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ESTUARINE SEDIMENT BEDS AS A RESERVOIR FOR HUMAN PATHOGENS: MONITORING TRANSPORT OF POPULATIONS OF ENTEROCOCCI AND *VIBRIO* SP. IN THE NEUSE RIVER ESTUARY

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ABSTRACT

Monitoring of microbial contamination of inland waters and estuaries usually focuses on the impacts of rainfall as the presumed major source. This study sought to quantify the potential for sediments to represent a significant source of bacterial populations during resuspension events. These events could be driven by wind without rain or by wind coincident with rainfall (storms) in shallow systems. The Neuse River Estuary is such a system and is the target of intense water quality monitoring for nutrient and bacterial dynamics. Changes in two bacterial groups (enterococci and *Vibrio sp.*) and particle suspensions were determined for the water column and sediment bed. Bi-weekly sampling at a station near New Bern, NC was complemented by samples from an *in situ* water sampling platform and collection of sediment cores during the summer of 2005. This platform was equipped with the capability to collect bottom water samples at high frequencies with sampling triggered by high turbidity events. Samples taken during the passage of Hurricane Ophelia captured the effects of storm surge followed by runoff. The net impact of the hurricane was contamination of the sediment bed with *Vibrio*, while concentrations of enterococci changed little from the significant levels found throughout the summer. The sediment bed represents an environment that may harbor these bacterial populations through periods of harsh conditions in shallow estuaries (low temperature, salinity changes, nutrient limits, sunlight). The results of this study reinforce the importance of high-frequency sampling and coring of surface sediments to complement water quality monitoring, especially for efforts targeting bacterial groups with potential human health impacts.

(fecal indicators, sediment transport, enterococci, *Vibrio*, estuaries, pathogens, hurricanes)

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SUMMARY

This study has made great progress in addressing a set of research questions targeting improved understanding of sediment-mediated transport of bacteria in the Neuse River Estuary:

- ***Do viable populations of enterococci and Vibrio exist in the sediment? Are they in a culturable state?*** At the study site (near New Bern, NC), significant populations of metabolically active organisms have been found in surficial sediments. The dynamics of these populations are particularly interesting. For enterococci (fecal indicator), a stable population was found in the sediments, representing a potential source for water column contamination during resuspension. Meteorological forcing altered the concentrations of *Vibrio* (genus of native bacteria that could include potentially pathogenic species) throughout the summer and fall. The advection and deposition of *Vibrio* at the study site, where significant concentrations are rare, represents a model for relocation of bacterial populations due to storms that has public health implications for other estuaries.

- ***Does sediment resuspension contribute significant amounts of enterococci and Vibrio to the water column through resuspension?*** Results show a clear linkage between turbidity and bacterial concentrations. Events where both increase are difficult to study without the *in situ* sampler and demonstrate the importance of time scales shorter than routine monitoring intervals (weeks). Results also reinforce the potential for wind (without rain) events to generate contamination not included in most event-response sampling for management and protection of public health.

- ***How does the particle loading that can occur after major precipitation events affect Vibrio populations?*** While *Vibrio* clearly depended on events, secondary fluxes (e.g., dissolved organic matter, nutrients) may exert some control on populations following rain and wind events. Observations at the study site deviated from our previous monitoring of *Vibrio* concentrations, as a function of environmental conditions. It appears that the combination of high salinity and resuspension at this site provided optimal conditions for *Vibrio* growth beyond established models. Following Hurricane Ophelia, the combination of high suspension (increased surface area for attachment) and low salinity due to runoff inputs (attachment induced due to stress) may be the mechanism responsible for the observed increase in sediment concentrations.

RECOMMENDATIONS

This project highlights the advances in our understanding of microbial transport attainable using *in situ*, adaptive sampling. Researchers that monitor aquatic environments are rapidly embracing the ability to observe systems on scales that are appropriate to both the processes and resources used in these environments. With respect to time, these scales are usually shorter than the frequency of monitoring measurements conducted using boats and personnel. While this study was a significant step forward in monitoring the Neuse River Estuary, the need remains to continue and expand *in situ* sampling.

Future monitoring efforts should also incorporate sediment sampling into the regularly scheduled and event-response efforts. The interaction between bacteria, suspensions, and weather are not sufficiently understood for effective prediction of concentrations and protection of the public. Without any measure of the resident bacteria in sediments, events (e.g., wind without rain) that cause significant resuspension may go unpredicted and possibly undetected. Based on our measurements, there is a high likelihood that this is occurring for enterococci in the upper Neuse.

There are many potentially useful measurements and advanced techniques not utilized during this project due to funding and/or time limitations. Elements that were not sampled but will be included in future TACO deployments are nutrients and dissolved organic matter concentrations. We have hypothesized in this study that the period of elevated *Vibrio* during September was due to the confluence of high mixing and salinity at the study site. These concentrations deviate significantly from models based on the whole estuary and may represent a bacterial response to a flux of dissolved resources from the sediment that, along with salt, provide unique conditions for growth beyond observations in similar salinity downstream.

Another method absent from this study is Quantitative Polymerase Chain Reaction (QPCR) for speciation of bacteria in both sediment and sediment-water interface samples. The goals for QPCR in analysis of these samples would be (1) identifying source-specific species composition of enterococci populations in sediments and (2) discrimination of *Vibrio* species (i.e. are pathogenic species present in quantities that pose a health risk?) composition during summer. Samples collected as part of this study have been archived for future work in these regards. Several obstacles must be cleared before these techniques will produce useful results. Sediment, even in suspension, significantly inhibits QPCR reactions and raises limits of detection. Currently, primers, probes, and optimized assays are still being developed for specific *Vibrio* species of interest in the Neuse River Estuary. Furthermore, controls are being developed to ensure absolute quantification of cells in estuarine samples.

INTRODUCTION

Estuaries are buffer zones between rivers and oceans, where many anthropogenic inputs are absorbed or modified. Several mechanisms could provide a sink for this material, including dispersal within the estuary, burial, wind-induced flushing, or storage in the sediment. While this range of mechanisms exhibits unique influences in each estuary depending on hydrographic forcing, the duration that pathogenic organisms remain viable and pose risk to public health critically depends on their deposition, lifestyle in the sediment, and resuspension. Microorganisms contained within anthropogenic inputs have the potential to persist, or even proliferate, in estuaries.

The Neuse River Estuary (NRE) is an ecologically and economically-important tributary of North Carolina's Albemarle-Pamlico Sound system. It is a shallow, bar-built estuary with minimal tidal influence and flows dominated by wind and river inflow. This estuary is experiencing a decline in water quality as a response to urban (approximately 1.5 million people) and agricultural growth in its watershed. The watershed also includes coastal communities that depend heavily on fishing, aquaculture, and tourism to sustain the economy as well as inland communities where farming and other businesses play an important role. We chose the NRE as a prime location for a study quantifying the potential for sediments to serve as reservoirs and a significant source of bacterial populations during resuspension events. The NRE typifies shallow, Atlantic and Gulf coastal plain estuarine ecosystems physically, chemically, and biologically. It is also an estuary that is experiencing rapid urbanization and agricultural development in its watershed, including confined animal feeding operations (CAFOs), which has led to the current increase in nutrient loading and subsequent eutrophication. It is a tractable estuarine ecosystem (~60 km along its axis) that is presently intensively monitored for water quality and habitat characteristics. It is the subject of several hydrologic, biogeochemical, and water quality modeling efforts that are germane to this project's research goals (e.g. Bowen and Hieronymus 2003). Finally, it is representative of many other river basins in the U.S., thus making findings in the NRE widely applicable.

The NRE has been widely studied in terms of water quality and monitored for a span of over 20 years thanks largely to the efforts of Dr. Hans Paerl through multiple projects such as NRE Modeling and Monitoring (MODMON) project (http://www.marine.unc.edu/neuse/modmon). This research has largely included study of chemical and nutrient inputs that affect phytoplankton dynamics and eutrophication and modeling of physical parameters and trophic structure. In more recent years there has been a growing emphasis on the study of additional microbial contaminants.

Most of North Carolina's key production of swine, chickens, and turkeys is located in the coastal plain region, including the Neuse River watershed. The swine population alone has increased from less than a million head in 1998 to over 12 million in 2002 and there are now more than 3 times as many swine as humans in the state's coastal watersheds (NC Dept of Environmental and Natural Resources). This industry generates about 10 times as much waste as the human population, with most of the waste released to the environment untreated. In addition, there are approximately 40 million turkeys and chickens raised in the state's coastal watersheds annually. A potentially serious problem is the increase in viral and bacterial pathogens that are transported from these upstream areas of the NRE downstream toward the coast. Bacterial and viral pathogens in hog waste are of major concern because many of these microbes are able to infect humans. Current treatment processes used in animal production are inadequate to prevent the release of potential human pathogens to the environment, which occurs by way of contaminated overland flow from spray irrigation and through groundwater. Other common sources of fecal contamination to the NRE are sewage treatment plant effluents, on-site wastewater treatment and manure spraying systems, agricultural animal manure runoff, sanitary wastes from boats, wildlife feces, and stormwater and other non-point source runoff.

Studies of water contamination in the NRE and other systems have noted increased fecal indicator concentrations in surface water during times of increased rainfall (Patz 1998; Ashford 1998; Mager 1996; Fries et al. in press), suggesting runoff as an important source of pollution. Recreational water quality standards rely on enumeration of indicator bacteria as proxies for the potential presence of fecal contamination. Studies have suggested that enterococci are an indicator of fecal contamination for several reasons: in recreational bathing waters, enterococcus concentrations correlate closely with the incidence of gastrointestinal illness (Dufour and Ballentine 1986) and fecal enterococci are resistant to chemical and physical stress and therefore persist in the marine environment (Kuhn et al. 2000; Frahm et al. 1998). Of the facultative anaerobic organisms common in human fecal flora, enterococci have been found in almost all subjects with a mean level of $8x10^8$ per gram feces (Klessen et al. 2000).

Sediment studies also benefit our understanding of the dynamics of bacterial populations not typically associated with contamination from anthropogenic sources. *Vibrio* species are naturally-occurring, heterotrophic bacteria ubiquitous in estuaries and coastal systems worldwide, including North Carolina. Some *Vibrios* have been shown to be human and animal pathogens (Center for Disease Control: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/). Among the most notable pathogenic species, *Vibrio cholerae* is the organism responsible for one of the world's recurring epidemics, cholera. *Vibrio vulnificus* and *Vibrio parahaemolyticus* are more commonly known as a cause of food borne illness, which occurs primarily through the consumption of contaminated shellfish. *V. vulnificus* can also cause skin infections when open wounds are exposed to seawater containing these bacteria (Strom and Paranjpye 2000). *Vibrio vulnificus* is of particular concern as it can develop into serious and life threatening infections, particularly in immunocompromised individuals. There are several hundred cases of *Vibrio* infections each year in the United States, including an average of 16 cases annually in NC from 2000 to 2005 (NC Dept of Health and Human Services: http://www.epi.state.nc.us/epi/) and five fatalities from infections in New Orleans following Hurricane Katrina (CDC 2005).

The ecology of *Vibrios* has been studied in estuaries throughout the world. Each *Vibrio* species has specific temperature and salinity requirements. In general, if the salinity requirements are met, the number of *Vibrio*s correlates strongly with temperature and increases in warmer months (Motes 1998). It has been shown that *Vibrio cholerae* demonstrate particle attachment to organic particles (Huo 1996). Other recent research suggests that the presence of particles may influence *Vibrio* populations. Louis et al. (2003) have shown that Susquehanna River inflow contributes to the variability in the occurrence of *V. cholerae* in Chesapeake Bay. This may be due to salinity changes or salinity may be an indicator of other changes in the system. Pfeffer (2003) found that *V. vulnificus* in the Neuse had positive correlations with temperature and turbidity, as well as other environmental parameters. To date, work on the sediment reservoirs of *Vibrio* have been directed at the roles of particle attachment providing a transport mechanism for sediment-water exchange (Randa et al. 2004). Our observations in the NRE support the possibility that a significant portion of *Vibrio* (~30%) are attached and this fraction is higher when environmental conditions (salinity) are unfavorable for growth or particulate loads are high (Hsieh et al. in revision).

Fecal bacteria have shown an affinity for fine particle attachment, providing advantages in survivability and increasing deposition to sediments. Bacterial mortality due to predation or environmental exposure is reduced for enterococci attached to particles (Davies and Bavor 2000; Jin et al. 2004). The ability of these organisms to survive longer than coliforms in sediments supports the use of enterococci as a fairly stable and conservative indicator of contamination (Craig et al. 2004). While particle attachment provides longer periods of viability, the time in suspension is reduced as particles settle to the bed. These particles have the potential to contaminate shellfish and create 'hot-spots' of sediment contamination (De Luca-Abbott et al. 2000). The sedimentation of new inputs of fecal contamination reinforces the importance of making accurate predictions of inputs due to resuspension of settled bacteria. Studies of bacterial survivability and exchange with coastal sediments benefit the design and execution of models predicting transport of pathogens (Steets and Holden 2003).

The NRE provides an optimal environment to assess the importance of sediment-derived populations of indicator and pathogenic bacterial species such as enterococci and *Vibrio* sp., respectively. There are significant levels of both in the NRE (Fries et al. in press; Hsieh et al. in revision) and in Pamlico Sound (unpublished data), and the range of salinity and temperature in the estuary is optimal for populations of both groups to remain viable in the water column for long periods of time (days to months), permitting transport and resuspension to occur. The connections between sediment and microbial transport may simply be due to particle attachment. Previous observations in NRE bottom water has demonstrated that approximately 30-40% of cells in both bacterial groups attach to particles that settle faster than 1.2 mm per hour (Fries et al. in press). This portion of the total population is subject to the movements of the particles or sediment.

Figure 1. Map of study site (Stn 30) on the Neuse River Estuary. Storm sampling area is region identified in previous studies as impacted by runoff and microbial contamination.

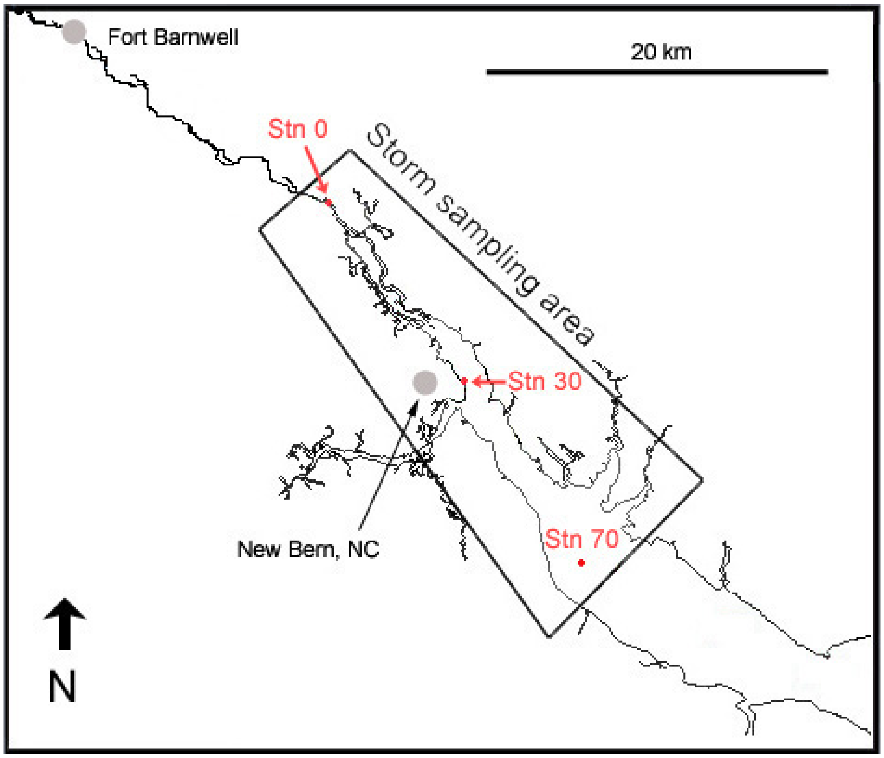
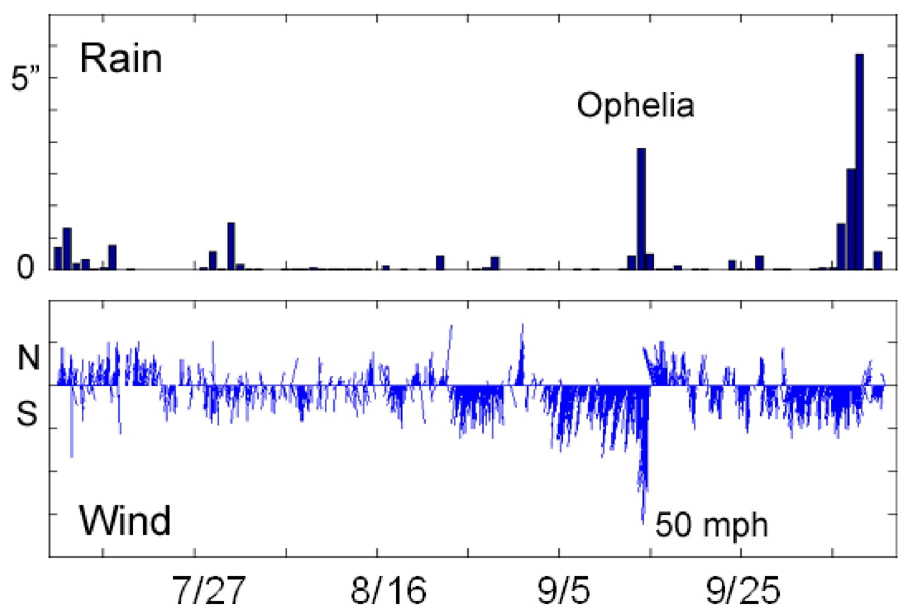


Figure 2. Meteorological forcing (daily rainfall and hourly wind) at study site from July to October 2005.



METHODS

Study Site

The site for this work was in the NRE in eastern North Carolina (Figure 1). Results from ongoing extensive water quality monitoring has established the importance of the section of the river near the city of New Bern as a transitional region between water columns dominated by runoff and resuspension and those dominated by phytoplankton (Fries et al. submitted). Understanding the interactions between particles and bacterial populations is a critical link in microbial transport models which need sedimentation terms for losses from the water column (Fries et al. in press). Following deposition, future resuspension events may represent a source of bacteria to the water column not often included in models or management frameworks.

Several periods of wind and rain were experienced at the study site during the summer of 2005 (Figure 2). These events strongly influenced the salinity in bottom waters, the vertical stratification, and the resuspension of sediments. Of particular note, Hurricane Ophelia passed the study site on September 14, with the highest winds (> 40 mph) and a total of 4.5 inches of rainfall. The approach of this storm subjected the area to 16 days of winds from the northeast (National Weather Service at New Bern Airport: http://www.erh.noaa.gov/mhx/f6.html). Given the alignment of this wind direction with the long axis of Pamlico Sound and the lower NRE, increases in salinity from a surge into the upper NRE was expected.

TACO Design

To capture the dynamics of resuspension, this study pursued a sampling strategy beyond the limitations of boat-based, fair-weather sampling often used for detection of microbial contamination. The Time-series Aquatic Contamination Observer (TACO) was designed and built as a research platform for acquisition of water samples *in situ* (Figure 3). Sampling was programmed on a regular schedule and based on instrument triggers using an ISCO 6712 sampler. A total of twenty four, 1 liter samples could be collected. For this study, a turbidity sonde (FTS-12) was used to trigger samples based on 10 minute averages of turbidity (NTU). Temperature and turbidity variance were also logged on the ISCO logger at 10 minute intervals.

Water Quality Measures

In addition to temperature and turbidity from the sonde connected to the sampler, water quality measures were also obtained from the samples themselves. Salinity was measured using a refractometer. Total suspended solids (TSS), particulate organic carbon (POC), and the ratio of POC to particulate nitrogen (CN) were measured using duplicate 50 ml samples on 0.7 μm glass fiber filters using a Carlo Erba NA1500. Particle size distribution was measured using a Beckman Coulter Multisizer III. From the distribution, the integrated fine particle volume (FPV) of particles from 3 to 60 μm in diameter was calculated. Complementary measurements of several water quality parameters for bottom water were also available from a United States Geological Survey monitoring station located 50 feet from the TACO (data available at http://waterdata.usgs.gov/nc/nwis/uv/?site_no=02092162).

Microbiological Measures

Enterococci were quantified using Enterolert™ media with Quanti-tray/2000® (IDEXX Laboratories) incubated at 41 °C for 24 hours. The most probable number (MPN) for each sample was calculated based on aggregate numbers of large and small positive wells (Hurley and Roscoe 1983). The method employed for *Vibrio* analysis was a hybrid dilution series based on our previous knowledge of concentrations as a function of salinity. Samples were filtered through 47 mm nitrocellulose (0.4 µm pores) filters, and then plated on Thiosulfate Citrate Bile Sucrose (TCBS) agar. Plates were incubated for 24 hours at 37 °C, after which time colonies were counted and recorded per dilution. Multiple sample volumes (factor of 5 between plates) were prepared with the central volume targeting 30 colonies on a plate, based on the salinity model published by Hsieh et al. (in revision). To maintain a minimum of 5 ml solution per filter, smaller volumes were diluted with sterile phosphate buffered solution (PBS). Only colonies that were green or yellow and exhibited relief from the surface of the filter were counted, and reported in colony forming units (CFU).

A common problem with microbiological analyses of water samples is the decay of concentrations in bottles stored *in situ* until recovery. Tests were conducted in the field using samples stored in the sampler during each deployment. When samples were recovered, duplicate samples were taken: one was taken back to the lab for analysis and the other left in the sampler until the next recovery (2-10 days). Following analysis of the stored sample, a decay rate was computed for each bacterial group and some particle measures (Table 1). Decay rates were only computed for samples that remained above the level of detection for the respective methods and dilutions. To complement this data, laboratory time series were also conducted using the same bottles stored at room temperature in the dark (similar to field conditions). These results were comparable to the field results and included in the average decay rates used. Given the large potential influence of this decay, samples stored longer than four days were excluded from analyses and not presented as part of this study. Decay of particle measures were neglected as they showed little decay through time.

Sediment Coring

Sediment sampling in the NRE occurred on seven occasions between July and October, 2005. Sediment cores were taken to a depth of six to 24 cm using a metal corer five cm in diameter. Cores were carefully transported back to the lab. Once in the lab, overlying water was withdrawn with a pipette and a portion was collected and analyzed as a water sample (procedures above) to check for accidental resuspension during transport and handling. The core was extruded and three sub-cores (1.54 cm^2) were collected with cut syringe barrels. The top 1 cm of sediment was sectioned from sub-cores and used for various analyses. Samples for total sediment CN using a Carlo Erba NA1500 were stored in a -20^{o}C freezer prior to analysis. Samples for microbiological analyses were resuspended in 25 ml sterile phosphate buffer solution (PBS). Sediment suspensions were analyzed as water samples for both IDEXX (1:100 dilution) and TCBS filter plating (0.1 and 0.5 ml diluted in PBS). MPN or CFU were expressed per gram of total surface sediment. Samples spent 30 to 90 minutes in suspension prior to being used in these analyses and fine sediment in both trays and filters was evenly distributed.

Resuspended sediment was used for grain size analyses as well. Grain size analyses of particles (>63 µm) were done using a series of sieves (63µm, 125 µm, 250 µm, 500 µm, and 1000 µm). Sieved sub-samples were dried over night at 55-60 °C and weighed. The fine fraction (<63 µm) was resolved using the particle size distribution (as described above). Particle counts were made from 1:400 dilution of sample (50 µl of sample in 20 ml diluent).

Figure 3. Schematic of TACO platform housing *in situ* sampler. Water depth at site was approximately 3 m during deployment.

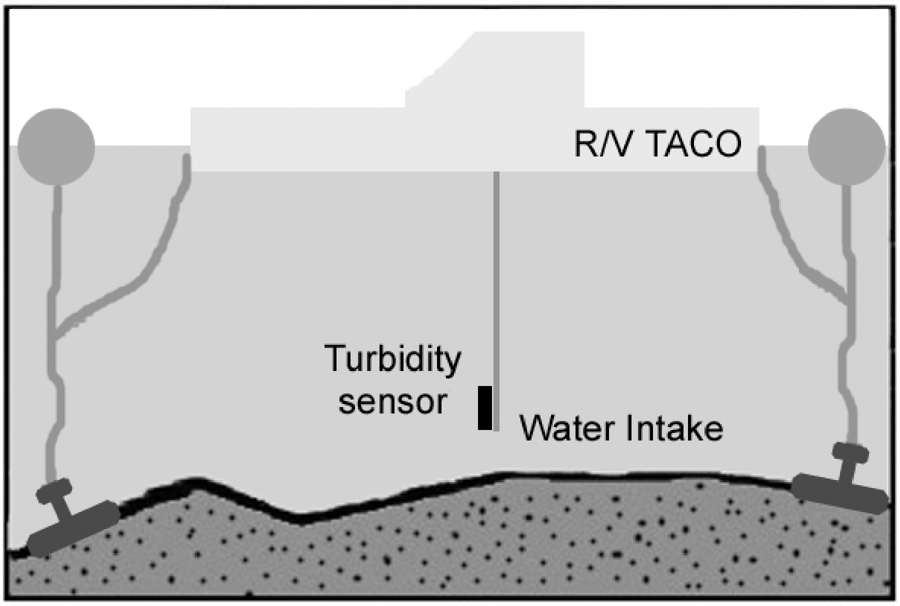
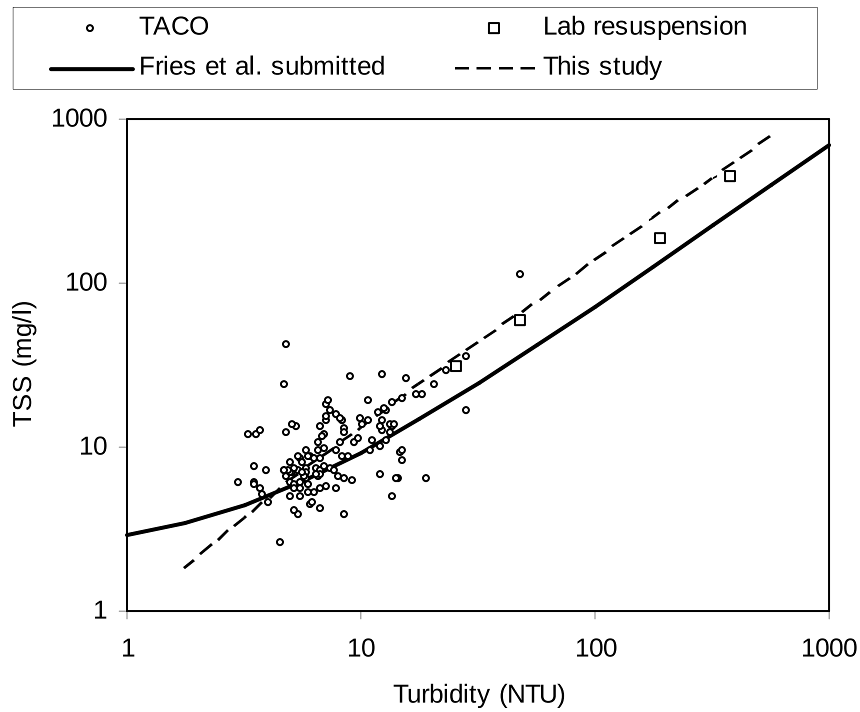


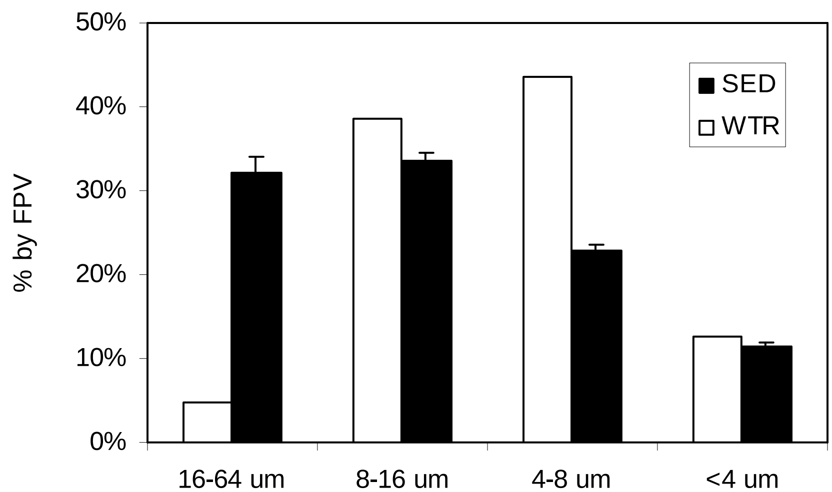
Table 1. Decay measurements (expressed as d^{-1}) based on field and laboratory incubations

Days		TSS	POC	Enterococci	*Vibrio*
2.0	LAB	-0.07	-0.09	-0.49	-0.39
4.0	LAB	-0.06	-0.02	-0.31	-0.36
4.9	TACO	0.07	0.06	-0.75	-0.36
6.3	TACO	0.03	0.02	-0.22	-0.47
6.8	TACO	0.04	-0.11	BLD	-0.12
6.9	TACO	0.01	-0.03	BLD	-0.33
7.0	TACO	-0.01	0.00	BLD	-0.51
7.0	TACO	0.00	-0.05	-0.36	-0.11
7.0	LAB	-0.02	NM	BLD	BLD
8.0	TACO	-0.01	-0.10	BLD	-0.11
8.0	LAB	-0.05	NM	-0.36	BLD
9.0	TACO	0.00	-0.11	BLD	-0.50
	TACO avg	0.02 ± 0.01	-0.04 ± 0.02	-0.44 ± 0.16	-0.31 ± 0.06
	LAB avg	-0.05 ± 0.01	-0.06 ± 0.03	-0.38 ± 0.05	-0.38 ± 0.01
	BOTH avg	0.00 ± 0.01	-0.04 ± 0.02	-0.41 ± 0.08	-0.33 ± 0.05

NM = not measured for this test
BLD = one or both time points were below level of detection for this test

Figure 4. Comparison of NTU-TSS and average particle size distribution with water quality results from same region of the Neuse





RESULTS

Turbidity and Resuspension

The particle suspensions sampled at TACO were different than those found in our parallel monitoring effort at the site (TSS = 0.70*NTU + 2.22: Fries et al. submitted) with respect to the resultant NTU-TSS curve (TSS = 1.38*NTU - 0.69; R = 0.73: Figure 4a). The suspensions in this study scattered less light for the same suspension mass (higher slope), consistent with larger particles from the sediment bed rather than those from the watershed. The NTU-TSS curve also matched a laboratory calibration of the instrument using a sediment sample (high TSS points in Figure 4a; not part of best fit calculation). Particle size distributions from cores were coarser than particles found in suspension (Figure 4b), consistent with the difference in scattering found. More details regarding the sediment fine fractions are presented below.

Validation of the use of turbidity as a resuspension proxy was found in the strong correlations between almost all suspension measures: TSS, NTU, FPV, and POC (Table 2). Using the slopes of linear regressions between these measures, several average quantities were calculated for the particle suspensions during the study. For example, the average organic carbon content of suspensions was 11.4 ± 0.4% and the average density was 1.3 ± 0.1 g ml^{-1}. The single metric that appeared somewhat independent of the others was CN.

Bacterial Populations

All three classes of bacteria measured in this study fluctuate over several orders of magnitude during the summer (Figures 5a and 6b). Comparisons between TSS, salinity, and bacterial concentrations show the influence of events that occur between monitoring events on the concentrations of both bacterial groups. Enterococci concentrations show some fluctuations where TSS peaks (i.e., resuspension) occurred (Figure 5). *Vibrio* concentrations were tightly related to salinity in bottom waters (Figure 6). For both bacterial groups, fluctuations captured *in situ* exceeded those in the bi-weekly monitoring data at the same site.

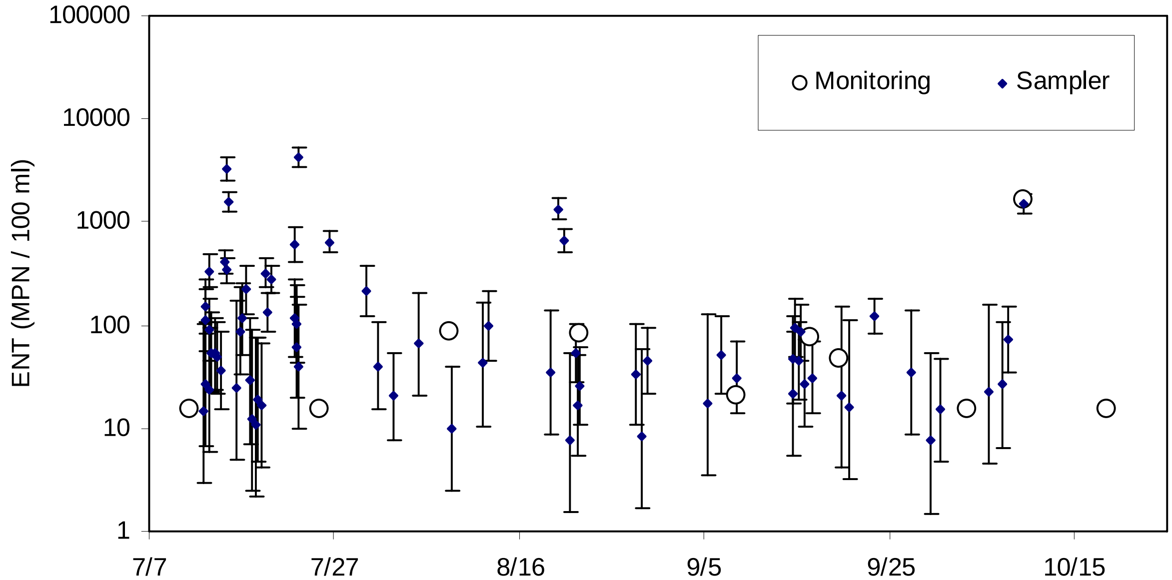
Correlations between bacterial populations and environmental parameters were sought to support links between concentrations, water conditions, and sediment resuspension (Table 3). FPV alone is presented for sediment concentration given the strong interrelationship between FPV, NTU, and TSS presented in Table 2. In general, correlation coefficients are smaller than those observed for suspensions, but the largest agree with previous observations as part of NRE monitoring. First, the strongest correlation found for enterococci concentrations was with CN, as reported by Fries et al. (in press) for the upper NRE. For *Vibrio*, salinity is the strongest predictor, as reported by Hsieh et al. (in revision) for surface waters in the entire NRE.

Sediment Cores

Sediment grain size distribution varies little through the summer (Figure 8). Given the dynamics observed, this was surprising; however, coring only took place on calm days which allowed fine particles to settle and be found on all coring dates. Cores contained less organic carbon (4.2 ± 0.3% by mass) than suspensions (11.4 ± 0.4%). Comparing these two fractions and assuming that POC is only derived from resuspension (assumption discussed below), the fraction of the surficial sediment being resuspended was 36.8% by mass. This compares well with the fine fraction (<63 μm) of the sediment samples (30 ± 11% by mass). This supports an assumption that most of the sediment-water exchange was the flux of fine particles carrying most of the POC and bacterial populations.

Sediment cores collected at the site provide a second set of time series for bacterial populations that do not strictly mirror water column fluctuations (Figure 9). Enterococci levels vary little through the summer with an average of 1494 ± 278 MPN per g sediment. *Vibrio* exhibited low concentrations in sediments until the period of wind in September. Following the storm, sediment concentrations increased significantly and remained high until the end of the program, after water column values started to fall. Of particular note, the levels found several months after the event (Feb 16, 2006) continue to be high for enterococci (852 MPN g^{-1}) and *Vibrio* (887 CFU g^{-1}).

Figure 5. Time series for enterococci and TSS as proxy for resuspension



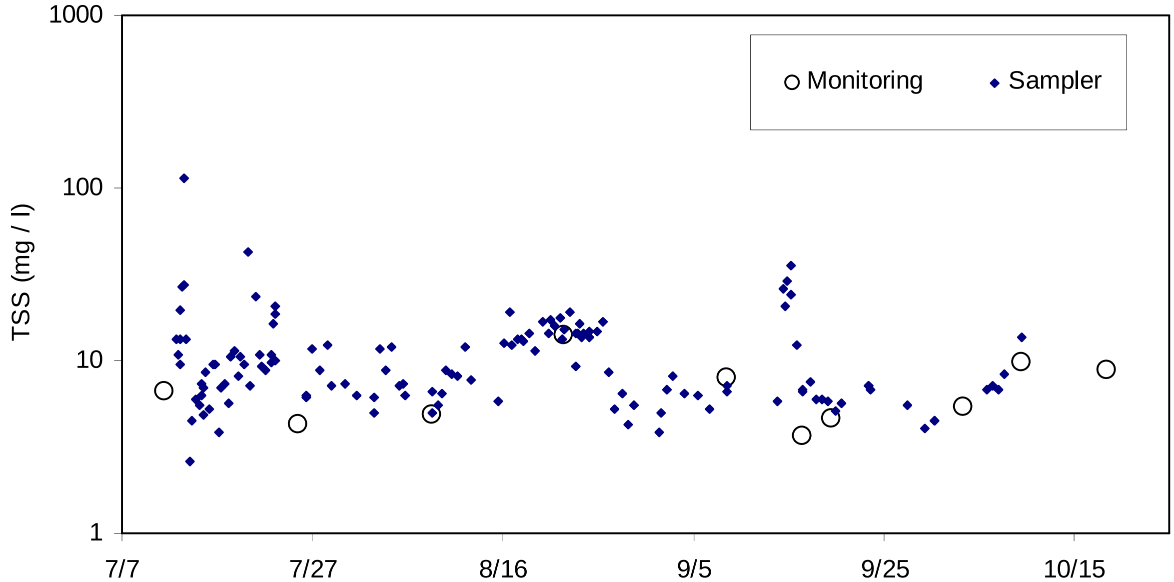
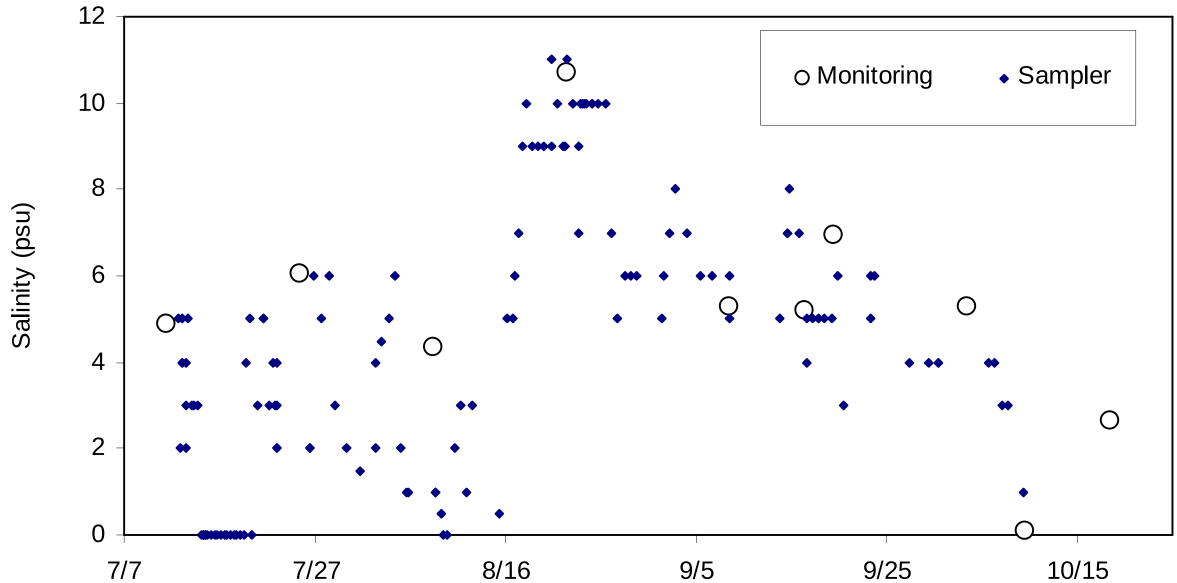


Figure 6. Time series for salinity and *Vibrio* concentration



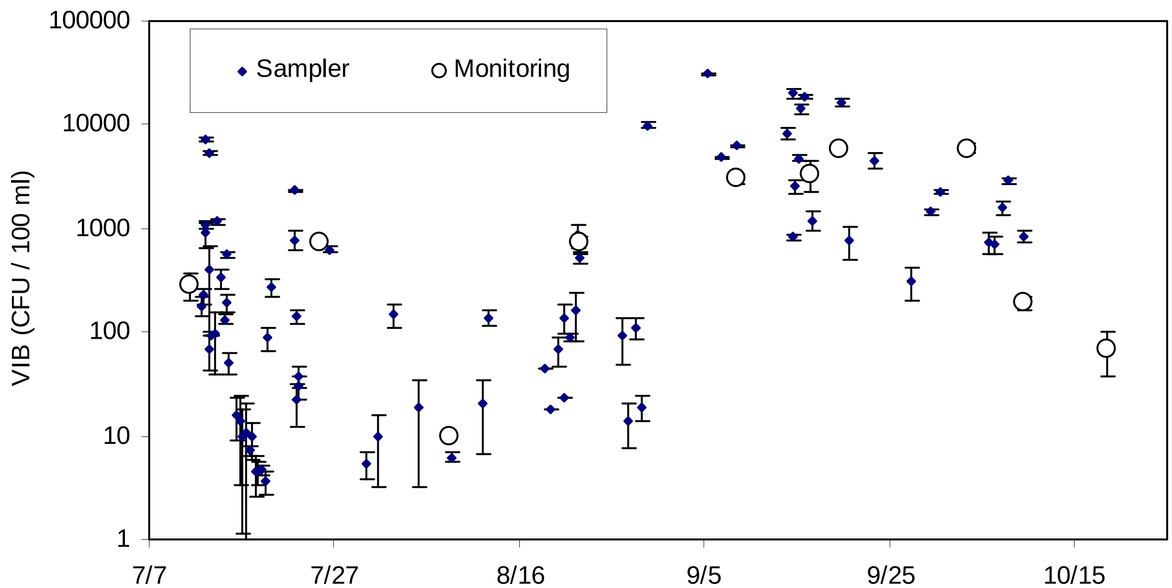


Table 2. Correlation table for particle suspension measurements. Bold values denote largest R values found.

	TSS	NTU	FPV	POC
NTU	**0.73**			
FPV	**0.58**	**0.45**		
POC	**0.83**	**0.63**	**0.50**	
CN	0.29	0.18	0.08	0.19

Table 3. Correlation table for bacterial and environmental measurements. Bold values denote largest R values found. ENT and VIB are concentrations of enterococci and *Vibrio*, respectively.

	Sal	FPV	CN	ln(ENT)
FPV	**0.44**			
CN	-0.06	0.08		
ln(ENT)	-0.20	0.11	**0.26**	
ln(VIB)	**0.43**	-0.01	-0.06	-0.05

Figure 7. Grain size distributions from all coring dates.

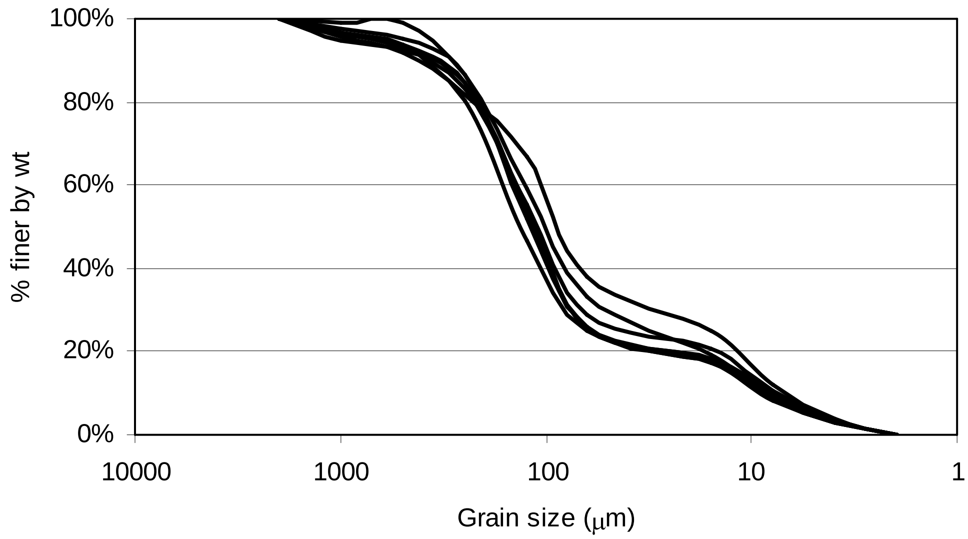
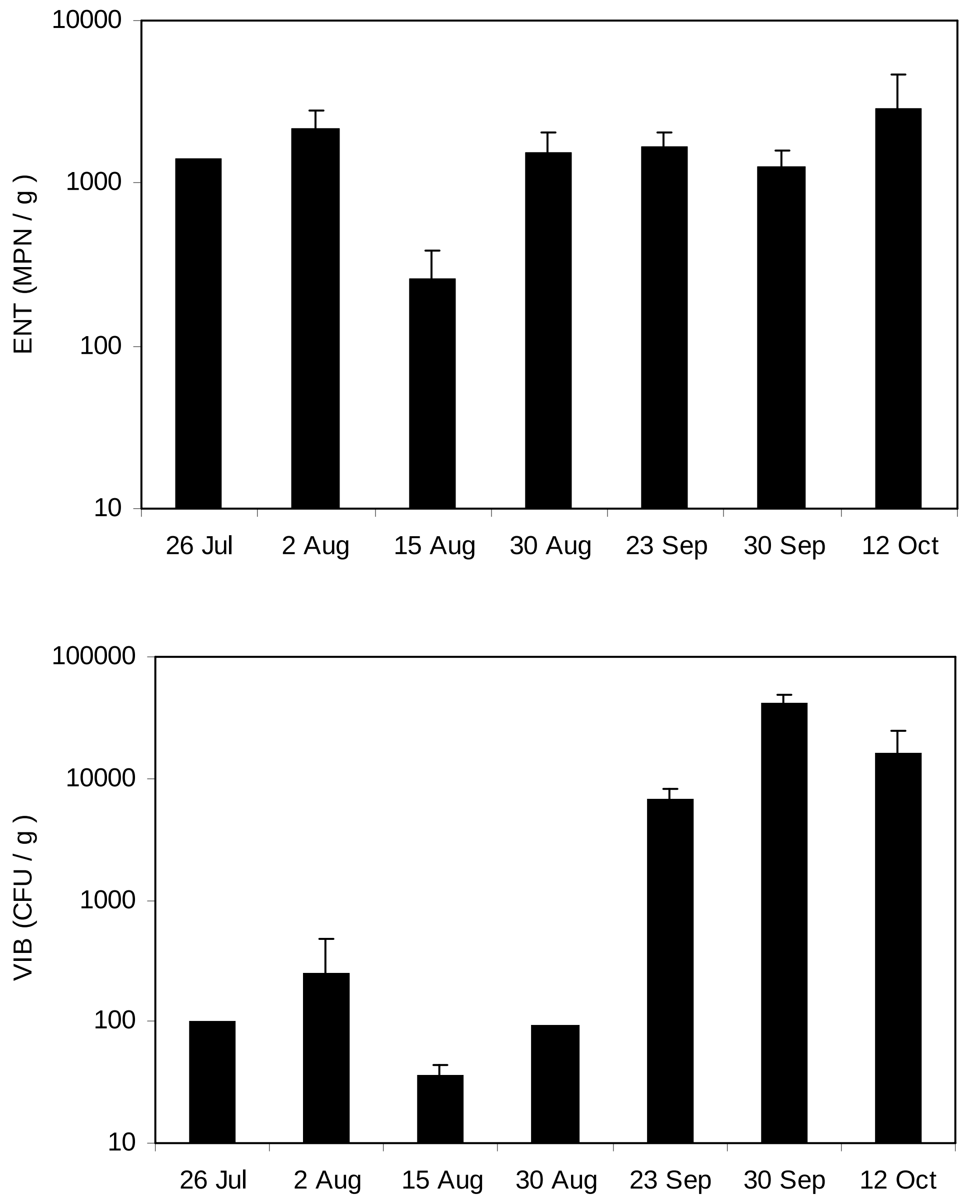


Figure 8. Core time series for both bacterial populations



DISCUSSION

Estuaries are dynamic places, and the NRE is no different. Our study area focused on the upstream reaches of the NRE, from stations 0 to 70, where we expected to see the largest stormwater runoff inputs and effects from upstream waters which are subject to heavy anthropogenic inputs. We have shown that enterococci can come from both the watershed or sediments, and signatures of attached particulate matter in the water column and sediments may allow us to create improved models of both indicator bacteria input and transport (Fries et al. in press). The levels of enterococci that were found in the NRE typically were either below or at the existing water quality thresholds (single measurement of 104 MPN per 100 ml for Tier 1 recreational waters in the State of NC). It is important to note that the water quality of the NRE is generally good and that episodic events (both wind and rain) are responsible for the majority of impaired waters in the estuary.

The case for sediments providing a constant source of enterococci can certainly be made based on the results of the sediment samples (Figure 8). The magnitude of this source, inferred from the fluctuations in bottom water, should be on the order of 100 to 1000 MPN per 100 ml. Based on the average sediment concentration (1494 MPN per g) and resuspension of 30% of the total sediment mass, an event with TSS of 100 mg/l would increase water concentrations only 50 MPN per 100 ml. While this level of contamination is significant compared to water quality standards, it falls short of the observed fluctuations. Reasons for this discrepancy include the advection of sediments from upstream areas with higher concentrations (e.g., closer to New Bern wastewater treatment plant) or increased metabolic activity during resuspension relative to sediment populations (more viable cells detected). Another possibility is the difference observed between resuspension and sediment fine fraction. The greatest difference between these particle size distributions was the fraction in the 16-64 μm range. Little mass of this size was found in suspension, but these particles made up 32% of sediment fines. Accounting for this difference increases the contamination estimate by a factor of 1.5 (to 75 MPN per 100 ml).

Differences in the particle size distributions between sediments and suspension counters the assumption that the sediment was the sole source of POC in suspension. Evidence for other sources of POC was found in the lower CN in suspension (4 to 14) than in the sediments (14 to 23). Likely sources of additional POC include runoff, algal cells, and bacterial biomass not associated with sedimentary sources. While the impact of these other sources were not seen in the total suspension measures, a ratio like CN would be far more sensitive to small inputs (in terms of mass) of high organic (low CN) particles. This result is consistent with the independence of CN with the other suspension measures (Table 2) and the stronger correlation between CN and enterococci, where changes in CN are more indicative of contaminated particle fluxes than changes in total particle mass or volume (Table 3).

While the mixture of particle types can not be resolved with the data collected in this study, there was quantitative evidence that a majority of the particles (FPV) at the sample site came from resuspension, rather than runoff. Fries et al. (submitted) used a model for wastewater particle size distributions (Ceronio and Haarhoff 2005) to distinguish runoff and resuspension in the NRE. The key statistic in this model (b) is analogous to the slope of the distribution with respect to diameter. Lower values of b describe distributions with peaks at large diameters (> 10 μm) and tended to describe suspensions in bottom waters (resuspended sediment) well. The time series data for FPV and b were in opposition (Figure 9), where large FPV corresponded with small b and vice versa. This pattern is consistent with particle suspensions that were predominately composed of resuspended sediment.

Vibrio concentrations in bottom water and sediment followed a different pattern than enterococci throughout the summer. The primary difference in these populations is the strong salinity dependence of *Vibrio* in the NRE (Pfeffer et al. 2003; Hsieh 2005) and in other shallow estuaries (Randa et al. 2004). This study differed from these others in terms of temporal sampling density at a single station, rather than spatial surveys with long periods between observations. Salinity still exhibited a large control on the *Vibrio* concentrations (ln(VIB) = 4.04 + 0.34*SAL; R = 0.43 : Figure 10) with larger coefficients than those reported for the NRE for surface waters during the summer of 2004 (ln(VIB) = 2.88 + 0.29*SAL : Hsieh et al in revision). The larger coefficients are not surprising given the large concentrations seen during the period of northeast winds before and during Hurricane Ophelia.

Although the salinity-*Vibrio* correlation is significant, there remains quite a bit of variability not explained by salinity alone. In fact, previous work on this subject (Hsieh 2005) has provided some evidence that the correlation with salinity may be tied into other factors that are strongly linked to salinity in the NRE. Examples include nutrient loads from runoff and interactions with algal populations that aggregate at salt fronts (Pinckney et al. 1998; Hsieh et al. in revision). These confounding factors are of less concern in the upper NRE; however, the mixing of the water column changes bottom water salinity and drives nutrient exchange with the sediments. The potential for confounding (and unmeasured) fluxes remains, although in a vertical rather than horizontal fashion. Future endeavors to sample *Vibrio in situ* must account for fluxes of dissolved constituents recognized to be important to microbial ecology.

Temperature dependence was not explored for two reasons. First, data from a single season provides little temperature variation for discerning trends. Second, the period of winds associated with Ophelia drove a cooling in water temperatures (Figure 11) coincident with vertical mixing, salinity increases, and rapid *Vibrio* concentration increases. Also, it is unlikely that temperature has a negative control on *Vibrio* populations given previous observations of positive relationships in this temperature range in the NRE (Pfeffer et al. 2003; Hsieh 2005).

The sediment *Vibrio* concentrations revealed an unexpected dynamic. Early in the summer, water column concentrations varied, while sediment concentrations remained low. The wind and hurricane period drove *Vibrio* increases in the water column that were unexpectedly reflected in the sediments. The difference in the two periods of high *Vibrio* was the long period of mixing and resuspension, followed by a significant runoff event. This succession describes a scenario where particle attachment and deposition act to store a large number of cells in the sediment bed. Previous observations in the NRE (Hsieh et al. in revision) have documented two trends in *Vibrio* attachment to particles. First, increased FPV was correlated with increased attachment. This describes an affinity to attach when more surface area is available and cell-particle contacts are more frequent. Second, decreased salinity was also correlated with increased attachment. This trend indicates that reduced salinity, which is unfavorable for growth and is controlled by runoff in the upper NRE, could induce attachment. Another possibility is the differential survival of attached organisms afforded refuge on particles as conditions worsen (water gets fresher). The sequence of bacterial growth (salinity from surge), resuspension (mixing from wind), then freshening water (runoff from rain) was a recipe for bacterial deposition in sediments. In this case, this deposition and storage occurred in a region of the Neuse where large *Vibrio* populations are rarely found.

Without the deployment of the TACO as part of this study and complementary sediment coring, the storage of *Vibrio* in the sediments of the upper NRE would have been difficult to detect. The storage of metabolically active cells has persisted at the site throughout the winter, as found in cores collected February 16, 2006. On several dates during routine NRE monitoring this past winter, *Vibrio* concentrations have been higher in bottom waters in the upper NRE than other stations further downstream (Figure 12). This was not observed in the winter of 2004-2005 and differs from our expectations based on salinity. It is possible that the effects of the winds associated with Hurricane Ophelia may extend through this coming summer and may impact the distribution of *Vibrio* in the NRE for some time.

The results of this study represent one component of the role of resuspension in estuarine water quality by uniquely combining observations of indicator and pathogenic microorganisms and particulate matter in the NRE. Given the size of the NRE and the many beneficial uses of its water to the communities around it, this project has the potential to demonstrate the importance of resuspension to water quality. For enterococci, a key indicator of fecal contamination, we have documented high concentrations of the bacteria not only in the water column after storms, but also in sediment cores. Similar results have been found for *Vibrio*. Our results demonstrate the importance of suspended material as a contributing factor to water quality measurements taken after heavy wind events. Fecal indicators (enterococci) and native bacteria (*Vibrio*) are not the only bacteria of public health interest that are likely to be found in the NRE. Other potential, more dangerous pathogens of concern include *Salmonella*, *Campylobacter*, *Yersinia*, *Listeria*, and possibly a range of enteric viral pathogens (Mark Sobsey, pers. comm.). It is clear that understanding the particle attachment characteristics of both bacterial pathogens and indicators is vital for successful management of our estuarine waters.

The NRE is a prime area for studying climatic events given the wide range of flow regimes to which the area is subject. During the fall of 1999, Hurricanes Dennis, Floyd, and Irene inundated coastal NC with up to 1 m of rainfall, causing a 100-500 year flood in the watershed of the Pamlico Sound (Paerl et al. 2001). During such intense events, NRE floodwaters can carry animal wastes, sewage and toxic chemicals via surface and groundwater downstream, contaminating water supplies in much of eastern NC. Bales (2003) observed a concurrent increase in diarrheal disease in flood-impacted areas, indicating the potential for microorganisms contained in these sources to adversely impact public health. These storms also greatly influence the water quality of the estuary through coastal inundation and storm surges, introducing higher salinity waters to regions of the estuary higher in the watershed (unpublished data). These events could have a lasting influence on these regions through the introduction and sedimentation of new microbial populations.

Through the support gained from this project, we have also been able to successfully design and build an autonomous sampling platform that permitted *in situ* sampling during times that boat use was impossible (heavy storms) and over important time scales (hours to days). The TACO sampling platform has already gained notoriety in the small field of estuarine sediment dynamics, and several other research groups have contacted us for a blueprint. In particular, researchers working in near-shore coastal environments that are heavily impacted by stormwater runoff are interested in building similar systems to characterize plume formation and dispersion. This study represents an important part of ongoing research demonstrating the importance of resuspension over short time scales in the NRE.

Figure 9. Time series of FPV and b statistic from particle size distributions

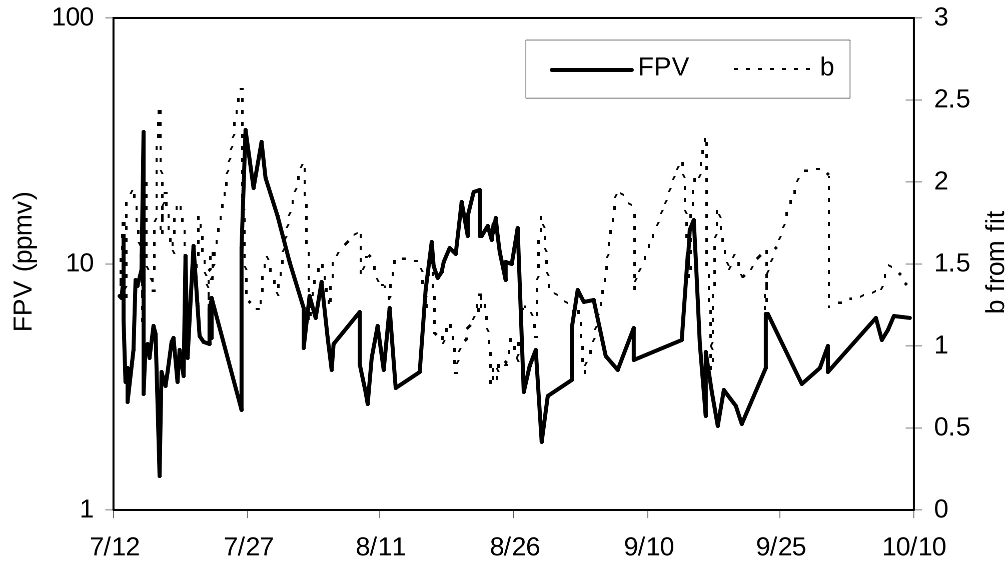


Figure 10. Comparison of *Vibrio* concentrations with salinity model

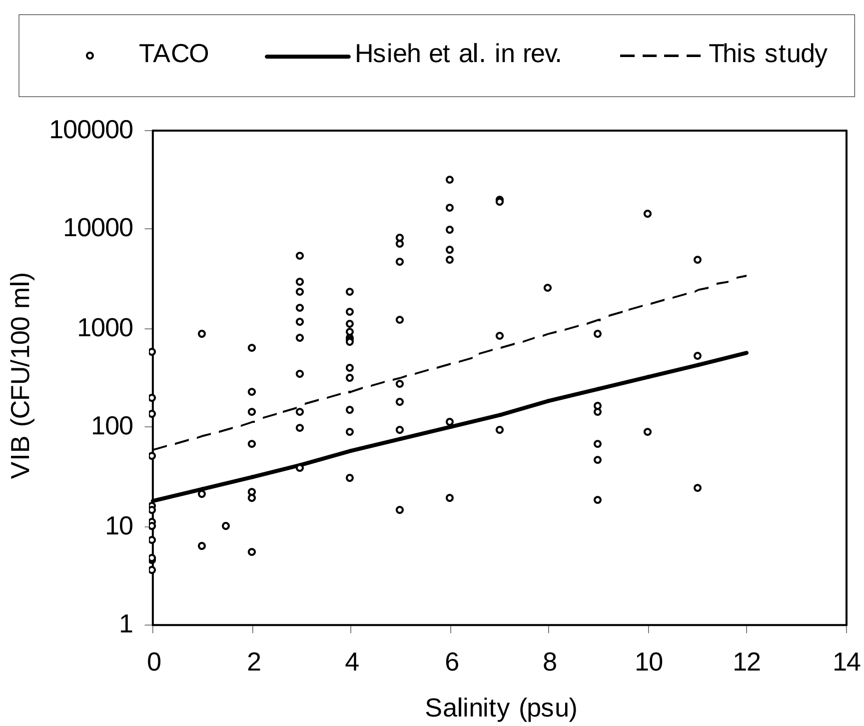


Figure 11. Temperature time series from TACO and USGS02092162 at study site.

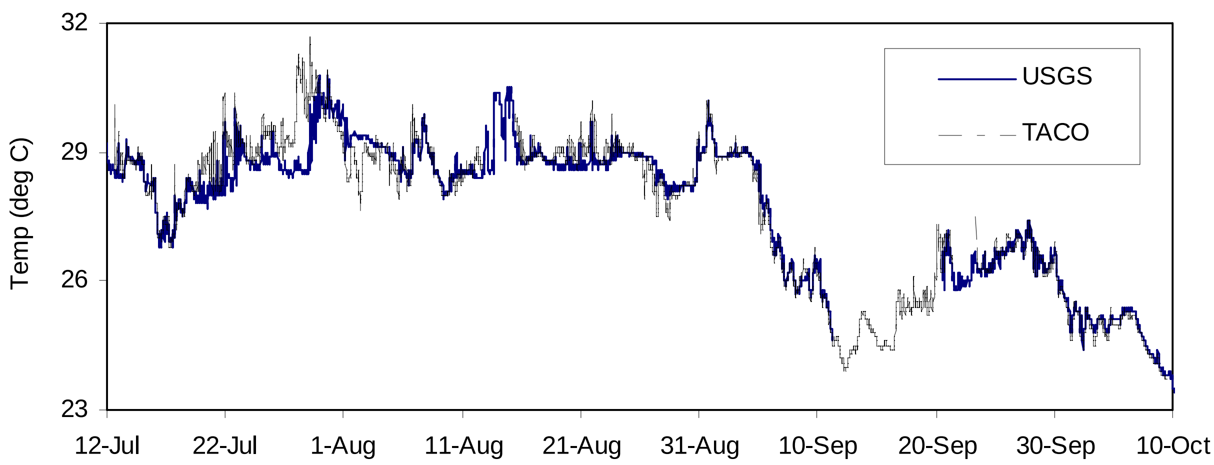
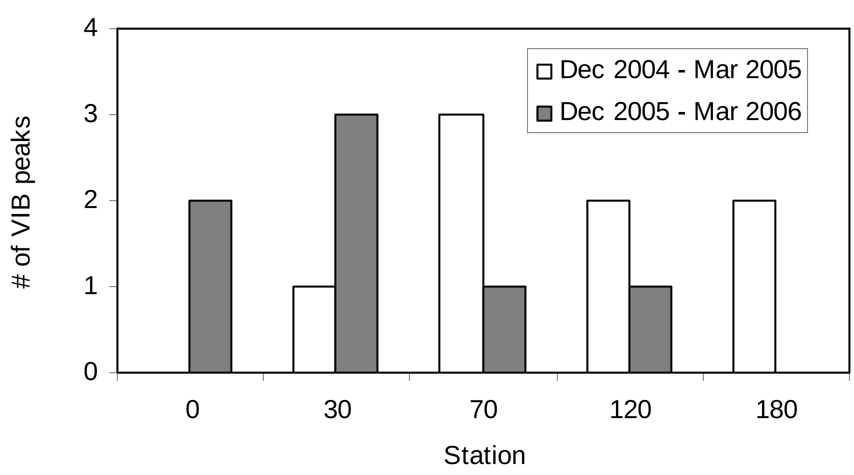


Figure 12. Frequency of peak *Vibrio* bottom water concentration found at stations in winter.



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