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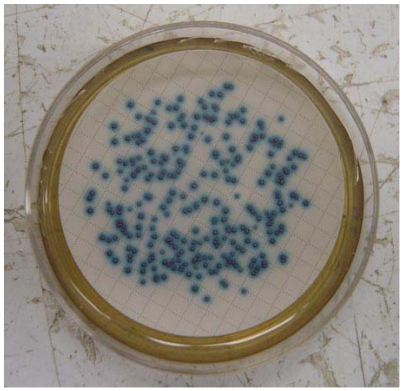
Urban Runoff Impact on the qPCR Signal of Enterococci and Other Alternative Fecal Indicators in a Tropical Beach

Marirosa Molina, Shayla Hunter, Emily White, Yong Jin Lee, Mike Cyterski, and Richard Zepp




Office of Research and Development
NERL/ Ecosystems Research Division

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Outline

- The Predictive Modeling Study
  - General Introduction and Objectives
- Luquillo details
  - Hypothesis
  - Possible sources of contamination
  - Study design and Methodology
- Results
- Summary and Conclusion

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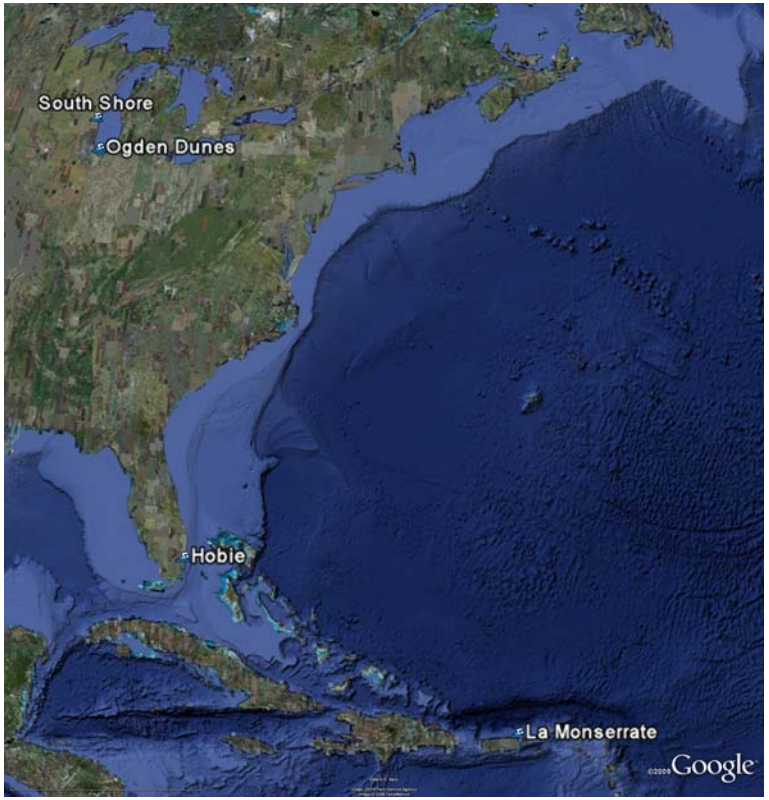
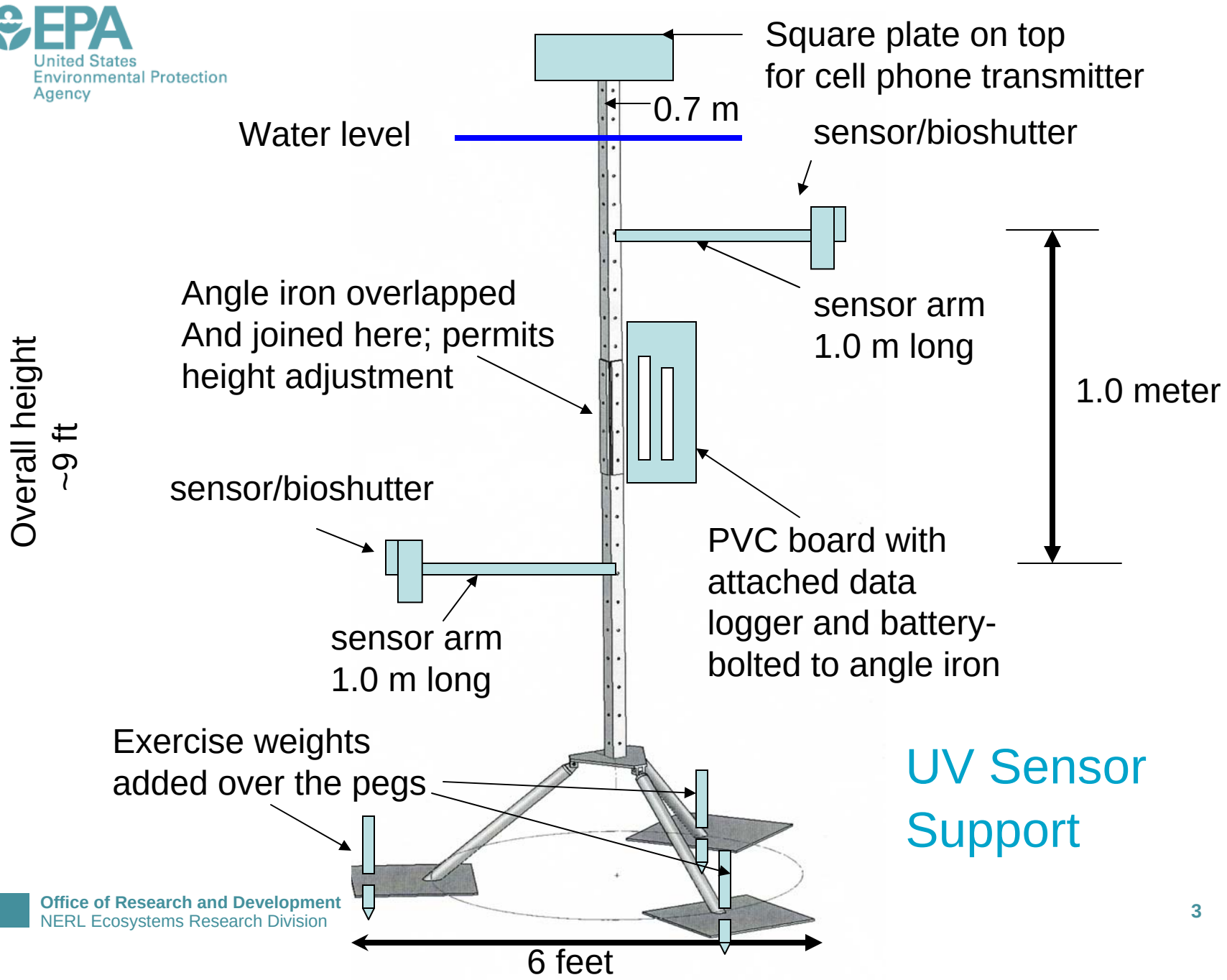
VB Predictive Modeling Study

- **Description and Objective of project:**
  - This study is part of several studies designed to evaluate the applicability of statistical modeling to a variety of marine and freshwater beaches.
  - Virtual Beach is a software tool used in the study to develop predictive models based on microbial data (dependent variables) and observations (independent variables) obtained from hydrometeorological and biogeochemical conditions at the beaches.





UV Sensor Support

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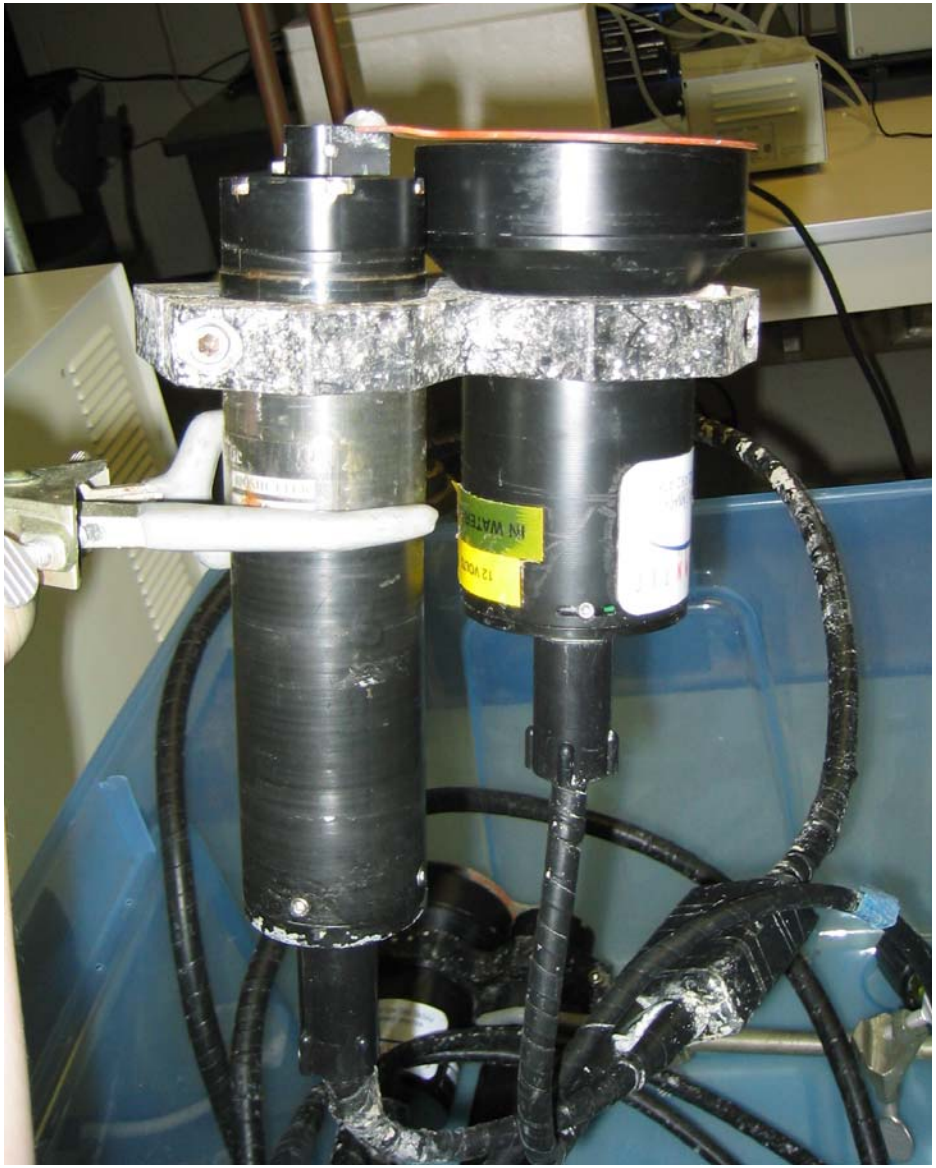
UV Sensors



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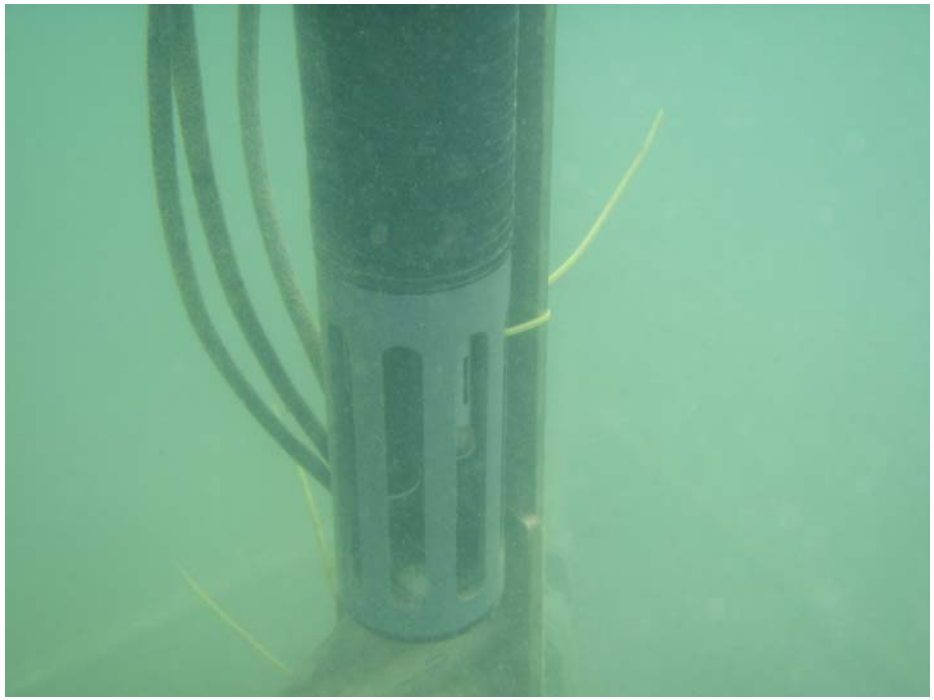

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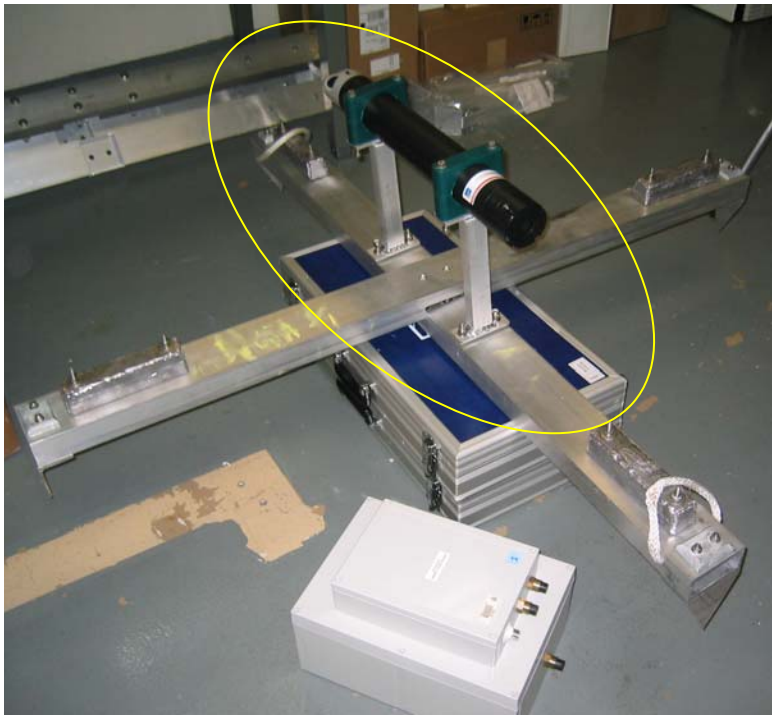
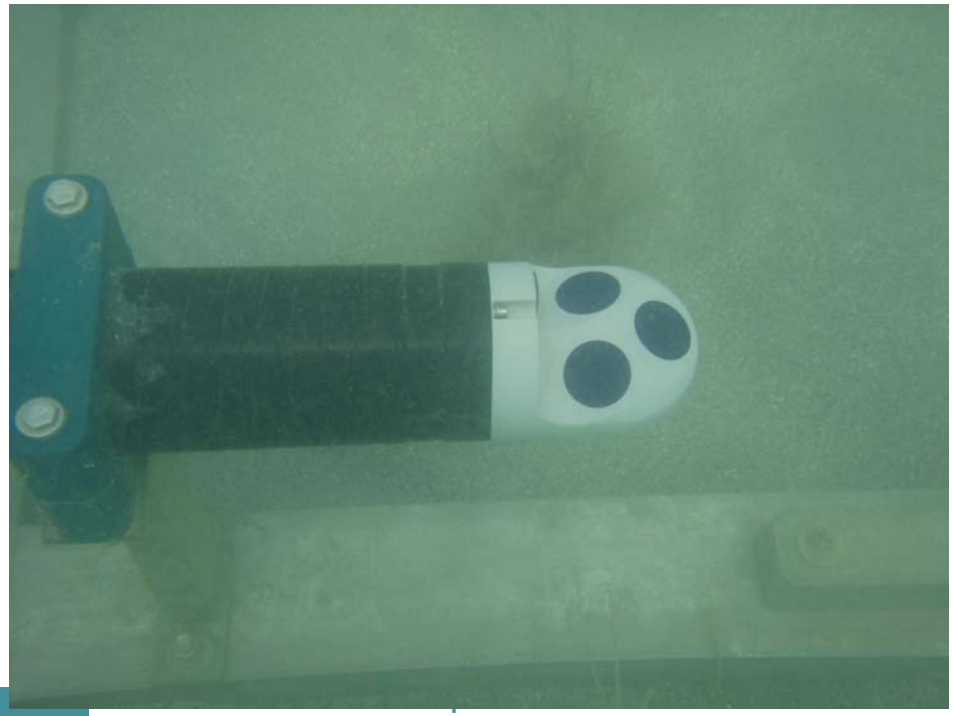
Acoustic Doppler Current Profiler (ADCP)





Other EPA/ERD presentations at this meeting

- **Predictive Modeling of a Fecal Indicator at a Subtropical Marine Beach**
  - R. G. Zepp, E. White, M. Molina, and M. Cyterski
    - Evaluation of the use of Virtual Beach Model Builder to develop models for predicting enterococci concentrations at Hobie Beach, Miami FL.

- **Optical Properties of Three Beach Waters: Implications for Predictive Modeling of Enterococci**
  - Emily M. White, Richard. G. Zepp, Marirosa Molina, and Mike Cyterski
    - Assessment of the optical properties of waters at a temperate freshwater beach (Milwaukee, WI), sub-tropical marine beach (Miami, FL), and a tropical marine beach (Luquillo, PR), during summer 2008 to determine the effect of light on the attenuation of FIBs.

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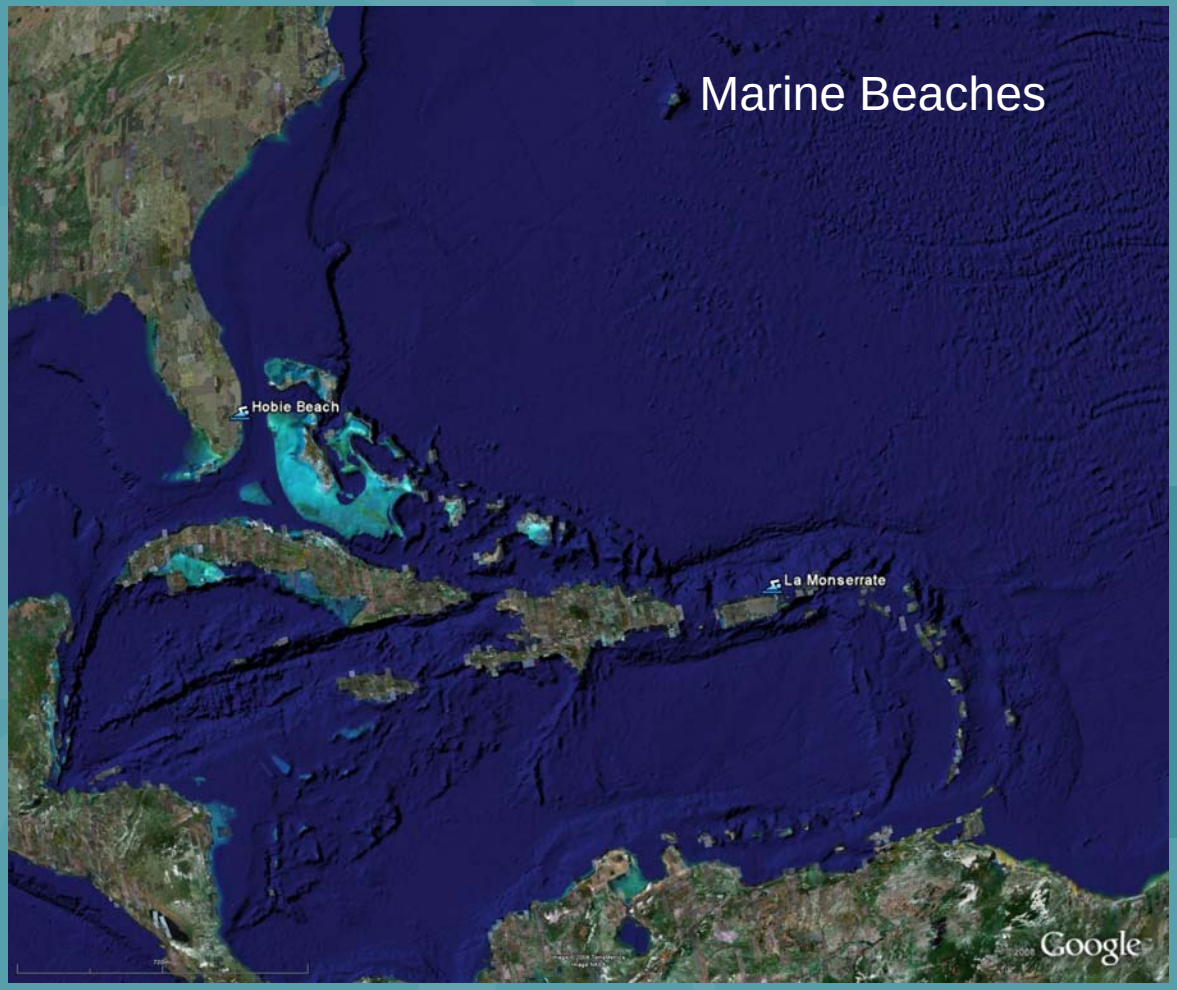

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Objectives of Study at Luquillo

- Determine the effect that non-point sources of runoff have on the concentration of enterococci at a recreational beach for both culturable and qPCR approaches.

- Hypothesis:
  - Because this is a runoff impacted beach, we expect enterococci concentrations to increase due to rain events

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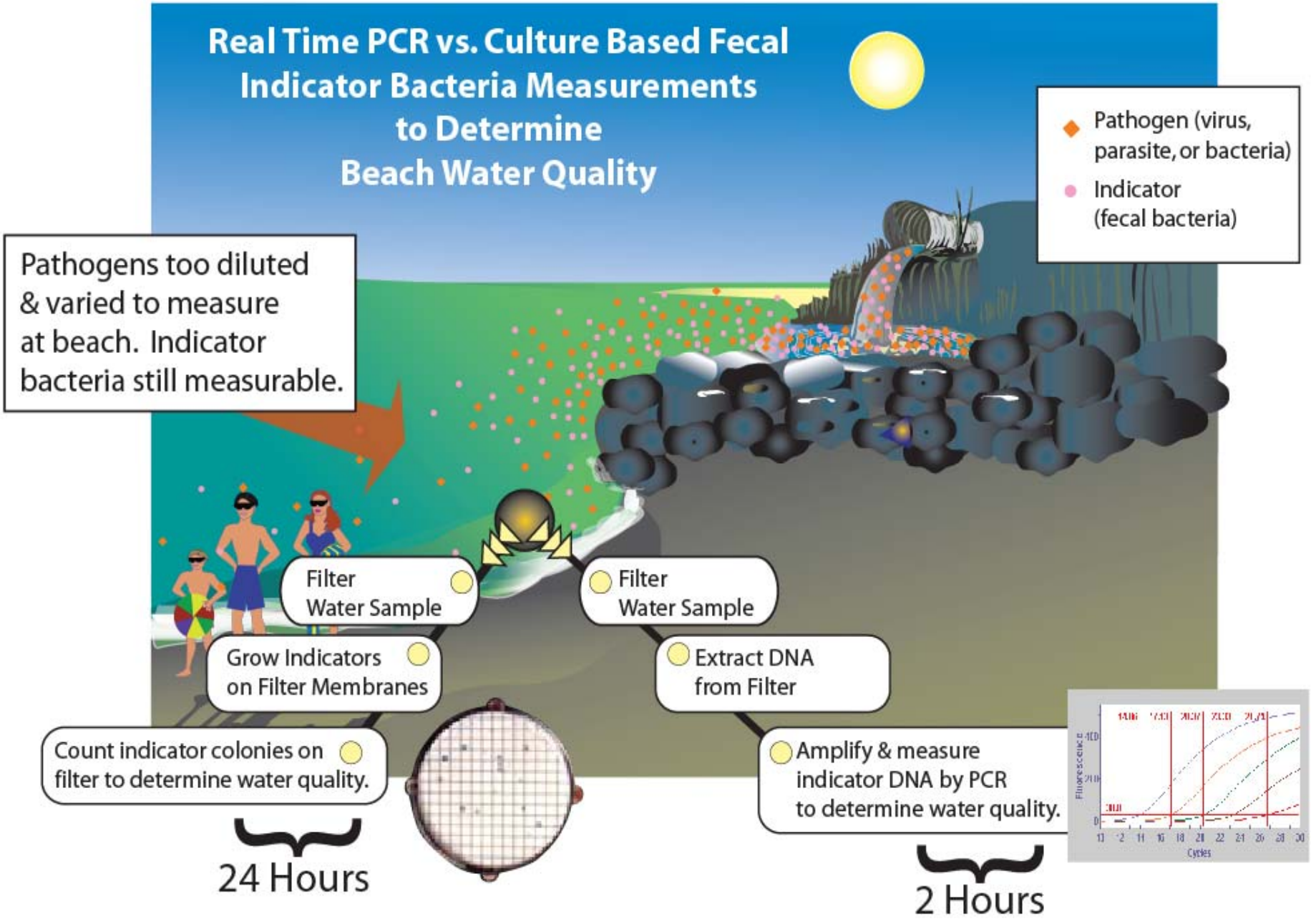
Methodology

- Water samples were collected by the PR Environmental Quality Board waist deep from three transects:
  - Once a day, three days a week for measurement of culturable enterococci.
  - Samples collected from June through October.
  - Filters also collected for enterococci qPCR analysis and archiving at EPA.
- Instruments were deployed at the beach to log data that were used to develop the models and establish relationships with enterococci concentrations.


Real Time PCR vs. Culture Based Fecal Indicator Bacteria Measurements to Determine Beach Water Quality
Pathogen (virus, parasite, or bacteria)
Indicator (fecal bacteria)
Pathogens too diluted & varied to measure at beach. Indicator bacteria still measurable.
Filter Water Sample
Filter Water Sample
Grow Indicators on Filter Membranes
Extract DNA from Filter
Count indicator colonies on filter to determine water quality.
Amplify & measure indicator DNA by PCR to determine water quality.
24 Hours
2 Hours
Fluorescence
Cycles

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qPCR Methods Used in Previous EPA Epidemiological Studies

Method	Epi Studies	Conclusion/Disposition
Enterococcus spp. by rDNA qPCR (Ludwig & Schleifer assay)	2003, 2004 Freshwater 2005, 2007 Marine	Adequate sensitivity Evaluation ongoing
Bacteroidales spp. by rDNA qPCR (Siefring et al. modified Kate Field lab assay)	2005, 2007 Marine	Adequate sensitivity Evaluation ongoing
Clostridium spp. by rDNA qPCR (Modified Rinttilä et al. assay)	2007 Marine	Adequate sensitivity Evaluation ongoing
E. coli by *uid*A gene qPCR (Modified Rachel Noble lab assay)	2007 Marine	Insufficient sensitivity Modifications being evaluated
Human-associated *Bacteriodes* by rDNA qPCR (Modified Rachel Noble lab assay)	2007 Marine	Sensitivity may be adequate Further evaluation TBD

Other EPA/ORD presentations at this meeting

- **Comparison of *Enterococcus* qPCR analysis results from fresh and marine water samples on two real-time Instruments**
  - Richard Haugland, Manju Varma, Robin Oshiro Jack Paar III, Mark Doolittle and Mano Sivaganesan
    - EPA Microbiological ATP Protocol analysis for comparability of *Enterococci* qPCR results obtained from Cepheid Smart Cycler and a new 96-well instrument.

- **QPCR Determined Fecal Indicator Bacterial Densities in Marine Waters from Two Recreational Beaches**
  - Eunice C. Chern, Kristen P. Brenner, Larry J. Wymer and Richard Haugland
    - Comparison of *Enterococci, E. coli, Bacteroidales* and *Clostridia* spp. total cell and qPCR target sequence density estimates in marine recreational waters.

- **Evaluation of holding time of freshwater and marine samples for qPCR analysis**
  - Larry J. Wymer, Richard Haugland, Jack Paar III, Mark Doolittle and Kevin Oshima
    - Effects of sample holding time on recoveries of qPCR target sequences from fecal indicator bacteria in diverse fresh and marine waters.

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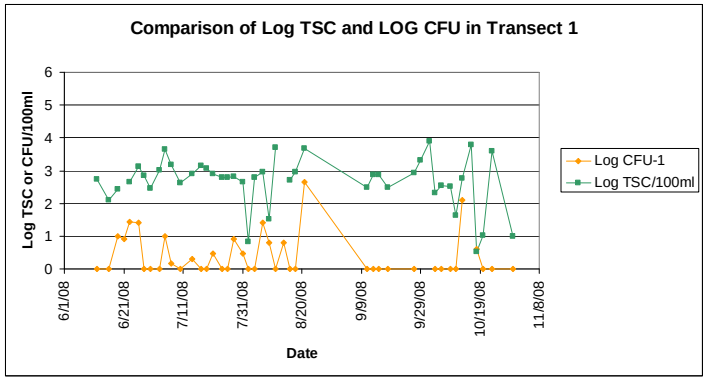
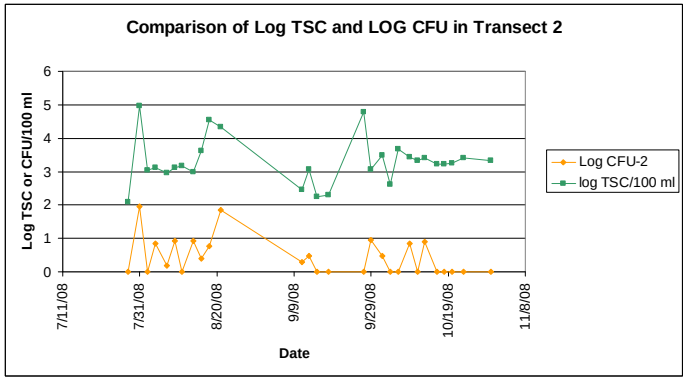
Multilinear Regression Model

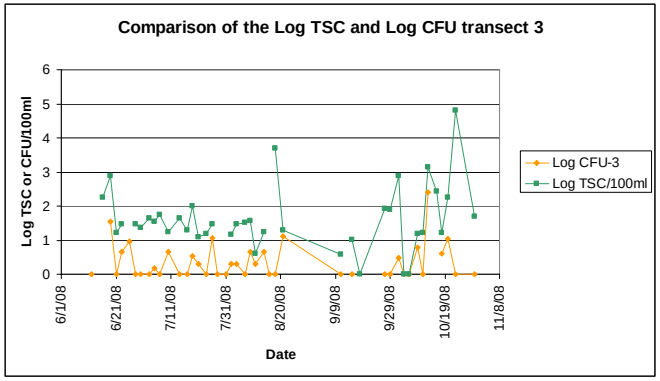
Ln Bact = 38.4; -246 U Comp Current; -2.74 ADCP water depth; -360843 Water Temp; +474 DOC: R^2 (adj)= 33.0%

Predictor	Coefficient	T	P	VIF
Constant	38.42	3.6	0.001	
Parallel Current	-245.91	-2.65	0.011	1.79
ADCP water depth	-2.7411	-2.92	0.006	1.037
Water temp	-360843	-2.53	0.016	2.301
DOC	473.8	2.48	0.018	2.725

Comparison of TSC and CFU in each beach transect relative to rain







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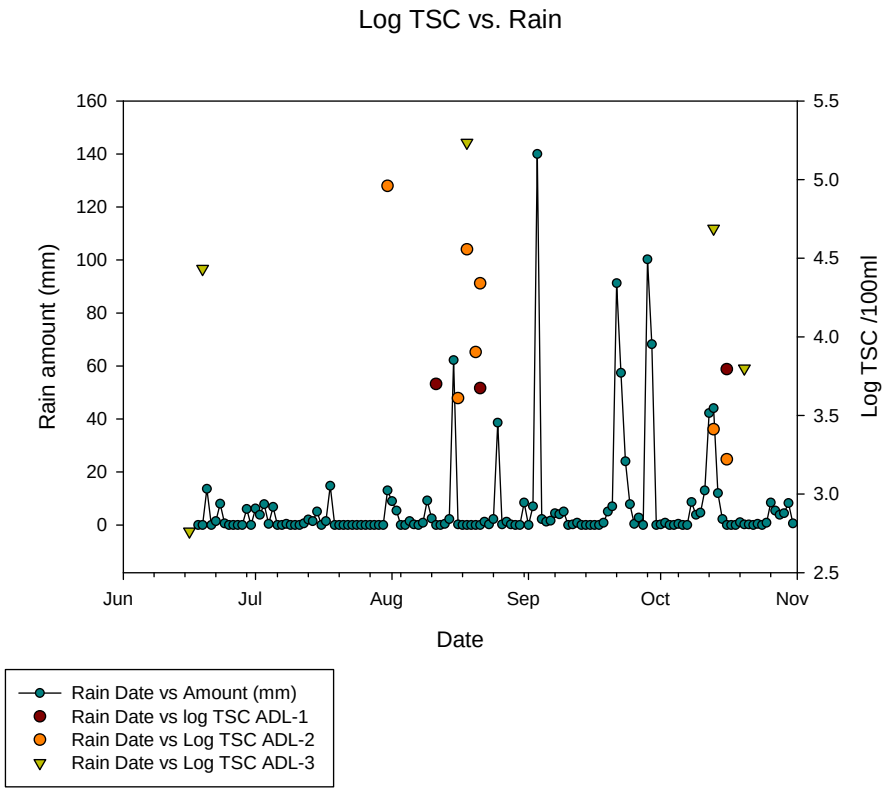
Log Enterococci TSC vs. Rain



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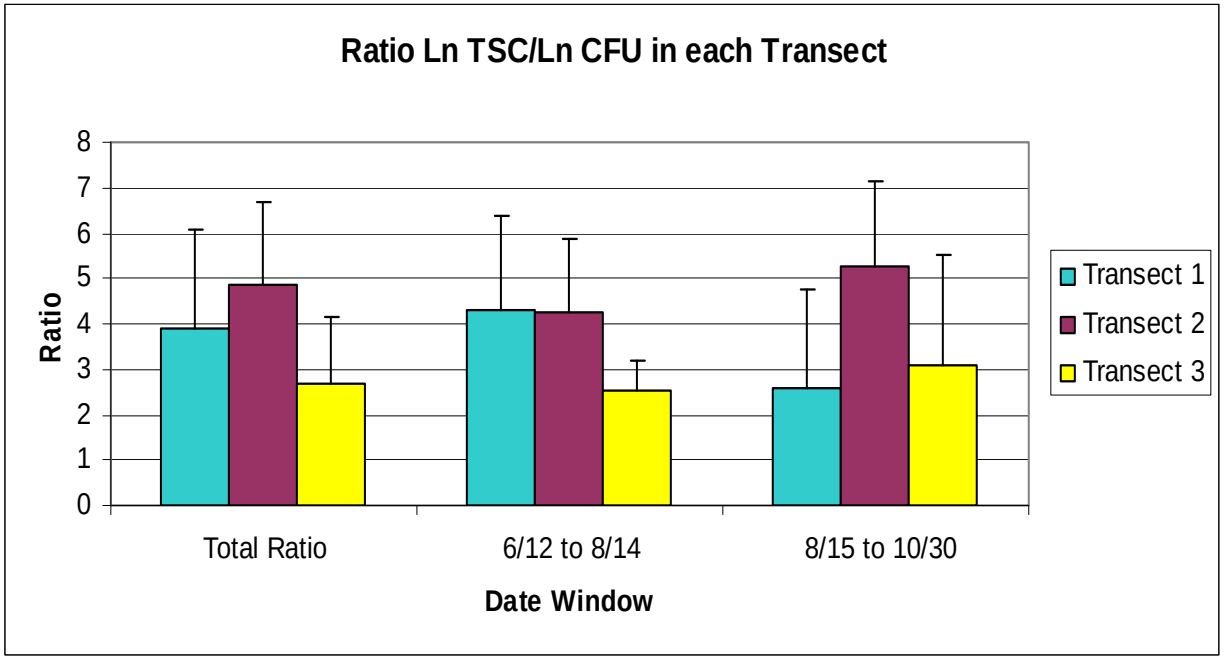
Ratio of TSC to CFU in each beach transect

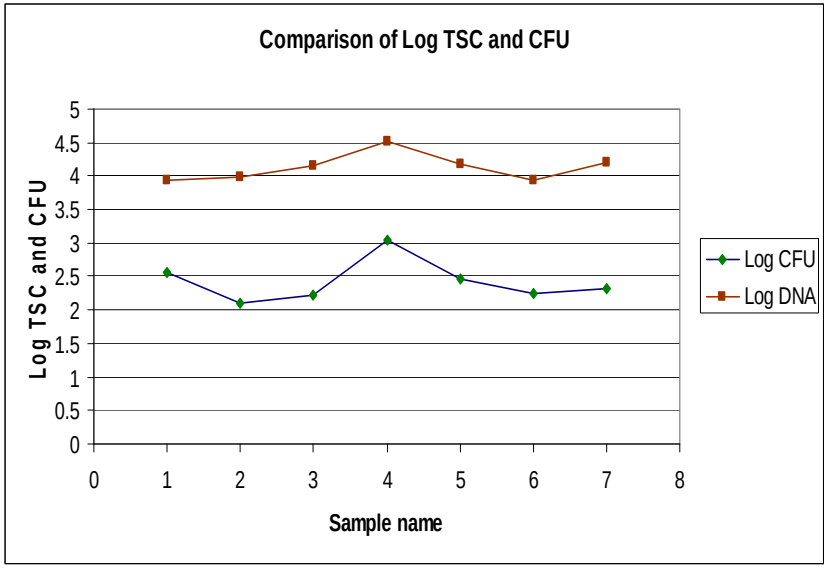


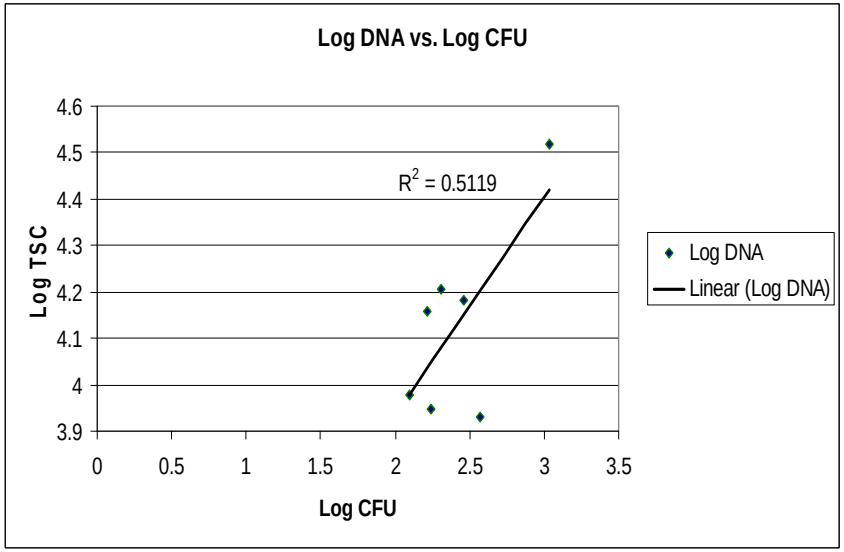
Enterococci Concentration and Presence of Bacteroidetes 16S rRNA marker

Stream Sampling Site	July CFU/ 100 ml	July Bac 32F	Nov CFU/ 100 ml	Nov Bac 32F
1	388	+	370	+
2	704	+	124	+
3	2450	+	164	+
4	TNTC	+	1085	-
5	1800	+	290	+
6	3800	+	175	-
7	780	+	205	-

Other 16S rRNA and metagenomic markers tested negative: HF183; CF128; CF 193; and Bird 1

Log Enterococci TSC Highly Correlates with Culturable Enterococci in Source Streams





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Summary and Conclusions

- Preliminary modeling results identified four parameters (parallel current, water depth, water temperature, and DOC) were responsible for explaining variability in bacterial concentrations
- The creek east of the beach served as a source to the closest sampling site (Station 1) but did not seem to influence bacterial concentrations at the other beach sites
- Ratios of enterococci DNA to culturable at the beach and other alternative markers provided evidence that the creek east of the beach was the main source of fecal indicators
- Most of the DNA signal observed at the source was from culturable enterococci

Thank You!

Acknowledgements

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- John Varner, Student Contractor