Grand</td>
<td>12854</td>
</tr>
</tbody>
</table>
Key Findings

• ddPCR lacks the shortfalls of qPCR and is quite useful technique for quantitative assessment of the markers.
• The bovine marker is related to the land use and crops, indirectly related to source of nutrients.
• During baseflow inverse relationship between bovine marker and nitrogen (TN, TDN and NOX), where more low intensity urban and septic tanks were associated with the nutrients.
• Nutrients are associated with crops including corn, estimated fertilizer applications.
• The bovine marker is found when it rains and coming in from runoff, nutrients are also related to rain.
Discussion

- We are beginning to understand the sources of fecal pollution in relationship to the landscape and climate factors (rain).
- The bovine marker could come from both manure and free ranging cattle, the pig marker may help elucidate this.
- *While E.coli* did have a relationship to phosphorous suggesting some relationship to fecal sources, the bovine marker was indirectly related to the nutrients.
- The transport of nutrients needs to be further evaluated in regard to the transport of the bacteria. Is accumulation in soils and/or sediments and later release delay the timing of the nutrient observations in the water column.
- We have identified 17 watersheds where the bovine marker was always present and 14 watersheds that did not have the marker or where it was present only once. These watersheds can be used to identify the impacts of agricultural management practices on water quality.
- Distinguish water quality impacts of fertilizer from those derived from human waste and animal waste/manure used on crop land
  - Focus efforts for water quality improvement
  - Elucidate relationships between agricultural characteristics, nutrient loading, and sources at the watershed scale to resulting water quality across the entire lower peninsula of Michigan.
Next Steps

• Porcine MST marker will be completed in the next few months
• Bird marker analysis will be completed by May
• Further ion analysis will be ongoing to address manure versus fertilizer
• Key counties and watersheds with various BMPs will be identified for future studies.
THANK YOU
Previous Results – *B. theta* and *E. coli* concentrations during base flow
Baseflow

B. Theta CART statistics

- B. theta related to increasing septic tank counts
- Then Buffer deciduous forest
- Then pH

Total PRE = 59.1%

μ = 5.15
n = 64

Septic Count > 1621.5
PRE = 36.4%

μ = 4.73
n = 19

 Buffer Deciduous Forest > 10.7%
PRE = 14.7%

μ = 5.05
n = 16

pH < 8.23
PRE = 8.0%

μ = 5.23
n = 11

μ = 5.62
n = 18

μ = 5.32
n = 45

PRE: Proportional Reduction Error, Percentage of Variance Explained
Previous Results
Baseflow CART – *E. coli*

\[
\text{Total PRE} = 61.0\% \\
\mu = 1.67 \\
n = 64
\]

\[
\text{Total } P > 19.0 \mu g/L \\
\text{PRE} = 47.6\% \\
\mu = 2.03 \\
n = 40
\]

\[
\text{Stream Temperature} < 12.2^\circ C \\
\text{PRE} = 13.4\% \\
\mu = 0.66 \\
n = 12
\]

\[
\mu = 1.47 \\
n = 12
\]

- *E.coli* related to Total phosphorous
- Then stream temperature

PRE: Proportional Reduction Error, Percentage of Variance Explained