Fecal Bacteria Indicators in Southeastern CT and their Implications for Recreation at Barn Island

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Introduction

Fecal Coliform are bacteria that originate in the gut of warm-blooded animals. Fecal Coliform tests are used as an indicator of fecal matter in waterways, which could expose people to dangerous pathogens. The bacteria may enter waterways through urban and agricultural runoff, faulty sewage systems, or ineffective water treatment facilities. Activities such as shellfish harvesting, drinking, and swimming may be restricted or banned when fecal coliform bacteria are present, so it is important to monitor and identify their presence and sources. While not all fecal coliform bacteria are harmful in-and-of-themselves, they indicate the contamination of water with fecal matter that could contain bacteria unsafe for public health.

Objectives

- Determine the abundance of fecal coliform bacteria in-and-around Southeastern CT and the Pawcatuck River Watershed (Barn Island).
- Compare our results to fecal coliform abundances observed by *CUSH* at Barn Island and Wequetequock Cove from 2008-2015. It should be noted that UConn's data was collected over one day, while *CUSH*'s data was averaged over five years.
- Determine sources of fecal bacteria contamination from DNA analysis.
- Evaluate public health and recreation status indicated by fecal coliform colony counts.

Methods

- Water samples were collected in sterile containers at various locations in Southeastern Connecticut in October 2015 (Fig. 1)
- Fecal coliform abundances were estimated using standard protocols (EPA Method 9132 modified for fecal coliforms in seawater). Water samples (100 mL of 1x and 10x dilutions) were filtered through a membrane and immersed in plates containing m-Endo broth (Millipore). Plates were incubated for 24 hours at 44.5 °C, after which emergent colonies were enumerated.
- Water samples (1 L) were filtered onto a 0.45 µm pore-size filters to retain particles for DNA analysis. Filters were flash-frozen in liquid nitrogen, and stored at -80˚C pending DNA extraction. Filters were re-suspended in an extraction buffer containing proteinase K, heated overnight at 60˚C in a water bath to extract DNA. Extracted DNA was purified from contaminant materials with a commercial kit (DNA Clean & Concentrator™-5, Zymo Research Corps). DNA was amplified using a commercial kit (SsoFast™ EvaGreen® Supermix) on a Step One Plus Real-Time PCR System™ (Applied Biosystems) following respective amplification protocols for three distinct primer sets:
  - Human-origin marker (HF183) *Bacteroides* spp. (Seurinck et al. 2005)
  - Goose-origin marker (GFD): *Helicobacter* spp. (Greene et al. 2002)
  - Cow-origin marker (CowM2): *Bacteroides* spp. (Shanks et al., 2008)

Results

- Fecal coliform abundances differed widely among sampling locations in October 2016, from as few as 1 cfu (colony forming unit) near Lord's Point in Stonington, to 2800 cfu near the sewage treatment plant in Groton (Fig. 1).
- At Barn Island, abundances ranged from 8 to 176 cfu at the time of sampling (Fig.1 inset).
- Mean observed fecal coliform abundances observed by *CUSH* in repeated samplings between 2008 and 2015 at Wequetequock Cove and Barn Island ranged from 5 to 239 cfu, with greater abundances generally observed in the cove (Fig. 1).
- Bird fecal indicator bacteria generally dominated at stations where targeted DNA was detected, including at the Groton sewage treatment plant (Fig. 2).
- Human fecal indicator bacteria were detected in Thames River (Groton), Avery Point, at some Mystic locations, and at Barn Island (Fig. 2)
- Cow fecal indicator bacterial were detected at some Mystic locations, and at Barn Island (Fig. 2).

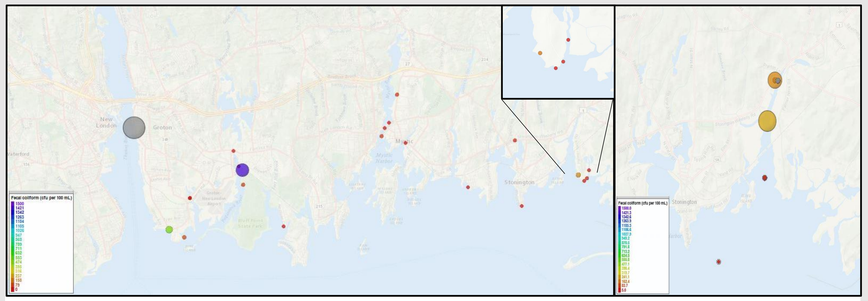

Figure 1: Fecal coliform counts (cfu) observed at sampling sites. Marker size is proportional to observed abundances (left) Fecal coliform detected in October 2015. (right) Average fecal coliform abundances (cfu) observed by *CUSH* in summer months between 2008 and 2015. Maps were created on GPS Visualizer freeware.

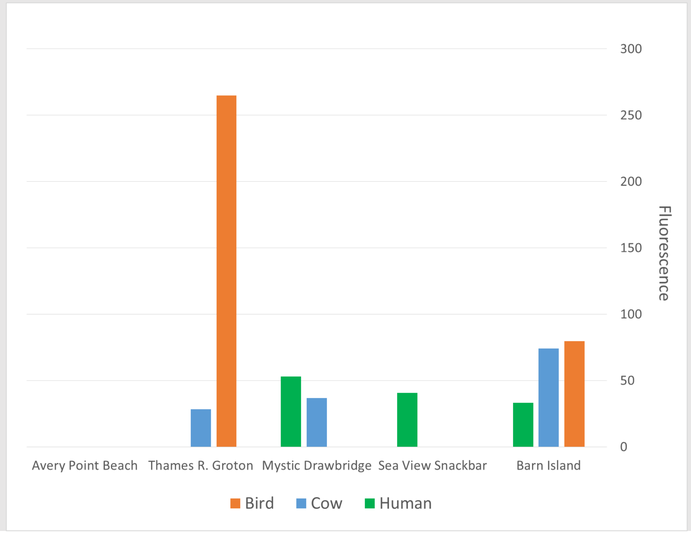

Figure 2: Detected fecal coliform indicator bacteria at sampling locations in southeastern Connecticut. The measured fluorescence is proportional to the concentration of double-stranded DNA at the last amplification cycle of the polymerase chain reaction. Only samples with ≥ 100 cfu per 100 mL were analyzed. Failure to detect specific indicator groups does not necessarily signal absence, and could be attributed to experimental error.

Conclusions

State of Connecticut guidelines regarding acceptable fecal coliform abundances are as follows:

- Bathing: Fecal coliform must not exceed than 200 colonies per 100 mL
- Shellfish Harvesting: Fecal coliform must not exceed 14 colonies per 100 mL

In this context, some of our sampling locations in 2016 appeared safe for both bathing and shellfish harvesting at the time of sampling. Some locations, however, were clearly above recommended safety guidelines, including the Thames River in Groton, a few locations in Groton and Mystic, and at one location off Barn Island.

Repeated measurements from *CUSH* indicate that fecal coliform abundances are generally too elevated in Wequetequock Cove to allow for either bathing or shellfish harvesting. Off of Barn Island, fecal coliform are generally too abundant for shellfish harvesting but adequate for bathing (Table 1).

At locations where the targeted fecal bacterial DNA was detected bird fecal indicators were generally dominant. Human and cow fecal indicators were also detected at a number of locations

DID FECAL COLIFORM LEVELS AT BARN ISLAND MEET STANDARDS ON 10/18/2016?

WATER QUALITY STANDARDS	QUALITY RESULTS
Shellfish Harvesting (≤ 14 cfu/100 mL) 	• **NOT MET on Western side (Wequetequock Cove)** • **MET on Southern side (Sandy Point/Little Narragansett Bay)**
Swimming/Bathing (≤ 200 cfu/100 mL) 	• **MET on Western side (Wequetequock Cove)** • **MET on Southern side (Sandy Point/Little Narragansett Bay)**

Table 1. Conclusions on whether or not Barn Island area meets water quality standards based on fecal coliform colony counts. Data from both UConn and *CUSH* was considered.

References

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