Enhancement of Beach and Water Quality Assessments for the Southern Florida Area Coastal Environment by Incorporation of Molecular Microbial Source Tracking

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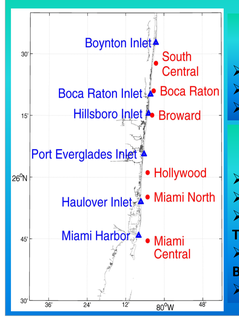
ABSTRACT

We report here a portion of several broader studies by the NOAA FACE program and the University of Miami Oceans & Human Health (OHH) Center to investigate land-based sources of microbial contaminants to recreational waters and the coastal environment. We tested the ability of certain Microbial Source Tracking (MST) methods to supplement culture techniques for improving measurement of human fecal pollution as a component of microbial contaminant discharge to South Florida coastal ecosystems, and to examine other potential contributors of Fecal Indicating Bacteria (FIB) to recreational beaches and coastal waters. Samples from recreational beaches, coastal inlets, and surface expression boils of treated wastewater ocean outfalls were enumerated for viable FIB by culture-based plate counts and IDEXX EnteroLert™ Chromogenic Substrate Assay, while a variety of genetic fecal markers targeting FIB specific to human, dog, and seabird hosts were enumerated by quantitative PCR assays. Elevated human-specific fecal markers were rarely detected in sand and water samples from the beaches tested, however all these beaches had background general enterococci populations in the sand, with greatest abundance typically just above the high tide line. Many beach samples had elevated gull fecal marker. Dog fecal marker was significantly lower at a dog beach with effective clean-up policies, as compared to an unenforced dog beach. Viable FIB were not often detected from treated wastewater outfalls, but significant periodic discharge was observed for protozoan pathogens and for genetic markers of total and human-source FIB. This study indicates that incorporation of MST can enhance water quality assessments to help management better address variations in microbial contaminant sources.

BACKGROUND

The coastal waters of South Florida provide critical fish and coral reef habitat. Concerns about the discharges have prompted legislation calling for expensive changes to how wastewater is disposed; however, the relative loadings of the various discharges into the area are poorly understood. The Florida Area Coastal Environment (FACE) Program was developed to provide an integrated analysis of physical, chemical, and biological oceanography of coastal areas near treated wastewater outflows, septic systems, inlets, and canals in order to ascertain the relative emitted nutrient and microbiological loads. Objectives of the FACE programs include characterizing the microbiological water quality near ocean outfalls and coastal inlets. Objectives of the UM NSF/NIEHS Oceans and Human Health Center, in collaboration with NOAA and the Florida Department of Health, includes microbiological characterization of sand and water quality at coastal marine recreational beaches.. The study area of the FACE Program and the OHH Beach studies covers 364 km of coastline in Miami-Dade, Broward, Palm Beach, and Brevard counties. The study area includes the following six treated wastewater plants (TWWPs) with coastal outfalls: Miami Central (MC), Miami North (MN), Hollywood (HWD), Broward (BWD), Boca Raton (BR), and South Central (SC), which together contribute ~1 million cubic meters (284 millions of gallons) per day to the region. In addition, this coastal area receives fresh water discharged through six inlets, from the Miami Harbor inlet in the south to the Boynton inlet in the north (Fig. 1).

The FACE Program



FACE Components

Outfall and Inlet Studies

- Ocean Chemistry
- Acoustics (currents)
- Microbiology

OHH Components

"Recreational Water Microbes"

- Indicators vs. Pathogens
- Human Exposure Risk
- Microbial Persistence, Fate, & Transport in Beach Sand & Water
- Background Fecal Indicating Bacteria (FIB) populations
- Indicator Host Sources

Figure 1: Locations of coastal inlets (triangles) and treated wastewater treatment plant outfalls (circles) in the FACE area of study. Further details of the outfalls and inlets in the study area are given below.

Ocean Outfalls

Table 1. Outfalls listed from north to south. The more southern outfalls have higher flow rates.

Name	Outflow MGD	Depth m	Length km	Diam m	Ports #
South Central	12.3	27.4	1.6	0.8	1
Boca Raton	10.7	27.4	1.6	0.9	1
Broward-N	36.5	32.6	2.2	1.4	1
Hollywood	39.5	28.3	3.1	1.5	1
Miami-Dade-N	80.6	32.9	3.6	2.3	12
Miami-Dade-C	104.6	30.5	5.7	2.3	5

Figure 2. Surface expression boils from wastewater ocean outfalls of Broward-N (left) & Miami-Dade-N (right).

Coastal Beaches

Ocean Inlets

NAME	Lat	Lon
Lake Worth Inlet	26.77	-80.03
Boynton Beach Inlet	26.55	-80.04
Boca Raton Inlet	26.34	-80.07
Hillsboro Inlet	26.26	-80.08
Port Everglades Inlet	26.09	-80.10
Bakers Haulover Inlet	25.90	-80.12
Miami Harbor Inlet	25.76	-80.13

Table 2. Inlets listed from north to south.

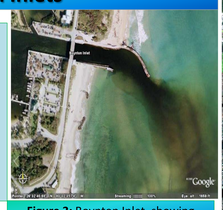


Figure 3: Boynton Inlet, showing discharge of humic-laden water into the coastal zone.

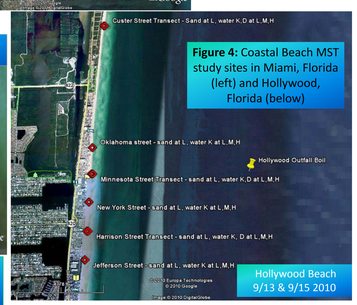


Figure 4: Coastal Beach MST study sites in Miami, Florida (left) and Hollywood, Florida (below)

L= low tide, M= mid tide, H=high tide, K= knee depth, W= waist depth

METHODS

Water and sand samples collected from outfall, inlet, and beach studies were analyzed for a variety of microbes. Viable enterococci FIB were enumerated using IDEXX Enterolert™ and mEI plate counts (EPA Method 1600). *Cryptosporidium* oocysts and *Giardia* cysts (protozoan pathogens) were determined by immunomagnetic separation and immunofluorescent microscopy (EPA Method 1623). Water samples for molecular analysis of viruses, bacteria, and source tracking markers (1 Liter) were processed by membrane filtration prior to nucleic acid extraction. Water samples for analysis of protozoans (>100 L) were processed using FiltaMax™ cartridges (IDEXX). RNA viruses (noroviruses and enteroviruses) were analyzed by real-time quantitative reverse-transcription PCR (qRT-PCR) or by endpoint PCR. Standard PCR or SybrGreen qPCR was used for detection of *Campylobacter jejuni*, *Salmonella* spp., *Staphylococcus aureus* (clfa), *Escherichia coli* O157:H7, and adenovirus. Real-time Taqman™ quantitative PCR (qPCR) was used to quantify enterococci, human-specific *Bacteroidales* HF8 gene cluster & Bac-UCD, human-specific *Methanobrevibacter smithii* , dog-specific *Bacteroides* (DogBac), and gull-specific *Catellicoccus marimammalium* (Gull2). Please see handout for more specific methods details.

PCR and qPCR

enterococci
Bacteroides spp. } **fecal indicators**

Human Bacteroidales BacHum-UCD marker
Human Methanobrevibacter smithii – nifH marker
Dog Bacteroidales – AOML DogBac marker
Gull Catellicoccus – AOML Gull2 marker
Human adenovirus – Hexon gene marker } **source tracking markers**

Salmonella spp.
Campylobacter jejuni
Staphylococcus aureus
norovirus
enterovirus
adenovirus
E. coli O157:H7 } **pathogens intestinal & dermal, bacteria & virus**

EPA-approved EnteroLert™ Chromogenic Substrate MPN Assay (IDEXX company) under a UV lamp. Fluorescent wells indicate specific presence of enterococci.

Bacteroides Bile Esculin Agar (BBE), a selective and differential medium for enumeration of anaerobic *Bacteroides* spp.

Real-Time Quantitative PCR Thermocycler for detection and enumeration of microbial source tracking markers (shown here is OneStepPlus™ instrument by ABI)

RESULTS

In general, low concentrations of viable enterococci were detected in the boils of coastal treated wastewater outfalls. For three sampling cruises conducted during 2006, 2007, & 2008, only one sample out of 18 (1/18) was over the EPA guidelines for recreational water quality (Fig. 5A). Viable *Bacteroides* spp., anaerobic bacteria associated with feces, were detected more often in these samples (Fig. 5B). *Giardia* cysts, *Cryptosporidium* oocysts, and the genetic signatures of human enterovirus, norovirus, and adenovirus were detected in certain samples (Fig. 5; Table 3; Table 4). Human fecal source tracking markers (Table 3; Fig. 5) and a variety of bacterial pathogens (Table 4, Fig 5) were detected via PCR.

In a study focused on the South Central outfall (Boynton), these FIB genetic signals were diluted out within ~1 km distance, for flow regimes both to the north and the south. No viable enterococci were detected near the outfall during this study, although viable enterococci and FIB markers were detected in the nearby intercoastal waterway. Interestingly, the Boynton Inlet appeared to be a source not only of enterococci (Fig 6) , but also a variety of microbial contaminants including pathogens and other FIB MST markers, to coastal seawater (Fig. 7).

In the Hollywood Beach Microbial Source Tracking Study (Tables 5-8) for 9/13/2010 and 9/15/2010, although the beach had frequent periodic postings for the prior two weeks, only 2 samples in were exceedance of single-grab limits by culture methods at the time of this study. Both Jefferson St. and Harrison St. also showed high levels of viable Bacteroidales on 9/15 by culture methods. The qPCR MST Assays indicated Minnesota St. Beach site with elevated human-source marker on 9/13, while Southern sites from Jefferson St. to New York St. showed very low levels of human marker on 9/15. No significant dog marker was observed even at Custer St. dog beach. Many sites showed elevated levels of gull-host-specific *Catellicoccus marimammalium* fecal marker. Large numbers of gulls and pigeons were observed during sampling at southern sites. Populations of Enterococci were observed in the sand for many samples.

The Crandon Beach MST Study also followed a period of repeated beach postings, but at the time of study no significant human marker was detected, however elevated levels of gull fecal marker were detected (Table 9).

Hobie Beach during the BEACHES epidemiology study showed frequent elevations of both dog and gull fecal maker and occasional elevations of human fecal marker. During a storm event in March 2008 (with 6+ hours of rainfall), extremely high levels of enterococci and of gull fecal marker were observed, along with elevation of dog marker, in both nearshore waters and in beach runoff water, however no significant human marker was seen in either the runoff or nearshore waters (Table 10). Substantial enterococci populations have frequently been observed in the sand of Hobie Beach, particularly just above the high tide line, for many years over the course of many separate studies.

In comparison of dog-host-specific *Bacteroides* fecal marker between two dog beaches, substantially more dog marker was observed at Hobie Beach in Miami than at Custer Street Beach in Hollywood (Tables 5-8, Fig 9, Table 10). Custer Street Beach actively enforces dog clean-up policies and provides clean-up supplies and disposal containers, while Hobie Beach does not.

outfall	location	*Salmonella* 2009	2008	2006	*C. jejuni* 2009	2008	2006	*S. aureus* 2009	2008	2006	adenovirus 2009	2008	2006	enterococci (GE/100 ml) 2009	2008	2006
Miami Central	surface	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	**5585**	**199**	(+)
	bottom	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(-)	**87**	**213**	(-)
Miami North	surface	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(+)	(-)	**1346**	**253**	(+)
	bottom	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	**9**	**18**	(-)
Hollywood	surface	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	**0.35**	**253**	(+)
	bottom	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	**BD**	**5**	(-)

Table 3: PCR detection of microbial contaminants and enumeration of enterococci for samples taken from surface boils and near-bottom depths during 2006, 2008, & 2009 (R/V Nancy Foster cruises). Higher enterococci concentrations were detected at the surface consistent with a buoyant plume; however, microbial contaminants were detected in some bottom samples (10/36). BD = below detection.

Location	Norovirus (GE/100ml)	*M. smithii* (GE/100ml)
Miami-C boil	235	$3.4x10^5$
Miami-N boil	2.3	$1.3x10^5$
Broward boil	6.3	$3.7x10^4$
Boca Raton boil	347	$2.7x10^4$
Hollywood boil	11	$3.0x10^5$
SC boil (Boynton)	nd	700
Deep water control	nd	nd

Table 4: Human viral pathogens of the norovirus group and the human source tracking marker *M. smithii* measured by qPCR from surface water collected from treated wastewater boils. GE = Genome Equivalents. (data from Feb. 2008 cruise aboard the R/V Nancy Foster. nd = not detected).

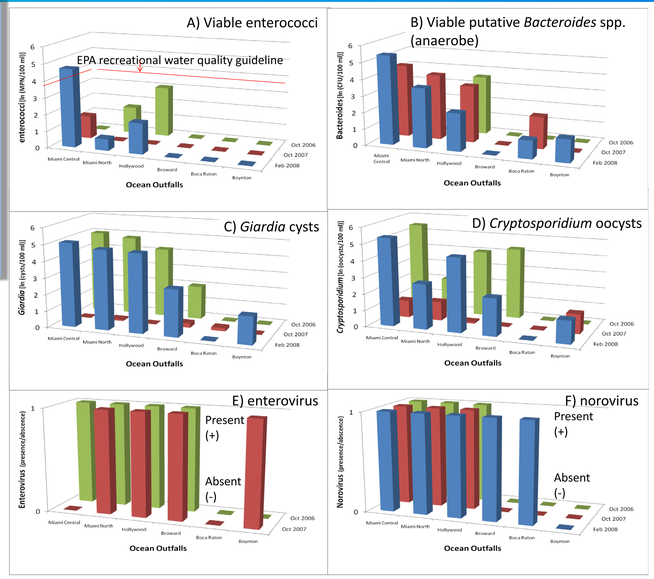


Figure 5: Selected microbiological results for seawater samples collected from treated wastewater boils. On the horizontal axis, the outfalls are listed from south (left) to north; which also tracks with the flow rates (see Table 1) . Note that "Boynton" is the South Central (SC) outfall. Some virus analyses were performed via quantitative RT-PCR (see Table 4).

Microbial Source Tracking For Hollywood Beach, Sept. 2010

Dashes indicate non-detects. Pink highlighted cells indicate substantially elevated values.
Red highlighted cells indicate values above regulatory criteria for single-grab samples.
cfu = "colony forming units", GEU = "Genome Equivalence Units", TSC = "Target Sequence Copies".

Sample Site Location	Site ID Label	Culturable putative Enterococci (mEI plate) cfu/100mL	Culturable putative Bacteroides (BBE plate) Cfu/100mL	General Enterococci (Entero1 qPCR) GE/100mL	General Bacteroidales (GenBac3 qPCR) GE/100mL	Human-Host Bacteroidales (BacHum-UCD qPCR) GE/100mL	Dog-Host Bacteroidales (DogBac qPCR) TSC/100mL	Gull-Host Catellicoccus (Gull2 qPCR) TSC/100mL
Harrison Street – waist (8:30am)	Ha-W1	5	72	1.0	197.5	1.0	-	47.0
Harrison Street – knee (8:35am)	Ha-K1	4	90	-	211.2	-	-	96.5
Minnesota Street – waist (9:06am)	Mi-W1	1	25	2.3	1332.5	44.0	-	60.5
Minnesota Street – knee (9:08am)	Mi-K1	4	115	5.5	1332.5	33.5	-	215.5
Custer Street – waist (9:20am)	Cs-W1	1	53	1.4	435.5	-	3.0	632.0
Custer Street – knee (9:22am)	Cs-K1	1	42	1.4	313.4	-	-	689.0

Table 5: qPCR MST for near-shore water at Hollywood Beach 9-13-2010

Sample Site Location	Site ID Label	Culturable putative Enterococci (mEI plate) cfu/gram of sand	General Enterococci (Entero1 qPCR) GE/gram of sand	General Bacteroidales (GenBac3 qPCR) GE/gram of sand	Human-Host Bacteroidales (BacHum-UCD qPCR) GE/gram of sand	Dog-Host Bacteroidales (DogBac qPCR) TSC/gram of sand	Gull-Host Catellicoccus (Gull2 qPCR) TSC/gram of sand
Harrison Street – sand (8:40am)	Ha-S	4	1.85	170.18	3.24	-	35.6
Minnesota Street – sand (9:10am)	Mi-S	16	2.97	70.30	-	-	24.32
Custer Street – sand (9:25am)	Cs-S	8	8.75	1454.32	-	35.78	41.80

Table 6: qPCR MST for sand just above high-tide line at Hollywood Beach 9-13-2010

Sample Site Location	Sample ID Label	Culturable putative Enterococci (mEI plate) cfu/100mL	Culturable putative Bacteroides (BBE plate) Cfu/100mL	General Enterococci (Entero1 qPCR) GE/100mL	General Bacteroidales (GenBac3 qPCR) GE/100mL	Human-Host Bacteroidales (BacHum-UCD qPCR) GE/100mL	Dog-Host Bacteroidales (DogBac qPCR) TSC/100mL	Gull-Host Catellicoccus (Gull2 qPCR) TSC/100mL
Jefferson Street – Low Tide – Knee (8:40am)	JH-L-K	9	TNTC	1.63	1101.90	-	-	2.60
Harrison Street – Low Tide – Knee (9:00am)	Ha-L-K	1	TNTC	4.28	2577.05	-	-	128.74
Harrison Street – Low Tide – Deep (9:10am)	Ha-L-D	4	18	-	60.34	-	-	-
New York – Low Tide – Knee (9:23am)	NY-L-K	2	41	2.40	61.00	-	-	78.1
Minnesota Street – Low Tide –Knee (9:40am)	Mi-L-K	4	63	4.87	125.42	-	-	8.77
Minnesota Street – Low Tide – Deep (9:44am)	Mi-L-D	4	57	2.25	59.44	-	-	6.55
Oklahoma Street – Low Tide – Knee (10:02am)	Ok-L-K	4	71	1.11	108.58	-	-	7.42
Custer Street – Low Tide – Knee (10:20am)	Cs-L-K	2	112	4.31	119.53	-	-	6.75
Custer Street – Low Tide – Deep (10:26am)	Cs-L-D	3	21	2	63.43	-	-	-
Jefferson Street – Mid Tide – Knee (12:00pm)	JH-M-K	**104**	110	35.76	321.07	4.6	-	27.12
Harrison Street – Mid Tide – Knee (12:20pm)	Ha-M-K	17	TNTC	2.85	456.03	2.8	-	28.05
Harrison Street – Mid Tide – Deep (12:25pm)	Ha-M-D	1	87	3.56	110.15	3.6	-	23.73
New York Street – Mid Tide – Knee (12:25pm)	NY-M-K	23	5	-	27.55	2.0	-	23.89
Minnesota Street – Mid Tide – Knee (13:01pm)	Mi-M-K	14	18	3.47	104.26	-	-	**330.37**
Minnesota Street – Mid Tide – Deep (13:05pm)	Mi-M-D	11	32	8.92	157.82	-	-	45.38
Oklahoma Street – Mid Tide – Knee (13:26pm)	Ok-M-K	14	13	4.67	44.76	-	-	-
Custer Street – Mid Tide – Knee (13:41pm)	Cs-M-K	8	5	6.82	90.20	-	-	6.58
Custer Street – Mid Tide – Deep (13:39pm)	Cs-M-D	4	22	3.26	39.38	-	-	16.65
Jefferson Street – High Tide – Knee (17:15pm)	JH-H-K	14	37	4.38	100.20	-	-	80.10
Harrison Street – High Tide – Knee (17:25pm)	Ha-H-K	9	60	10.21	120.80	-	-	86.79
New York Street – High Tide – Knee (17:40pm)	NY-H-K	**138**	22	70.52	55.99	-	-	**385.25**
Minnesota Street – High Tide – Knee (17:55pm)	Mi-H-K	6	81	8.89	96.00	-	-	39.18
Oklahoma Street – High Tide – Knee (18:18pm)	Ok-H-K	5	25	-	30.26	-	-	11.00
Custer Street – High Tide – Knee (18:33pm)	Cs-H-K	5	28	2.86	47.36	-	-	7.52

Table 7: qPCR MST for near-shore water at Hollywood Beach 9-15-2010

Sample Site Location	Sample ID Label	Culturable putative Enterococci (mEI plate) cfu/g sand	General Enterococci (Entero1 qPCR) GE/g sand	General Bacteroidales (GenBac3 qPCR) GE/g sand	Human-Host Bacteroidales (BacHum-UCD qPCR) GE/g sand	Dog-Host Bacteroidales (DogBac qPCR) TSC/g sand	Gull-Host Catellicoccus (Gull2 qPCR) TSC/g sand
Jefferson Street – sand (8:40am)	JH-L-S	12	4.28	9.14	1.76	-	24.32
Harrison Street – sand (9:10am)	Ha-L-S	4	5.50	170.18	-	-	18.86
New York Street – sand (9:23am)	NY-L-S	4	6.26	305.58	-	-	-
Minnesota Street – sand (9:50am)	Mi-L-S	14	7.00	305.88	-	-	21.28
Oklahoma Street – sand (10:00am)	Ok-L-S	-	-	14.18	-	-	-
Custer Street – sand (10:20am)	Cs-L-S	22	21.68	42.92	-	-	7.66

Table 8: qPCR MST for sand just above high-tide line at Hollywood Beach 9-15-2010

For more information, please contact Dr. Chris Sinigalliano at NOAA-AMOL, 4301 Rickenbacker Causeway, Miami, FL 33149. Office Phone: 305-361-4384, Cellular Phone: 954-801-7789, email: christopher.sinigalliano@noaa.gov

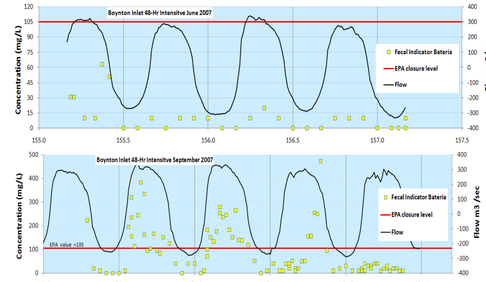


Figure 6: Concentration of enterococci ("fecal indicator bacteria") at the mouth of the Boynton inlet over two complete tidal cycles for June 2007 (upper panel) and September 2007 (lower panel). The EPA water quality guideline for recreational waters is shown by the red horizontal line for reference.

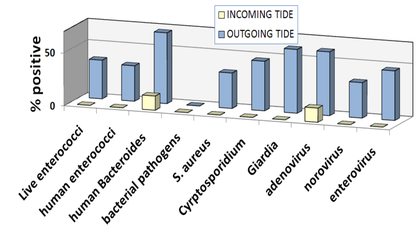


Figure 7: Detection of microbial contaminants for incoming vs. outgoing tides during a 48-hr intensive study at the Boynton inlet. Data show the percentage of samples showing positive detection for microbial contaminants out of 15 discrete time points. "Bacterial pathogens" is a composite for *C. jejuni*, *Salmonella* spp., and *E. coli* O157:H7.

Microbial Source Tracking For Crandon Park Beach, Miami, Sept. 2010

Sample Site Location	Sample Date and Time	Viable Enterococci by plate count Cfu/100mL	General Enterococci (Entero1 qPCR) GE/100mL	General Bacteroidales (GenBac3 qPCR) GE/100mL	Human-Host Methanobrevibacter smithii (Ms qPCR) GE/100mL	Dog-Host Bacteroidales (DogBac qPCR) TSC/100mL	Gull-Host Catellicoccus (Gull2 qPCR) TSC/100mL
Crandon #1	9/3/2010 10:15am	10	6.6	555.9	-	-	74
Crandon #1	9/3/2010 12:15pm	68	9.6	102.6	-	-	896
Crandon #12	9/7/2010 10:00am	42	90.2	7418.2	-	-	60.5
Crandon #12	9/9/2010 10:00am	14	52.2	2848.2	-	-	215.5

Table 9: qPCR MST for near-shore water at Crandon Beach

Microbial Source Tracking during BEACHES Epidemiology Study, Hobie Beach, Miami

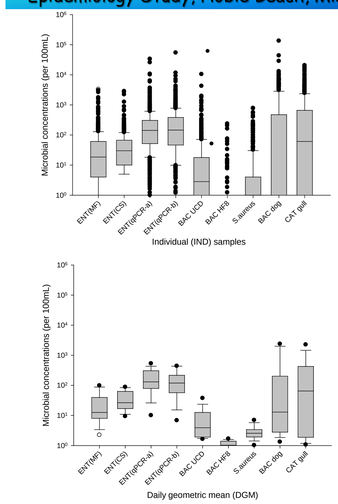


Figure 8: qPCR MST for near-shore water at Hobie Cat Beach during the "Beach Environmental Assessment and Characterization Human Exposure Study" (BEACHES) Epidemiology Study in 2008.
Microbial concentrations in a) individual samples, and b) Daily Geometric Mean levels . The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (or error bars) above and below the box indicate the 90th and 10th percentiles. The solid circles outside the whiskers mark results outside the corresponding range. (n=668 over 15 days of monitoring from December 2007 to June 2008)

Sample Site Location	Sample Date and Time	Viable Enterococci by plate count Cfu/100mL	General Enterococci (Entero1 qPCR) GE/100mL	General Bacteroidales (GenBac3 qPCR) GE/100mL	Human-Host Bacteroidales (BacHum-UCD qPCR) GE/100mL	Human-Host Methanobrevibacter smithii (Ms qPCR) GE/100mL	Dog-Host Bacteroidales (DogBac qPCR) TSC/100mL	Gull-Host Catellicoccus (Gull2 qPCR) TSC/100mL
Hobie # 1	3/8/2008	**2,080**	76,700	72,400	2	-	75	4,000
Hobie # 2	3/8/2008	**833**	1,200	36,900	-	-	262	3,010
Hobie # 1 - runoff	3/8/2008	**25,200**	347,000	680,000	-	-	50	126,000
Hobie # 2 - runoff	3/8/2009	**115,000**	214,000	240,800	-	-	-	213,000

Table 10: qPCR MST for near-shore water and beach runoff water at Hobie Cat Beach during a storm event on March 8, 2008 (6 hr rainfall =12 mm of rain with active beach run-off to surf zone, forming natural beach drainage ditches.)

DISCUSSION & CONCLUSIONS

These data suggest that the treated wastewater outfalls studied in this area were not a significant source of viable enterococci to coastal waters. Seawater samples taken from the boils yielded protozoan cysts & oocysts, and the genetic signatures of enterococci, human viruses, human bacterial pathogens (e.g., *S. aureus*), and human source tracking markers (e.g., *M. smithii*, human Bacteroidales). There was a tendency to detect more microbial contaminants from the more southern outfalls, consistent with the higher effluent flows.

Interestingly, these data indicated that inlets can be an important source of microbial contaminants to this coastal area. This finding highlights the need to assess the coastal zone in a cohesive manner, especially if the data will be used to determine the impacts of land-based pollutants, anthropogenic water discharges, for guidance in the operation and development of water and sewer infrastructure, and for the formulation of science-based regulation. Overall it appears that the inlet studied here presented a substantial but variable source of contaminants to the adjacent coast.

It is presumed that the genetic signatures detected from the enterococci and other microbial contaminants from the wastewater outfalls were from successfully disinfected cells rather than from viable but non-culturable cells (VBNC), as VBNC cells (and perhaps intact dead cells) could serve as source for gene transfer. Even if microbial contaminants in effluent are successfully disinfected, the export of this genetic information (including potential antibiobtic resistance markers, pathogenicity genes, and other genes of concern) to the native microbial populations of the coastal zone may still be of some public and ecosystem health concern given the prevalence of horizontal gene transfer in the environment. However, to ensure the protection of human, animal, and ecosystem health, these hypotheses warrant further investigation.

Microbial Source Tracking (MST) Studies of Crandon Beach, Hobie Beach, and Hollywood Beach showed relatively little evidence of human-source fecal contamination even after a period of repeated frequent exceedances of traditional fecal indicator exposure guidelines and beach notifications. However, these beaches demonstrated frequent elevations of gull fecal marker, and a dog beach with little enforcement of pet clean up rules showed more frequent contamination by dog fecal markers. While these types of animal fecal contamination sources may be sporadic or seasonal they may confound assessment of potential health risk when such assessments are only based on culture data of traditional indicators. These animal sources of fecal indicators and pathogens may also represent "low hanging fruit" for remediation by beach managers where relatively inexpensive education, outreach, policy enforcement, and good beach hygene may potentially moderate the impact of such fecal contaminants. MST combined with traditional monitoring during beach criteria exceedance events may also help clarify the assessment of potential relative human health risk and potential contributing factors to such exceedance events to better protect the environment, public health, and economy.

Acknowledgements

We thank the AOML FACE team and all ship personnel. Acknowledgement is given to BCS Labs, Inc. for analysis of *Cryptosporidium* oocysts and *Giardia* cysts. We thank the NOAA Hollings Marine Laboratory, Charleston SC, and Dr. Jill Stewart at the University of North Carolina for analysis of *M. smithii* and RNA viruses for selected samples. This research was carried out in part under the auspices of the Cooperative Institute for Marine and Atmospheric Studies (CIMAS), a joint institute of the University of Miami and the National Oceanic and Atmospheric Administration, cooperative agreement #NA17RJ1226. This research was also supported in part by the NSF/NIEHS Oceans and Human Health Center at the University of Miami (NSF award OCE0432368/0911373) and NIEHS award P50 ES12736).