Microbial Source Tracking at Two Beaches (MD & DE)

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Scope of Work

Microbial source tracking (MST) incorporates various techniques in the identification of sources of fecal contamination in water samples. Based on MST information, fecal sources can be targeted for reduction which would then be expected to decrease fecal loading. Recent studies using genetic markers for pollution source tracking in coastal waters have shown to be successful in identifying non-point sources of pollution. One of the most promising techniques includes the use of quantitative PCR (qPCR) utilizing DNA probes specific for fecal bacteria (e.g. *Bacteroides* spp.) originating from a specific source. The current study involves the collection of water samples from selected sampling sites where previous monitoring studies have shown high levels of fecal indicator organisms, particularly after a rain event, and the analysis of those samples using qPCR and DNA probes specific for human and/or other likely sources of contamination. The results of this proposed work will help guide remediation efforts designed to reduce the levels of fecal contamination in the study areas.

This study will address 2 major questions:
1) Are there any human sources of fecal contamination at the selected sites in Maryland and Delaware?
2) Can we confirm the most likely source, other than human, using genetic markers?

Monthly samples will be collected for 12 months at each beach and submitted to Salisbury University for genetic testing (human, poultry litter, ruminant and gull markers), and for enterococcus concentrations using the IDEXX Enterolert™ method. Water quality parameters, wind, weather, and tide observations will be recorded at each sampling event.

Results from this study may be used for Quantitative Microbial Risk Assessment (QMRA) in the future related to site-specific criteria for the beach.

Study Sites

Laboratory Methods

Water

One liter water samples collected monthly at each station

Enumeration of Fecal Indicators

Enterococci MPN/100 ml Enterolert™ and the Quanti-Tray® method of Idexx Laboratories

DNA Analysis

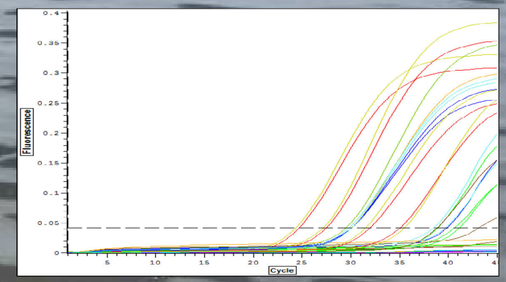
100 ml aliquots of each water sample are filtered through nitrocellulose filters and stored at -80°C prior to analysis.

DNA is extracted from the filters using a DNA isolation kit (MoBio Labs, Carlsbad, CA).

qPCR reactions are run using 1X TaqMan Environmental Master Mix 2.0 (Applied BioSystems, ThermoFisher Scientific) on a 48 well MJ Mini™ Thermal Cycler and MiniOpticon™ Real-Time PCR System (BioRad, Hercules, CA). Data analysis is conducted using the MJ Opticon Monitor Software, version 3.1.

Results

- First samples collected in December 2015
- To date most samples contained low numbers of enterococci with the exception of the December reading for the Delaware Beach site (1333 MPN/100 ml) and the January values for the Maryland Beach (≥ 2419 MPN/100 ml).
- Preliminary analysis resulted in no detection of the human genetic marker (HF183) at the Delaware Beach and a very low copy number of the HF183 marker at the Maryland Beach.
- The analysis of samples for additional genetic markers is still in progress.



MD Beach: FIB Data (Geometric mean)

Date	Dunn Creek Enterococci	Dunn Creek E.coli	Comment
6/4/2015	687		after 3 days of rain event
6/16/2015	556	>2005*	after rain event

6/4/2015	1252		after 3 days of rain event
6/16/2015	667	>2005*	after rain event
6/22/2015		>2005*	dry event (WC2 only)
6/24/2015		>2005*	after rain event (WC2 only)

Date	Cove Road Enterococci	Cove Road E.coli	Comment
5/19/2015	487		after rain event
5/27/2015	1		re-sample
6/3/2015	6		after 2 days of rain event
6/16/2015	23	1169	after rain event
6/22/2015	62	451	dry sample
6/24/2015	2	244	after rain event
7/14/2015	430	10	after rain event
7/16/2015	10		re-sample
8/11/2015	123		after rain event, high tide
8/13/2015	10		re-sample

*values in geometric mean value exceeded maximum detection level

Field Methods

12-month Project
Monthly: Dec 2015 – Nov 2016

Water Collection
2 X1,000 ml samples at ankle-depth

Acknowledgements

This investigation was funded by the Maryland Department of the Environment under a grant by the U.S. EPA's Beaches Program.

We gratefully acknowledge the support and participation of the Maryland Department of the Environment and the support of Salisbury University and the Henson School of Science & Technology.