QUANTITATIVE ANALYSIS OF FECAL CONTAMINATION IN STORMWATER CONVEYANCE SYSTEMS AND THE EFFECTS OF STORM DRAIN DISCHARGE ON BEACH WATER QUALITY IN WRIGHTSVILLE BEACH, NC.

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ABSTRACT

Kellen Christine Lauer: Quantitative analysis of fecal contamination in stormwater conveyance systems and the effects of storm drain discharge on beach water quality in Wrightsville Beach, NC.
(Under the direction of Rachel T. Noble)

Fecal contamination in stormwater runoff is a concern for public health in coastal beach communities. Historical data collected by the Town of Wrightsville Beach has previously indicated that fecal indicator bacteria (FIB - *Escherichia coli (E. coli)* and *Enterococcus* spp.) concentrations frequently exceeded USEPA recommended water quality standards during and after storm events. Using both culture-based methods and quantitative PCR (qPCR), water samples from the storm drain systems of two problem watersheds were analyzed for FIB concentrations in addition to quantification of specific sources of fecal contamination from humans, gulls and dogs. Human and gull fecal contamination were both frequently quantified during the storm events (n=16). Significant correlations were observed between 1 hour antecedent rainfall and the human-associated fecal *Bacteroides* marker ($r = 0.17$, $p<0.05$, $n=149$), indicating the potential for future real-time beach management decisions to be made based on rainfall. An across beach study was then conducted to assess the dispersion of the stormwater plume during a typical storm event. The data demonstrated that significant levels of contamination were observed up to 200 m downcoast of the point of discharge, including quantified human and gull fecal contamination in the receiving waters. These results provide a valuable platform for the Town of Wrightsville Beach to mitigate sources of fecal contamination and prioritize strategies for improved public health notification.

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CE	Cell equivalents
C_T	Cycle threshold
E. coli	*Escherichia coli*
FIB	Fecal indicator bacteria
IMS	Institute of Marine Sciences
MPN	Most probable number
MST	Microbial source tracking
NEC	Negative extraction control
NTC	No template control
PC	Polycarbonate
SPC	Specimen processing control
TWB	Town of Wrightsville Beach
qPCR	Quantitative polymerase chain reaction
USEPA	United States Environmental Protection Agency

INTRODUCTION

Fecal contamination of recreational bathing waters is a concern for many coastal communities. Fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*, a subset of the fecal coliforms) and *Enterococcus* spp. are used globally as proxies of the presence of important viral and bacterial pathogens. A range of studies have shown that increased FIB concentrations can be indicative of higher rates of illnesses for beachgoers who have been exposed to contaminated water or beach sand (e.g. Haile et al., 1999; Colford et al., 2007; Wade et al., 2008; Heaney et al., 2012). Although heightened FIB concentrations can stem from a variety of sources, including leaking sewage infrastructure and wildlife, stormwater runoff is a major contributor of FIB and pathogens to coastal receiving water bodies (e.g. Ahn et al., 2005; Brownell et al., 2007; Reifel et al., 2009; Zhang et al., 2013).

When North Carolina coastal recreational waters are determined to be unsafe, a beach advisory must be issued to warn the public about the increased risk of illness from exposure to pathogens in the water or beach sands. Water quality standards are established by the USEPA, and are adopted by the states. The two types of water quality standards are a single sample threshold and a 30-day geometric mean. Standards are set based on acceptable risk for swimming-related illness rates per 1,000 primary contact recreators. The State of North Carolina only uses *Enterococci* as an indicator of marine water quality, and currently uses standards from the 1986 published water quality criteria (USEPA, 1986). However, new suggested standards were published in by the USEPA in 2012 that have not yet been adopted for use in North Carolina (USEPA, 2012). All standards are summarized in Table 1. If a water sample is found to

have FIB concentrations in exceedance of either the single sample threshold or the geometric mean, the beach will be posted and another sample will be taken. This process is repeated until the FIB concentrations have decreased enough to fall below the threshold, and the beach notification will be taken down.

Table 1: USEPA water quality standards from 1986 and 2012. The USEPA recommended standards for both *Enterococci* and *E. coli* are presented, even though only *Enterococcus* spp. are used to manage recreational waters in the State of North Carolina.

	1986 Water Quality Criteria			2012 Water Quality Criteria		
Indicator	Single sample threshold (MPN per 100 mL)	30-day geometric mean threshold (MPN per 100 mL)	Number of illnesses per 1,000 swimmers	Single sample threshold (MPN per 100 mL)	30-day geometric mean threshold (MPN per 100 mL)	Number of illnesses per 1,000 swimmers
Enterococci	104	35	19	110	30	32
E. coli	235	126	8	320	100	32

The Town of Wrightsville Beach (TWB) has a history of being proactive to protect the public, most recently banning the smoking of cigarettes on town beaches. Wrightsville Beach is a popular vacation destination year round, but the population swells from around 2,500 to over 15,000 during the summer months (Imperial & Powell-Williams, 2006). Therefore, protecting the health of residents and visitors is of the utmost importance to town managers. In the past six years, an assessment of water quality during wet and dry weather conditions was conducted at the initiative of TWB managers. Town officials specifically noted that beach water quality during dry weather was excellent. However, during wet weather the areas proximal to the storm drain outfalls (sound side of the island, see Figure 1) were noted to be contaminated on a consistent basis, but with varying levels of FIB contamination (Dellies & Babin, personal communication).

Figure 1. Aerial view of the barrier island encompassing the Town of Wrightsville Beach.

Based upon this initial data collection, a collaborative study was designed to understand the dynamics of stormwater discharge related contamination. One major objective was to characterize the contamination stemming from stormwater discharge following localized, intense summer storm events. These events may be very small and might only last an hour or two, but they are problematic because many vacationers will return to the beaches within minutes after the weather clears. Current methods used for quantifying FIB concentrations can take 24-96 hours to obtain results, causing a large delay from sample collection to beach posting. Therefore, it is important to have a better understanding of the effects of stormwater discharge on delivery of fecal contamination to the receiving waters in Wrightsville Beach in an attempt to develop an early warning system for beachgoers. To this end, a range of storm events were studied so as to assess the impact of storms on water quality in the context of presumptive rainfall advisories.

The watersheds in Wrightsville Beach are extremely small and rectangular in size, with the majority of stormwater discharge conveyed to the sound side of the island. Due to the fact that each storm drain empties a small, tractable watershed, identification of the potential sources of fecal contamination in this system was manageable.

The major sources of fecal contamination in Wrightsville Beach include leaking sewage infrastructure, pets (dogs and cats) and sea birds (predominantly seagulls). Since Wrightsville Beach is a mostly residential barrier island with shallow groundwater tables, and therefore a shallow unsaturated zone for the coexistence of stormwater and sewage conveyance systems, inputs of human fecal contamination from damaged sewage infrastructure and/or illicit connections between pipes could be a potential source.

Another potential source of fecal contamination in this area is contamination from animal feces, particularly dogs and seagulls. When dog feces are not picked up, whether on a beach or around the neighborhood, they have the potential to be washed into the storm drains and receiving waters during a storm event, contributing to the increased bacteria load. Gulls represent another potential source; they are common along the beaches and parking lots of Wrightsville Beach, and there is a bird sanctuary on the north end of the island to protect nesting shorebirds and provide untouched habitat for their survival. Dogs and gulls have been found to be the major animal sources of fecal contamination in several studies of beach water quality (e.g. Edge & Hill, 2007; Jiang et al., 2007; Wright et al., 2009; Zhu et al., 2011).

The aims of this study were three-fold. The first aim was to conduct microbial contaminant assessments over a range of wet weather conditions, along with characterization of the FIB concentrations and sources of fecal contamination in two tractable watersheds in Wrightsville Beach (Iula St. and Snyder St. watersheds, Figure 2). Both FIB and molecular

microbial source tracking (MST) markers were used to determine the magnitude and sources of fecal contamination in the two watersheds. The second aim was to utilize the data generated over a range of storm conditions to determine if relationships to rainfall-based parameters were observed. The third objective of this study was to conduct a focused investigation on beach water quality in dry and wet weather in a single watershed. An across beach study during a single summertime storm assessed spatial distribution of MST markers when there was active discharge from storm drains along the sound side beach receiving waters. The overall goal of this study was to gain a more complete assessment of the impact of stormwater-based contamination on beach receiving waters, with the aim of improved and timely notification of the public and prioritization of the possible mitigation strategies for the sources of fecal contamination.

MATERIALS AND METHODS

Site characterization

The Town of Wrightsville Beach is a 5.4 km^2 barrier island community in New Hanover County, North Carolina (Figure 1). Just off the coast of Wilmington, it is a popular beach vacation destination. A year round population of about 2,500 people swells to an estimated 15,000 on weekends in the summer months, and up to 45,000-50,000 on holiday weekends (Imperial & Powell-Williams, 2006; Wrightsville Beach History). Forty-eight percent of the land in Wrightsville Beach is residential and 22% is undeveloped, mostly wetlands and Areas of Environmental Concern. Commercial land usage accounts for 12% of Wrightsville Beach, and land for recreational purposes accounts for 8% (Imperial & Powell-Williams, 2006).

Wrightsville Beach is bordered by the Atlantic Ocean to the southeast and Banks Channel, which separates the barrier island from the mainland, to the northwest. Banks Channel is tidally flushed, with a maximum tidal range of 223 cm. Snyder St. and Iula St. watersheds are approximately 2.5 km and 1.5 km, respectively, from Masonboro Inlet, the nearest connection to the Atlantic Ocean. Banks Channel is heavily used for recreation including kite surfing, wind surfing, boating, swimming and kayaking. It is also heavily used for fishing, shellfishing and navigational purposes. Due to pollution from urban runoff and marinas, the more northerly reaches are not suitable for shellfish harvesting for human consumption, and have been restricted in part since 1947 (NC Division of Marine Fisheries, Shellfish Sanitation Section historical data). The southern portions of Banks Channel are suitable for shellfish harvesting, but this area is subject to closures if bacterial pollution is too high (Classification on Shellfish Growing Waters,

NCAC). Such increases in bacteria concentrations have often been linked to storm drain discharge during and after storm events.

Small, tractable watersheds line Wrightsville Beach from north to south, delineated by storm drains which occur every hundred meters or so. These storm drain outfalls empty onto the sound side beaches, delivering stormwater runoff into Banks Channel. The storm drain infrastructure includes over 14,000 m of piping, manholes and outfall pipes, of which the town owns only 42.5%. The rest is owned by North Carolina Department of Transportation, New Hanover County and private entities. There are also 152 m of open ditches and 762 linear meters of sheet flow area (Imperial & Powell-Williams, 2006).

The two individual watersheds were chosen for this study because of previously noted exceedances of beach water quality standards for *Enterococcus* spp. after storm events (Figure 2). Each watershed contains at least one stormwater conveyance pipe emptying onto the beach along Banks Channel. The Snyder St. watershed encompasses the Blockade Runner Beach Resort and its parking lot, as well as a few residential homes. The Iula St. watershed encompasses a large paved parking area for beach access and a few residential homes.

Figure 2. Map of the locations of the Snyder St. and Iula St. watersheds on Wrightsville Beach and sampling points along each of the storm drains.

Sample collection and processing

A total of 16 storm events, classified as having active precipitation and observed discharge from drain outfalls of varying amounts and durations, were sampled between July 2011 and August 2012. Table 2 lists the dates on which sampling occurred and the analyses that were carried out on the samples from each date. Grab samples were collected in 500 mL acid-washed polypropylene (Nalgene^{TM}) bottles from inside the two storm drains (I-D1, I-D2, I-D3, S-D1, S-D2, S-D3 and S-D4) and at each drain outfall (I-Discharge and S-Discharge). At the time of sampling, water temperature (°F), air temperature (°F), wind speed (mph) and wind direction (cardinal directions) were measured, and any missing information was gathered from the NOAA Tides & Currents Wrightsville Beach monitoring station (Station 8658163, located on

Johnnie Mercer's Pier). Samples were transported back to Wrightsville Beach Public Works on ice and processed upon return, within regulatory holding times of water samples (USEPA, 2002a, b). The weather station used for hourly antecedent rainfall was located on the roof of the Wrightsville Beach Public Works building. This weather station is hosted by Weather Underground with the station ID KNCWRIGH3. The rain gauge is a MK III (RainWise Inc., Trenton, ME) and it uses Weather View 32 v70 software (Weather Information Systems, Amity, OR).

Table 2. Dates of sampling events, weather conditions and analyses carried out on each sample collected in 2011 and 2012. An X indicates that analysis was not performed on that sample.

Date	Wet/ Dry	*E. coli*	*Enterococcus*	Fecal *Bacteroides*	BacHum	HF183	Gull-2	DogBac
7/27/2011	Wet	✓	✓	✓	✓	✓	X	✓
8/18/2011	Wet	✓	✓	✓	✓	✓	X	✓
8/26/2011	Wet	✓	✓	✓	✓	✓	✓	✓
9/20/2011	Wet	✓	✓	✓	✓	✓	X	✓
10/19/2011	Wet	✓	✓	✓	✓	✓	X	✓
3/19/2012	Wet	✓	✓	✓	✓	✓	✓	✓
5/16/2012	Wet	✓	✓	✓	✓	✓	✓	✓
5/30/12 AM	Wet	✓	✓	✓	✓	✓	✓	✓
5/30/12 PM	Wet	✓	✓	✓	✓	✓	✓	✓
6/12/2012	Wet	✓	✓	✓	✓	✓	✓	✓
6/13/2013	Wet	✓	✓	✓	✓	✓	✓	✓
7/10/2012	Wet	✓	✓	✓	✓	✓	✓	✓
8/1/2012	Wet	✓	✓	✓	✓	✓	✓	✓
8/8/2012	Wet	✓	✓	✓	✓	✓	✓	✓
8/22/2012	Wet	✓	✓	✓	✓	✓	✓	✓
9/18/2012	Wet	✓	✓	✓	✓	✓	✓	✓

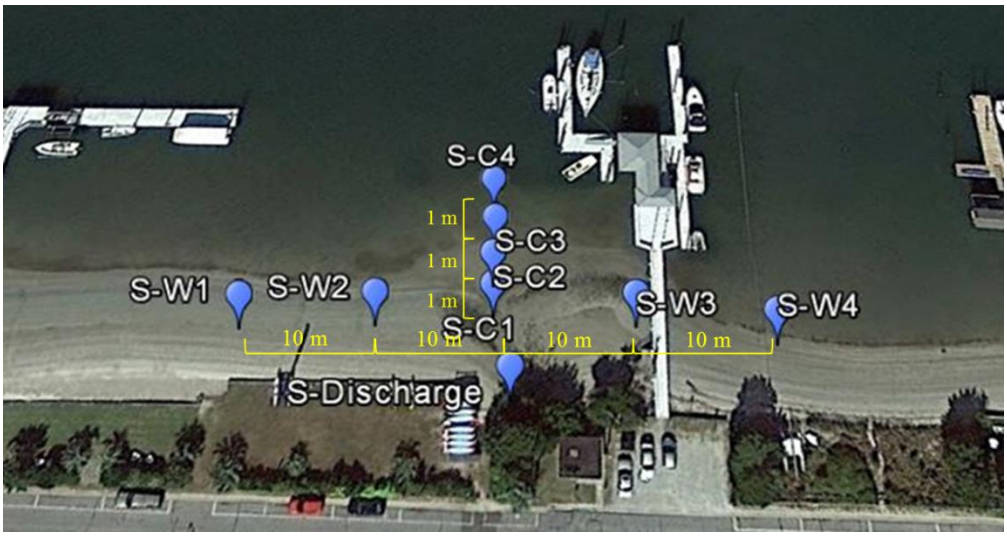
FIB concentrations were determined using Defined Substrate Technology™ as per manufacturer guidelines (IDEXX Laboratories, Inc., Westbrook, ME). *E. coli* and *Enterococcus* concentrations were quantified using Colilert®-18 and Enterolert®, respectively, using the most probable number (MPN) Quanti-tray®/2000 tray system. MPN calculations were completed

using the IDEXX MPN Generator Software Program 3.2, downloaded from the IDEXX Laboratories website, which uses the Thomas MPN equation to calculate FIB densities (Thomas, 1942). All water samples were diluted 1:10 prior to analysis with deionized water. In addition, duplicate subsamples of water were filtered in volumes of 100 mL through 0.4 μm, 47mm diameter polycarbonate (PC) filters (HTTP-04700, Millipore Corp., Bedford, MA) for later use in quantitative PCR (qPCR) analysis. Filters were stored in sterilized 2 mL polypropylene screw cap tubes with 0.3±0.01 g of 0.1 mm zirconia/silica beads (BioSpec Products, Inc., Bartlesville, OK), henceforth referred to as bead tubes, at -20°C for less than two weeks. The bead tubes containing filters were transported to the laboratory at UNC's Institute of Marine Sciences (IMS) in Morehead City, NC on dry ice and stored at -80°C until used for analysis.

In 2013, an across beach study was conducted to determine the effects of the Snyder St. drain outfall on the water quality of the surrounding beach. During the summer months, grab samples were collected upcoast and downcoast of the outfall and out into Banks Channel using the same methods listed above. There were six days when dry weather samples were collected, and one storm event was sampled. These sampling events, and analyses carried out on each sample, are listed in Table 3. Dry weather beach samples were collected 10 m and 20 m to either side of the pipe outfall at a depth of 0.3 m. In addition, four grab samples were collected in a line straight out into Banks Channel at one meter intervals (Figure 3a). Samples were processed using the same methods as described above.

Table 3. Dates of sampling events, weather conditions and analyses carried out on each sample collected in 2013. An X indicates that analysis was not performed on that sample.

Date	Wet/ Dry	*E. coli*	*Enterococcus*	Fecal *Bacteroides*	BacHum	HF183	Gull-2	DogBac
7/8/2013	Dry	✓	✓	X	X	X	X	X
7/11/2013	Dry	✓	✓	X	X	X	X	X
7/29/2013	Dry	✓	✓	X	X	X	X	X
8/5/2013	Dry	✓	✓	X	X	X	X	X
8/8/2013	Dry	✓	✓	X	X	X	X	X
8/15/2013	Wet	✓	✓	✓	X	✓	✓	X
9/9/2013	Dry	✓	✓	X	X	X	X	X



(a)

(b)

Figure 3. Sampling locations and distances between samples from the Snyder St. watershed during (a) dry and (b) wet weather sampling events. The star in (b) indicates the location of another storm drain outfall.

On August 15, 2013 beach water quality was characterized after a typical summer storm event. Grab samples were collected along the beach at a depth of 0.3 m at 200 m, 150 m, 100 m and 50 m downcoast of the pipe outfall, and 10 m and 50 m upcoast of the outfall. The four samples straight out into Banks Channel were collected from the same locations as the dry weather sampling (Figure 3b). Samples were transported directly to IMS on ice and processed immediately upon return in the same manner as described above.

Specimen processing control and standard preparation

For qPCR analysis, a specimen processing control (SPC) was used in order to measure the amount of sample loss during sample processing and matrix inhibition by adding a known amount of DNA at the beginning of the extraction step to each sample, calibration standard and a blank containing a PC filter which became the negative extraction control (NEC). Salmon Testes DNA (Sigma-Aldrich, St. Louis, MO) was added to buffer AE (QIAGEN, Valencia, CA) at a final concentration of 120 ng per 600 μL and is hereafter referred to as the Extraction Buffer. The primers and probe target a segment of the ribosomal RNA gene operon, internal transcribed spacer region 2 of chum salmon, *Oncorhynchis keta* as described in Haugland et al. 2005.

For the fecal *Bacteroides* qPCR assay, a genomic calibration standard was prepared from *Bacteroides thetaiotamicron* (ATCC 29148, Manassas, VA) as described in Converse et al., 2009. Briefly, *B. thetaiotamicron* was grown anaerobically in an overnight culture at 37°C in Cooked Meat Medium (BD Diagnostic Systems, Sparks, MD). Cell counts were obtained using SYBR Green (Invitrogen, Carlsbad, CA) as described in Noble & Fuhrman, 1998. Volumes of culture containing 83,000 cells were filtered onto PC filters and stored in bead tubes at -80°C for use as single-use cell standards.

Plasmid standards were used for BacHum, HF183, Gull-2 and DogBac qPCR assays. Standards were synthesized by GenScript (Piscataway, NJ). Gene sequences relating to the target sequences were synthesized and inserted into a linearized pUC57 vector which was cloned into DH5α competent cells. Plasmids containing the insert were extracted using Wizard® *Plus* SV Minipreps DNA Purification System (Promega Corp., Madison, WI). Plasmids were linearized using Eco R1 digestion and verified via a 1% agarose gel in Tris-Acetate-EDTA buffer. The weight of purified plasmids was then determined spectrophotometrically (Nanodrop 2000c, Thermo Scientific, Waltham, MA). Nanograms of purified plasmids were converted to copy number by using a copy number calculator available from SciencePrimer (http://scienceprimer.com/copy-number-calculator-for-realtime-pcr) which requires the amount of DNA (ng), and length of target (bases) to carry out the calculation. Linearized plasmids were diluted and stored at a concentration of $1x10^8$ copies per µL at -20°C in single-use aliquots.

DNA extraction

All unknown samples and positive and negative controls were extracted by a crude bead beating approach, and some crude extracts were further purified using a modified version of the GeneRite extraction kit DNA EZ RW04 (North Brunswick, NJ). For crude bead beating, 600 µL of Extraction Buffer was added to each bead tube containing either sample or control. The tubes were placed in a 48-place Mini-Bead Beater™ (BioSpec Products Inc., Bartlesville, OK) and homogenized for two minutes. The tubes were spun at 12,000 x *g* in a microcentrifuge for one minute to pellet the filter and beads. As much supernatant as possible was removed without disturbing the pellet and added to a 1.7 mL low retention microcentrifuge tube (GeneMate, ISC BioExpress, Kaysville, UT). The supernatant was spun for an additional minute at 12,000 x *g* to pellet any debris that was captured in the initial transfer. Four hundred microliters of supernatant

was removed and added to a new 1.7 mL low retention microcentrifuge tube. The crude extracts were processed immediately, and could be stored at 4°C for up to one week. For samples that were further purified using the DNA EZ RW04 kit, the crude extract (typically 400 µL) was added to two times its volume of Binding Buffer (not to exceed 800 µL). Half of this solution was added to a DNAsure™ column which was placed in a 2 mL collection tube and centrifuged at 6,000 x *g* for one minute. The flow through was discarded and this process was repeated with the remaining crude extract and Binding Buffer solution. The column was transferred to a new collection tube and 500 µL of Washing Buffer was added to the column. The column was spun at 6,000 x *g* for one minute, and the flow through was again discarded. The wash step was repeated for a second time in the same manner. The column was spun again at 6,000 x *g* for one minute to remove all traces of Washing Buffer. The column was then transferred to a new collection tube and 50 µL of Elution Buffer was added directly to the center of the column, which was allowed to sit at room temperature for one minute. The tube was spun for one minute at 6,000 x *g* to elute the DNA. Eluted DNA was processed immediately and could be stored at -20°C for up to six months.

qPCR analyses

qPCR was used to quantify fecal *Bacteroides* spp. and *Catellicoccus marimammalium* concentrations present in the samples. All assays were optimized for the CFX96™ Real-Time System (Bio-Rad Laboratories, Inc., Hercules, CA) platform using OmniMix® HS Lyophilized PCR Master Mix (Cepheid, Inc., Sunnyvale, CA) for fecal *Bacteroides,* BacHum, and Gull-2 assays, and SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories, Inc., Hercules, CA) for DogBac and HF183 assays. Primers and probes were synthesized by Biosearch Technologies (Novato, CA). All reactions had a total volume of 25 µL which included OmniMix® beads

reconstituted in nuclease-free water or Supermix, primers, probe and 5 µL unknown or control. Each group of samples was run with the following: standard curve made from a three- or four-fold serial dilution of the extracted calibration standard in nuclease-free water, the NEC that was extracted with the samples being analyzed and a no template control (NTC) which contained only master mix reagents and nuclease-free water. All samples, standards and controls were run in duplicate, and 2 out of a total of 28 NTCs run came up positive. Table 4 describes the cycling conditions for each assay that was used and Table 5 contains individual assay information. Fecal *Bacteroides*, BacHum, and Gull-2 assays utilized the TaqMan® chemistry for quantification, while HF183 and DogBac were SYBR Green-based assays.

Table 4. Cycling conditions for each MST assay on the Bio-Rad CFX96™ Real-Time System.

	(1) Initial Denaturation		(2) Denaturation		(3) Annealing with the Optics On			Melt Curve			
Assay	Temp (°C)	Time (minutes)	Temp (°C)	Time (minutes)	Temp (°C)	Time (minutes)	Repeated cycles (2&3)	Holding temp (°C)	Holding Time (minutes)	Final temp (°C)	Temp increments (°C)
SPC	94	2:00	94	0:30	60	0:45	40				
Fecal *Bacteroides*	94	2:00	94	0:30	60	0:45	40				
BacHum	94	2:00	94	0:15	62	1:00	40				
Gull-2	94	2:00	94	0:15	62	1:00	40				
HF183	98	2:00	98	0:02	55	0:05	40	60	0:10	95	0.2
DogBac	98	2:00	98	0:10	60	0:30	40	60	0:01	95	0.2

Table 5. MST assay information.

Assay Name	Primers and Probe	Primer/Probe Sequence 5' to 3'	Concentration (μM)	Reference
Fecal *Bacteroides*	BFDFor	CGTTCCATTAGGCAGTTGGT	1	Converse et al., 2009
	BFDRev	CGTAGGAGTTTGGACCGTGT	1	
	BFD TM FAM	FAM-CTGAGAGGAAGGTCCCCCACATTGGA-BHQ-1	0.1	
BacHum	BacHum-160f	TGAGTTCACATGTCCGCATGA	1	Kildare et al., 2007
	BacHum-241r	CGTTACCCCGCCTACTATCTAATG	1	
	BacHum-193p TM FAM	FAM-TCCGGTAGACGATGGGGATGCGTT-BHQ-1	0.1	
HF183	HS183 For	ATCATGAGTTCACATGTCCG	0.25	Seurinck et al., 2005
	HS Rev Seurinck	TACCCCGCCTACTATCTAATG	0.25	
Gull-2	Gull For	TGCATCGACCTAAAGTTTTGAG	1	Sinigalliano et al., 2010
	Gull Rev	GTCAAAGAGCGAGCAGTTACTA	1	
	Gull TM FAM BHQ	FAM-CTGAGAGGGTGATCGGCCACATTGGGACT-BHQ-1	0.1	
DogBac	DogBac DF475F	CGCTTGTATGTACCGGTACG	0.4	Sinigalliano et al., 2010
	Bac708R	CAATCGGAGTTCTTCGTG	0.4	
SPC	SPC For	GGTTTCCGCAGCTGGG	1	Haugland et al., 2005
	SPC Rev	CCGAGCCGTCCTGGTCTA	1	
	SPC TM FAM	FAM-AGTCGCAGGCGGCCACCGT-BHQ-1	0.1	

For the SYBR Green assays, HF183 and DogBac, a melt curve was run to confirm the presence of amplified target DNA as opposed to non-specific amplicons. Samples were only considered positive if their melt peak temperature fell within a range of temperatures within one degree of the calibration standard melt peak temperature. For HF183 this range of temperatures

was 77.5°C -78.5°C, and for DogBac this range of temperatures was 84.0°C – 85.0°C. A standard curve with an acceptable efficiency between 90% - 110% (calculated as $E = (2 - 10^{-1/-slope}) * 100$) and an R^2 greater than 0.995 could not be obtained for the DogBac assay in this study. Therefore, the DogBac assay was only used to determine the presence or absence of dog fecal contamination in samples. Master curves for each assay used for quantification were created in Microsoft Excel® by combining all of the points from a minimum of four standard curves that were run with different sets of samples. The trend line function was used to get a linear regression of the cumulative points. Master curves are presented in Figure 4.

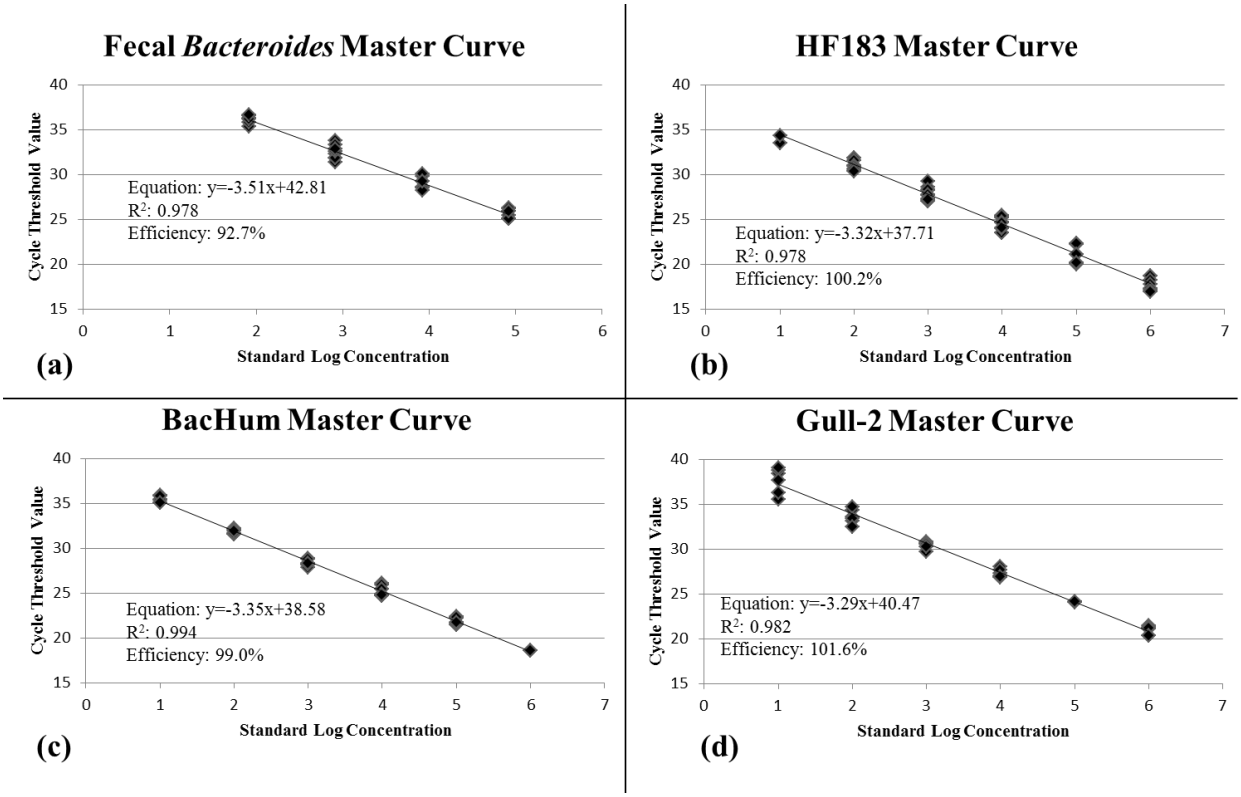


Figure 4. Master curves for (a) fecal *Bacteroides*, (b) HF183, (c) BacHum, and (d) Gull-2 with the equation of the line, R^2, and efficiency reported.

Data analysis

All microbial measurements were log transformed prior to statistical analyses in order to reduce skewness. Once log transformed, all statistics were performed in JMP® 10 (SAS Institute

Inc., Cary, NC). However, normality testing using the Shapiro-Wilk test showed that these data were not normally distributed, so non-parametric analyses were used when needed. The alpha level of significance accepted for all statistical tests was set at $\alpha = 0.05$. Wilcoxon/Kruskal-Wallis rank sum tests were performed to determine if there were differences in FIB and molecular marker concentrations between the Snyder St. and Iula St. watersheds, and between dry and wet weather samples in the Snyder St. across beach assessment. Bivariate correlations between antecedent rainfall and each FIB species and molecular marker were carried out for each site, each watershed, and all storm samples combined to determine the effects of rainfall-related parameters on bacteria concentrations. Bivariate correlations were also carried out to determine the effects of distance from the drain discharge on bacteria concentrations during the across beach Snyder St. storm event analysis.

RESULTS

Stormwater sampling FIB analysis

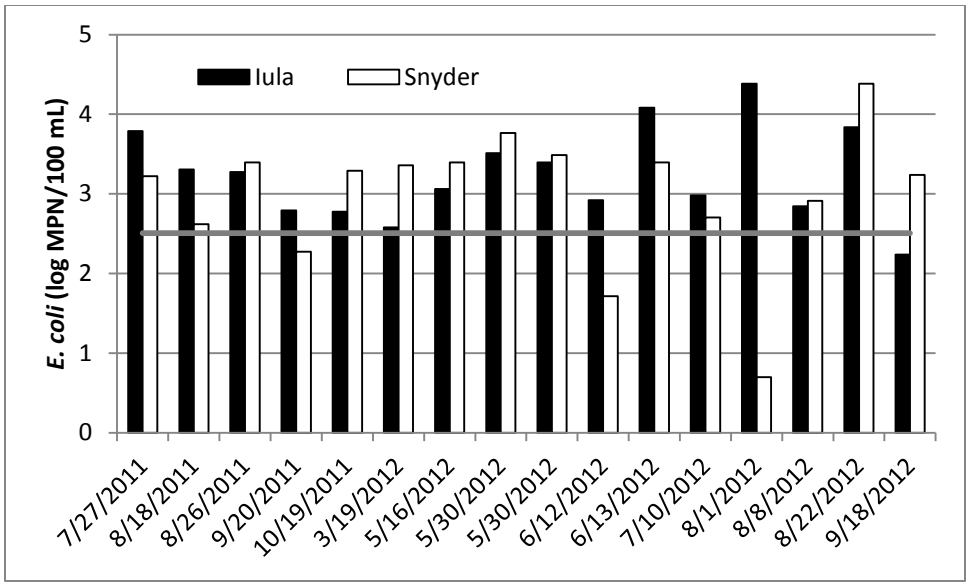
There were 16 storm events assessed from July 27, 2011 to September 18, 2012, providing a wide range of precipitation events representative of the region. Water samples collected from the storm drains during all storm events were high in FIB concentrations, exceeding 24,196 MPN per100 mL for *E. coli* in both watersheds and for *Enterococcus* in the Snyder St. watershed. The maximum *Enterococcus* concentration measured in the Iula St. watershed was 15,531 MPN per100 mL. Throughout the sampling, both *E. coli* and *Enterococcus* values spanned the entire quantifiable range of the Quanti-tray®/2000 system. This ranges from non-detects as <10 MPN per 100 mL which were given a value of 5 MPN per 100 mL, to all positive wells as >24,196 MPN per 100 mL which were given a value of 24,197 MPN per 100 mL. This corresponds to a log concentration range of 0.7 MPN per 100 mL to 4.4 MPN per 100 mL. The mean and range of FIB concentrations for each storm sampled are in Table 6.

The concentrations of FIB in the storm drains exceeded USEPA recommended recreational water single sample thresholds for *E. coli* (320 MPN per 100 mL) in 89.3% of the samples and for *Enterococcus* (104 MPN per 100 mL) in 92.1% of the samples. FIB were an order of magnitude greater than the recommended single sample threshold in 30.7% and 35.0% of the samples for *E. coli* and *Enterococcus*, respectively. Eight of the 140 samples (5.7%) were over 100 times the *Enterococcus* single samples threshold, demonstrating the potential for certain storm events to result in severe water quality degradation. Log *E. coli* concentrations greater than 100 times the single sample threshold (4.5 MPN per 100 mL) could not be

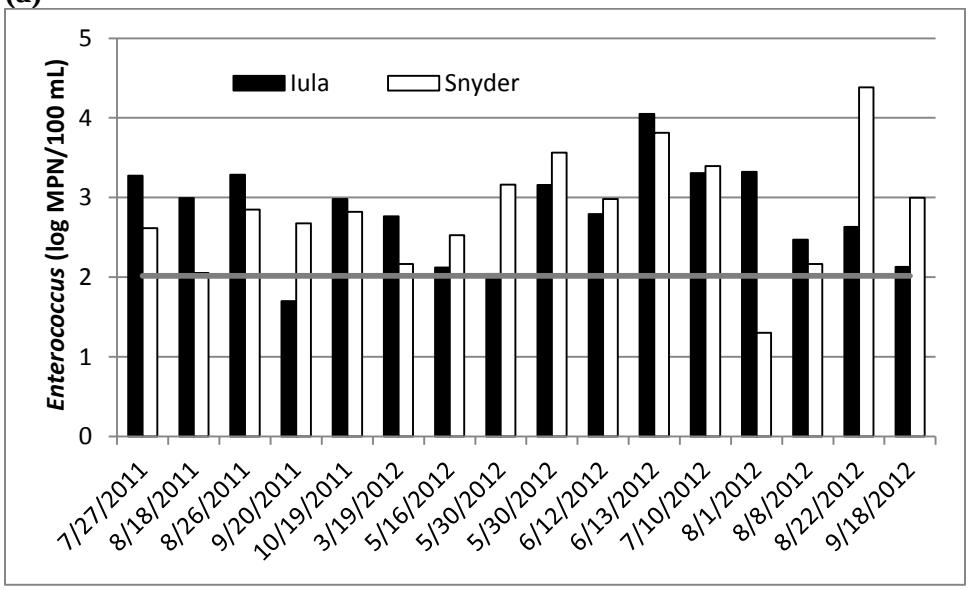
quantified with the methods used in this study, as the maximum quantifiable log concentration of the Quanti-tray®/2000 system is 4.4 MPN per 100 mL. At a maximum, 6.4% of *E. coli* samples were above the quantification limit and had the potential to have values over 100 times the *E. coli* single sample threshold. Water sampled from the discharge points of both pipes exceeded the standards for *E. coli* 87.5% of the time, and for *Enterococcus* 93.8% of the time. The FIB concentrations in this discharge for both watersheds are presented in Figure 5. The mean log concentrations of *E. coli* and *Enterococcus* in the discharge from the Snyder St. drain were 2.8 MPN per 100 mL and 2.7 MPN per 100 mL, respectively. For the Iula St. drain the mean log concentrations of FIB in the discharge were 3.2 MPN per 100 mL for *E. coli* and 2.8 MPN per 100 mL for *Enterococcus*. The mean concentrations between the two watershed were not significantly different for either *E. coli* ($p = 0.64$) or *Enterococcus* ($p = 0.85$).

Table 6. Mean values and ranges of FIB concentrations for each storm event sampled in 2011 and 2012.

Date	Mean *E. coli* (log MPN per 100 mL)	*E. coli* range (log MPN per 100 mL)	Mean *Enterococcus* (log MPN per 100 mL)	*Enterococcus* range (log MPN per 100 mL)
7/27/2011	3.7	3.2-3.9	3.3	2.6-4.0
8/18/2011	3.0	2.6-3.3	2.5	1.7-3.2
8/26/2011	3.3	3.2-2.5	2.8	2.5-3.3
9/20/2011	3.3	2.3-3.9	2.4	1.5-3.3
10/19/2011	3.0	2.5-3.3	2.8	2.2-3.0
3/19/2012	3.1	2.6-3.6	2.5	2.2-2.8
5/16/2012	3.4	2.9-4.4	2.3	2.1-2.5
5/30/12 AM	3.7	3.4-4.1	2.5	2.0-3.2
5/30/12 PM	3.4	3.0-3.9	3.3	2.8-3.8
6/12/2012	2.6	0.7-3.8	3.0	2.2-3.7
6/13/2012	3.6	2.7-4.2	3.8	3.4-4.2
7/10/2012	2.5	0.7-3.3	3.1	0.7-3.7
8/1/2012	3.1	0.7-4.4	2.6	0.7-3.5
8/8/2012	3.1	2.8-3.8	2.5	2.2-3.3
8/22/2012	4.1	3.6-4.4	3.5	2.2-4.4
9/18/2012	2.7	0.7-4.4	2.1	0.7-3.1



(a)



(b)

Figure 5. Mean (a) *E. coli* and (b) *Enterococcus* concentrations in drain discharge waters of each storm sampled. Grey lines indicate the USEPA recommended single sample thresholds for each bacteria type.

SPC analysis

The SPC was added in equal amounts to all samples and positive and negative controls (NEC), with the exception of the NTC. The NEC, which was a blank PC filter that was carried through the extraction process along with the samples, was used as a positive SPC and thus its cycle threshold (C_T) value was used as guidance for determining whether samples were

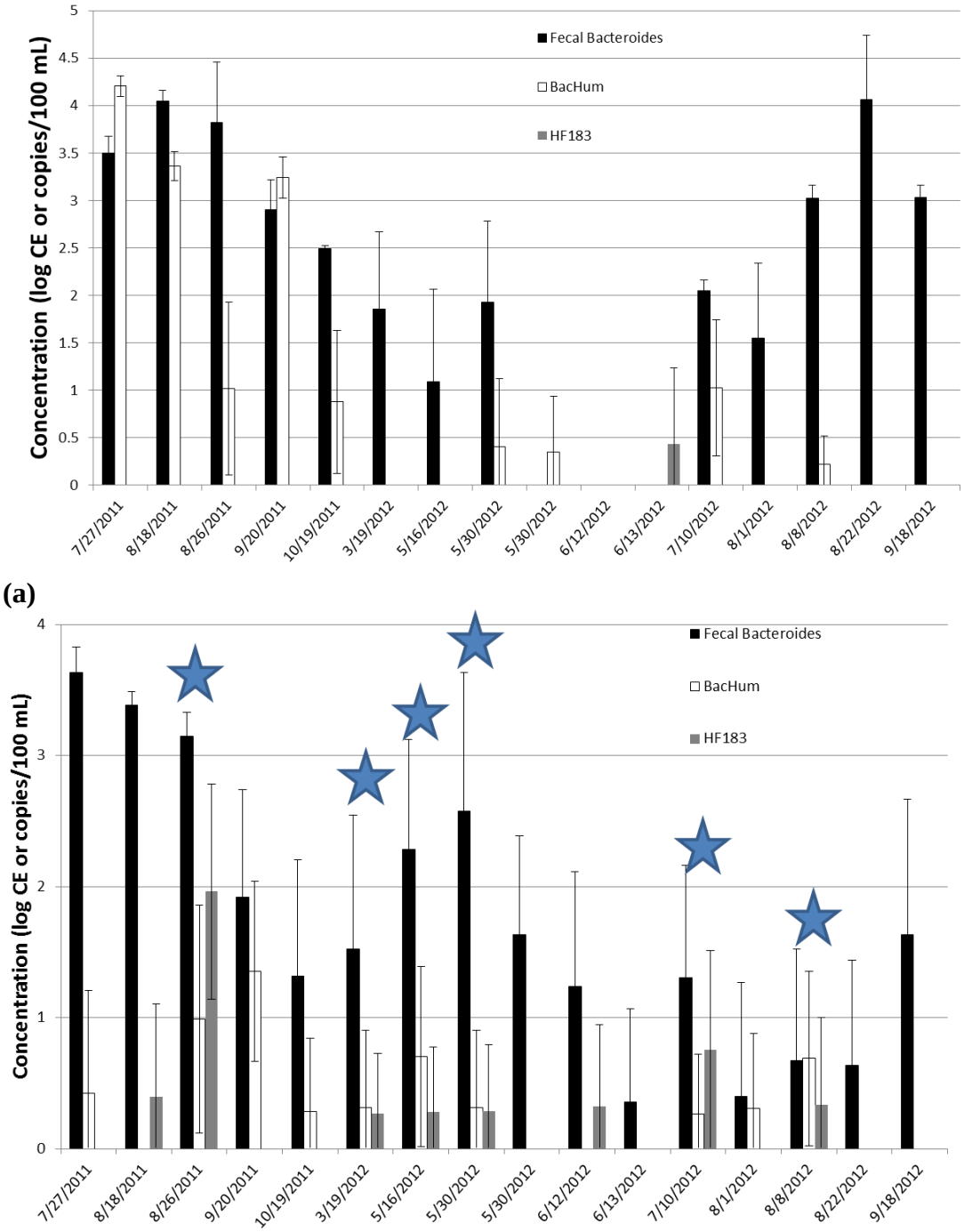
inhibited. qPCR reactions can be inhibited by substances which can interfere with the amplification efficiency of the DNA polymerase. In other words, if the C_T value differed by a delay greater than 2.32 C_T (equal to half a log difference in concentration), then the sample was considered inhibited and would be diluted according to how delayed the C_T value was (Cao et al., 2012). After bead beating and GeneRite extraction, all storm samples from 2011-2012 were initially diluted 1:10 with nuclease-free water before SPC analysis, which was run as a separate assay from that of the target (fecal *Bacteroides*, BacHum, HF183, Gull-2 or DogBac) assays. Of 140 samples processed, only 10 samples (7%) showed inhibition at the 1:10 dilution and were diluted 1:10 further in an attempt to sufficiently reduce the concentration of the inhibitors in the sample. This gave them a final dilution of 1:100. Three of those samples did not have inhibition completely removed by the 1:100 dilution, so they were run in three serial dilutions: 1:100, 1:1,000 and 1:10,000. This would show a linear response (3.3 C_T difference between each 10-fold dilution) if no inhibition was present or show a delayed response for those that sill showed inhibition. Samples from the 2013 storm event were diluted 1:5 with nuclease-free water after bead beating and GeneRite extraction. Only one sample required a final dilution of 1:10, which successfully relieved the inhibition.

Stormwater sampling MST analysis

Rather than relying on a single marker of human fecal contamination, a multiple and tiered marker quantification scheme covering a range of marker specificities and sensitivities was utilized. The markers utilized in this study, from least specific to most specific for human contamination (Layton et al., 2013) were 1) fecal *Bacteroides*, 2) BacHum and 3) HF183. There was no instance where all three human markers were positive in a single sample from the Iula St. watershed. However, all three markers were positive in nine samples out of 76 (11.8%) collected

from the Snyder St. watershed. Across both watersheds, 29 other samples (22.1%) from 10 of the storm events had two of the three human markers present. There were wide ranges of concentrations of each marker quantified throughout the sampling effort, with extremely high levels measured in certain samples. Quantified fecal *Bacteroides* log concentrations ranged from 1.3 - 6.7 cell equivalents (CE) per 100 mL. Quantified BacHum log concentrations ranged from 0.3 – 4.5 copies per 100 mL. Lastly, quantified HF183 log concentrations ranged from 2.3 – 3.4 copies per 100 mL. When all storm samples were considered together in a Wilcoxon/Kruskal-Wallis rank sum test, there was no difference in the mean concentration of the fecal *Bacteroides* marker between the two watersheds. The mean BacHum marker concentration was significantly higher in the Iula St. watershed than in the Snyder St. watershed ($H=5.64$, $p = 0.0175$). However, the mean HF183 marker concentration was significantly higher in the Snyder St. watershed than in the Iula St. watershed ($H=7.20$, $p = 0.0073$). Figure 6 shows the incidences of measured human contamination in both watersheds.

Dog fecal contamination, as measured using the DogBac qPCR marker, was ephemeral in both time and space. The DogBac marker was only detected four times, in samples from three different sites and three different storms, over the entire sampling effort. Because no acceptable standard curve could be generated, the assay tested for presence or absence of dog fecal material in a sample, and therefore was not quantitative. However, this assay also suffers from a lack of sensitivity as determined in the laboratory (Blackwood, personal communication). This means that using the presented sampling approach, a significant amount of dog fecal material would have to be present to generate a positive response. Dogs could potentially be a source of fecal contamination to this system, but as the methods did not permit the assessment to be quantitative, no statement can be made as to how trivial or significant it is.



(a)

(b)

Figure 6. Geometric means of human markers measured in all sites of the (a) Iula St. and (b) Snyder St. watersheds for each storm sampled. Stars in (b) indicate the days when all three human markers were detected in a single sample. Error bars are standard error of all four or five sites.

The Gull-2 marker, measuring *Catellicoccus marimammalium* concentrations, was quantified in all 12 storm events in which it was analyzed and was frequently measured in every

single drain site within both watersheds. In the Iula St. watershed the Gull-2 marker was quantified in 58% of the 48 samples collected, with log concentrations ranging from 1.2 – 4.3 copies per 100 mL. However, the highest concentrations of gull fecal contamination were measured in the Snyder St. watershed, at the two sites closest to the Blockade Runner. Seventy-six percent of the 60 samples were positive for the Gull-2 marker, and log concentrations ranged from 1.2 – 6.5 copies per 100 mL (Figure 7). The mean log concentration of the Gull-2 marker measured in all samples from the Iula St. pipe was 1.1 copies per 100 mL. For the Snyder St. pipe this mean log concentration was 2.7 copies per 100 mL, which is 35 times higher. This difference in mean concentrations was found to be significant using a Wilcoxon/Kruskal-Wallis rank sum test (H=10.31, p = 0.0013). The mean and range of values for each MST marker in each storm event are summarized in Table 7.

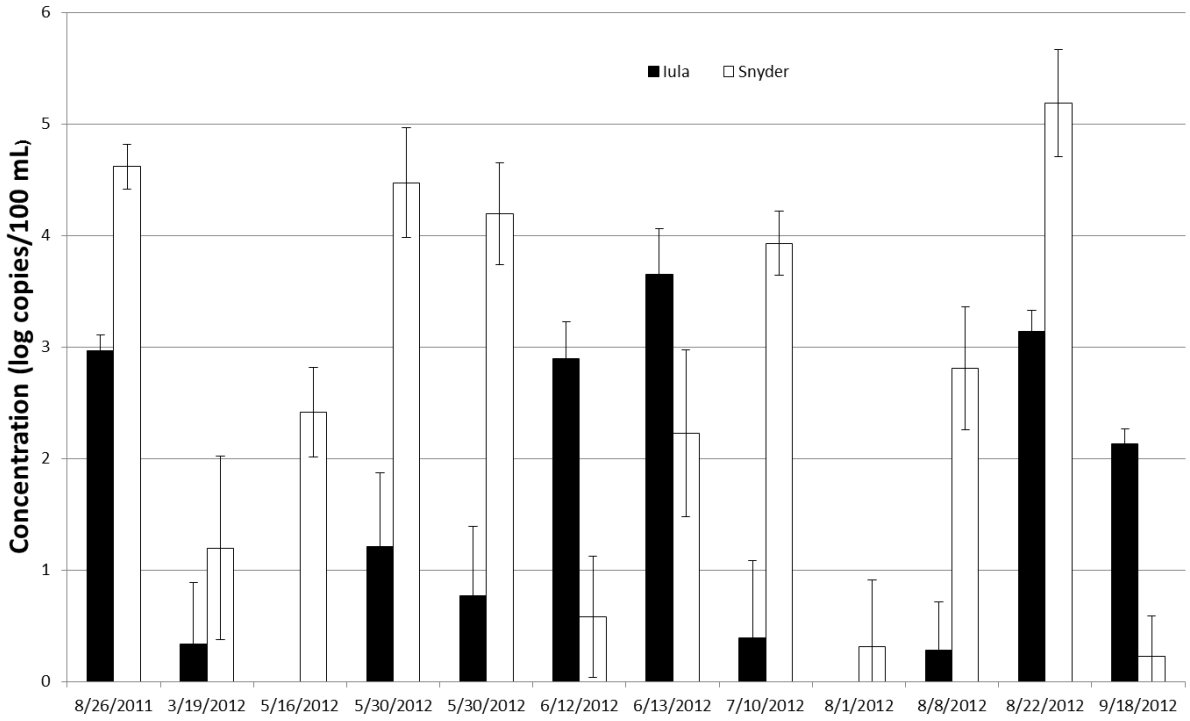


Figure 7. Geometric means of gull contamination measured in all sites of each watershed for each storm sampled. Error bars are standard error of all four or five sites.

Table 7. Mean values and ranges of all MST marker concentrations for each storm sampled in 2011 and 2012.

Date	Mean fecal *Bacteroides* (log CE per 100 mL)	fecal *Bacteroides* range (log CE per 100 mL)	Mean BacHum (log copies per 100 mL)	BacHum range (log copies per 100 mL)	Mean HF183 (log copies per 100 mL)	HF183 range (log copies per 100 mL)	Mean Gull-2 (log copies per 100 mL)	Gull-2 range (log copies per 100 mL)	DogBac detected (# of samples)
7/27/11	3.6	3.1-4.2	2.5	0.0-4.5	0.0	0.0-0.0			No
8/18/11	3.7	3.1-4.2	1.7	0.0-3.8	0.4	0.0-2.8			No
8/26/11	3.6	2.9-5.8	1.5	0.0-3.5	1.2	0.0-3.4	3.8	2.6-4.9	No
9/20/11	2.7	0.0-3.5	2.5	0.0-3.7	0.0	0.0-0.0			No
10/19/11	2.1	0.0-3.9	0.8	0.0-2.9	0.0	0.0-0.0			No
3/19/12	2.3	0.0-4.8	0.3	0.0-2.3	0.3	0.0-2.3	1.2	0.0-3.7	No
5/16/12	2.3	0.0-4.8	0.6	0.0-2.7	0.3	0.0-2.5	1.4	0.0-3.3	No
5/30/12 AM	2.9	0.0-6.7	0.7	0.0-2.9	0.3	0.0-2.5	3.2	0.0-5.5	1 of 9
5/30/12 PM	1.1	0.0-4.7	0.3	0.0-2.4	0.0	0.0-0.0	2.9	0.0-4.9	No
6/12/12	1.0	0.0-4.7	0.0	0.0-0.0	0.3	0.0-3.1	1.8	0.0-3.6	No
6/13/12	0.4	0.0-3.6	0.0	0.0-0.0	0.4	0.0-3.2	3.2	0.0-4.3	No
7/10/12	2.0	0.0-4.4	1.0	0.0-3.2	0.7	0.0-3.1	2.5	0.0-4.9	2 of 9
8/1/12	1.4	0.0-4.4	0.3	0.0-2.9	0.0	0.0-0.0	0.3	0.0-3.0	No
8/8/12	2.0	0.0-4.4	0.7	0.0-2.9	0.4	0.0-3.3	1.9	0.0-4.3	1 of 9
8/22/12	2.5	0.0-6.2	0.0	0.0-0.0	0.0	0.0-0.0	4.3	2.7-6.5	No
9/18/12	2.7	0.0-4.8	0	0.0-0.0	0	0.0-0.0	1.2	0.0-2.5	No

Relationships to rainfall

Certain markers showed weak but significant correlations with antecedent rainfall totals. The following relationships correlate all storm samples collected over the course of the study to the antecedent rainfall totals for each storm event. Fecal *Bacteroides* marker concentrations had a significant positive relationship ($r(149)=0.17$, $p=0.039$) with 1 hour antecedent rainfall. The Gull-2 marker was significantly correlated to 1 hour ($r(149)=0.21$, $p=0.008$), 6 hour ($r(149)=0.23$, $p=0.005$), 12 hour ($r(149)=0.22$, $p=0.006$), 18 hour ($r(149)=0.23$, $p=0.005$) and 24 hour ($r(149)=0.31$, $p=0.0001$) antecedent rainfall. *Enterococcus* concentrations were

significantly correlated with 24 hour (r(149)=0.21, p=0.011) and 48 hour (r(149)=0.21, p=0.010) antecedent rainfall. On the contrary, there was a significant negative relationship between fecal *Bacteroides* marker concentrations and 24 hour (r(149)=0.25, p=0.002) and 48 hour (r(149)=0.25, p=0.002) antecedent rainfall. Significant relationships are summarized in Table 8.

Table 8: Significant relationships between MST marker concentration and antecedent rainfall totals. Green shaded boxes are positive relationships and red shaded boxes are negative relationships.

		1 hour antecedent rainfall	6 hour antecedent rainfall	12 hour antecedent rainfall	18 hour antecedent rainfall	24 hour antecedent rainfall	48 hour antecedent rainfall
Fecal *Bacteroides*	N	151				151	151
	r-value	0.17				0.25	0.25
	p-value	0.039				0.002	0.002
Gull-2	N	151	151	151	151	151	
	r-value	0.21	0.23	0.22	0.23	0.31	
	p-value	0.008	0.005	0.006	0.005	0.0001	
Enterococcus	N					151	151
	r-value					0.21	0.21
	p-value					0.011	0.01

Across beach Snyder St. dry weather and storm event assessment

Due to the higher prominence of human and gull contamination measured in the Snyder St. watershed, a spatial examination of the beach surrounding the drain discharge point was carried out between July and September 2013 to determine the effect that the storm drain discharge would have on beach locations where people would be actively recreating. Sampling upcoast and downcoast of the drain outfall and straight out into Banks Channel took place on multiple occasions in dry weather and an across beach storm sampling event occurred on August 15, 2013.

During the six dry weather sampling events, 48 water samples were collected from Banks Channel, and there was never an instance of FIB exceedance of the single sample threshold for

recreational waters for either *E. coli* or *Enterococcus*. FIB were below the detection limit of the Quanti-tray®/2000 system (10 MPN per 100 mL) in 12 of 48 samples (25.0%) for *E. coli* and 25 of 48 samples (52.1%) for *Enterococcus*. These samples were given a value of 5 MPN per 100 mL for statistical analysis.

The storm event sampled on August 15, 2013 was a typical summer storm for Wrightsville Beach. Rainfall began just before 4:00 AM and continued until 12:00 PM, and storm drains were actively discharging into Banks Channel. The average rainfall rate during the storm was 3.3 mm/hr and the total amount of rainfall in this time period was a moderate 26.4 mm. Samples from around the Snyder St. drain were collected just as the rain was ending so that measurements explained the impacts of the storm event as a whole, and samples would be indicative of what beachgoers are exposed to when swimming immediately after the storm.

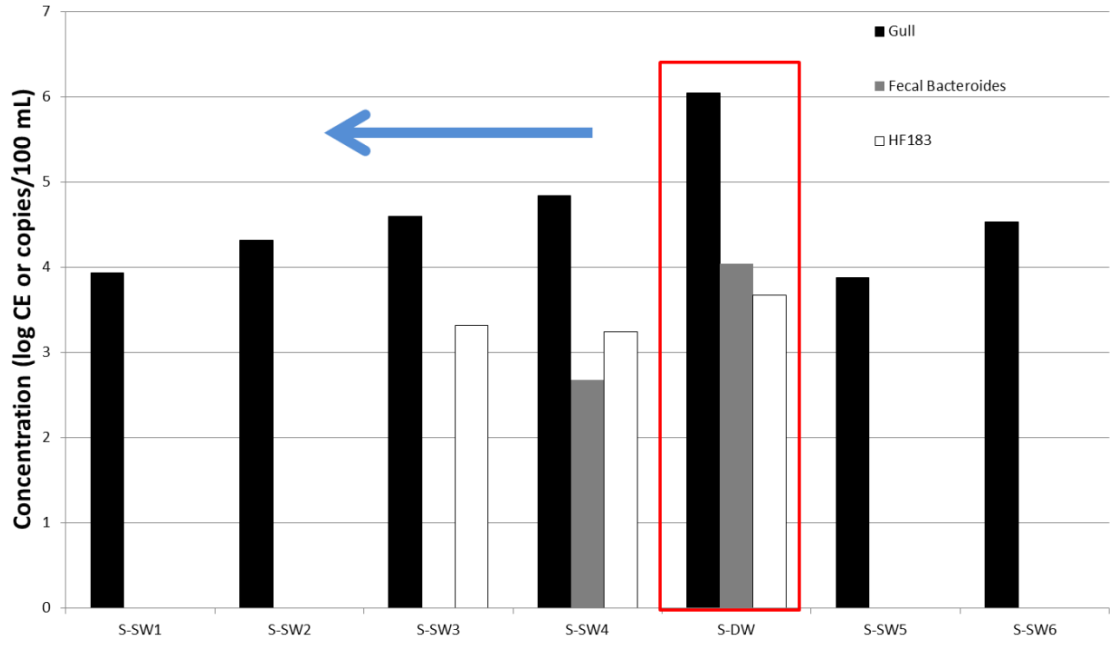
In the drain discharge, the *E. coli* log concentration was 3.4 MPN per 100 mL and the *Enterococcus* log concentration was 3.1 MPN per 100 mL (over 10 times the single sample threshold). Even higher log concentrations of *Enterococcus* were measured at the three sites in Banks Channel closest to the drain outfall (3.7, 3.6 and 3.6 MPN per 100 mL). These storm samples along the beach were significantly higher than the dry weather samples that had also been collected along the beach (Wilcoxon/Kruskal-Wallis rank sum test, H=26.7 for both *E. coli* and *Enterococcus*, $p<0.001$) There was a significant linear decrease in *E. coli* ($r(3)=0.95$, $p=0.047$) in the downcoast samples as the distance from the drain outfall increased, at a rate of -8.5 MPN per m. However, this relationship was not significant for *Enterococcus* ($p = 0.25$) as concentrations did not begin to decrease until 150 m downcoast. Even with the observed decrease in FIB concentrations, every water sample collected (up to 200 m downcoast and 50 m upcoast) remained above the single sample threshold for both *E. coli* and *Enterococcus*.

Human fecal contamination was quantified in the drain discharge through positive results for multiple human-associated markers, fecal *Bacteroides* and HF183, at log concentrations of 4.0 CE per 100 mL and 3.7 copies per 100 mL, respectively (Figure 8). This human contamination was also quantified at the four sites closest to the discharge point, the two nearest downcoast sites (S-SW4 and S-SW3, up to 100 m downcoast) and the two nearest sites straight out from the drain into Banks Channel (S-SC1 and S-SC2). The human markers were then below detection limits at sites farther from the discharge.

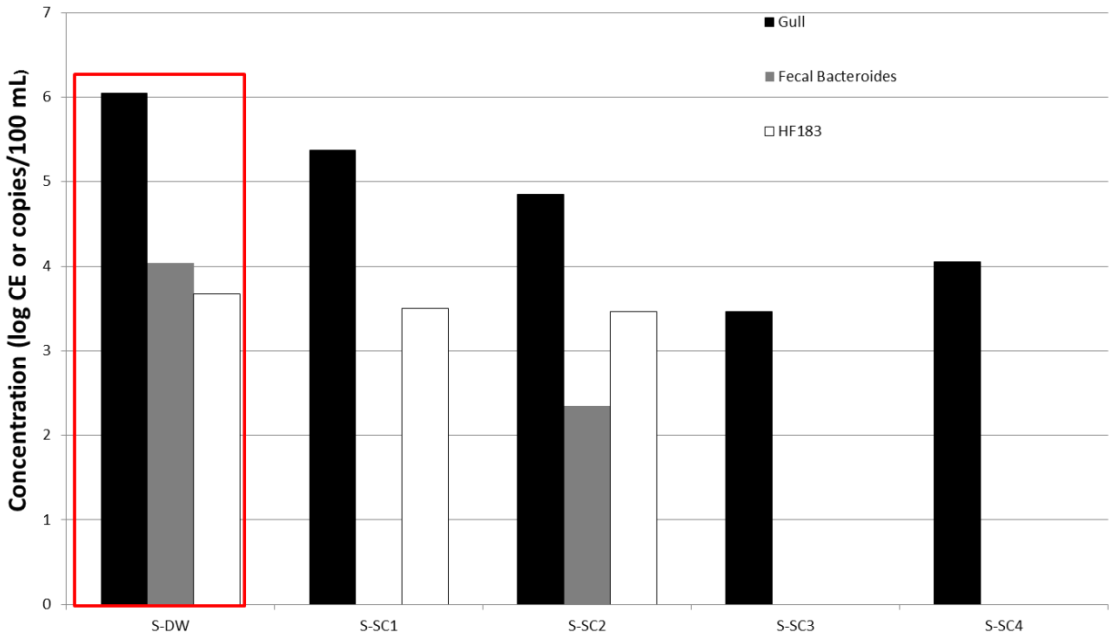
Log transformed gull contamination was quantified to be 6.0 copies per 100 mL of drain discharge, and measured in all water samples taken upcoast (up to 50 m) and downcoast (up to 200 m) of the storm drain, and all sites straight out into Banks Channel (Figure 8a and b). Because all sites were positive for gull contamination, this indicates that the Snyder St. storm drain is not the only source of gull contamination to Banks Channel. However, the log concentration of the Gull-2 marker at the nearest upcoast site (3.9 copies per 100 mL) was over 100 times less than the log concentration in the discharge. There was also a significant linear decrease in the Gull-2 marker concentration at successive downcoast sites from the drain outfall (at a rate of -433 copies per m) as the discharge was diluted ($r(3)=0.99$, $p=.005$).

FIB and Gull-2 marker concentrations were measured in gull feces collected from Wrightsville Beach during the time of this study (Lauer et al., in prep.). The 10 individual gulls sampled had a mean Gull-2 marker log concentration of 11.7 copies per g of feces. Comparing that concentration to the measured Gull-2 marker concentration in the drain discharge during this storm event, it equates to $2.0x10^{-5}$ g of gull feces per 100 mL of drain discharge water. The log concentration of *Enterococcus* in $2.0x10^{-5}$ g of feces as measured in the study would be 0.1 MPN. With a measured *Enterococcus* log concentration of 3.1 MPN per 100 mL of drain

discharge, gull fecal contamination only accounts for 0.1% of the *Enterococcus* quantified in this storm event.



(a)



(b)

Figure 8. Mean human and gull MST marker concentrations measured (a) along the beach upcoast and downcoast of the Snyder St. outfall and (b) out into Banks Channel on August 15, 2013. Storm drain discharge is contained in the red box, and water in Banks Channel was flowing in the direction of the blue arrow in (a). Each bar is the average of duplicate samples from each site.

DISCUSSION

Storm events of varying intensity in Wrightsville Beach lead to orders of magnitude increases in FIB concentrations in the storm drain systems and in receiving waters of Banks Channel. Ubiquitously high FIB concentrations after storm events have also been measured in previous studies conducted in North Carolina (e.g. Hathaway et al., 2010; Parker et al., 2010; Stumpf et al., 2010; Converse et al., 2011; Hathaway & Hunt, 2010), demonstrating that this is not a unique problem to Wrightsville Beach. However, FIB exceedances of 10 or 100 times the USEPA recommended single sample threshold were commonly observed throughout the drain system and discharge waters. Such high concentrations of bacteria pose a threat to the health of those who visit the sound side beaches after a storm event.

Perhaps an explanation for such high FIB concentrations measured in the storm drains and receiving waters is that there is bacteria growth in the persistent, deposited sediment inside the storm drain itself. Offering a sheltered environment protected from sunlight, the sediment inside of the storm drain may provide a habitat suitable for the persistence or growth of FIB outside of their hosts. Many studies have shown the ability for FIB to persist and reproduce in sediment environments (e.g. Marino & Gannon, 1991; Anderson et al., 2005; Ferguson et al., 2005; Lee et al., 2006; Pote et al., 2009). This hypothesis merits more research into the possibility of a reservoir population of FIB that builds up inside the drain during dry weather, and is then flushed out during a storm. Initial results from a study of storm drain sediment sampled from the Snyder St. drain in dry weather indicate that there may in fact be some persistence and growth of *Enterococcus* in the drain. Log concentrations of *Enterococcus*

remained high and averaged 3.9 MPN per100 g dry weight throughout all dry weather sampling events. *Enterococcus* speciation results showed that *Enterococcus casseliflavus,* a species that is known to be plant-associated, was the most common *Enterococcus* species quantified in the drain sediment (Lauer, unpublished data). This indicates that perhaps a portion of the measured *Enterococcus* concentrations in the storm drain may not be associated with fresh fecal material.

Even though there might be increasingly elevated FIB concentrations in the storm drains due to persistence or growth, it is not to say that growth is the sole cause of these high concentrations measured during the storm events. Despite the fact that the human markers were ephemeral in this study, each marker was detected over the entire length of the sampling period in both watersheds. Therefore, we know that human contamination is one frequent contributor to the FIB signal measured during storm events. Sauer et al. found similar results in a 2011 study of human contamination in storm drain outfalls around Milwaukee, WI. All 45 drains studied were positive for human contamination at least once, with positive results measured in each drain ranging from 11%-100% of storm events. It appears that some sort of human sewage contamination is inevitably present in densely settled areas.

Gull feces were confirmed to be the most widespread contributing source of contamination to this system. The Gull-2 marker was measured at least once in every storm event, and was quantified in 67.6% of the total samples. Gulls can carry a number of human pathogens such as *Campylobacter* spp. and *Salmonella* spp. (Lévesque et al., 2000; Albarnaz et al., 2007; Kinzelman et al., 2008), and the Gull-2 marker concentration has been shown to positively correlate with the amount of *Campylobacter* spp. in gull feces (Lu et al., 2011). Therefore, high concentrations of gull contamination in receiving waters can increase the threat of illness to swimmers. Controlling the gull population and deterring beachgoers from feeding

gulls through public education are both strategies which could be put into place in an attempt to reduce the fecal contamination from the gulls. A study published by Converse et al. in 2012 showed that using highly trained dogs to chase and deter gulls from a Lake Michigan beach resulted in a 50% decrease in gulls and a 38% and 29% reduction in *Enterococcus* and *E. coli* concentrations, respectively. Measures were taken to ensure that there was no additional fecal contamination from the dogs used in this study.

Higher mean concentrations of gull contamination in the Snyder St. watershed might be attributable to the locations of garbage dumpsters in the parking lot of the Blockade Runner. If gulls are congregating around these dumpsters to feed, and they then leave their feces behind in the parking lot, it would explain the higher rate of detection and larger concentrations of the Gull-2 marker quantified in the Snyder St. drain. Perhaps a gull survey or installation of a webcam could be used to determine if in fact there is a convergence of gulls around these dumpsters. If so, the Blockade Runner could then implement measures to reduce gull scavenging with the aim of decreasing the input of gull feces into the storm drain system beneath their parking lot.

Since it was not possible to quantify the DogBac marker in this study, the overall impact from dog fecal contamination is still unknown. However, in the presence/absence nature that the DogBac marker was detected, it was present in less than three percent of the samples. On the occasion that a dog was to defecate on the beach, it would be a concentrated amount of bacteria in a localized area. Over the course of this study, no dog feces were ever observed on the beach, and dogs are banned from being on the beach strand from April 1 through September 30. The Town of Wrightsville Beach also strives to educate residents and visitors on the potential hazards of pet waste being delivered to the water in an effort to maintain a clean and safe community.

Finding significant positive relationships between antecedent rainfall and FIB and MST marker concentrations is an important result of this study. Current methods for FIB quantification can take 24-96 hours for results to become available, which does not allow for a timely warning to be issued to the public on the state of the beach water quality. By the time the results are obtained, the beach management decision reflects the water quality conditions on the previous day, not the current conditions experienced by beachgoers. A potential solution to this problem using the results from this study could be implementing a real-time beach management decision to presumptively post the beaches based on rainfall amounts. Fecal *Bacteroides* marker concentrations, which were correlated with 1 hour antecedent rainfall, have previously been shown to be a predictor of illness occurrence after exposure to contaminated waters and sand (Wade et al., 2010; Heaney et al., 2012). After about 12 mm (or 0.45 inches) of rainfall in one hour there were consistently high concentrations of the fecal *Bacteroides* marker in samples, as only two samples were a non-detects above this amount of rainfall. Half an inch of rain in one hour could serve as a threshold for town managers to make a decision to post the beaches or issue a warning to the general public of suspected elevated bacteria concentrations. Such a policy would be more protective of public health since a decision could be made immediately, before most public exposure would occur, as opposed to waiting 24 hours for the results of culture-based methods.

Other significant positive relationships include gull fecal material and all time points of antecedent rainfall up to 24 hours, indicating immediate and continuous delivery of gull fecal contamination throughout even a prolonged storm event. This supports our findings of persistent and widespread gull contamination throughout both watersheds, independent of storm conditions. Although regrowth has been suggested as a possible explanation for exceedingly high

Enterococcus concentrations, the significant relationships between measured *Enterococcus* and 24 and 48 hour antecedent rainfall highlight the importance of storms in delivering this contamination to the receiving waters, regardless of the source. Surprisingly, a negative relationship with the fecal *Bacteroides* marker and 24 and 48 hour rainfall was discovered in these data. This relationship could indicate a flushing of built-up bacteria from the storm drains with prolonged rainfall in a 24 or 48 hour period. Once the bacteria that has been deposited into the storm drains during dry weather is flushed out into receiving waters, the input of fresh fecal contamination may no longer be high enough to maintain the elevated bacteria concentrations measured in the first few hours of a storm event.

The focused study on beach water quality surrounding the Snyder St. storm drain outfall in 2013 found water quality to be excellent during dry weather. Of all 48 samples collected over the six dry days sampled, no water sample was over the single sample threshold for either *E. coli* or *Enterococcus*. Although Banks Channel is lined with docks and boat slips, illicit dumping of boat holding tanks does not appear to be an issue that is negatively impacting the water quality in this area. These results demonstrate that water quality issues in Wrightsville Beach are driven by stormwater runoff conveying contaminants to the receiving waters via storm drain systems.

Even though there are many storm drain outfalls along the sound side beaches of Banks Channel, the results from the across beach storm event sampling indicate that the Snyder St. storm drain has a particularly large impact on downcoast water quality. There is an additional storm drain outfall approximately 80 m downcoast from the Snyder St. drain, occurring between the first and second downcoast sample sites (labeled in Figure 3b). However, even with the addition of discharge from this pipe, there was still a significant decrease in *E. coli* and Gull-2 marker concentrations from the Snyder St. drain discharge in successive downcoast samples. The

presence of human contamination in the storm drain discharge and surrounding area of Banks Channel is a cause for concern for swimmers in the vicinity of the drain outfall, although dilution does appear to reduce human markers below quantifiable levels by 150 m downcoast. There was no more human contamination introduced by the additional storm drain in the sample area, again pinpointing the source of the problems to the Snyder St. drain.

Although the Gull-2 marker concentration did show a significant decrease as the distance downcoast of the drain outfall increased, the two samples upcoast of the drain were also positive for the Gull-2 marker. Since there was no indication from FIB concentrations or human contamination that these sites were impacted by the Snyder St. discharge, it is assumed that gull fecal contamination is not exclusively delivered into Banks Channel from the storm drains. However, concentrations of gull contamination upcoast of the drain were over 100 times less than the concentration measured in the discharge.

In this August 2013 storm event, gull feces only explained 0.1% of the *Enterococcus* concentration measured in the drain discharge. This was only at one point in time, so measuring discharge throughout an entire storm event would allow for a more complete understanding of FIB and feces loading over the length of the storm event. There was confirmed human contamination in the discharge which would contribute to the *Enterococcus* concentrations as well; however, that might not be enough to explain the remaining 99.9% of the measured *Enterococcus*. More research into the *Enterococcus* contributions from human fecal contamination and any other possible sources would be needed to determine if these findings give support to the notion of *Enterococcus* growth within the storm drain environment.

What is most worrisome about these results is that the samples were taken at the very end of the storm event, which was a very typical summer storm. With sunshine returning and

temperatures rising, it is likely that beachgoers would return within an hour or two to enjoy the rest of the summer day. The beach-going public might not be aware of the dramatic decrease in water quality, and go swimming immediately. The sound side beaches along Banks Channel are also more protected from wave action, and might be more heavily used by young children and those involved in water sports and open water swimming. These groups could then be exposed to any pathogens which were just delivered by the storm drains into Banks Channel. This warrants further examination of pathogen transport in relation to time and storm patterns in order to determine how long and at what locations people are at the greatest risk of exposure to these pathogens.

CONCLUSIONS

- When storm drains were not actively discharging, there were no FIB exceedances ever measured in Banks Channel.
- Storm events of almost any size in Wrightsville Beach cause a rapid and extreme spike in FIB levels which are delivered into the receiving waters of Banks Channel.
- Fecal contamination from gulls is a widespread problem in Wrightsville Beach, while human contamination has an ephemeral presence and dog contamination is not as well understood.
- Implementing a presumptive beach advisory notification system based on rainfall may be the most effective way to protect public health by keeping beachgoers out of the water when the water quality has been degraded because of a storm event.
- During the across beach storm event study, water up to 200 meters downcoast of the storm drain still had FIB threshold exceedances and gull fecal contamination, confirming the extensive influence of storm drain discharge on the water quality in Banks Channel.

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