A High-Throughput MFQPCR Approach to Simultaneously Quantify FI Bacteria, MST Markers, and Pathogens of Lake Superior Beaches

Elizabeth Hill, advised by Dr. Satoshi Ishii and Dr. Chan Lan Chun
University of Minnesota, Water Resources Science

Abstract

A total of 118 samples were collected roughly bi-weekly from five beaches in Duluth and Two Harbors, MN. Finished effluent samples were collected from two wastewater treatment facilities. The overall objective of this work is to simultaneously quantify fecal indicator bacteria (FIB), microbial source tracking (MST) markers, and pathogens in a single water sample using microfluidic qPCR (MFQPCR). Later, this data will be used to generate a quantitative microbial risk assessment (QMRA) workflow to estimate the risk of pathogen infection in humans with given MST and FIB concentrations in the environment. Data show preliminary trends in average total coliform and E. coli concentrations, total nitrogen and phosphorus, and absolute bacterial quantification by 16S rRNA. Upon completion, this project is expected to make significant contributions to the fields of recreational water quality and risk assessment monitoring.

Introduction

- Exposure to sewage-contaminated waters is a known human health concern.
- Fecal indicator bacteria (FIB) tests are used for recreational water quality monitoring; studies suggest FIB concentration is not a reliable predictor of pathogen presence (Harwood et al., 2005) or human health risks (Colford et al., 2007).
- FIB are ubiquitous in the digestive tracts of most animals (Harwood et al., 2017), and cannot elucidate source of fecal contamination.
- Naturalized strains of FIB may persist in the environment, potentially confounding the relationship between FIB and fecal contamination (Ishii et al., 2006).
- Microbial Source Tracking (MST) can pinpoint major sources of fecal contamination in the environment by identifying host-specific fecal microorganisms (Scott et al., 2005). However, relationship between MST markers and pathogens in recreational waters is unknown.
- This project seeks to address a need to understand the relationship between MST markers, FIB, and pathogens in a single water sample. By using high-throughput MFQPCR, 96 genes of interest can be quantified in 96 samples simultaneously.

Methods

- Pump 10 - 100 L of water through REXEED-25S membrane filter (Asahi Kasei Medical Co.) to capture biomass. Record physicochemical data in the field, collect water samples for additional lab analysis.
- Backflush filters with 500 mL sterile backflushing solution to retrieve biomass. Flocculate and concentrate via centrifugation.
- Duplicate samples: half receive PMA dye treatment (Biotium, Inc.) to differentiate between live and dead cells.
- Extract DNA/RNA from samples using the AllPrep PowerViral DNA/RNA Kit (Qiagen).
- Quantify 16S rRNA by conventional qPCR and SBYR Green Master Mix (Bio-Rad).
- *Starting October 2021*, quantify a suite of MST markers, pathogens, and FI bacteria by MFQPCR using TaqMan reagents and probes (Applied Biosystems).

Fig. 1: Collecting a sample with the membrane filter.

Fig. 2: Backflushing the filter.

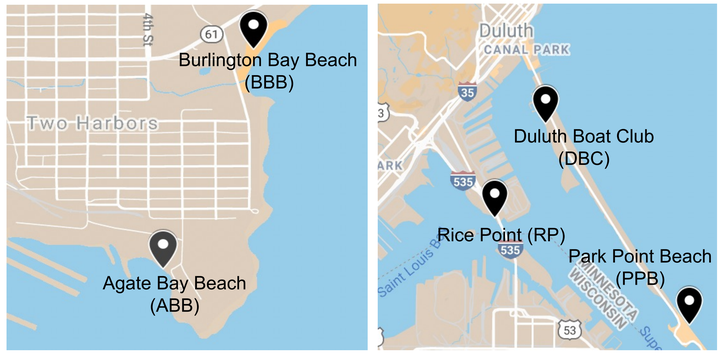
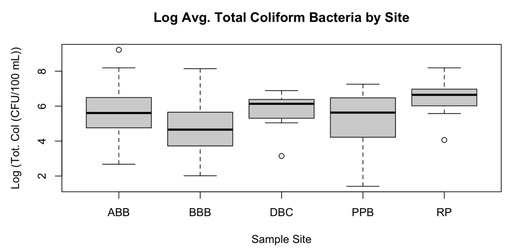


Fig. 3-4: Location of beach sites in Two Harbors (left) and Duluth (right) MN.

Results

Concentrations of total coliform bacteria and E. coli by Colilert methods (IDEXX) are shown in **Figure 5**. There was no significant relationship between total coliform bacteria or E. coli concentration and month, suggesting little time variability throughout the sample collection season. Summaries of TN/TP data are shown in **Figure 6**. Gene copy data for 16S rRNA is summarized in **Figure 7**. Based on nutrient and FIB concentration, and bacterial abundance by 16S rRNA, it appears that Rice Point and Duluth Boat Club may be more polluted than Park Point Beach in Duluth. Similarly, it appears that Agate Bay may be more polluted than Burlington Bay in Two Harbors.



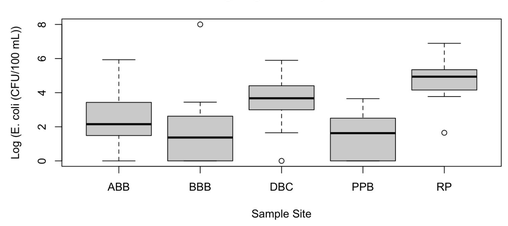
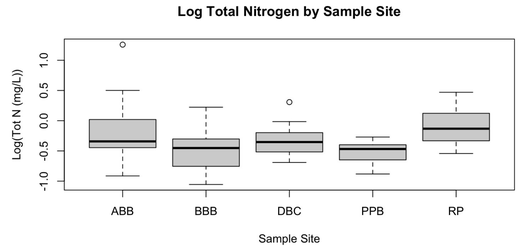


Fig. 5: Average total coliform bacteria and E. coli by site.



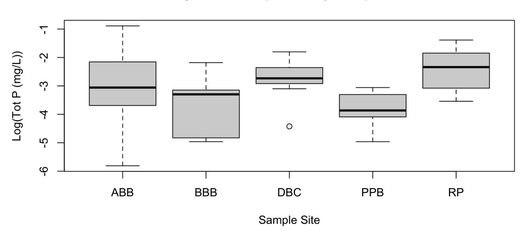


Fig. 6: Total phosphorus and total nitrogen concentration by site.

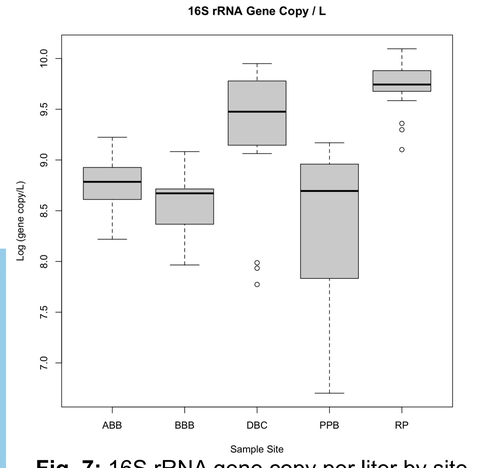


Fig. 7: 16S rRNA gene copy per liter by site.

Conclusion

Additional information and analysis is needed to draw conclusions from these trends. MFQPCR to quantify MST markers, FIB, and pathogens will be conducted starting in mid-October. Following this, data will be analyzed to assess the relationship between the concentration of pathogens, MST markers, and FIB, as well as the relationship of these data with physicochemical parameters, weather conditions, and other variables. The overall goal of this project is to use the resulting dataset to generate Quantitative Microbial Risk Assessment (QMRA) models to estimate the risk of pathogen-related human health outcomes with known water quality parameters, FIB and MST marker concentrations.

Acknowledgements

Support for this work comes from the National Science Foundation and the Moos Graduate Research Fellowship in Aquatic Biology. Special thanks to Max Brubaker, Caitlin Graeber, Collin Krochalk, Elizabeth Jarocki, Adelle Keppers, Alexis Ward, Braeden Cox, and Tamara Walsky for assistance with field sampling and sample processing.