Q-397

Assessing the Impact of Urban Runoff in Recreational Beaches in South Carolina and Florida Using Culturable and QPCR Fecal Indicators

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ABSTRACT:
Urban/suburban runoff carries a variety of pollutants that often includes bacterial pathogens and indicators of fecal contamination. The objective of this study was to assess the microbial water quality of recreational beaches impacted solely by urban runoff through the use of culturable (enumeration of enterococci and *Pseudomonas aeruginosa)* and molecular (end-point PCR and qPCR for *Escherichia coli*, enterococci and *Bacteroidales*) methodologies. At each of three South Carolina beaches and two Florida beaches, water samples and physico-chemical parameters were collected from three to five locations perpendicular to the shoreline. Sampling was also conducted at several locations in the ditch (swash) or storm drain stream directly impacting each SC beach. No storm drain discharge directly affected the FL beaches. Results indicate that although swash-associated (SA) beaches (i.e., SC beaches) had a higher concentration of enterococci (25-163 CFU/100 ml) than beaches with no direct urban drain inputs (12-20 CFU/100 ml), the counts did not always correlate with the high swash counts (163-654 CFU/100 ml). *P. aeruginosa* was detected in low concentrations during baseflow conditions (2-12 CFU/100 ml) only in SA-beaches. Enterococci and *P. aeruginosa* concentrations went up at the SA-beaches transects as the result of rain episodes of over 0.50 inches. Regression analysis indicated a poor correlation between the qPCR enterococci 1 assay and the enterococci culture method across all sampling sites. Most of the detectable qPCR enterococci values were observed at the beach transects after storm events. Data suggest that correlations between qPCR and culture-based approaches can change dramatically from one beach site to another suggesting that site specific factors such as physico-chemical properties of the water matrix and the presence of a swash zone are important factors. High concentrations of fecal indicators in urban runoff sources (ditches) seem to impact beach waters when the beach is not protected by extensive pervious surfaces (i.e., long sandy shorelines) and/or transport is facilitated by measurable storm events.

INTRODUCTION

The Environmental Protection Agency (EPA) recommended recreational water criteria are based on epidemiology studies of publicly owned treatment works (POTW) impacted waters. A complete assessment of recreational criteria must include the investigation of non-POTW impacted recreational waters to determine whether potential health risks are different than those in recreational waters associated with POTWs. Therefore, there is a need to monitor recreational waters impacted primarily by urban/suburban runoff, which may carry a variety of pollutants including bacterial pathogens and indicators of fecal contamination. Urban runoff is defined as storm water from rain, snowmelt or irrigation that flows over the land surface and is not absorbed into the ground, instead flowing into streams or other surface waters or land depressions, including the possible discharges of storm water or storm water runoff. The primary objective of this study was to perform preliminary microbial monitoring (i.e., enumeration of enterococci and *Pseudomonas aeruginosa* and qPCR analysis of a variety of indicators) to initially assess the water quality of marine, non-POTW impacted beaches affected by urban runoff. The marine waters selected for this study were not known to be impacted by: (1) discharges from POTWs or combined sewer overflows (CSO); or (2) identified discharges of untreated human waste from sanitary sewer systems. Therefore, the study was designed to perform preliminary assessment of a variety of beaches that could lead to the collection of additional data that will allow for the determination of a relationship between human illness and fecal indicators originating from urban runoff in the absence of human-related wastes.

STUDY SITES

- Five marine beaches: three in South Carolina and two in Florida.
- The South Carolina beaches include: Canes Patch Swash (CPS) and Withers Swash (WS) in Myrtle Beach, and Surfside Swash (SS) in Surfside.
- The Florida beaches include: Silver Beach (SB) and Florida Shores (FS), north of Daytona Beach.

Figure 1. Beach and ditch sampling locations at Canes Patch Swash in Myrtle Beach, South Carolina

Figure 2. Beach and ditch sampling locations at Surfside Swash in Surfside, South Carolina.

Figure 3. Beach and ditch sampling locations at Withers Swash in Myrtle Beach, South Carolina.

Figure 4. Beach sampling locations at Florida Shores in Daytona Beach, Florida.

Figure 5. Beach sampling locations at Silver Beach in Daytona Beach, Florida.

Figure 6: Surfside Swash meeting with the Atlantic Ocean looking north in Surfside, South Carolina.

METHODS

Water sampling
At each of the three South Carolina beaches **(Figures 1 through 3)**, the sampling area was divided into five segments, each located perpendicular to the shoreline, with approximately 100 m between each segment. The sampling area was selected by identifying the main ditch or storm drain affecting the bathing zone. A total of five segments, each located 100 m apart, were sampled at each of the three South Carolina beaches. In Florida, the beaches were divided into three segments approximately 100 m apart **(Figures 4 - 5)**. Three water samples were collected from one location per transect at waist deep (approximately 1.0 m deep, 0.3 m below the surface) in 500 mL, pre-sterilized polycarbonate bottles and then composited into a 2 L pre-sterilized polycarbonate bottle. This composite sample was used for enterococci, *Pseudomonas* and qPCR sample analyses.

In addition to sample collection at each of the five beach segments, sampling was also conducted in the swash associated with the beaches in SC **(Figures 1-3 and Figure 6)**. The open ditch or stream was divided into three segments, each between 100 and 300 m apart. A reducing agent (three tablets of sodium thiosulfate ($Na_2S_2O_3$)) was added to prevent the continuation of bactericidal action and to reduce any strong oxidants that may have been present in the sample. Samples were placed in a cooler immediately after collection and maintained at < 4˚C on wet ice.

A 500 mL portion of each of the composited water samples was removed from the 2 L polycarbonate bottle for qPCR analysis. The remaining portion of the composite sample (1,000 mL) was filtered for enumeration of culturable enterococci and *Pseudomonas aeruginosa*.

Site-specific ancillary data were collected during sampling visits at each of the five beaches.

Culturable and qPCR analysis

Membrane filtration (EPA Method 1600) was used to determine culturable enterococci levels and ASTM Method D5246-92 (2004) was used to determine *Pseudomona aeruginosa* levels. DNA was extracted from filters using the DNA-EZ kit (GeneRite, North Brunswick, NJ) following the manufacturer's protocol. QPCR analysis was performed using the assays included in Table 1 as described in Shanks et. al., 2009 and Seifring et al., 2008. The assays identified the following targets: HumM2, BsteriF1, BuniF2, HF183 = human; Entero 1= enterococci; GenBac3=General bacteroidales; EPA23S = *E. coli;* CowM2 = cattle.

Figure 7: Florida Shores Beach looking south in Daytona Beach, Florida.

Table 1: Calibration curves and performance characteristics

Assay	Calibration Equation	AE	R^2	Range of Quantification	Precision	Reference
HumM2	Y=39.9-3.54X	0.92	0.993	10 to 1x10^5	1.31	[1]
BsteriF1	Y=39.5-3.51X	0.93	0.993	10 to 1x10^5	2.23	[2]
BuniF2	Y=38.9-3.48X	0.94	0.992	10 to 1x10^5	1.75	
HF183	Y=38.7-3.47X	0.94	0.985	10 to 1x10^5	1.31	[3, 4]
Entero1	Y=37.8-3.59X	0.90	0.987	10 to 1x10^5	0.97	[5]
GenBac3	Y=38.7-3.48X	0.94	0.993	10 to 1x10^5	1.10	[6]
EPA23S	Y=39.4-3.48X	0.94	0.992	10 to 1x10^5	1.51	[7]
CowM2	---	---	0.995	10 to 1x10^5	1.63	[8]

"R-squared" denotes the coefficient of determination representing the proportion of variability in the data set accounted for by the linear model. "AE" indicates amplification efficiency" is equal to10 $^{(1/\text{slope})}$-1. Range of quantification is reported in copies of target DNA for each respective qPCR assay. "Precision" denotes the mean percent coefficient of variation across the ROQ.

RESULTS

Assessment of Microbial Water Quality through the use of Culturable and qPCR Bacterial Indicators

Swash-associated (SA) beaches (i.e., SC beaches) had a higher concentration of enterococci and *Pseudomonas aeruginosa* than beaches with no direct urban drain inputs (FS and SB). *P. aeruginosa* concentrations were generally low at all beach sites and were significantly higher at the ditch relative to the beach (exception for CPS). No significant difference was observed between culturable and qPCR enterococci levels at both the ditch and the beach for CS and CPS, except for WS. General Bacteroidales signal was significantly higher at the ditch relative to the beach for all three SC Beaches; while there were no significant difference in the *E. coli* qPCR signal for all sampling locations.

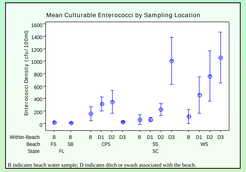


Figure 8: Mean enterococci concentrations at each of fourteen sites sampled in the vicinity of Daytona Beach, Florida and Myrtle Beach, South Carolina

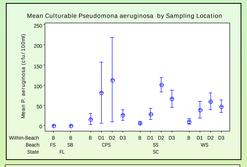


Figure 9: Mean *Pseudomonas* concentrations at each of fourteen sites sampled in the vicinity of Daytona Beach, Florida and Myrtle Beach, South Carolina.

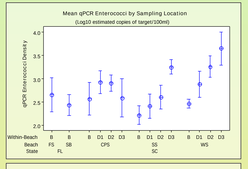


Figure 10: Mean enterococci qPCR concentrations at each of fourteen sites sampled in the vicinity of Daytona Beach, Florida and Myrtle Beach, South Carolina.

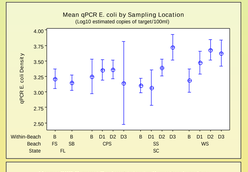


Figure 11: Mean E. coli qPCR concentrations at each of fourteen sites sampled in the vicinity of Daytona Beach, Florida and Myrtle Beach, South Carolina.

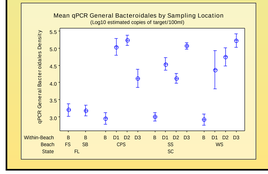


Figure 12: Mean General Bacteroides qPCR primer concentrations at each of fourteen sites sampled in the vicinity of Daytona Beach, Florida and Myrtle Beach, South Carolina.

Correlation Between Culturable and QPCR Enterococci Measures

These correlations represent the data both before and after the removal of measurements below the Range of Quantification (ROQ). For Surfside Beach, Surfside D1, and Withers Beach, after removal of the below-ROQ data points, no more than three data points remained, so the correlation coefficient was highly suspect and the confidence interval could not be calculated. The results indicate correlations with coefficients (r) ranging from -0.2 to 0.85 depending on the beach. Removing the below-ROQ points, in general, decreased the correlation between these two variables, with a few exceptions. The confidence interval obviously widens with the lower n, too. The overall linear regression for all beaches had an R^2 of 0.38

Figure 13: Correlation coefficients between culturable enterococci (Entero culture) and Entero1 QPCR at the 14 different sampling sites.

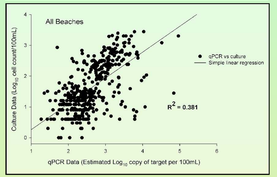


Figure 14: Relationship between qPCR and culturable enterococci for all beaches

Number of Birds Affects the Relationship between Culturable and qPCR Enterococci

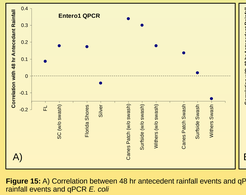
To investigate what factors affected the strength of the correlations, a linear regression approach was taken. A forwards stepwise regression determined that the number of birds was the most important factor that determined the strength of the correlation between QPCR and culturable data, followed by conductivity of the water. Both regression coefficients were negative, meaning that as the number of birds and conductivity increased, the correlation coefficient between QPCR and culturable data decreased

Table 2: Forward Stepwise Regression Results

Predictor	Coefficient	SE Coefficient	T	P
Constant	0.783	0.084	9.32	0.003
Total Birds	− 0.00124	0.00055	− 2.25	0.110

Relevant Environmental Variables that Affect the Densities of Culturable and qPCR Indicators

Rainfall (antecedent 48 hr rainfall events) had a distinct effect on the qPCR signal of enterococci and *E. coli*, with correlation coefficients of up to 0.45. In contrast, rainfallcorrelated weakly (r = -1.5 to 0.25) with culturable enterococci, *Pseudomonas* and general Bacteroidales.



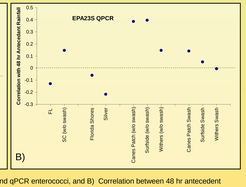


Figure 15: A) Correlation between 48 hr antecedent rainfall events and qPCR enterococci, and B) Correlation between 48 hr antecedent rainfall events and qPCR *E. coli*

Current parallel to the shore (Current U) resulted in the most important parameter for both culturable and qPCR enterococci for all SC beaches, and only for the qPCR enterococci in the Florida beaches, highlighting the importance of transport parallel to the shore as a contributor to high enterococci levels in these systems. In FL, meterological parameters (wave height, wind, cloud coverage) were important explanatory variables affecting culturable levels; while the presence of birds explained the variability affecting the qPCR signal. In the SC beaches, rain and wind along with current were important parameter affecting the level of the qPCR signal.

Table 2: Most important environmental variables predicting the concentration of culturable (top) and qPCR (bottom) enterococci densities at each of 14 sampling sites in the vicinity of Daytona, Fl and Myrtle Beach, SC. Regressors were done using the generic algorithm in Virtual Beach 2.0 for finding a best model and using the adjusted R^2 metric as the objective function.

Culturable Entero

Beach	Sampling Location	Adjusted R-Square	48hr Rainfall, mm	Air Temp, C	Water Temp, C	Cloud Cover	Wind U	Wind V	Current U	Current V	Wave Height, m	Total Birds	pH	Turbidity, NTU	Salinity, ppt	Conductivity, mS
Florida Shores	Beach Water	0.75						+				+				-
Silver Beach	Beach Water	0.68			+				-				+	-		
Canes Patch	Beach Water	0.52	+						-	-	-					
Surfside	Beach Water	0.84	+						-		-	+				+
Withers	Beach Water	0.8									+				+	-
Total Appearances per Independent Variable			2	0	1	0	3	2	3	0	2	1	1	1	2	1

Entero 1 QPCR

Beach	Sampling Location	Adjusted R-Square	48hr Rainfall, mm	Air Temp, C	Water Temp, C	Cloud Cover	Wind U	Wind V	Current U	Current V	Wave Height, m	Total Birds	pH	Turbidity, NTU	Salinity, ppt	Conductivity, mS
Florida Shores	Beach Water	0.74	+									+	+			
Silver Beach	Beach Water	0.54		-	+	+						-				-
Canes Patch	Beach Water	0.85	+					-	-							
Surfside	Beach Water	0.91	+		-		-							+		
Withers	Beach Water	0.62										-	+	-		-
Total Appearances per Independent Variable			3	1	2	1	1	1	1	0	3	2	1	1	1	1

CONCLUSIONS

- Results indicate that in beaches dominated by birds, the correlation between culturable and qPCR enterococci seems to be poor. For example, bird numbers in SC were lowest at SS Beach which exhibited the largest correlation coefficient between these two measures. We hypothesize that bird fecal material is mostly deposited on sand, exposing the material to UV and other possible contributors to fast bacterial decay, leaving behind the qPCR signal, but no culturable cells and therefore, lowering the correlation between the two signals. The fact that the model indicated that birds were one of the explanatory variables in FL beaches supports this conclusion.
- Parallel current to the shore was the most important variable overall at SC beaches for culturable enterococci, while rainfall was important for qPCR enterococci, indicating that local channels contribute with viable enterococci, and rainfall might be washing out the qPCR signal from dead cells on impervious surfaces into the water. During baseflow periods, in the presence of a local contamination source (swashes in SC beaches), the qPCR signal has a good correlation with culturable measurements which indicates that most of the qPCR signal is contributed by live cells. This conclusion is also supported by the fact that there were no significant differences between the culturable and qPCR enterococci at the swashes relative to the beach during baseflow periods.
- Significant difference in the concentration of the general Bacteroidales at the swash relative to the beach suggest that the sources of each type of contamination are either greatly diluted during transport, or their sources are independent to each other. In contrast, the concentration of the *E. coli* and enterococci qPCR signals suggests that the swashes might be the main contributors of this signal.
- The fact that the variability of each type of measure (culturable vs. qPCR) is explained by different types of explanatory variables indicates that the fate and transport processes governing the behavior of each signal are not in sync and better characterization of the effects of environmental variables on the behavior of the molecular signal is needed to fully understand the correlation with the culturable information as well as its relationship to health effects.

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