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Bacterial source tracking guides management of boat head waste in a coastal resort area

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ABSTRACT

Fecal contamination of water bodies causes a public health problem and economic loss. To control such contamination management actions need to be guided by sound science. From 2007–2009 a study was undertaken to determine the sources of fecal bacteria contamination to the marine waters adjoining the Town of Wrightsville Beach, North Carolina, USA. The research effort included sampling for fecal coliform and *Enterococcus* bacteria, sampling for optical brighteners, dye studies, and use of molecular bacterial source tracking techniques including polymerase chain reaction (PCR) and terminal restriction fragment polymorphism (T-RFLP) fingerprinting of the *Bacteroides–Prevotella* group. Of the 96 samples collected from nine locations during the study, the water contact standard for *Enterococcus* was exceeded on 13 occasions. The T-RFLP fingerprint analyses demonstrated that the most widespread source of fecal contamination was human, occurring in 38% of the samples, with secondary ruminant and avian sources also detected. Optical brightener concentrations were low, reflecting a lack of sewage line leakage or spills. A lack of sewer leaks and lack of septic systems in the town pointed toward discharge from boat heads into the marine waters as the major cause of fecal contamination; this was supported by dye studies. Based on these data, the Town initiated action to have the U.S. Environmental Protection Agency declare the coastal waters (out to 3 nautical miles), the nearby Atlantic Intracoastal Waterway and its tributaries a no-discharge zone (NDZ) to alleviate the human fecal pollution. The Town garnered supporting resolutions from other local communities who jointly petitioned the North Carolina Department of Environmental and Natural Resources. This State regulatory agency supported the local government resolutions and sent an application for an NDZ to the EPA in April 2009. The EPA concurred, and in February 2010 the coastal waters of New Hanover County, NC, became the first marine area on the U.S. eastern seaboard between Delaware and the Florida Keys to be declared a no-discharge zone.

1. Introduction

Environmental degradation of a recreational area can lead not only to ecological impacts, but can cause economic loss as well. This is especially true when public health is involved; for example, contaminated beach water can cause a significantly increased incidence of illness among beachgoers including vomiting, diarrhea, fever, etc. (Alexander et al., 1992). Gastrointestinal illness and eye, ear and respiratory infections contracted from beach waters can also lead to a significant economic burden (Dwight et al., 2005). Eating shellfish contaminated with fecal microbes causes various illnesses to consumers (Wittman and Flick, 1995), and closures of shellfishing beds to harvest from fecal contamination leads to significant economic losses (Mallin, 2009). In resort areas, people recreate on and within local water bodies in multiple ways, such as swimming, surfing, diving, water skiing, boating, kayaking, fishing, wildlife tours, etc. One such area is Wrightsville Beach, North Carolina, USA, which has been considered a resort area since 1899, and has had an operational yacht club since 1856. In recent years waters within town limits have had an increasing number of water contact warnings posted by the North Carolina Division of Environmental Health's Shellfish Sanitation and Recreational Water Quality Office, based on excessive fecal bacteria indicator (*Enterococcus*) counts. Because these postings are distributed not only locally but also to news organizations within inland cites (where many visitors of

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Wrightsville Beach reside) this has led to concern from town administrators and business owners about potential loss of visitor revenues. Because of these concerns the Town of Wrightsville Beach partnered with the University of North Carolina Wilmington in an effort to determine the source(s) of the fecal contamination in order to properly direct management efforts designed to reduce the microbial contamination and subsequent postings.

The Town of Wrightsville Beach is located on a barrier island complex in New Hanover County, adjacent to the City of Wilmington (Fig. 1). It is fronted by the Atlantic Ocean with a series of sounds, including Banks Channel, Motts Channel and the Atlantic Intracoastal Waterway (ICW) lying adjacent to and within the town (Fig. 1). Land use within the town is primarily residential, including second homes, seasonal and weekend rentals, hotels and commercial establishments. Land within the town is fully developed, although large salt marsh areas occur both within town limits and nearby on the mainland and spoil islands in the ICW. While some shellfishing areas within town limits are open to harvest, others are closed due to excessive fecal coliform bacteria counts. The town has a year-round population of 2600 and a summer population of approximately 15,000, with elevated visitor populations on holidays as well. The town hosts five marinas, with numerous boats at semi-permanent mooring. Harbors and marinas in general have been shown to be focal points of fecal contamination, with concentrations of fecal contamination indicator microbes increasing during the higher occupancy of holiday weekends (Guillon-Cotard et al., 1998; Sobsey et al., 2003); increases in boat usage in general in relatively confined estuarine areas have also increased the microbiological contamination of the water (Faust, 1982). Wrightsville Beach is a popular stopping point in autumn for boaters passing south on the ICW to winter in warmer climes (often referred to as snowbirds) and those returning boaters in the spring. Sewage is piped onshore and treated at the Lower Cape Fear Public Utility Authority's Southside Wastewater Treatment Plant, located along the shores of the Cape Fear River within the City of Wilmington. There are no active septic systems on the island, causeway, and mainland areas within the town limits.

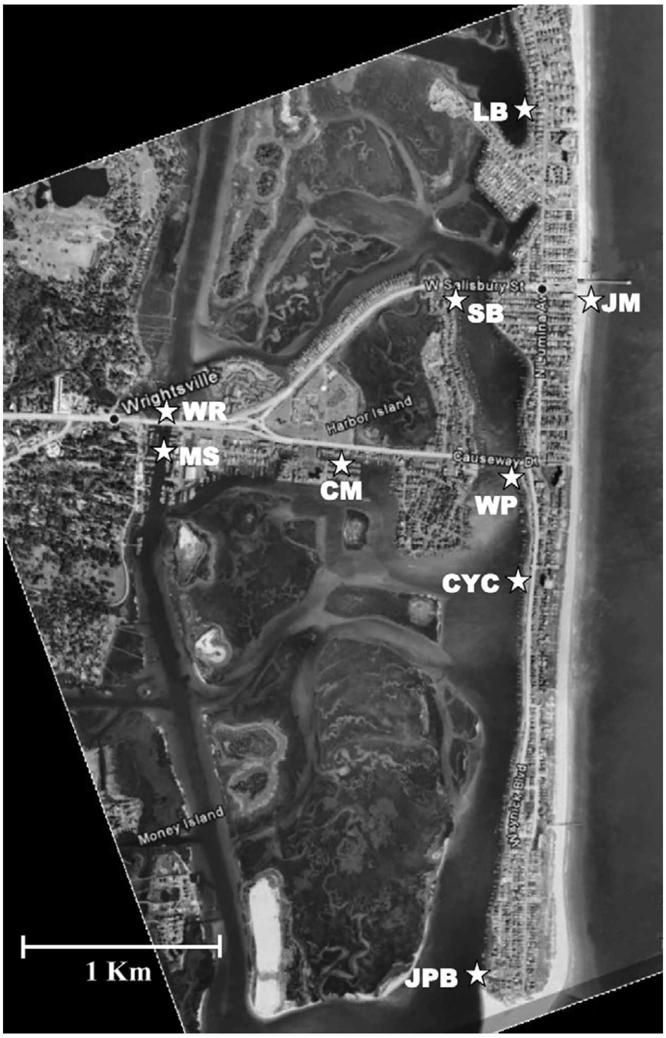

Fig. 1. Map of Wrightsville Beach and surrounding waterways showing sampling stations.

A number of sources of fecal microbial contamination to other regional waterways have been identified in previous research. These include urban and suburban stormwater runoff (Mallin et al., 2000, 2009), poorly-functioning or improperly placed septic systems (Cahoon et al., 2006), large-scale sewage spills (Mallin et al., 2007), and smaller sewage spills and leaks (Tavares et al., 2008). Human fecal contamination has been found at several mainland tidal creek locations using molecular methods detecting host specific *Bacteroides–Prevotella* groups (Spivey, 2008). Thus, one objective of the research portion of this study was to assess the microbiological water quality at a variety of locations in town waters, looking for any particular loci of elevated pollutant activity ("hot spots") and assess fecal contamination in light of various environmental parameters. The second objective was to use DNA-based techniques to determine the source or sources of the fecal contamination causing the regulatory postings. The objectives were pursued by sampling the waters for two commonly-used fecal contamination indicators, fecal coliform bacteria and *Enterococcus* bacteria; sampling for optical brighteners; and sampling for accompanying field parameters including water temperature, salinity and turbidity. Molecular microbial source tracking was conducted using polymerase chain reaction (PCR) and T-RFLP fingerprinting of *Bacteroides–Prevotella* group. The overall goal of these two objectives was to provide management guidance for town administrators and regulatory agencies to reduce fecal contamination of the waters of the Town of Wrightsville Beach.

2. Methods

2.1. Sampling design

Nine sites were sampled throughout the course of the study (Table 1). Some stations were chosen based on previous standard violations as determined by State agencies, while other stations were chosen based on human boating activity or dockages. Station WB-CM was located in a commercial marina off Motts Channel, Station WB-WP was at a public-access boat dock in a small town park on Banks Channel near the commercial district where boaters can tie up temporarily, Station WB-CYC was from the dock of the Carolina Yacht Club on Banks Channel, Station WB-JPB was at the U.S. Coast Guard dock on Banks Channel, Station WB-WR was at the boat launch ramp operated by the North Carolina Wildlife Resources Commission on the ICW, Station WB-JM was in the surf on the ocean beach near a popular pier, Station WB-SB was from a private multi-boat dock along the northern reaches of Banks Channel, Station WB-LB was a small private dock on Lollipop Bay, a relatively enclosed bay near the northern portion of Wrightsville Beach, and Station WB-MS was on a dock located at the Wrightsville Yacht Club on the ICW. The beach water station WB-JM was only sampled for seven months; owing to its consistently low fecal indicator counts sampling for the remaining five trips was conducted at WB-MS.

Table 1
Description and GPS coordinates of sampling sites at Wrightsville Beach, North Carolina.

WB-CM: Causeway Marina. This was sampled from the end of the docks at Seapath Marina on the causeway.
GPS: N 34 12.716 W 77 48.279
WB-WP: Wynn Plaza, sampled from boat dock.
GPS: N 34 12.502 W 77 47.819
WB-CYC: Carolina Yacht Club, sampled from the end of dock.
GPS: N 34 12.105 W 77 48.093
WB-JPB: Sampled from Coast Guard dock at the end of Jack Parker Boulevard.
GPS: N 34 11.357 W 77 48.765
WB-JM: Johnny Mercer's Pier, sampled from the surf.
GPS: N 34 12.849 W 77 47.302
WB-LB: Lollipop Bay, sampled from a dock at a private residence on N Lumina Ave.
GPS: N 34 13.486 W 77 47.128
WB-SB: Salisbury Bridge, sampled from a private dock on Salisbury Street near Salisbury Bridge.
GPS: N 34 13.000 W 77 47.702
WB-WR: Wildlife Ramp, samples were taken from the end of a dock.
GPS: N 34 13.114 W 77 48.687
WB-MS: Marina Street, samples taken from the dock of the Wrightsville Yacht Club.
GPS N 34 13.017 W 77 48.770.

While on-site, samples were taken for water temperature, salinity, turbidity, and dissolved oxygen using a YSI 6920 multi-parameter water quality probe (sonde) linked to a YSI 650 MDS display unit. The collection schedule for the various biological and chemical parameters sampled is shown on Table 2.

2.2. Fecal coliform bacteria and Enterococcus analyses

Samples were collected for fecal coliform bacteria and *Enterococcus* bacteria at the above locations. In the laboratory fecal bacteria counts were performed using membrane filtration; Method 9222 D for fecal coliform bacteria and Method 9230 C for *Enterococcus* (as in Standard Methods, APHA, 1998), with fecal bacteria results reported as colony-forming units (CFU)/100 mL of water.

2.3. Optical brightener measurement

Optical brighteners are compounds that are added to laundry detergents, adsorb to clothing and form a light reflective layer creating the appearance of whiter whites and brighter colors. These compounds are excited by light in the near UV range (360–365 nm) and emit light in the blue range (400–440 nm). After light absorption, fluorescence is given off during a second excited state and can be measured by a fluorometer. Since household plumbing systems combine wastewater from toilets and washing machines, the presence of optical brighteners and fecal coliform bacteria in a waterway may indicate an input of human origin (Hartel et al., 2007a). Optical brightener samples were collected by filling Nalgene 125 mL opaque collection bottles 10 cm below the surface facing into the stream (Tavares et al., 2008). Collection bottles were acid washed and triple rinsed before sampling. Samples were refrigerated in the dark at 8 °C and analyzed within 8 days.

Samples were analyzed using a laboratory fluorometer (Turner Model 10-AU-000). A kit was added to the fluorometer that included a lamp (10–049) emitting near UV light at 310–390 nm, a filter (10-069R) for the 300–400 nm light range, and finally a 436 nm filter was added to greater decrease background fluorescence (Hartel et al., 2007a). A standard curve was created using serial dilutions from 100 mg of Tide detergent (Procter and Gamble, Cincinnati, Ohio) in 1 L of deionized water. When the fluorometer was adjusted to an 80% sensitivity scale, the fluorometric value of 100 was equal to 100 mg of Tide in 1 L of deionized water. The standard curve demonstrated a linear relationship between the fluorometric response and detergent-sourced optical brighteners up to a reading of 100. Following field collections, each sample was read on the fluorometer in triplicate at room temperature after 10 s to minimize degradation of optical brighteners by UV light (Hartel et al., 2007b; Tavares et al., 2008). For optical brightener determination, the samples were allowed to warm to room temperature for approximately 30 min. Each sample was shaken and poured into a cuvette (about 1/3 full). The cuvette was then placed in the fluorometer modified as above for optical brightener measurement.

Table 2
Collection matrix for the various sampled parameters collected between August 2007 and January 2009. WQ = water temperature, salinity, turbidity; FC = fecal coliform bacteria; ENT = *Enterococcus*; OB = optical brighteners.

Month	WQ	FC	ENT	OB
August 2007	X	X		
November 2007	X	X	X	
December 2007	X	X	X	
February 2008	X	X	X	X
March 2008	X	X	X	X
April 2008	X	X	X	X
June 2008	X	X	X	X
August 2008	X	X	X	X
October 2008	X	X	X	X
November 2008	X	X	X	X
December 2008	X	X	X	X
January 2009	X	X	X	X

2.4. Bacterial source tracking techniques

Water samples from each station were collected in autoclaved 500 mL Pyrex glass bottles. The samples were transported on ice and allowed to sit no longer than 6 h before filtration. Upon return to the lab, 500 mL of water were filtered on Whatman GF/F 47 mm filters (pore size of 0.7 μm) using autoclaved glassware for DNA extraction. DNA was extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories), with some modification for a filter extraction. A portion of the filter was ground using a Power Bead Tube and tissue grinder, and then the extraction was completed per manufacturer's instructions. PCR was first conducted with the 16s universal primer pair of 27F and 1522R (Suzuki and Giovanni, 1996) and then with fluorescently labeled [6′FAM] Bac32F and Bac708R primers to amplify 16S rRNA genes of *Bacteroides–Prevotella* groups as described by Bernhard and Field (2000). The amplified products were gene cleaned using a GENECLEAN® Turbo Kit (Q-BIO gene) following manufacture's instruction. The purified PCR products were quantified with a Qubit Fluorometer (Invitrogen). Approximately 20 ng of the amplicons were digested with a restrictional enzyme (AciI). The digested samples were analyzed with the Genetic Analyzer 3700 (Applied and Biosystems) for T-RFLP analysis. Identification of T-RFs with different fragment sizes was conducted using Microbial Community Analysis 3 (MiCA) T-RFLP Analysis Phylogenetic Assessment Tool (PAT) (http://mica.ibest.uidaho.edu/pat.php). The source of bacterial contamination was predicted based on the specific hosts of *Bacteroides–Prevotella* group. A seagull-specific PCR primer pair called gull-2 (Lu et al., 2008) was added toward the end of the study and run with all DNA samples.

2.5. Statistical analyses

The data sets were analyzed for basic summary statistics in Excel. Correlation analysis (also using Excel) was performed between fecal coliform and *Enterococcus* counts to test for their utility as comparable indicators in these brackish waters. The New Hanover County area has previously been determined to have a non-point source (i.e. stormwater runoff) pollution problem in its local waterways (Mallin et al., 2000; Spivey, 2008) and in some cases pollutant loads including fecal bacteria have been strongly correlated with rainfall (Mallin et al., 2009). Therefore, fecal coliform bacteria and *Enterococcus* concentrations were correlated against various physical and hydrological parameters including temperature, salinity, and rainfall (for the day of sample + the day before). Rain that fell on the day of sampling but after the crews had sampled was not included in this analysis. Additionally, one data point (an unusually high fecal bacteria count from Station WB-JPB) was excluded based on the high optical brightener readings and our assessment that this was a sewage-related spill (see within). Rainfall data were obtained from the following website: http://www.wunderground.com/history/d. A probability (p) < 0.05 was used for significance for all analyses.

2.6. Dye study

The Town of Wrightsville Beach, in partnership with the University of North Carolina Wilmington, the North Carolina Department of Environment and Natural Resources, and the New Hanover County Sheriffs Department conducted a dye study in March 2009 in town waters to obtain a better understanding of the currents in the study area. On the outgoing tide, researchers in a boat spread approximately four kg of powdered fluorescent green dye (disodium fluorescein) across the middle portion of Banks Channel at a location just downstream of the causeway bridge near Station WB-WP (Fig. 1). At the same time researchers in a second boat added about 2.75 kg of red rhodamine WT dye as powder at a location in Motts Channel outside of the entrance to the commercial marina where Station WB-CM was located (Fig. 1). Observers were stationed on the roof of a seven-story hotel located near Station WB-CYC, which provided an excellent view of both channels (Fig. 1), and photographed the dye passage. During the operation personnel from the New Hanover County Sheriff's Department flew overhead and filmed the dye movement.

3. Results and discussion

3.1. Fecal bacteria concentrations and distribution

Field results of fecal coliform and *Enterococcus* sampling are presented in Table 3. The North Carolina single-sample standard for recreational contact waters (freshwater) is 400 CFU/100 mL. Fecal coliform samples were all below this standard in the twelve months we sampled except for a large April 2008 peak of 1000 CFU/100 mL at WB-JPB, off the Coast Guard dock (Table 3). The US EPA guideline, and North Carolina single-sample standard for *Enterococcus* for human contact waters is 104 CFU/100 mL. This standard was exceeded on four occasions at WB-WP, three times at WB-LB and WB-JPB, twice at WB-CYC and once at WB-SB. The highest counts occurred in November 2008 during a rain event where we obtained *Enterococcus* counts of 1000 CFU/100 mL at WB-CYC and 1025 CFU/100 mL at WB-LB. Additionally, in April 2008 we had a count of 925 CFU/100 mL at WB-JPB (Table 3). The four stations with the highest geometric mean *Enterococcus* counts during the course of our study were WB-LB (65 CFU/100 mL), WB-JPB (50 CFU/100 mL), WB-WP (43 CFU/100 mL) and WB-CYC (42 CFU/100 mL). Thus, our sampling indicated fecal contamination at a variety of locations throughout the Wrightsville Beach area, although WB-WP, WB-LB, WB-JPB and WB-CYC appeared to be impacted most frequently and by the greatest numbers of *Enterococcus*.

There was a highly significant statistical correlation between fecal coliform bacteria and *Enterococcus* counts for the period August 2007 through January 2009 ($r = 0.502$, $p < 0.001$); thus, both indicators serve as a proxy for fecal bacteria in these waters. However, the correlation coefficient r is not overly strong, geometric mean *Enterococcus* counts were considerably higher than the fecal coliform counts, and there were many more *Enterococcus* excursions above water quality standards. E*nterococcus* is longer-lasting in the marine environment and as others have determined (Cabelli et al., 1983) is a more appropriate marine indicator due to fecal coliform salinity sensitivity.

3.2. Optical brightener concentrations

Optical brightener samples were generally low at all sites and in all months, ranging from 1.7 to 4.7, with an overall average of 3.1. These data (based on previous work, Hartel et al., 2007a,b; Tavares et al., 2008) indicate the lack of sewage pipe leaks in this area (there are no operating septic systems in Wrightsville Beach, according to town staff). However, the April 2008 sample at WB-JPB was 8.2, which leads us to believe a sewage spill of some sort was responsible for the high fecal coliform bacteria and *Enterococcus* counts at that site in April (Table 3).

3.3. Sources of fecal contamination

3.3.1. Human fecal contamination

The T-RFLP analysis showed that all months except February and October 2008 had T-RFs of human specific *Bacteroides–Prevotella* group as an indication of human fecal contamination (Table 3). Of the months analyzed, human fecal contamination was detected on seven occasions at WB-CYC, six occasions at WB-CM and WB-WR, and five occasions at WB-LB. Human fecal contamination was found on four occasions at WB-SB and WB-WP and three times at WB-JPB

Table 3
Number of samples collected (n), fecal coliform (FC) and *Enterococcus* (ENT) bacterial counts (as geometric mean and range), number of excursions over water quality standards,[a] and number of recognizable source determinations in Wrightsville Beach area waters, August 2007–January 2009.

Station	n	FC count	Excursions	ENT count	Excursions	Human	Ruminant	Avian	Rat	Gull
WB-CM	12	5 (2–53)	0	22 (8–56)	0	6	2	2		
WB-WP	12	11 (1–47)	0	43 (8–410)	4	4	1			
WB-CYC	12	4 (1–27)	0	42 (2–1000)	2	7	2	1	1	
WB-JPB	12	8 (1–1000)	1	50 (4–925)	3	3				
WB-JM	7	5 (1–107)	0	7 (3–13)	0	1				
WB-LB	12	3 (1–64)	0	65 (17–1025)	3	5		2		1
WB-SB	12	7 (2–76)	0	34 (14–128)	1	4	1			
WB-WR	12	8 (1–360	0	34 (16–96)	0	5	1	1		2
WB-MS	4	16 (9–27)	0	20 (10–35)	0	1	1			

[a] Excursions are when fecal coliform bacterial counts exceed the instantaneous standard of 400 CFU/100 mL, and when *Enterococcus* counts stantaneous standard of 104 CFU/100 mL.

(including the suspected spill situation). There was no consistent seasonality to the human signals in that the largest number of sites showing human fecal contamination was seven in March 2008, then six each in April, November and December 2008. Human signals of fecal contamination were the most abundant and widespread of any of the potential sources detected (Table 3). Notably, human fecal contamination was found in 62% (8/13) of the samples that exceeded the *Enterococcus* water contact standard.

The Town of Wrightsville Beach Public Works contracted a private company to video the sanitary sewer system in the area and found no leakage. As mentioned, there are no active septic systems within the town. Lack of these potential sources of human waste, coupled with the locations where human signals were frequently detected lead us to assume that boat-borne fecal sources have been entering area waters, as has been found in other locations (Faust, 1982; Sobsey et al., 2003). Certainly boat use is very frequent at the Carolina Yacht Club (WB-CYC), Causeway Marina (WB-CM) and Wildlife Ramp (WB-WR) sites, as well as the Wynn Plaza (WB-WP) area. However, human fecal contamination was widespread and signals appearing in one location may have originated even hundreds of m away and carried by the strong tidal currents throughout this area.

3.3.2. Dye movement and fecal bacteria

The dye study proved to have direct applicability to potential movement of human fecal bacteria in the Wrightsville Beach channels. Both the green and red dyes were easily visible from the hotel rooftop. Distinct patches of dyed water were observed to travel over two km in little over an hour with the tidal currents before dispersal; presumably, entrained particulate matter including fecal bacteria would also be transported far from their point of origination before significant deposition or dilution. For instance, during the ebb tide, the red dye deposited in Motts Channel moved downstream to Banks Channel, where it did not immediately intermingle with the green-dyed water moving down Banks Channel, but moved along the west shore of Banks Channel for several hundred m before being carried across the channel to the shoreline near WB-JPB (Fig. 1). The implications of this are that, on the ebb tide, fecal bacteria sourced from a marina in Motts Channel could easily be carried to Station WB-JPB, to be detected later by researchers (we detected human signal on three occasions at that location). We note that Sobsey et al. (2003) found significant fecal contamination as much as 300 m from suspected marina sources.

We also observed that in Banks Channel the largest mass of green dye moved rapidly down the center of the channel, but tendrils of the dyed water were seen to slow near shore and actually form eddies when encountering the outermost supports of dock structures. Green dye was observed to be retained in dock locations well after the main mass of dyed water passed down-channel toward the inlet beyond WB-JPB. Thus fecal bacteria sourced from boat heads located well up-channel could readily become entrained around dock structures down channel, and settle to the sediments where they could later be released to overlying waters by boat docking activity or human and animal waders.

3.3.3. Animal fecal contamination

None of the T-RFs were identified from the *Bacteroides–Prevotella* group living in dog and other canidae during those periods at any of the sites. The Town of Wrightsville Beach has an active, and apparently successful pet waste control program with pet waste bags in dispensers located along popular walking routes and fines for violators. However, we on occasion (June, November and December) found avian fecal contamination (at WB-CM, WB-WR and WB-CYC), and seagull-specific fecal contamination signals were found at WB-LB in November 2008 and at WB-WR in November and December 2008 (Table 3). Ruminant fecal contamination was found in February at WB-WR; June at WB-CM and WB-WR, October at WB-SB, and in the rainy periods of November and December 2008 ruminant fecal contamination was detected in a broad variety of locations including WB-CM, WB-WP, WB-WR, WB-LB, WB-SB, WB-CYC and WB-MS (Table 3). There are no domestic ruminants within Wrightsville Beach so these presumably originated from deer utilizing nearby marshes and spoil islands, with their fecal material carried by currents to those sampling sites. Evidence of rat fecal contamination was detected in June at WB-CYC as well. Some locations detected mixed human and other fecal contamination signals.

3.4. Environmental factors related to fecal contamination

There was a significant inverse statistical relationship between salinity and overall fecal coliform counts ($r = -0.368$, $p < 0.001$) and also between salinity and overall *Enterococcus* counts ($r = -0.263$, $p = 0.013$). That fact that these relationships are both statistically significant may point toward stormwater runoff as a second source of fecal bacteria to these waters. Also, the correlation analyses indicate that fresher water is related to higher fecal bacteria counts, suggesting rainfall as potential driver.

The relationship between rain and overall *Enterococcus* counts was not statistically significant (Table 4). However, there was a highly significant positive correlation between rainfall on the day of sampling plus that of the previous day and overall fecal coliform bacteria counts ($r = 0.299$, $p = 0.003$). Individually several stations showed significant correlations between either fecal coliform or *Enterococcus* counts and rainfall (Table 4), including WB-CYC, WB-JPB, WB-WR and WB-SB. At WB-LB there was a high correlation coefficient ($r = 0.504$) but the probability value was not statistically significant. These correlation data overall indicate that there remains a problem of stormwater runoff being a second source of fecal bacteria to the waters around Wrightsville Beach.

4. Management actions

Boating activity has been linked to fecal bacterial pollution in a number of studies. This previously has been accomplished by using elevated indicator bacteria counts in the water (Faust, 1982;

Table 4
Correlations between fecal coliform bacteria counts, *Enterococcus* counts, and total rainfall on day of sampling plus the previous day, August 2007–January 2009. Stations WB-JM and WB-MS are excluded due to reduced sampling effort. Data presented as correlation coefficient *r*/probability (*p*). Bolded results are statistically significant at $p < 0.05$. Correlation results for all dates combined represent seven stations × 12 sampling dates. Correlation results for individual station fecal coliforms represent 12 sampling dates and for individual station *Enterococcus* represent 11 sampling dates.

	Fecal coliforms	*Enterococcus*
All sites combined	**r = 0.299**	$r = 0.168$
	p = 0.003	$p = 0.121$
WB-CM	$r = 0.445$	$r = -0.083$
	$p = 0.147$	$p = 0.807$
WB-WP	$r = 0.368$	$r = 0.027$
	$p = 0.225$	$p = 0.937$
WB-CYC	**r = 0.814**	$r = 0.422$
	p = 0.001	$p = 0.187$
WB-JPB	**r = 0.736**	$r = -0.169$
	p = 0.010	$p = 0.649$
WB-LB	$r = 0.504$	$r = 0.303$
	$p = 0.090$	**p = 0.366**
WB-SB	$r = -0.027$	**r = 0.665**
	$p = 0.933$	$p = 0.025$
WB-WR	**r = 0.668**	**r = 0.607**
	p = 0.018	**p = 0.048**

Sobsey et al., 2003) or in shellfish (Guillon-Cotard et al., 1998). We have utilized a multifaceted approach to assess sources of fecal contamination to the waters of a popular resort area.

Our sampling, source tracking, and dye study data indicated that human waste flushing/dumping in the area around Wrightsville Beach was a major source of fecal bacterial contamination to these waters. Since maritime vessels appear to be the most likely source of human fecal material, the local governments sought to prohibit all boat sewage discharges in the area waters to prevent degradation of the water quality and reduce the risk of human illness from inadequately treated sewage. In August 2008, the Town of Wrightsville Beach's Board of Aldermen adopted a resolution seeking New Hanover County and the State of North Carolina's support in petitioning the U.S. Environmental Protection Agency to establish a No-Discharge Zone (NDZ) for New Hanover County's coastal waters. The NDZ would completely prohibit the discharge of sewage, whether treated or not, from all vessels in the coastal waters of New Hanover County, extending out 3 nautical miles from the coast. It also included all of the tributaries within the county as well as the Atlantic Intracoastal Waterway. All of the coastal municipalities (Kure Beach, Carolina Beach and the City of Wilmington) within New Hanover County adopted similar resolutions.

The North Carolina Department of Environmental and Natural Resources supported the local government resolutions and sent an application for an NDZ to the EPA in April 2009. The EPA Region 4 concurred with the State's findings and placed the proposed NDZ on the Federal Register in September 2009 for public comment. The measure successfully made it through the public comment period and in February 2010 this area became the first NDZ within the marine waters of the coastal United States located between Delaware and the Florida Keys. The U.S. Coast Guard will have the primary responsibility for enforcing the NDZ. However, any State law enforcement officer will also have enforcement authority.

5. Conclusions

The outcome of this study demonstrates that positive environmental management actions can occur when research, management, and the regulatory community partner. The scientific research findings were embraced by administrators and elected officials, who took the case to the state and federal regulatory communities, which then created the first NDZ in this large coastal region. Such actions should reduce the human health microbiological risk in these waters, as well as the potential loss of tourism. This coastal region has experienced a significant loss in shellfishing revenues due to bed closures in the past two decades (Mallin, 2009); hopefully actions such as these will alleviate this problem as well.

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