Seasonal Dynamics of Male Specific Coliphage and Enteric Viruses in Bivalve Shellfish within Wastewater Treatment Facility Safety Zones

Thomas Howell, Spinney Creek Shellfish, Inc. Eliot, ME
Milford, February 2014
Study Goals and Objectives

- Safely re-open near-shore New England growing areas that are permanently closed to shellfish aquaculture and harvest due to proximity to WTP outfall using depuration and relay strategies.


- Collect American oyster and hard-shelled clams samples in Taunton River (MA)
  - Evaluate fecal coliform (FC), male-specific coliphage (MSC), norovirus (NoV), and adenovirus (AdV) monthly to determine seasonal persistence/dynamics
  - Evaluate FC, MSC, NoV, and AdV in depuration trials to determine temperature/seasonal rates of indicator/pathogen depuration.

- Validation and NSSP acceptance of MSC method for quahogs (2012 ISSC San Antonio, TX)
What is Male-specific Coliphage (MSC)?

- Male-specific Coliphage is a bacteriophage (virus) of *E. coli* bacteria.
- MSC presents in municipal sewage consistently in high numbers ($10^4$ – $10^5$ PFU/100ml).
- MSC is an RNA virus similar in size, shape, and disinfection resistivity to enteric viruses of concern, particularly NoV.
- MSC (FRNA Bacteriophage) has proven to be an effective indicator of viral persistence in soft-shelled clams and Pacific-oyster meats.
- MSC has proven to be an appropriate modeling organism for the determination of treatment plant performance w.r.t. enteric viruses by evaluating influent and effluent wastewater samples.
Why the Need for Using MSC?

- Municipal Wastewater Treatment plants are designed, operated, monitored, and regulated around bacterial indicators.
- Many studies have shown that enteric viruses are not easily deactivated by chlorination and other disinfection methods.
- Typically, secondary chlorine disinfection WTPs demonstrate 90% to 99% (1 to 2 log) reduction in viruses under normal operational conditions. (Bacterial disinfection >99.9999%).
- Pathogenic viruses of concern are difficult to enumerate in wastewater. Practical methods do not exist.
- The enumeration methods for MSC for wastewater and shellfish meats are easy, fast, rugged, and inexpensive.
An Example of Degradation of WTP Viral Performance with Increased Flow

<table>
<thead>
<tr>
<th>Yarmouth WTP after a 3 Inch Rainfall Event</th>
<th>Flow MGD</th>
<th>FC/100ml</th>
<th>MSC/100ml</th>
<th>MSC Log Reduction</th>
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</table>
A year-long study in the Taunton River (MA) showed relatively low MSC levels in all samples

- MSC and FC levels were too low to see any seasonal persistence patterns in American oysters and quahogs
- MSC in soft-shelled clams harvested close to the Fall River and Somerset outfall were not particularly high

Conclusions:
- The Taunton River is really not all that contaminated and recent water/quality seems to confirm
- 30 years of public investment in treatment plant upgrades and watershed management efforts have resulted in marked water quality and environment improvement
Moved to Salem Sound (MA) and Three Rivers (NH–ME)

Experiments were set up to investigate *species-specific differences in bio-accumulation* using soft-shelled clams as a control species

- Marblehead Harbor – clean quahogs from the Taunton River were transplanted in close proximity to native soft-shelled clams
- Salem Harbor – native soft-shelled clams, European oysters were present
- Three Rivers – native American oysters and soft-shelled clams
FRNA Bacteriophage and Norovirus Outbreaks in Pacific Oysters in England (David Lees, 2000)
FRNA Bacteriophage, E. coli and % Positive Norovirus in Pacific Oysters in Ireland (William Dore, 2009)

**FIGURE 1.** Geometric mean values of FRNA bacteriophages (□) and E. coli (○) and percentage of samples positive for NoV (●) in oysters per month of sample collection.
e Rivers Site in Eliot, ME
Soft-shelled Clam - American oyster Intercomparison

Oct 2
17°C
F+Coliphage and Fecal Coliform in American Oysters from the Gulf (Burkhardt & Calci 2000)

FIG. 1. Geometric mean bioaccumulation of fecal coliforms and F+ coliphage by *C. virginica* in Gulf Coast estuarine water assessed with respect to season and temperature.
rest River Park Site in Salem Harbor, MA
Soft-shelled Clam - Oyster Intercomparison
Interim Conclusions from Our Study Comparing with Other Studies

- Cold-water adapted species such as soft-shelled clams, Pacific oysters, and European oysters demonstrate strong seasonal MSC patterns (probably mussels as well).

- Non cold-water adapted species such as American oysters and quahogs are anomalous because they stop pumping at water temperatures below 10°C.

- American oysters and quahogs demonstrate similar seasonal MSC as cold-water adapted species in southern waters where water temperatures do not drop below 10°C.

- American oysters and quahogs can trap MSC for the winter months if significant MSC contamination is present in the environment prior to when water temperature falls below 10°C.

- FC indicators die-off when American oysters and quahogs stop pumping over the winter period.
Sea Grant Project Partners and Participants

- Steve Jones, Ph.D, UNH
- Bill Burkhardt, Ph.D and Joey Marchant, FDA (Dauphin Island)
- Mike Hickey and Jeff Kennedy, MA DMF
- Greg Sawyer and James Rossignol, Taunton River MA DMF
- Greg Bettencourt, Devon Winkler, Dave Roach, Salem Sound MA DMF
- Kohl Kanwit, ME DMR
- Mike Chambers, Ph.D, UNH Extension
- Tom and Lori Howell, Laura Stadig, Spinney Creek Shellfish