Impacts of rainfall on the water quality of the Newport River Estuary (Eastern North Carolina, USA)

Angela D. Coulliette and Rachel T. Noble

ABSTRACT

The Newport River Estuary (NPRE), an important North Carolina (NC) shellfish harvesting area, has been experiencing alterations to the land-water interface due to increasing population and coastal development. Water quality degradation in the estuary over the last decade has led to an increase of shellfish harvesting area closures, and has been postulated to be due to non-point source contamination in the form of stormwater. Water samples were taken in the NPRE ($n = 179$) over a range of weather conditions and all seasons from August 2004 to September 2006. Fecal coliform (FC), as estimated by *E. coli* (EC), and *Enterococcus* (ENT) concentrations (MPN per 100 ml) were examined in relation to rainfall levels and distance from land. The relationships among the fecal indicator bacteria (FIB) and environmental parameters were also examined. The data revealed a significant increase in FC concentrations after measured rainfall amounts of 2.54 cm (general threshold) and 3.81 cm (management action threshold). However, higher than expected FIB concentrations existed during conditions of negligible rainfall (< 0.25 cm), indicating a possible reservoir population in the sediment. Overall, stormwater runoff appears to be adversely impacting water quality in the NPRE.

Key words | *E. coli*, *Enterococcus*, estuaries, fecal indicator bacteria, rainfall, water quality

Angela D. Coulliette (corresponding author)
Department of Environmental Sciences and
    Engineering, School of Public Health,
University of North Carolina at Chapel Hill,
Chapel Hill 27599, North Carolina,
USA
Tel.: 252.726.6841
Fax: 252.726.2426
E-mail: angiecou@email.unc.edu

Rachel T. Noble
Institute of Marine Sciences,
University of North Carolina at Chapel Hill,
Morehead City, NC 28557,
USA

INTRODUCTION

Estuaries, the transition zone between rivers and oceans, are complex aquatic systems which are utilized globally for commercial (fishing and shellfishing), recreational (swimming and boating), and industrial (transporting goods, mining, and dredging) purposes. In addition to these economically important activities, the 2000 United States (US) Census Bureau Brief reported a growing trend of Americans moving to estuarine areas with 53% of the US population now living in coastal counties (Perry *et al.* 2001). The surrounding land-water interface of estuaries is undergoing rapid modification due to escalating coastal populations and subsequent development. An increase in impervious surfaces (i.e. parking lots, paved roadways, rooftops, driveways) and the clearing of previously-forested land directly impact estuarine water quality. Receiving waters associated with such modifications are impacted by

higher volume and higher transit speed of stormwater runoff. As a result, these waters have degraded water quality and an increase of potential health concerns.

Estuarine water quality is regulated by the Clean Water Act (National Research Council 2004) and a waterbody is designated as impaired when the acceptable level or concentration of a water quality indicator is exceeded. Fecal indicator bacteria (FIB), such as fecal coliforms (FC), *E. coli* (EC) and/or *Enterococcus* (ENT), are commonly used as proxies of potential pathogenic microorganisms by both recreational and shellfish harvesting water quality management programs. Fecal coliforms are the recommended and commonly applied FIB for managing water quality of shellfish harvesting waters at the state and/or national level (NSSP 2005). According to the US Environmental Protection Agency (USEPA) 303(d) List, of

the 726 impaired waterbodies in NC, 341 are listed as impaired based upon FC criteria for either recreational contact or use for shellfish harvesting (USEPA 2004).

The Newport River Estuary (NPRE) is a NC coastal estuarine system (453.25 km^2) within the White Oak River Basin (Figure 1, NCDENR-SSS 2005). The NPRE is one of the many waterbodies which have been placed on the 303(d) list due to exceedance of the FC standards for shellfish harvesting waters. The degradation of the water quality of the NPRE and subsequent status as an "impaired waterbody" is coincident with increased levels of storm-water runoff due to clearing of land, coastal development, and associated population growth. A reported 13% increase in population from 1990 to 2000 in the NC counties surrounding the NPRE (NCSD 2000) has led to increased levels of anthropogenic influence from coastal development and degraded water quality. Tourism is an additional stressor, as the NC Department of Commerce reported NC 8th in the nation, with coastal activities as a top choice for visiting the state (NCDC 2004). At a local level, the economy is dependent on the NPRE for recreational use, boating, and commercial and recreational shellfish harvesting (responsible for 3.63% of the total NC shellfish profit ($675,537) from 1996 to 2006; NCDMF). Since 1986, the NPRE has experienced a 9% increase in shellfish harvesting area closures with a total of 32.9% of the areal extent of the estuary being closed (Figure 1(b); conditionally approved-closed or prohibited) (NCDENR-SSS, Patricia Fowler pers. communication).

Identifying the cause and understanding the decline of water quality in the NPRE is of fundamental importance, and the microbiological water quality of the NPRE has not been adequately studied. Shellfish harvesting waters are currently managed by determining FC concentrations and by extrapolating weather conditions to establish a classification status (i.e. approved, conditionally approved-open, conditionally approved-closed, prohibited). Generally, a minimum of six sets of samples are collected randomly each year during 'open' status (dry weather or negligible rainfall) and analyzed for FC. Additional sampling efforts are conducted only to reopen shellfish harvesting areas that have been closed due to rainfall and resultant runoff (amounts exceeding 3.81 cm of rainfall occurring within 24 hours). This sampling occurs only when an adequate

number of days (3 – 5 d) have passed with dry weather to permit the hydrograph of typical storms to return to baseline. Therefore, with the current sampling program, characterization of estuarine water quality following storm events does not occur. Remediation of degraded water quality can only be initiated after sufficient research has been conducted to characterize and quantify the microbial contaminants in the estuary.

The goal of our research has been to conduct an estuary-wide assessment of FIB concentrations and impacts of stormwater runoff on the NPRE. Specifically, our research objectives were to (1) characterize microbial water quality of the entire estuary by enumerating FIB and measuring environmental parameters over a large geographical area, (2) relate FIB findings to rainfall and distance to land to assess the impact of stormwater runoff, and (3) utilize measurements of FIB to begin to identify potential hot spots for future work to determine sources of fecal contamination to the estuary.

MATERIALS AND METHODS

Newport River estuary

The NPRE (Figure 1) is located north of Morehead City and Beaufort and is in an area classified as Area E-4 by NC Department of Environment and Natural Resources-Shellfish Sanitation Section (NCDENR-SSS 2005). This estuary has an average depth of 1 m and is a well-mixed system with an average residence time of 6 days or 12 tidal cycles, with flushing stemming from the Atlantic Ocean controlled through the Beaufort Inlet (Kirby-Smith & Costlow 1989). The surrounding land-uses consist of approximately 45% forestland, 38% wetlands, 9% residential, 5% bays/estuaries, and 3% cropland (NCDENR-SSS 2005). There are also two point-source discharges (wastewater treatment plants). Associated with varied land-uses are sources of fecal contamination including wildlife (deer, raccoon, bear, or waterfowl), small farm operations (horse, cow, hog), and agricultural drainage (animal biosolids application). The most likely sources of human contamination are subdivision stormwater runoff, septic tank failure, and treated waste-water from the Morehead City and Newport Wastewater Treatment Plants (NCDENR-SSS 2000, 2005).

(a)
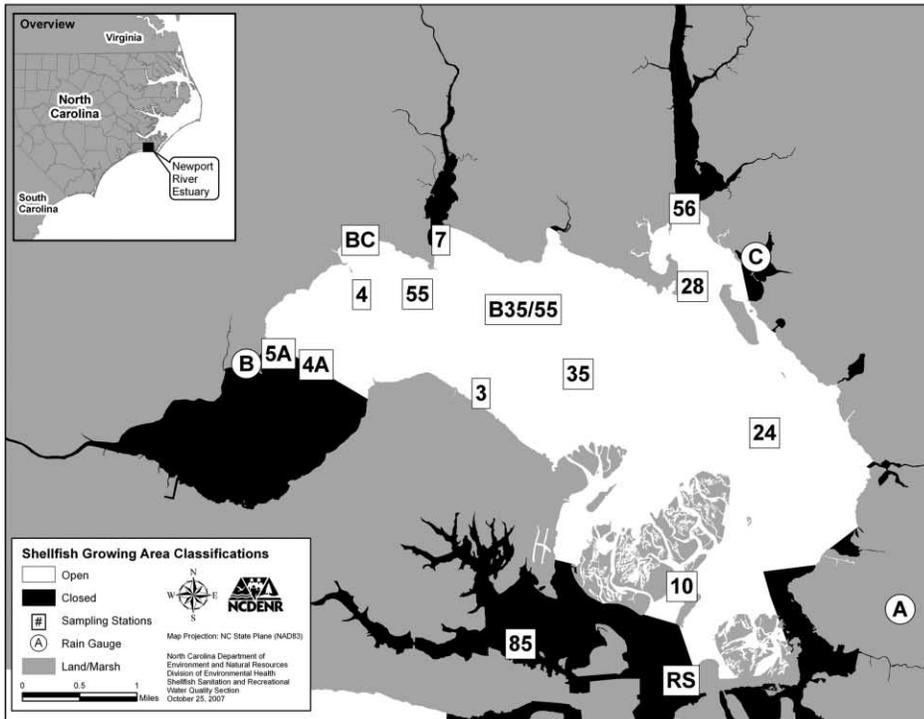
(b)

Figure 1 │ Newport River Estuary (NPRE) (a) NPRE sampling stations and rain gauges during the study period August 2004 to September 2006 (b) NPRE closed areas to shellfish harvesting according to decade.

Sampling locations

Sampling sites were chosen based on existing NCDENR-SSS stations and NCDENR-SSS sanitary surveys (2000). Our goal was to select sites that (1) were spatial distributed across the NPRE, (2) were in high priority shellfish harvest areas (i.e. areas where commercial and recreational shellfish harvesting is prevalent), and (3) were proximal to runoff from land. Figure 1(a) shows the location of the sampling sites, while Table 1 describes the sampling sites and the land-use proximal to each site. The distance criteria used for the designation of the "close to land" and "distant from land" sites was < 0.25 km and > 0.25 km, respectively.

Sample collection

Between September 2004 and August 2006, a total of 179 surface water samples were collected from the 16 sites listed in Table 1. Sampling occurred at least three times a season. Seasons were defined as winter (December 21st to March 20th), spring (March 21st to June 20th), summer (June 21st to September 20th), and fall (September 21st to December

20th). However, additional efforts were made to collect samples across varying weather conditions and a range of storm sizes to produce a robust dataset. One litre samples were collected within 3 hours of low tide in order to collect samples with minimal dilution from marine waters (NPRE is too shallow to navigate at peak low tide). The samples were collected in sterilized containers following sampling techniques outlined in standard methods (APHA (American Public Health Association) 2005). After collection, samples were placed on ice and transported immediately to the University of North Carolina at Chapel Hill, Institute of Marine Sciences in Morehead City, NC for processing.

Fecal indicator bacteria analyses

All samples were tested for EC and ENT using the defined substrate technology test kits, Colilert®-18 and Enterolert® (IDEXX® Laboratories, Westbrook, ME). Conversion of positive wells from these tests to a MPN value was conducted following Hurley & Roscoe (1983). Although literature cites false-positives occurring in tropical and subtropical marine

Table 1 | NPRE sampling stations and neighboring land-uses. The land-uses noted are general descriptions relative to the NPRE. Those noted with an asterisk (∗) indicate a higher density of development for residential or industrial purposes (i.e. "developed") while all other sites are referred to as "undeveloped"

	Approximate distance (km)	Land-use
Close in proximity to land (< 0.25 km)		
3 west crab point*	0.18	low density residential, forested
5A closure line	0.15	low density residential, forested
29 ware creek*	0.24	residential
56 closure line core creek	0.16	low density residential
85 calico creek*	0.11	light industrial (including marina), low density residential
Brickyard (BC)	0.16	low density residential
Recreation site (RS)*	0.08	light industrial, low density residential
Distant to land (> 0.25 km)		
4 turtle rock	0.74	low density residential, forested
4A telephone/cable crossing	0.66	low density residential, forested
7 harlowe creek	0.32	low density residential, forested
10 marker #36	1.46	light industrial
24 marker #30	1.30	low density residential
28 core creek*	0.32	low density residential
35 middle river	1.26	no nearby land use, in center of estuary
Between 35/55 (B35/55)	0.80	no nearby land use, in center of estuary
55 white rock	0.66	low density residential, forested

and estuarine waters (Pisciotta et al. 2002), studies conducted in NC coastal estuarine waters have not demonstrated any measurable rate of false-positive results using Colilert®-18 for E. coli enumeration (Noble et al. unpublished). In addition, previous analyses of estuarine water samples taken throughout eastern NC have shown that 93% of the FC are EC ($n = 3020$, Kirby-Smith and Noble, unpublished data). Thus for the purposes of this study we consider our EC measurements to be conservative representations of FC concentrations.

Sample concentrations were $\log_{10}$ transformed prior to all statistical analyses. The percent of samples exceeding the standard was calculated by comparing the number of samples exceeding the limit to the total number of samples. Normality tests were assessed for the datasets (Howell 2002; Salkind 2004). Independent samples t-test was used to examine significant differences (alpha (α) = 0.05, two-tailed) between FIB concentrations in comparison to land-use, where Levene's test for equality of variances determined whether equal variances were or were not assumed ($\alpha = 0.05$, two-tailed). Rainfall category comparisons and seasonality regarding FIB was determined using the one-way ANOVA with the post-hoc comparison Bonferroni. A significant relationship was determined with respect to an alpha (α) of 0.05 (two-tailed).

As mentioned previously, the goal for this study was to conduct sample collection over a wide range of weather conditions (regardless of 'open' or 'closed' status of shellfish harvesting waters and independent of shellfish harvesting water management guidelines). For statistical analysis, we applied one of the currently used thresholds for shellfish water quality management; the geometric mean threshold of 14 FC MPN per 100 ml. Although not currently designated for recreational use, the NPRE is actively utilized for boating, sailing, and other forms of recreation. Thus, the "Tier 1" single sample threshold of 104 ENT MPN per 100 ml was applied as a means to compare this estuarine waterbody with other recreational waters.

Environmental parameter measurements

Turbidity (NTU), salinity (based upon the practical salinity scale), dissolved oxygen (mg/l), and temperature (°C) were measured at each site using a calibrated multi-probe instrument (YSI Inc., Yellow Springs, OH). The relation-ships between FIB and selected environmental parameters were examined. Normality tests were assessed for each environmental parameter dataset and determined which significance test was conducted (Howell 2002; Salkind 2004). If datasets were normal before or after $\log_{10}$ transformation, the Pearson product moment correlation (PP) was used to assess significance. If the datasets did not have normal distributions, then the Spearman Rank (SR) analysis was used to assess significance. Turbidity was $\log_{10}$ transformed to achieve normality, while raw scores were used for all other environmental parameters. A significant relationship was determined with respect to an alpha (α) of 0.05 (two-tailed).

Due to the heterogeneous nature of rainfall in coastal NC, daily rainfall data were collected from three rain gauges, situated for full coverage of the NPRE (Figure 1). Rain gauge "A", located at the Michael J. Smith Field Airport in Beaufort NC, is maintained by the National Ocean and Atmospheric Association (NOAA) National Climatic Data Center (NCDC) and is available online (http://www.ncdc.noaa.gov/oa/ncdc.html). Rain gauge "B", located in Mill Creek in Newport, NC, is maintained by volunteers for NCDENR-SSS. Rainfall gauge "C", located in Ware Creek in Beaufort, NC, is maintained by a volunteer from Duke University Marine Laboratory. For comparison of FIB to rainfall levels, data from the closest rain gauge was used for each site. In addition, due to sampling constraints via boating during foul weather, a 48 hour rainfall total was used for analyses comparing rainfall to FIB concentrations.

RESULTS

Rainfall caused a significant increase in FC concentrations at a rain threshold of 2.54 cm (1.00 in; Figure 2). Statistical analyses revealed average FC concentrations of 111.8 MPN/100 ml and 221.0 MPN/100 ml for the >2.54 cm (general threshold, $n = 61$) and >3.81 cm (1.50 in, management action threshold, $n = 15$) rainfall categories, respectively. These FC concentrations were significantly higher ($F_{(4,316)} = 9.4$, $p < 0.001$) than those for the lesser rainfall categories. The threshold categories of >2.54 cm and >3.81 cm exceeded the 14 MPN/100 ml FC limit for
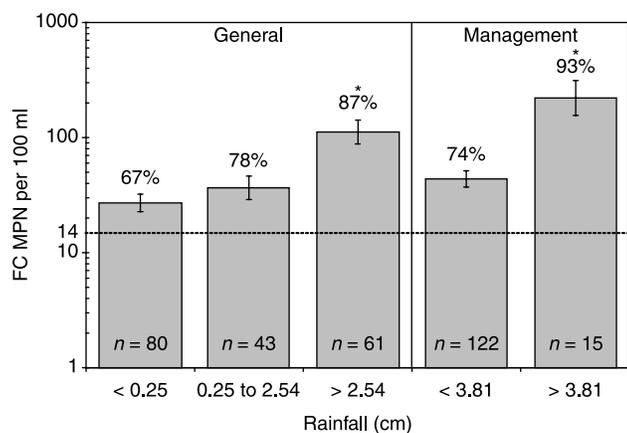
Figure 2 | FC concentrations found in the NPRE according to general rainfall categories of <0.25 cm, >0.25 to <2.54 cm, and >2.54 cm; and then in regards to the management action plan of <3.81 cm and >3.81 cm. The percent of samples exceeding the FC standard limit of 14 MPN or CFU per 100 ml (horizontal dotted line) within that rainfall category are marked above its respective column. Asterisks over the bar indicates a significant difference as compared to the other categories and the error bars are ± 1 standard error.

Figure 3 | Mean FIB concentrations at NPRE sampling sites categorized by distance from land (close $=$ <0.25 km, distant $=$ >0.25 km). The geometric mean threshold for FC (14 MPN/100 ml) is shown by the bar over the left pairs of columns, while the single-sample threshold for ENT (104 MPN/100 ml) is shown by the bar over the right pairs of columns. The error bars are ± 1 standard error.

shellfish harvesting waters 87% and 93% of the time, respectively. The rainfall categories of <0.25 cm (0.10 in, $n = 80$), 0.25 to 2.54 cm ($n = 43$), and <3.81 cm ($n = 122$) exceeded the FC limit greater than 67% of the time. The average FC concentrations for these categories were 27.0, 36.6, and 43.8 MPN/100 ml, respectively.

FC concentrations were significantly higher (average of 78.0 MPN/100 ml) when there was some rainfall (>0.25 cm) in comparison to negligible amounts of rain (<0.25 cm, average of 25.8 MPN/100 ml) ($t_{177} = -4.4$, $p < 0.001$) (Figure 3). Regardless of rainfall category, the average FC values exceeded one of the currently used thresholds for shellfish harvesting waters. ENT demonstrated a similar relationship with rainfall where >0.25 cm resulted in a significantly higher average of 33.9 MPN/100 ml, while <0.25 cm averaged 13.1 MPN/100 ml ($t_{165.9} = -3.3$, $p = 0.001$). Unlike FC, all average ENT concentrations for each category were below the single-sample recreational water quality standard (ENT: 104 MPN/100 ml).

There was no significant difference between FIB concentrations at those sites close to land (<0.25 km) versus those sites distant from land based upon rainfall (>0.25 km, Figure 3). When data was separated according to the general categories of "developed" (residential and industrial, $n = 76$) and "undeveloped" (forested, $n = 103$),
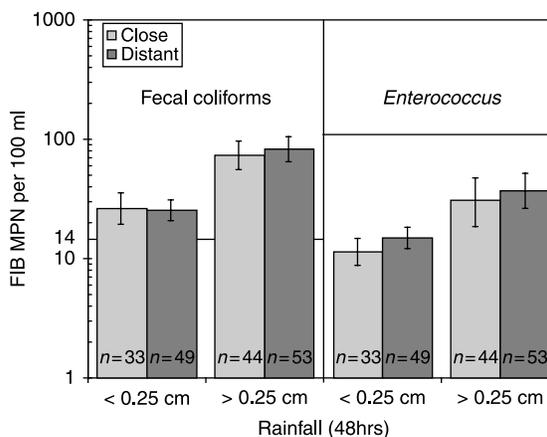
there was no significant different between FC concentrations ($t_{177} = 0.763$, $p > 0.05$) (see Table 3 for sites designated as "developed" and "undeveloped"). However, ENT concentrations did reveal significantly higher concentrations in "undeveloped" areas ($n = 103$; $t_{177} = 3.04$, $p < 0.005$).

Observations from all stations represented a wide range of turbidity (0 to 87.1 NTU; $n = 146$), salinity (2.0 to 35.4; $n = 170$), DO (3.6 to 14.4 mg/l; $n = 169$), rainfall (0 to 7.0 cm in 24 hours and 0 to 14.6 cm in 48 hours; $n = 179$). FC concentrations exhibited positive significant relationships with turbidity and rainfall (48 hours), while also having significant inverse correlations with salinity and DO (Table 2). ENT had a similar positive correlation with turbidity, and negative relationships with salinity and DO. Although temperature was measured ($n = 173$, range: 9.9 to 32.0°C), a large portion of sampling events occurred in the summer months (52.9%) and any observed correlations would be biased. Seasonality was therefore examined, considering temperature to be a major factor. The analysis showed that FC concentrations were significantly lower in the winter (8.4 MPN/100 ml) and significantly higher in the summer (91.4 MPN/100 ml) ($p < 0.001$, $F_{(3,175)} = 17.6$), as compared to all other seasons (Figure 4). Seasonally, ENT concentrations showed no statistical differences with the concentrations ranging from 11.0 to 30.0 MPN/100 ml.

Table 2 | Correlations of environmental parameters and FIB in the NPRE. Statistically significant values were determined for all correlations ($\alpha = 0.05$, two-tailed) using the Pearson product moment correlation (PP), except for rainfall correlations (24 and 48 hours) when Spearman rank correlation coefficient (SR) analysis was used

|	Turbidity (NTU)	Salinity	DO (mg/1)	Rainfall (24 hours)	Rainfall (48 hours)	FC MPN/100 ml	ENT MPN/100 ml
Turbidity(NTU)	1.00	–	–	–	–	–	–
Salinity	−0.321 $p = 0.01$	1.00	–	–	–	–	–
DO (mg/1)	−0.324 $p = 0.01$	0.178 $p = 0.05$	1.00	–	–	–	–
Rainfall (24 hours)	0.199 $p = 0.05$	−0.084	−0.064	1.00	–	–	–
Rainfall (48 hours)	0.232 $p = 0.01$	−0.042	−0.149	0.640 $p = 0.01$	1.00	–	–
FC MPN/100 ml	0.127 $p = 0.05$	−0.400 $p = 0.01$	−0.496 $p = 0.01$	0.048	0.179 $p = 0.05$	1.00	–
ENT MPN/100 ml	0.208 $p = 0.01$	−0.608 $p = 0.01$	−0.277 $p = 0.01$	0.099	0.117	0.554 $p = 0.01$	1.00
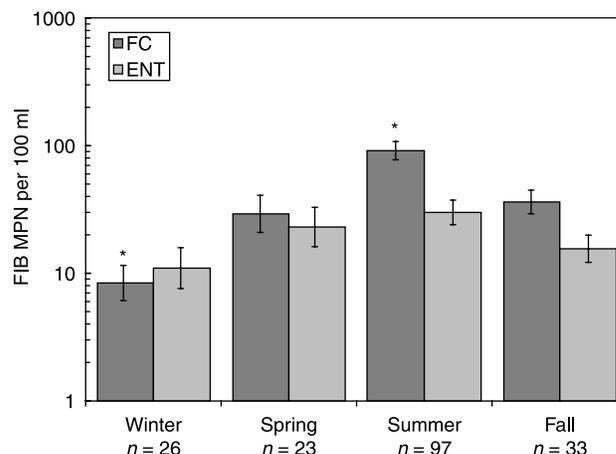
Figure 4 | Seasonal concentrations of FIB in the NPRE. Asterisks over the bar indicates a significant difference as compared to other seasons and the error bars are ± 1 standard error.

Environmental parameters were further examined for confounding relationships, and turbidity appeared to be correlated with most other parameters (Table 2). Turbidity had significant inverse relationships with salinity and DO, while having a significant positive relationship with rainfall (24 and 48 hours).

DISCUSSION

Non-point source pollution, such as stormwater runoff, is a serious issue for NC coastal water quality. Alterations to the land-water interface (i.e. deforestation, impervious surface coverage) are major contributors to this problem. The gradual increase in NC populations is associated with the degrading water quality, although the problem has not reached the magnitude of other coastal states (CA, FL; Noble *et al.* 2000; Lipp *et al.* 2001; Perry *et al.* 2001; Ackerman & Weisberg 2003; Shehane *et al.* 2005). The 13% population growth in neighboring watersheds surrounding the NPRE in the past decade (NCSD 2000) and associated changes to the land-water interface are likely impacting the water quality of this ecologically and economically important estuary, especially during times of heavy rainfall.

Results indicate that rainfall is a significant factor in the contribution of fecal contamination via stormwater runoff to the NPRE. After 2.54 cm (1 inch) there are significantly

higher EC and ENT concentrations as compared to no rainfall (Figures 2 & 3). Our findings agree with other research reports from coastal NC, which describe the increasing impacts of stormwater runoff in the context of land and hydrological modifications, as well as impervious surface coverage (Mallin *et al.* 2000; Kirby-Smith & White 2006). Further evidence demonstrating the impact of stormwater runoff is provided by the significant relationships between FIB concentrations and the freshwater-input related environmental parameters (Table 2). Fecal indicator bacteria had significant correlations with turbidity, salinity, and DO. In addition, these parameters had strong relationships with each other. Stormwater runoff, while introducing FIB into the NPRE, also causes increasing turbidity due to the transport of particulate matter from land sources (scouring) and the resuspension of bottom sediments which occur with high flow or strong winds during rainfall events. Runoff (i.e. freshwater input) also creates a decrease in salinity and an increase in DO due to freshwater inputs and associated biological activity, respectively. Similar correlations with turbidity and salinity were found in coastal NC (Mallin *et al.* 2000). One Florida (FL) study showed similar salinity relationships (Lipp *et al.* 2001), while another FL study did not show the same trend (Shibata *et al.* 2004). The contradicting study (Shibata *et al.* 2004) was in a beach location where salinity did not fluctuate with the tides.

In addition to the observed relationship between rainfall and fecal contamination, there were also unexpectedly high concentrations of FIB during periods of negligible rainfall (<0.25 cm (0.10 in), Figures 2 & 3). This observation indicates a background signal, most likely due to a reservoir population in the sediment, and suggests that FIB may be persisting in the benthos of the NPRE. This phenomenon has been documented in similar sub-tropical and tropical watersheds (Desmarais *et al.* 2002; Byappanahalli & Fujioka 2004; Shibata *et al.* 2004; Fries *et al.* 2008). Studies conducted in northern temperate regions also reveal the persistence and survival of EC through freezing winters with subsequent growth in the warmer months (Whitman & Nevers 2003; Ishii *et al.* 2006; Whitman *et al.* 2006). Ongoing work analyzing reservoir EC and ENT populations in sediments of the NPRE show a contribution of 2.45 to 762.71 and 2.45 to 1072.67 MPN per gram ($n = 4$;

dry weight), respectively (Coulliette & Noble unpublished data). Similar results were observed in the nearby Neuse River Estuary with particle attached ENT (Fries *et al.* 2008). Given the shallow, well-mixed nature of the NPRE and the turbidity values observed over the course of this study, sediment-attached FIB may be partially responsible for the baseline signal during dry weather.

Distance to land was not found to be a significant factor in determining FIB concentrations in this study. Sampling sites for this study were primarily chosen from historically sampled locations, secondarily to have spatially representative sampling locations in shellfish harvesting areas, and finally, based on distance to land. Thus, the lack of statistically significant difference between the FIB concentrations at sites close and distant from land was a likely product of sites being too far from land. In addition, this lack of distinction was further confounded by the fact that sampling via boat did not allow for immediate sampling after rain events for safety. Samples were taken within 48 hours of rain events but tidal flushing and wind mixing may have dispersed FIB signals by that time. Future examinations of land-based runoff will include sampling in tributaries, sampling throughout the duration of storms, and measuring flow to quantify microbial contaminant loading rates.

The seasonal analyses revealed atypical FC concentrations (Figure 4), as historical data shows higher concentrations of FC during the winter versus the summer months. This anomaly may be due to the abnormally high rainfall levels during the study period, as compared to the State Climate Office of North Carolina measurements from a nearby monitoring station (Station: 315830 Morehead City 2 WNW, rainfall levels being measured since 1948). The winter months during the study period had 2.13 cm less rainfall as compared to normal levels. The summer months during the study had 5.77 cm more rainfall as compared to normal levels. Spring and fall also had higher levels of rainfall as compared to normal levels with 2.83 cm and 13.04 cm more rainfall, respectively. Collectively, the study period represents FIB concentrations during 'wet' conditions.

This study did not address potential sources of fecal contamination. Previous sanitary surveys conducted by NCDENR-SSS indicate that animals and humans are

both likely contributors of fecal contamination to the NPRE (NCDENR-SSS 2005). The large avian community, in addition to livestock and wildlife, may be responsible for contributing various fecal pathogens, such as *Cryptosporidium* spp. (Jellison *et al.* 2007) and *Campylobacter* spp. (Dixon 2001; Waldenstrom 2007). Neighboring wastewater treatment plants can also become overburdened during times of heavy rainfall or during disinfection failure, and can release human fecal contamination into the estuary. However, during the study period, the treatment plants did not become overburdened to our knowledge.

The NCDENR-SSS has the complex task of managing the NPRE by taking into account poor estuarine water quality (often associated with rainfall), livelihoods of local shellfish harvesters, and overall public safety. Through the NCDENR-SSS program, only FC sample concentrations during 'open' status are used to classify shellfish harvesting waters, and this historical data indirectly aids in determining the rainfall threshold used for managing shellfish harvesting areas in the NPRE. The current rainfall threshold (management action threshold) of 1.5 in (3.81 cm) of rainfall in 24 hours, established in 1994 for the NPRE, is used to close shellfish harvesting waters. Our research demonstrates that the current rainfall limit may not be adequately protective of human health, and that a more stringent limit should be considered.

This is the first intensive study conducted on the water quality of the NPRE. Future work will incorporate this data into ongoing modeling efforts intended to assist TMDL development, as well as determine sources of fecal pollution through molecular approaches and sediment studies. Data from the TMDL models are being utilized for the development of probabilistic models of fecal contamination transport. Molecular techniques will be used to identify and quantify human versus non-human sources (Boehm *et al.* 2003; Noble *et al.* 2006). Resulting data will be used to partition the sources of fecal contamination and to determine microbial contaminant loading rates. The incorporation of modeling efforts, the partitioning of fecal contamination, and the quantification of microbial loading are integral to understanding and characterizing the major sources of fecal pollution to the NPRE. Hopefully these efforts will lead to the design of effective Best Management Practices (BMPs) for future restoration of the estuary.

CONCLUSIONS

Based on our fecal contamination data, the NPRE is experiencing water quality degradation. Stormwater runoff appears to be a main contributor of fecal pollution; however the existence of a persisting bacterial indicator population deserves further investigation. Application of molecular fecal indicator tools (i.e. *Bacteroides*) and pathogen testing will be needed to determine whether the high concentrations of FIB are indicative of a human health threat. The combination of research and governmental efforts will hopefully allow the remediation of this estuary by identifying problem areas and utilizing BMPs for restoration.

ACKNOWLEDGEMENTS

REFERENCES

Ackerman, D. & Weisberg, S. B. 2003 Relationship between rainfall and beach bacterial concentrations on Santa Monica Bay beaches. *J. Water Health* **1**(2), 85–89.

APHA (American Public Health Association) 2005 *Standard Methods for the Examination of Water and Wastewater*, 21st Edn., Baltimore, Maryland, USA.

Boehm, A. B., Fuhrman, J. A., Mrse, R. D. & Grant, S. B. 2003 Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. *Environ. Sci. Technol.* **37**, 673–680.

Byappanahalli, M. & Fujioka, R. 2004 Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. *Water Sci. Technol.* **50**(1), 27–32.

Desmarais, T. R., Solo-Gabriele, H. M. & Palmer, C. J. 2002 Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl. Environ. Microbiol.* **68**(3), 1165–1172.

Dixon, B. 2001 Natural might not be healthier. *Lancet Infect. Diseases* **1**(5), 360.

Fries, J. S., Characklis, C. W. & Noble, R. T. 2008 Sediment-water exchange of *Vibrio* sp. and fecal indicator bacteria: Implications for persistence and transport in the Neuse River Estuary, North Carolina, USA. *Water Research* **42**(4-5), 941–950.

Howell, D. C. 2002 *Statistical Methods for Psychology*, 5th edition. DUXBURY, Pacific Grove, CA, USA.

Hurley, M. A. & Roscoe, M. E. 1983 Automated statistical analysis of microbial enumeration by dilution series. *J. Appl. Microbiol.* **55**, 159–164.

Ishii, S., Ksoll, W. B., Hicks, R. E. & Sadowsky, M. J. 2006 Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior Watersheds. *Appl. Environ. Microbiol.* **72**(1), 612–621.

Jellison, K. L., Distel, D. L., Hemond, H. F. & Schauer, D. B. 2007 Phylogenetic analysis implicates birds as a source of *Cryptosporidium* spp. oocysts in agricultural watersheds. *Environ. Sci. Technol.* **41**(10), 3399–3400.

Kirby-Smith, W. W. & Costlow, J. D. 1989 *The Newport River Estuarine System*. UNC Sea Grant College Publication UNC-SG-89-04.

Kirby-Smith, W. W. & White, N. 2006 Bacterial contamination associated with estuarine shoreline development. *J. Appl. Microbiol.* **100**, 648–657.

Lipp, E. K., Farrah, S. M. & Rose, J. B. 2001 Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. *Marine Pollut. Bull.* **42**(2), 286–293.

Mallin, M. A., Willams, K. E., Esham, E. C. & Lowe, R. P. 2000 Effect of human development on bacteriological water quality in coastal watersheds. *Ecol. Appl.* **10**(4), 1047–1056.

National Research Council 2004 *Indicators for Waterborne Pathogens*. The National Academy Press, Washington, D.C., USA.

NCDC 2004 *North Carolina Department of Commerce*. http://nccommerce.com/tourism

NCDENR-SSS 2000 *E-4 Shoreline Survey*. North Carolina Department of Environment and Natural Resources, Shellfish Sanitation Section.

NCDENR-SSS 2005 *E-4 Shoreline Survey*. North Carolina Department of Environment and Natural Resources, Shellfish Sanitation Section.

NCDMF (North Carolina Division of Marine Fisheries) *Trip Ticket Program*. Unpublished data from 1972 to 2006.

NCSD (North Carolina State Demographics) 2000 http://demog. state.nc.us/frame_start.html

Noble, R. T., Dorsey, J. H., Leecaster, M., Orozco-Borbon, V., Reid, D., Schiff, K. & Weisberg, S.B. 2000 A regional survey of the microbiological water quality along the shoreline of the Southern California Bight. *Environ. Monit. Assessment* **64** 435–447.

Noble, R. T., Griffith, J. F., Blackwood, A. D., Fuhrman, J. A., Gregory, J. B., Hernandez, X., Liang, X., Bera, A. A. & Schiff, K. 2006 Multitiered approach using quantitative PCR to track sources of fecal pollution affected Santa Monica Bay California. *Appl. Environ. Microbiol.* **72**(2), 1604–1612.

NSSP 2005 *Guide for the Control of Molluscan Shellfish*. U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration.

Perry, M. J., Mackun, P. J., Baker, J. D., Joyce, C. D., Lollock, L. R. & Pearson, L. S. 2001 *Population Change and Distribution: 1990 to 2000. Census 2000 Brief*. U.S. Department of Commerce, Economics and Statistics Administration, U.S. Census Bureau., April 2001.

Pisciotta, J. M., Rath, D. F., Stanek, P. A., Flanery, D. M. & Harwood, V. J. 2002 Marine bacteria cause false-positive results in the colilert-18 rapid identification test for *Escherichia coli* in Florida waters. *Appl. Environ. Microbiol.* **68**(2), 539–544.

Salkind, N. J. 2004 *Statistics for People Who (Think They) Hate Statistics*, 2nd edition. SAGE Publications, Thousand Oaks, CA, USA.

Sattenspiel, L. 2000 Tropical environments, human activities, and the transmission of infectious diseases. *Yearbook Phys. Anthropol.* **43**, 3–31.

Shehane, S. D., Harwood, V. J., Whtilock, J. E. & Rose, J. B. 2005 The influence of rainfall on the incidence of microbial feacal indicators and the dominant sources of faecal pollution in a Florida river. *J. Appl. Microbiol.* **98**, 1127–1136.

Shibata, T., Solo-Gabriele, H. M., Fleming, L. E. & Elmir, S. 2004 Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. *Water Res.* **38**, 3119–3131.

USEPA. 2004 *Final North Carolina Water Quality Assessment and Impaired Waters List* (2004 Integrated 305(b) and 303(d) Report). North Carolina Department of Environment and Natural Resources, USA.

Waldenstrom, J., On, S. L. W., Ottvall, R., Hasselquist, D. & Olsen, B. 2006 Species diversity of campylobacteria in a wild bird community in Sweden. *J. Appl. Microbiol.* **102**(2), 424–432.

Whitman, R. L. & Nevers, M. B. 2003 Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan Beach. *Appl. Environ. Microbiol.* **69**(9), 5555–5562.

Whitman, R. L., Nevers, M. B. & Byappanahalli, M. N. 2006 Examination of the watershed-wide distribution of *Escherichia coli* along Southern Lake Michigan: an integrated approach. *Appl. Environ. Microbiol.* **72**(11), 7301–7310.