Cryptic nutrient sources and elevated $\delta^{15}N$ Johnson Bay

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High nutrients and human/animal wastes are a problem in Maryland’s coastal bays

- Water quality declining with thresholds exceeded (Wazniak et al, 2007)
- Broad surveys have identified ‘hotspots’
  - Broad survey in 2004 (Fertig, 2009)
  - Focus in on hotspots in 2006 (Fertig 2009)
- Johnson Bay hotspot, but land use doesn’t suggest any obvious sources
Johnson Bay watershed rural, protected, clay soils & sediments
Previous studies found 'hotspots' of potential human and/or animal wastes

Fertig et al. 2009. Estuaries and Coasts
Johnson Bay well mixed, but has areas of consistently higher and lower nitrogen.
Nitrogen could enter Johnson Bay in multiple ways – which is most important?

Which hypothesis is correct?

- Land Use?
- Groundwater?
- Import?
Physical and chemical variables vary temporally, not spatially in Johnson Bay.

**a) Physical**
- pH: $p < 0.05$, $n = 70$, $(-0.71, 0.03)$
- Temperature: $(-0.70, -0.11)$
- Dissolved Oxygen: $(-0.69, -0.52)$
- Secchi depth: $(-0.07, -0.89)$
- Salinity: $(0.40, -0.78)$

**b) Chemical**
- Nitrite+Nitrate: $(-0.69, -0.48)$
- Phosphate: $(-0.69, -0.53)$
- Total Nitrogen: $(0.64, -0.64)$
- Total Phosphorus: $(0.85, -0.37)$

Bacterial, viral abundance higher inshore; potential denitrification higher offshore.
Potential shift in relationship between N loading and TN, chlorophyll over time

This study
Nitrogen could enter Johnson Bay in multiple ways – which is most important?

Which hypothesis is correct?

Land Use?

Groundwater?

Import?

Data → ?
Nitrogen could enter Johnson Bay in multiple ways - which is most important?

Which hypothesis is correct?

- Land Use?
- Groundwater?
- Import?

Data → ?
Aquatic microbes can be indicators of water

• Sensitive to environmental change
• Fecal coliforms and enterococci indicate waste inputs
• Antibiotic resistance assay (ARA) indicative of microbes derived from livestock or humans exposed to antibiotics
Sampled along four creeks of varying TN and land use in June and July

<table>
<thead>
<tr>
<th>Creek</th>
<th>TN (mg/L)</th>
<th>Land Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boxiron</td>
<td>0.98</td>
<td>Forest, wetland</td>
</tr>
<tr>
<td>Scarboro</td>
<td>1.7</td>
<td>Marsh, poultry houses</td>
</tr>
<tr>
<td>Powell</td>
<td>7.6</td>
<td>Poultry houses, poultry litter fertilized agriculture, residential draining</td>
</tr>
<tr>
<td>Swan Gut</td>
<td>6.7-7.8</td>
<td>Poultry houses, poultry litter fertilized agriculture, residential draining</td>
</tr>
</tbody>
</table>
Hypotheses

- Nutrient concentrations will be greatest in Swan Gut and Powell followed by Scarboro and Boxiron due to known gradients in TN and land use intensity.

- Bacterial and VLP abundance will be positively correlated with each other (more strongly than with chlorophyll a), with nutrient loading, increasing intensity of land use, and temperature, and negatively correlated with salinity.

- Bacterial and VLP abundance will be greatest in Powell and Swan Gut followed by Scarboro and Boxiron and will increase from downstream to upstream in all creeks, primarily due to nutrient gradients, tidal flushing, and temperature.

- Enterococci will be most abundant in Powell and Swan Gut followed by Scarboro and Boxiron due to the use of poultry manure, presence of poultry houses, and lack of feeding operations in the respective watersheds.
Summer 2010

- Measured water quality parameters
- Determined total bacterial, total coliform, enterococci, and VLP abundances
- Looked for correlations between water quality parameters, land use, and bacterial and viral abundance
- Took samples for ARA & Del N
Assessing bacterial, viral, and fecal coliform abundances

Patel et al., 2007

SYBR Green I epifluorescence microscopy
- 0.02 μm Anodisc filter
- Nikon Eclipse E800, blue light excitation
Assessing bacterial, viral, and coliform abundances

Patel et al., 2007

SYBR Green I epifluorescence microscopy
- 0.02 μm Anodisc filter
- Nikon Eclipse E800, blue light excitation

Total coliforms
- 0.45 μm gridded filter
- m FC Agar
- Incubate 24h at 44.5°C

Enterococci
- 0.45 μm gridded filter
- m E Agar
- Incubate 24-48h at 35°C
Total nitrogen and phosphorus were consistent over time
Water quality standards have two recreational use designations:

- **Primary contact recreation:**
  “recreational activities involving a significant risk of ingestion of water, including wading by children, swimming, water skiing, diving, and surfing.”

- **Noncontact recreation**
  “aquatic recreational pursuits not involving a significant risk of water ingestion: including fishing, commercial and recreational boating, and limited body contact incidental to shoreline activity.”
Enterococci are abundant and above EPA standards

- Abundance highest at Powell mid and mouth, also highest TN

- Lack of change following rain (July) vs dry sampling (June) suggests potential consistent source.

- Boxiron and Scarboro head sources most influenced by rainfall

- EPA primary contact Enterococci
  - 35 col/100 ml (geometric mean
  - 89 col/100 ml (single sample max)
  - 168 for non contact recreation
Total coliforms also high with similar patterns to enterococci

- Powell mid and mouth high similar to enterococci and TN
- Boxiron head high compared to enterococci
- Scarboro and Powell head, and Boxiron mid most influenced by rainfall

EPA primary contact total coliforms: 200 col/100 ml (geometric mean 400 col/100 ml (single sample max)
Total bacterial abundance is more consistent than fecal bacteria

- Also must be considered on a site by site basis
- Bacterial and VLP abundance comparatively low at Powell mid, mouth
- Bacteria in Powell not as influenced by rainfall as fecal bacteria
- Bacteria don’t indicate same types of inputs as fecal bacteria
Virus Like Particles (VLPs) also consistent but low in Powell

- Powell VLPs an order of magnitude lower
- May be too many bacteria for viruses to keep up
- Boxiron mid and mouth, Powell mouth most affected by rain
- Rainfall influences differ from both fecal and total bacteria
All bacterial and VLP parameters respond at localized spatial and temporal scales.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>num df</th>
<th>den df</th>
<th>F</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Enterococci</td>
<td>6</td>
<td>36</td>
<td>8.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total Coliforms</td>
<td>6</td>
<td>36</td>
<td>17.38</td>
<td>&lt;0.0001</td>
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<tr>
<td>Bacterial Abundance</td>
<td>6</td>
<td>12</td>
<td>10.67</td>
<td>0.0003</td>
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<tr>
<td>VLP Abundance</td>
<td>6</td>
<td>12</td>
<td>4.00</td>
<td>0.0196</td>
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<tr>
<td>VLP:Bacteria</td>
<td>6</td>
<td>12</td>
<td>10.90</td>
<td>0.0003</td>
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<tr>
<td>Enterococci</td>
<td>9</td>
<td>53</td>
<td>7.39</td>
<td>&lt;0.0001</td>
</tr>
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<td>9</td>
<td>55</td>
<td>7.55</td>
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<td>Bacterial Abundance</td>
<td>6</td>
<td>12</td>
<td>5.97</td>
<td>0.0043</td>
</tr>
<tr>
<td>VLP Abundance</td>
<td>6</td>
<td>12</td>
<td>8.01</td>
<td>0.0012</td>
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<tr>
<td>VLP:Bacteria</td>
<td>6</td>
<td>12</td>
<td>6.49</td>
<td>0.0031</td>
</tr>
</tbody>
</table>
Bacterial abundances in creeks higher than Johnson Bay proper

Needham, 2007

Current study
Bacterial abundances and VLP abundances high compared to other systems

<table>
<thead>
<tr>
<th>Location</th>
<th>System Type</th>
<th>Bacterial Abundance (x10^7)</th>
<th>VLP Abundance (x10^8)</th>
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<tr>
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<td>Estuarine</td>
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<td>Estuarine</td>
<td>1.28-2.83</td>
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<td>Estuarine</td>
<td>0.49-1.28</td>
<td>0.23-1.59</td>
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<td>Swan Gut Creek</td>
<td>Estuarine</td>
<td>0.99-2.67</td>
<td>0.98-2.54</td>
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<tr>
<td>Maryland Coastal Bays (Needham, 2007)</td>
<td>Estuarine</td>
<td><strong>0.22-2.63</strong></td>
<td>0.92-2.48</td>
</tr>
<tr>
<td>Moreton Bay and Noosa River, Australia</td>
<td>Estuarine</td>
<td>~0.05-2.4</td>
<td>0.05-3.0</td>
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<td>(Hewson et. al, 2001)</td>
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<td></td>
</tr>
<tr>
<td>Chesapeake Bay (Wommack et. al, 1993)</td>
<td>Estuarine</td>
<td>ND</td>
<td>0.026-1.4</td>
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<tr>
<td>Lake Saelenvannet, Norway (Tuomi et. al, 1997)</td>
<td>Estuarine</td>
<td>0.15-0.60</td>
<td>0.2-3.0</td>
</tr>
<tr>
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<td>Open Ocean</td>
<td>0.021-0.21</td>
<td>~0.019</td>
</tr>
<tr>
<td>Key Largo, FL (Paul et. al, 1993)</td>
<td>Coastal</td>
<td>~0.0036-0.017</td>
<td>~0.015-0.12</td>
</tr>
<tr>
<td>Lake Plusse, Germany (Bergh et. al, 1989)</td>
<td>Freshwater</td>
<td>ND</td>
<td>2.54</td>
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VLP abundances are more consistent with previous study.

Bacterial abundances and VLP abundances high compared to other systems

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Viruses did not correlate with bacteria but are with chlorophyll $a$ in July

June: Correlation coefficient = 0.3626
   $P > 0.05$

July: Correlation coefficient = 0.3836
   $P > 0.05$

June: Correlation coefficient = 0.3279
   $P > 0.05$

July: Correlation coefficient = 0.6626
   $P < 0.001$
Bacterial and VLP parameters correlated with NH$_4$ and NO$_x$ in June

- None correlated with total nitrogen
  - Except enterococci in July
- Fecal coliforms no longer correlate with either in July

<table>
<thead>
<tr>
<th></th>
<th>NH4</th>
<th>NOx(2&amp;3)</th>
<th>TN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>June</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococci</td>
<td>0.58**</td>
<td>0.64***</td>
<td>0.42</td>
</tr>
<tr>
<td>Total Coliforms</td>
<td>0.43*</td>
<td>0.53**</td>
<td>0.38</td>
</tr>
<tr>
<td>Total Bacteria</td>
<td>-0.60**</td>
<td>-0.49*</td>
<td>-0.06</td>
</tr>
<tr>
<td>VLP</td>
<td>-0.54**</td>
<td>-0.65***</td>
<td>-0.32</td>
</tr>
<tr>
<td>VLP:Bacteria</td>
<td>-0.35</td>
<td>-0.52*</td>
<td>-0.37</td>
</tr>
</tbody>
</table>

| **July** |      |          |       |
| Enterococci | -0.16  | 0.15     | 0.63** |
| Total Coliforms | -0.03  | 0.20     | 0.16  |
| Total Bacteria | -0.56** | -0.62** | -0.18 |
| VLP     | -0.46*  | -0.66*** | -0.28 |
| VLP:Bacteria | -0.13  | -0.32    | -0.14 |

Significance level
* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$
Bacterial abundances correlate with environmental parameters

June
Chlorophyll $a$, temperature, pH, and salinity correlated…
  – Negatively with fecal bacteria
  – Positively with total bacteria

July
Number of correlations decrease
  – No temperature or pH correlations
  – Enterococci and chlorophyll $a$
  – Total bacteria and salinity

Left to right: Samples from Swan Gut head, mid, mouth
Methods: Antibiotic Resistance Analysis

Note several colonies growing on the plate with the penicillin. Most studies use 10 or more antibiotics.
## Methods: Antibiotic Resistance Analysis

### Surface Water Sampling Stations

<table>
<thead>
<tr>
<th>Station</th>
<th>Number of Resistant Tests</th>
<th>Number of Tests</th>
<th>MAR Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>04-01</td>
<td>1</td>
<td>90</td>
<td>0.0111</td>
</tr>
<tr>
<td>04-01A</td>
<td>1</td>
<td>110</td>
<td>0.00909</td>
</tr>
<tr>
<td>04-02</td>
<td>0</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>04-03</td>
<td>0</td>
<td>110</td>
<td>0.0</td>
</tr>
<tr>
<td>04-04</td>
<td>0</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>04-06</td>
<td>0</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>04-07</td>
<td>0</td>
<td>100</td>
<td>0.0</td>
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<tr>
<td>04-08</td>
<td>1</td>
<td>100</td>
<td>0.0100</td>
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<tr>
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<td>0.0</td>
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<tr>
<td>04-16</td>
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<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>04-17</td>
<td>0</td>
<td>90</td>
<td>0.0</td>
</tr>
<tr>
<td>04-18</td>
<td>0</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>04-25</td>
<td>0</td>
<td>100</td>
<td>0.0</td>
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<tr>
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<tr>
<td>04-29</td>
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<td>0.1700</td>
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<td>04-30</td>
<td>0</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>GC-1</td>
<td>13</td>
<td>100</td>
<td>0.1300</td>
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<tr>
<td>MC-5</td>
<td>4</td>
<td>70</td>
<td>0.0571</td>
</tr>
</tbody>
</table>

**Average** 0.0265

### Sewage System Lift Stations

<table>
<thead>
<tr>
<th>Station</th>
<th>Number of Resistant Tests</th>
<th>Number of Tests</th>
<th>MAR Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA 302</td>
<td>10</td>
<td>140</td>
<td>0.0714</td>
</tr>
<tr>
<td>EPA 303</td>
<td>12</td>
<td>120</td>
<td>0.1000</td>
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<tr>
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<tr>
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<td>150</td>
<td>0.0933</td>
</tr>
<tr>
<td>EPA 306</td>
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<td>110</td>
<td>0.0909</td>
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<tr>
<td>EPA 307</td>
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<td>0.0</td>
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<tr>
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<tr>
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<tr>
<td>LS 72</td>
<td>10</td>
<td>140</td>
<td>0.0714</td>
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</table>

**Average** 0.0710

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Sampling Stations:
- 0
- 0 - 0.011
- 0.011 - 0.057
- 0.057 - 0.073
- 0.073 - 0.17

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![Map showing sampling stations]
Methods: Antibiotic Resistance Analysis

Resistance Pattern Cluster Analysis

- Surface Water Samples
- Sewage Samples
- Station No. 04-29

Isolate ID
Hypotheses

- Nutrient concentrations will be greatest in Swan Gut and Powell followed by Scarboro and Boxiron due to known gradients in TN and land use intensity.
• Nutrient concentrations will be greatest in Swan Gut and Powell followed by Scarboro and Boxiron due to known gradients in TN and land use intensity.

Swan Gut lower than expected
Powell was indeed the highest in terms of nutrients
Scarboro and Boxiron were fairly similar but fit hypothesized gradient
Hypotheses

- Nutrient concentrations will be greatest in Swan Gut and Powell followed by Scarboro and Boxiron due to known gradients in TN and land use intensity.
- Bacterial and VLP abundance will be positively correlated with each other (more strongly than with chlorophyll $a$), with nutrient loading, increasing intensity of land use, and temperature, and negatively correlated with salinity.

Viruses did not correlate significantly with bacteria, but did correlate with chlorophyll, after rain in July
Bacteria and VLP did correlate with ammonium and nitrate
Patterns with salinity did not follow expected trend = hot spots
Hypotheses

• Nutrient concentrations will be greatest in Swan Gut and Powell followed by Scarboro and Boxiron due to known gradients in TN and land use intensity.

• Bacterial and VLP abundance will be positively correlated with each other (more strongly than with chlorophyll a), with nutrient loading, increasing intensity of land use, and temperature, and negatively correlated with salinity.

• Bacterial and VLP abundance will be greatest in Powell and Swan Gut followed by Scarboro and Boxiron and will increase from downstream to upstream in all creeks, primarily due to nutrient gradients, tidal flushing, and temperature.

Values were highest in Powell but independent of upstream-downstream gradient; Swan Gut lower than expected.
Hypotheses

- Nutrient concentrations will be greatest in Swan Gut and Powell followed by Scarboro and Boxiron due to known gradients in TN and land use intensity.
- Bacterial and VLP abundance will be positively correlated with each other (more strongly than with chlorophyll a), with nutrient loading, increasing intensity of land use, and temperature, and negatively correlated with salinity.
- Bacterial and VLP abundance will be greatest in Powell and Swan Gut followed by Scarboro and Boxiron and will increase from downstream to upstream in all creeks, primarily due to nutrient gradients, tidal flushing, and temperature.
- Enterococci will be most abundant in Powell and Swan Gut followed by Scarboro and Boxiron due to the use of poultry manure, presence of poultry houses, and lack of feeding operations in the respective watersheds.
- Highest concentration in Powell - but also high in Scarboro and Boxiron after the rain; Swan Gut did not fit the pattern.
Conclusions

- System contamination exhibits spatial complexity with localized affects
- High fecal bacterial contaminants and nutrient loadings suggest anthropogenic inputs
- Bacterial abundances correlated with environmental parameters including ammonium and nitrate
- Creeks leading into Johnson Bay exceed EPA standards for primary recreational contact and non-contact recreation in many instances.
Future research

• Connect high nutrient loads and bacterial abundances to specific inputs using:
  – GIS
  – Del-N results
  – Antibiotic resistance assays results

• Role of viruses in system

• Effects of rain events

• Extend analyses to Chesapeake tributaries
Thanks to the crew...

WYE Research Inst.:
Dan Fisher
Lance Yonkos

NOAA Oxford Cooperative Lab:
A.K. Leight
Phytoplankton preference

$NH_4^+ > \text{Urea} \gg NO_3^-$

Uptake (V x 1000 h$^{-1}$)
Delmarva Peninsula: TN increases with load, & Chincoteague Bay is intermediate.

- Hog Island Bay, VA
- Chincoteague Bay, MD
- Delaware Inland Bays, DE

Graph:
- Y-axis: Total Nitrogen Concentration (µM)
- X-axis: Average Annual Total Nitrogen Load (gN m⁻² yr⁻¹)
- Equation: \( y = 5.8x + 24.5 \)
- \( R^2 = 0.99 \)
Fecal bacteria are positively correlated with each other...

June: Correlation coefficient = 0.8075
P>0.001

July: Correlation coefficient = 0.7920
P<0.001
Johnson Bay water quality improved over long-term
...but were negatively correlated with total bacteria in June

**Enterococci**
Correlation coefficient = -0.7130  
P<0.001

**Total coliforms**
Correlation coefficient = -0.5873  
P<0.01