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Effect of Environmental Parameters on the qPCR Signal of Enterococci in Tropical Waters

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Abstract

Fecal contamination is the major source of pathogens in recreational waters. The need for quick public notifications has expanded the interest in the use of a rapid, quantitative polymerase chain reaction method (qPCR) to determine enterococci density. However, very little information is available on the fate and transport of the enterococci qPCR signal under a variety of environmental scenarios. In this study, we focused on the relationship among the enterococci qPCR signal, culturable enterococci and environmental parameters in marine tropical waters. Sampling was conducted from June thru October, 2008 at a beach located in Luquillo, Puerto Rico. Samples were collected three times per week at waist deep and processed for enterococci densities using membrane filtration and qPCR. Hydrometeorological and biogeochemical variables were collected on a 24 hour basis using automated equipment installed at the beach. The culturable enterococci information indicated that the highest densities were detected closer to a mangrove channel draining a sanitary sewer pumping station. Culturable and qPCR enterococci densities were not linearly correlated (r2= 0.05) in the beach water column indicating that outputs from each methodology cannot be explained by the same environmental variables. However, these two measurements were highly correlated (r2=0.7) in the streams adjacent to the beach, indicating that most of the qPCR signal was contributed by culturable enterococci. Using a statistical modeling approach, a preliminary model was developed to identify the variables that can best predict enterococci concentrations. Results indicated that current parallel to the shore from east to west, low tide, low water temperature, and DOC concentration were the best predictors for bacterial concentration. This study identified environmental parameters that best describe the variability in the enterococci numbers; and provided a basis for the development of more accurate predictive models that can be used as alternative tools for fast assessments of recreational water quality.

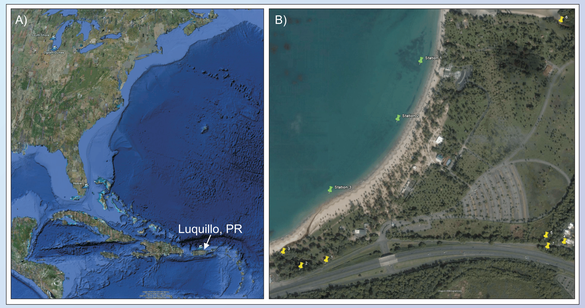

Figure 1: Study site (A) and sampling (B) locations

Objective

This study is part of a larger study designed to evaluate the applicability of statistical modeling to a variety of marine and freshwater beaches. Predictive models are developed using a software tool called Virtual Beach and are based on microbial data (dependent variables) and observations (independent variables) obtained from hydrometeorological and biogeochemical conditions at the beaches.

Methodology

- Water samples were collected waist deep from three stations:
  - Once a day, (10 a.m.), three days a week for measurement of culturable and qPCR enterococci.
  - Samples were collected from June through October, 2008.
- Instruments were deployed at the beach to log water quality parameters that were used to develop the models and establish relationships with enterococci concentrations.
- A fast QPCR procedure (30 min) using entero 2 primers as described by Haughland et al. 2005 and Siefrieng et al. 2008 was applied after bead beating filters. Culturable enterococci densities were obtained by membrane filtration using US EPA Method 1600.

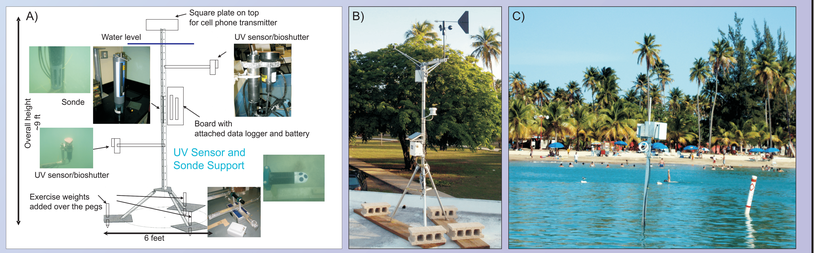

Figure 2: Instruments deployed at beach; A)Instruments installed under water; B)Weather Station; C)Tower in water with remote data transmitter

Sonde: pH, turbidity, salinity, chlorophyll, temperature, dissolved oxygen
Acoustic Doppler Current Profiler (ADCP): current direction and speed, wave height
Weather Station: rain amount, air temperature, wind speed and direction, barometric pressure, solar irradiation, photosynthetic active radiation (PAR)

RESULTS

Multilinear Regression Model

Ln Bact = 38.4 -246 (U Comp Current) -2.74 (Ln Water Depth) -360843 (Water Temp) +474 (DOC) r^2(adj)= 0.33

Modeling Results

A preliminary model indicated that four main parameters explain 33% of the variability in the bacterial concentration.

Higher bacterial concentration is explained by:
- Current parallel to the beach from east to west
- Shallow water depth
- Cooler water temperature

Predictor	Coeff.	T	P
Parallel Current	-245.91	-2.65	0.011
ADCP water depth	-2.7411	-2.92	0.006
Water temp	-36084	-2.53	0.016
DOC	473.8	2.48	0.018

Figure 3: Model indicates that stream to the east is responsible for bacterial contamination at the beach.

Bacterial Results

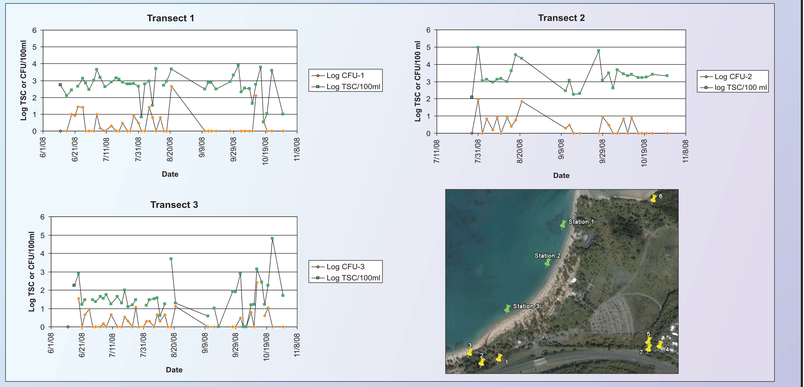

Figure 4: Comparison of Log TSC and LOG CFU in culturable and qPCR enterococci at each sampling station from June thru October 2008.

Both culturable and qPCR enterococci numbers exhibit a high degree of variability. Station 1 and Station 3 exhibit higher variability during rain events. Culturable enterococci correlated with rain (r^2=0.7).

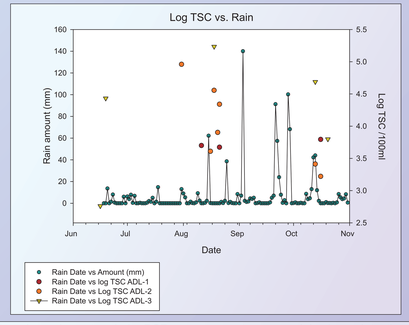

Figure 5: Log enterococci target sequence copies vs. rain in Luquillo, PR from June thru October 2008.

Summer can be divided into a dry and a wet period; qPCR measurements were mainly above detection limit during the wet period.

Figure 6: Sampling locations in streams adjacent to beach

Table 1: Enterococci densities and source specific DNA markers identified in runoff streams adjacent to beach

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	+

Two streams fed by storm water runoff could potentially bring contamination to the beach. Stream water samples exhibited extremely high enterococci concentration and presence of Bacteroidales spp. 16S rRNA Markers. General bacteroidales marker was present in all stations. CF 128 (16srRNA ruminant marker) was positive for Nov samples 5 and 7; other 16S rRNA and metagenomic markers were not identified: HF183, HF 134, CF 193, and Bird 1.

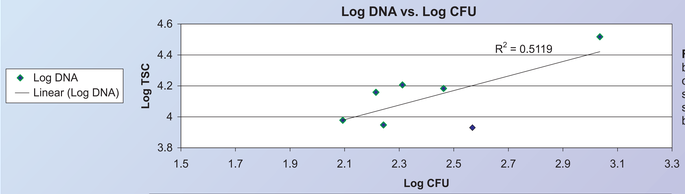

Figure 7: Relationship between enterococci concentration and target sequence copies in streams adjacent to beach.

Log Enterococci TSC highly correlates with culturable enterococci densities in source streams.

SUMMARY AND CONCLUSIONS

- Preliminary modeling results identified four parameters (parallel current, water depth, water temperature, and DOC) responsible for explaining variability in bacterial concentrations.
- The creek east of the beach served as a source of contamination to the closest sampling site (Station 1) but did not seem to influence bacterial concentrations at the other beach stations. This result is also supported by modeling data.
- Ratios of enterococci DNA to culturable enterococci at the beach provided evidence that the main source of contamination to the beach comes from the stormwater-fed streams.
- Most of the DNA signal observed at the source streams was from culturable enterococci.

REFERENCES

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