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IS THERE A RELATIONSHIP BETWEEN PHOSPHORUS AND FECAL MICROBES IN AQUATIC SEDIMENTS?

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ABSTRACT

This study examined the distribution and concentration of fecal indicator bacteria in sediments in diverse aquatic habitats in southeastern North Carolina, and examined their relationship to different measures of sediment phosphorus, hypothesized to limit aquatic bacteria, and other parameters, using both observational and experimental approaches. Sediment concentrations of fecal coliform, streptococcus, and enterococcus bacteria varied from 0 to 10^3-10^4 Colony-Forming Units (CFU) cm^{-2}. Concentrations of indicator bacteria types were significantly correlated with each other, but not with sediment phosphorus. Average concentrations of fecal coliform and fecal enterococcus bacteria, if totally suspended in one meter of water, were frequently higher than relevant regulatory standards for human body contact (coliforms: 200 CFU/100 ml; enterococcus: 33 CFU/100 ml) and significantly higher than shellfishing standard (coliforms: 14 CFU/100 ml). Thus, the concentrations of fecal indicator bacteria were problematic at many times and places in southeastern North Carolina in which exposure of the public to these contaminants is likely. Multiple regression analysis showed an effect of rainfall within the previous 24 hours, indicating that storm water runoff drove recruitment of fecal indicator bacteria to aquatic sediments. Fecal bacteria counts in a tidal creek following a large sewage spill attenuated within a few days in the water column but persisted much longer in sediments, and rose again after runoff from a rain event.

Experimental evaluations of the responses of fecal indicator bacteria to added phosphorus and organic substrate revealed that fecal bacteria responded positively to added P when background P levels were low, but organic carbon more generally stimulated growth of these bacteria populations. Preliminary evidence indicates that storm water runoff may be elevated in bio-available organic carbon, which should be considered in evaluating the effects of storm water pollution.

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SUMMARY AND CONCLUSIONS

Populations of fecal indicator bacteria and the pathogens whose presence they indicate are widely distributed and frequently very abundant in shallow aquatic sediments. Measures of fecal indicator bacteria in the overlying water column are far more frequently conducted, however. Water quality regulations are currently based on water column concentrations of indicator bacteria, even though sediments represent a significant reservoir and may support survival and growth of these contaminants. This study examined the distribution and concentration of fecal indicator bacteria in sediments from a diverse set of aquatic habitats in southeastern North Carolina, examined their relationship to different measures of sediment phosphorus, a macronutrient hypothesized to limit aquatic bacteria, as well as other parameters, using a combination of observational and experimental approaches. Finally, major sewage spills in one tidal creek watershed provided an opportunity to test ideas about sediment-associated fecal indicator bacteria and the factors supporting them.

The concentrations of fecal indicator bacteria (fecal coliforms, fecal streptococcus, and fecal enterococcus) were measured in sediments from a diverse set of aquatic habitats in southeastern North Carolina, including 76 sites in fresh water drainages in the Lower Cape Fear River basin and in fresh water and estuarine watersheds, at boat ramps and adjacent beaches, and in detention ponds in New Hanover County. Concentrations of fecal coliform, fecal streptococcus, and fecal enterococcus bacteria varied from 0 up to 10^3-10^4 Colony-Forming Units (CFU) /cm^2 of sediment. Concentrations of these indicator bacteria types were significantly correlated with each other. Our study is the first of which we are aware quantifying fecal enterococcus in aquatic sediments. Our results show that levels of this indicator are relatively higher than those of fecal coliforms, and the correlation between the two indicators gives confidence that enterococcus concentrations are similarly useful in indicating the presence of actual pathogens. Average concentrations of fecal coliform and fecal enterococcus bacteria, if totally suspended in a 1-m deep water column, were frequently higher than relevant regulatory standards for human body contact (200 CFU/100 ml for coliforms, 33 CFU/100 ml for enterococcus) and significantly higher than the standard for shellfishing waters (14 CFU/100 ml for coliforms). Thus, the concentrations of fecal indicator bacteria were indicative of problematic levels of contamination at many times and places in southeastern North Carolina, including situations in which exposure of the public to potentially harmful levels of these contaminants is likely.

It was originally hypothesized, based on published literature and previous studies in our laboratories, that concentrations of the macronutrient, phosphorus (P), in sediment matrices might limit survival and growth of sediment populations of fecal indicator bacteria. When sediment P as persulfate-labile P was measured and compared to simultaneous measures of sediment-associated fecal coliforms and enterococcus, no significant relationships were found. Concentrations of persulfate-P in sediments frequently exceeded the relatively low levels previously found to limit bacterial survival and growth. When sediment P was extracted and fractionated using a combination of techniques, including the standard Mehlich-III soil test extraction technique as well as

acid-extraction and combustion techniques, comparisons of fecal coliforms and enterococcus concentrations measured simultaneously with the P fractions similarly revealed no usefully significant relationships. Thus it was concluded that levels of sediment P generally exceeded those that limit fecal indicator bacteria populations in aquatic sediments in this region.

Authigenic P was the dominant portion of sediment P in most samples, and was positively correlated with M3-SRP values, which suggested an interaction of anthropogenically added P with natural or added soil minerals, especially calcium, which may be added to soils as lime or land plaster (gypsum). This fraction is not part of the soil test P evaluated and reported by the N.C. Division of Agronomy. The general predominance of authigenic P in these sediment samples indicated that conventional soil test procedures fail to measure most of the P in this landscape much of the time. If the correlation between M3-SRP and authigenic P reflects a chemical transformation of M3-SRP to authigenic P by reaction with soil minerals, then actual export of added P from agricultural fields might also be underestimated by use of conventional soil test P measures.

Pair-wise regressions of fecal indicator bacteria concentrations against other parameters from intensive sampling in the Bradley Creek watershed in New Hanover County revealed effects of salinity and temperature on fecal coliform concentrations in sediments, but multiple regression analysis showed an effect of rainfall within the previous 24 hours. The latter result indicated that storm water runoff was an important mechanism driving recruitment of fecal indicator bacteria to aquatic sediments. The significant negative relationship between salinity and fecal coliform concentrations in sediments also indicated a relationship between runoff and bacterial recruitment to sediments.

Experimental evaluations of the responses of fecal indicator bacteria to combinations of added phosphorus and organic substrate revealed that fecal bacteria did respond positively to added P when background P levels were low; however, field sites where sediment P levels were that low were largely restricted to forested areas. Urbanization of forested areas, or transformation to agriculture will likely cause increased sediment P levels that would remove P limitation of fecal bacterial growth. Experimental organic carbon additions more frequently and more generally stimulated growth of these bacteria populations. Preliminary evidence indicates that storm water runoff may also be elevated in bio-available organic carbon, so this may be an additional factor to be considered in evaluating the effects of storm water runoff on bacterial contamination in surface waters and sediments.

Major sewage spills in the Hewletts Creek watershed in the summer of 2005 provided an additional opportunity to study recruitment and survival of sediment-associated fecal indicator bacteria. Results of an intensive monitoring effort after the first major spill, on July 1, 2005, showed that the concentrations of fecal indicator bacteria attenuated faster in the water column than in sediments, and that bacteria in the sediments survived at higher relative levels longer than in the water column. The sudden introduction of large

amounts of fecal contamination to the watershed and subsequent large increases in sediment concentrations supported the statistically significant relationship between runoff events and sediment concentrations described above. Sediment bacteria information was used to justify continuing closure of the Hewletts Creek basin to swimming, and supports the argument that sediment contamination by fecal bacteria after such spills should be considered by public health officials and regulations.

RECOMMENDATIONS

Our research, along with our studies of the July 1 sewage spill demonstrated that following a major pollution incident where human or animal waste is involved, sampling the water column for fecal bacteria is not sufficient to obtain a complete picture of the system in terms of human health issues. Large quantities of the polluting bacteria settle to the sediments and remain viable for weeks to months, and are subject to resuspension in the water column after a mixing event. Fecal bacteria on or in the sediments are largely protected from UV radiation, a principal means of death or deactivation in the water column. Also, the sediments contain carbon, nitrogen, and phosphorus, key nutrient for survival and growth. We recommend that regulatory authorities devise sampling and assessment plans for pollution incidents that consider sediment-associated fecal bacteria. Furthermore, sediment sampling of fecal indicators should be included in normal monitoring programs by regulatory agencies, as these sediment-associated fecal microbes represent a readily available source of pollution to the overlying water column.

During the sediment phosphorus sampling portion of this project it became evident that standard laboratory procedures for sediment P analysis underestimate an important component of sediment P, authigenic P. The general predominance of authigenic P in these sediment samples indicates that conventional soil test procedures fail to measure most of the P in this landscape much of the time. If the correlation between M3-SRP and authigenic P reflects a chemical transformation of M3-SRP to authigenic P by reaction with soil minerals, then actual export of added P from agricultural fields might also be underestimated by use of conventional soil test P measures. We recommend that regulatory agencies and researchers revise soil P tests so that they will account for all major fractions of P in soils.

Introduction

Microbial contamination of surface waters, particularly by fecal material, is one of the most challenging problems facing environmental managers, necessitating closures of large areas of otherwise economically valuable waters to shellfishing and recreational uses. North Carolina currently has over 300,000 acres of estuarine waters closed to shellfishing (N.C. Shellfish Sanitation Section records), and 40,000 acres conditionally closed to shellfishing following rain events (Mallin et al. 2001a). Selected recreational areas receive periodic bathing advisories owing to microbial contamination (N.C. Shellfish Sanitation Section records). The potential threats to human health represented by these closures add to the economic damage they actually cause. Remediation of this contamination is clearly important for economic and public health reasons (Maiolo and Tschetter 1981).

Research indicates that P is the key limiting factor for bacterial populations in many aquatic ecosystems, including lakes (Currie 1990; Morris and Lewis 1992), the coastal ocean (Bjorkman and Karl 1994; Cotner et al. 2000), and salt marshes (Sundareshawar et al. 2003). Kirchman (1994) found that heterotrophic bacteria are responsible for a large portion of inorganic P uptake in lakes, much more than for ammonia uptake. Mallin et al. (2001b, 2002a, 2004) demonstrated P stimulation of ATP, heterotrophic bacteria, and BOD in two blackwater streams and two blackwater rivers in the NC coastal plain. Nitrogen inputs directly stimulated the autotrophs (phytoplankton), but not the heterotrophs. A recent study by an M.S. student in the Marine Science program at UNC Wilmington examined the effects of sediment P levels on fecal coliforms in relatively undeveloped estuarine watersheds and in experimental microcosms (Rowland 2002). At field sites, increases in persulfate-oxidizable P levels ("persulfate-P") correlated with increasing sediment fecal coliform levels. Manipulations of persulfate-P levels in experimental microcosms across the same range of concentrations observed in the field studies yielded similarly significant correlations with fecal coliform populations (Fig. 1). These results suggested that P may limit fecal microbial populations in shallow aquatic sediments, at least when sediment P levels are otherwise low.

Phosphorus pollution of surface waters is widespread (Correll 1998), but nitrogen pollution has received more attention owing to problems with algae blooms (Bricker et al. 1999). Sources of P pollution include domestic and wild animal wastes, human wastes, and fertilizers. Mechanisms of P loading to surface waters include both permitted and improper point discharges of wastes and non-point runoff. Our previous research has shown strong positive correlations between total suspended solids and P in wet detention ponds (Mallin et al. 2002b). Research has shown that human uses of fertilizers and animal wastes have elevated soil levels of P (Cahoon and Ensign 2004) and increased discharges from land to coastal waters (Liu et al. 1997; Daniel et al. 1998; Sharpley and Tunney 2000). Cahoon (2002) showed that fertilizer use in residential neighborhoods was linked to elevated sediment P concentrations in the Bradley Creek watershed, where elevated sediment fecal coliform levels also occurred.

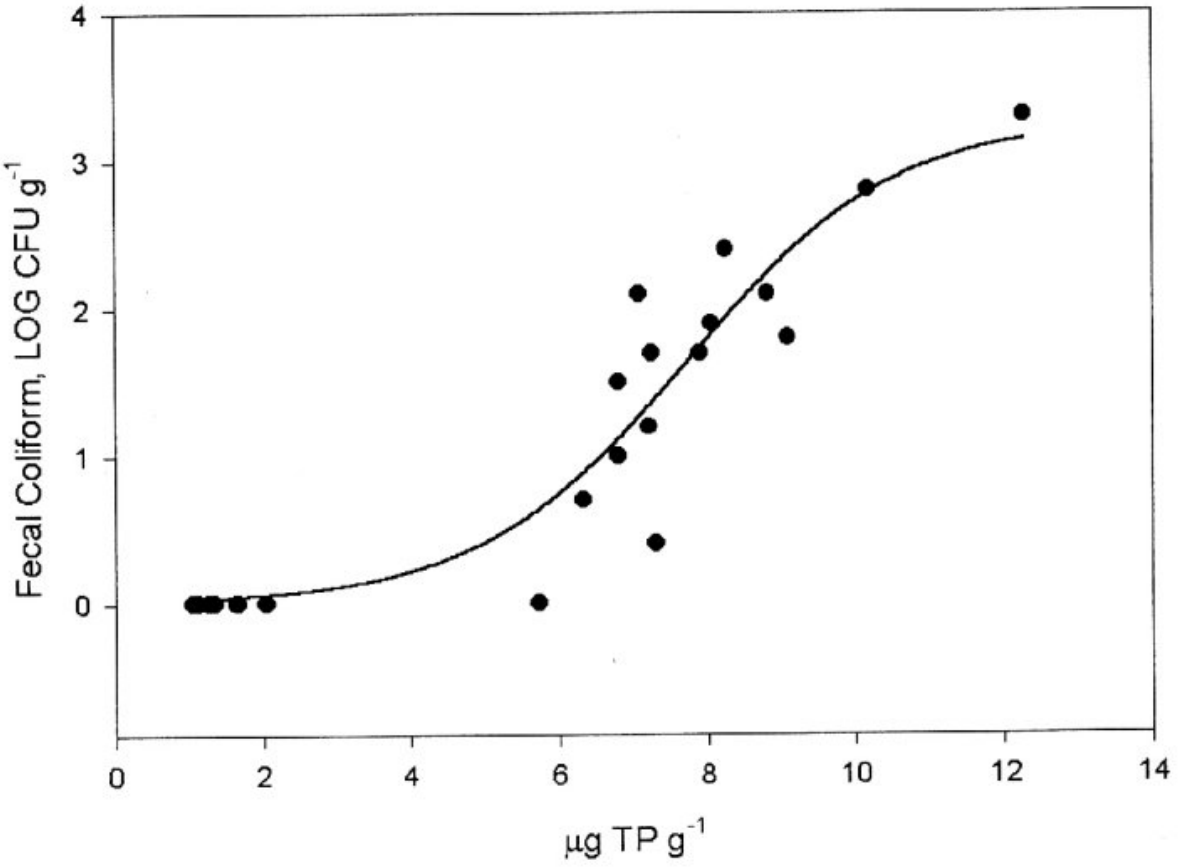

Fig. 1. Response of fecal coliform bacteria in sediment microcosms to added P as KH_2PO_4. For regression F= 72.7, df=2,22, $p < 0.0001$. Total P analyzed as persulfate P (Valderrama, 1981). Figure from Rowland (2002).

Phosphorus (P) pollution is well known to contribute to eutrophication problems in North Carolina's waters. A phosphate detergent ban in 1989 (N.C. D.E.M. 1991) and other measures to limit point and non-point source discharges of pollutants have implicitly recognized the potential for phosphorus to cause water quality problems. Recent increases in soil P levels in much of eastern North Carolina, caused by application of animal manures and use of commercial fertilizers, have resulted in a stronger emphasis on agricultural P management, evidenced by North Carolina's promulgation of a "Phosphorus Loss Assessment Tool" (PLAT; Osmond et al. 2005). However, the rationale for these management measures has been control of algal blooms. Only recently has the importance of P limitation of bacteria in aquatic ecosystems been recognized.

Objectives

We originally hypothesized that phosphorus concentrations limit the concentrations of fecal indicator microbes in aquatic sediments, and proposed to use both observational and experimental approaches to test this hypothesis. Therefore, we identified two main and several corollary objectives:

1. Determine the relationship between sediment phosphorus levels and sediment concentrations of fecal indicator bacteria (fecal coliforms, streptococci, and enterococci) in several different watersheds in eastern NC:

- Sample in a wide range of habitats to provide a sufficient range of parameter values to detect a relationship if one exists.
- Describe the concentrations and variability in fecal indicator concentrations.
- Determine the relationship between sediment fecal indicator concentrations and P levels.
- Define the components or fractions of "sediment P" using different extraction and measurement techniques.
- Determine the relationships, if any, between fecal indicator concentrations and other potentially relevant parameters.

2. Determine experimentally the response of fecal microbial concentrations in sediments to manipulated variations in phosphorus and organic substrate availability.

An event we could not foresee when we wrote our original proposal, in the form of a major sewage spill and subsequent runoff event, provided an opportunity to evaluate the response of populations of sediment fecal indicator bacteria to sudden fecal contamination uncoupled from storm water runoff. On Friday, July 1, 2005 the middle branch of Hewletts Creek at Pine Grove Road was subjected to a raw sewage spill of 3,000,000 gallons (~11,360 m^3). This occurred when a 60 cm force main coupling repair burst apart. This line carried sewage from Wrightsville Beach to a pump station on Bradley Creek, then to a pump station (#34) on Hewletts Creek (near the breach) then on to the Wilmington South Side Wastewater Treatment plant on River Road (the plant discharge is near Channel Marker 54 in the Cape Fear River Estuary). This line had been built in the mid 1980s using EPA funds. A citizen called the City at approximately 6:20 AM with a complaint; city workers were on site at 7:10 AM and found an obvious major leak. The workers turned the pump off but sewage continued to flow into the creek. During the course of the day they dug down 2-3 m to find the problem, finally finishing a temporary repair at 10:30 PM. The workers estimated that the spill had begun about 5:00 AM; thus the sewage spill occurred over a near-18 hour period (Hugh Caldwell, City of Wilmington, personal communication). Some waste flowed into the creek or nearby swamp forest, and some flowed into the nearest storm drains, which drain directly into Hewletts Creek. Both the North Carolina Division of Water Quality (DWQ) and the N.C. Shellfish Sanitation Section were alerted that morning, and as a result the N.C. Division of Marine Fisheries closed the creek and a large section of the Intracoastal Waterway (ICW) to shellfishing, and Shellfish Sanitation

closed that area to swimming. This section of the ICW encompassed the area between the Wrightsville Beach Bridge and ICW Channel Marker 141 near Peden Point, including all tributaries between.

We requested and received an extension of our funded research project to take advantage of the opportunity this spill event presented to study fecal contamination of sediments, adding another objective to our original set:

3. Determine the response of sediment populations of fecal indicator bacteria to human sewage spills and runoff events.

Methods and Materials

Sampling Locations: Sampling was conducted at several sets of locations in southeastern North Carolina. One set of locations was distributed through portions of Duplin and Pender counties, and included several sites sampled on a monthly basis since 1995 by the Lower Cape Fear River Program (LCFRP), directed by co-PI Mallin (www.uncw.edu/cmsr/aquaticecology/lcfrp/) in addition to 48 sites selected as representative of natural and man-made drainage features (Fig. 2). These were fresh water sites in mostly rural locations and were sampled once or twice for this project. Another set of sampling locations included 11 public and private boat launching ramps and five nearby shoreline locations for comparison, where people come into direct contact with water and sediments, throughout New Hanover County, the most urbanized of North Carolina's 20 coastal counties (Fig. 3). These sites were sampled several times on a monthly basis for this project. Another set of locations sampled for this project included a large number of stream and pond sites in central New Hanover County (Fig. 4), sampled once or twice for this project. Most of these stream locations were also sampled on a monthly basis by the City of Wilmington Watersheds/New Hanover County Tidal Creeks Program (www.uncw.edu/cmsr/aquaticecology/TidalCreeks/), also directed by co-PI Mallin, but a number of detention ponds were included for the purposes of this project. A set of sites in the Bradley Creek watershed, including several also sampled monthly by the New Hanover County Tidal Creeks Program, was sampled intensively (monthly since January, 2003) as part of previous research projects and for this project (Fig. 5). Finally, we sampled in the Hewletts Creek watershed in response to a major sewage spill in the middle branch of that creek in July, 2005 and again in September, 2005 (Fig. 6).

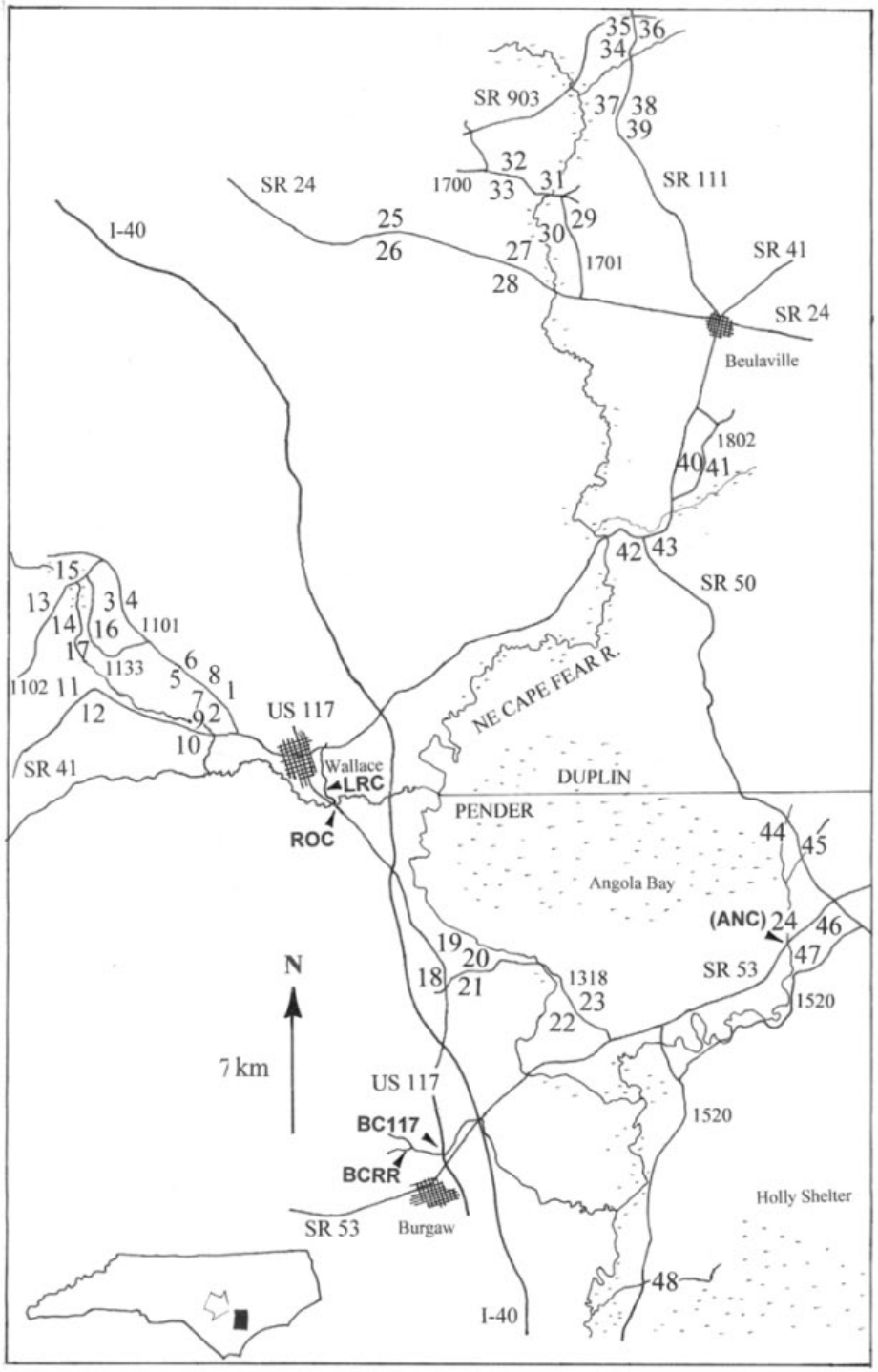

Fig. 2. Map of portions of Duplin and Pender counties in southeastern North Carolina, showing drainage feature sites sampled for sediment P fractionation (numbered 1-48), LCFRP sites sampled for sediment fecal indicator bacteria and persulfate P (Angola Creek=ANC, Burgaw Creek at Wright St.=BCRR, Burgaw Creek at US 117=BC117, Little Rockfish Creek at NC 11=LRC, Rockfish Creek at US 117=ROC), as well as adjacent roadways, towns and other features.

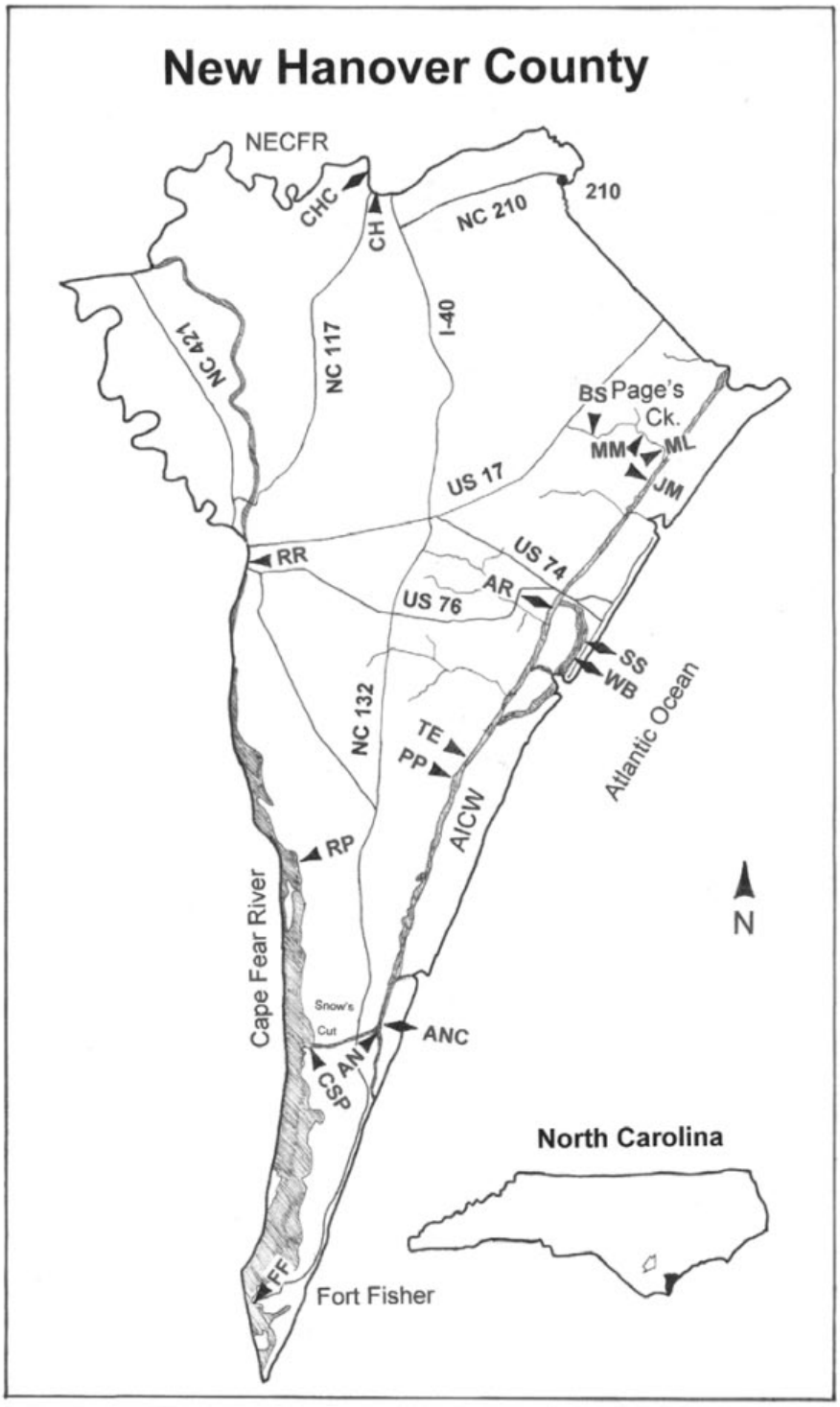

Fig. 3. Map of New Hanover County in southeastern North Carolina, showing sampling locations at boat ramps (double letter names, triangles), nearby control beach sites (triple letter names ending in “c”, diamonds), an experimental site at the intersection of NC 210 and Prince George Creek (“210”, solid circle), as well as major roads and geographic features.

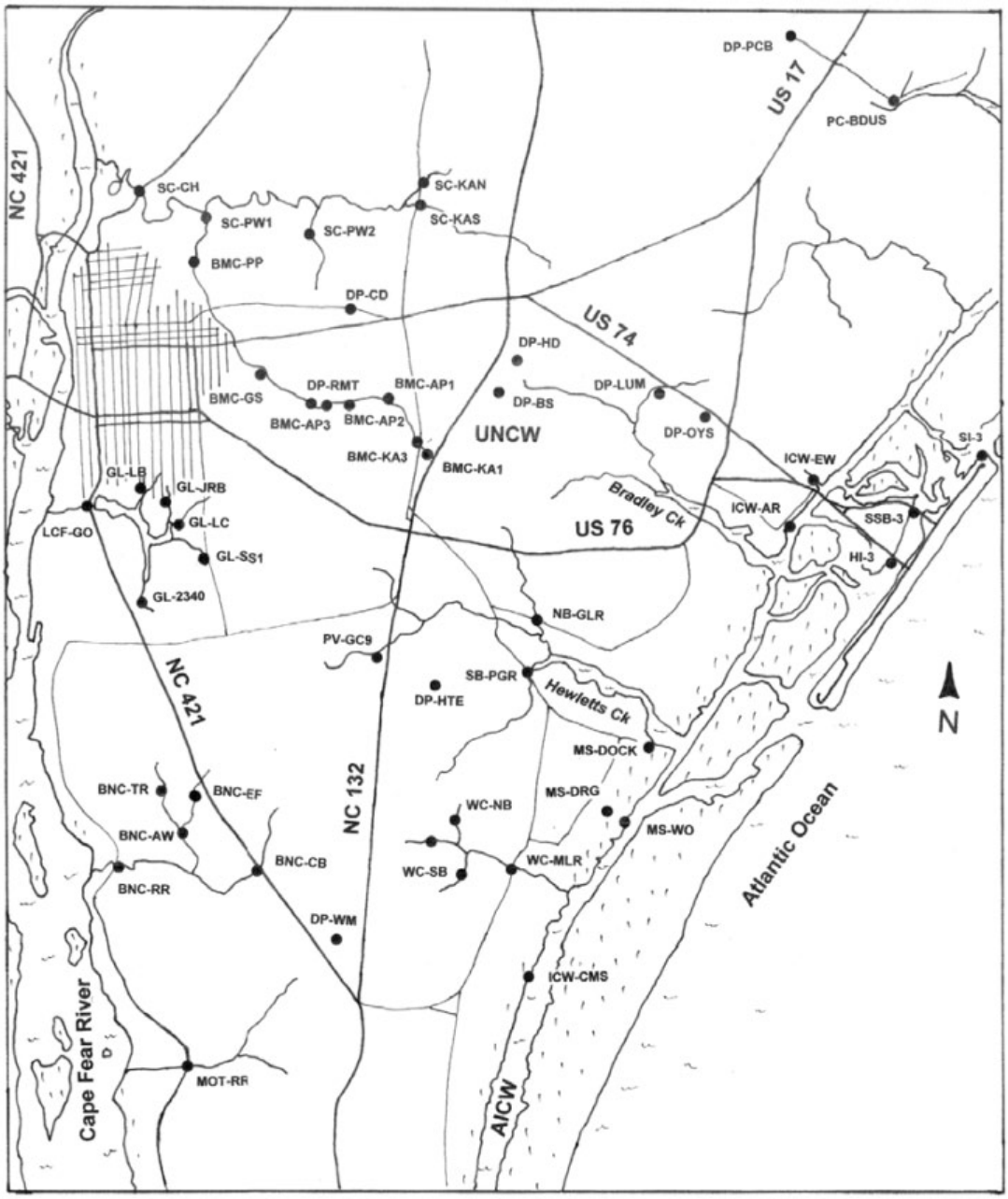

Fig. 4. Locations in central New Hanover County sampled for fecal indicator bacteria and persulfate P concentrations. Sites are grouped and named by watershed (BMC=Burnt Mill Creek, BNC=Barnard's Creek, GL=Greenfield Lake, HI=Harbor Island, ICW=IntraCoastal Waterway, MOT= Mott's Creek, MS=Marine Science lease sites, PC=Pages Creek, SC=Smith Creek, SI=Shell Island, SSB=Salisbury Street Bridge, WC=Whiskey Creek) or as detention ponds (DP); Sites in Bradley and Hewletts creek watersheds are shown in Figs. 4 and 5, respectively.

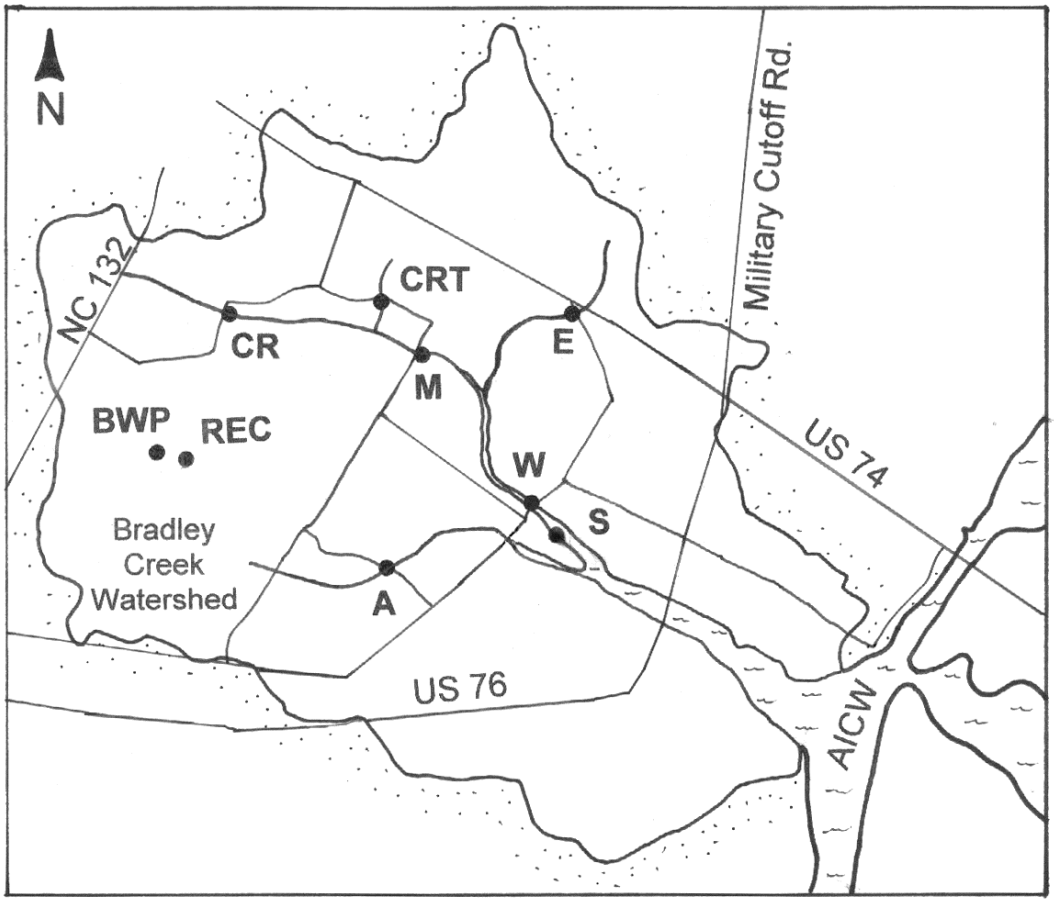

Fig. 5. Map of Bradley Creek watershed, showing long-term locations at which sampling for sediment fecal indicator bacteria and relevant environmental parameters took place (A, CR, E, M, S, and W), sites from which samples for experimental studies of fecal indicator responses to limiting substrates were obtained (BWP, CRT, E, and REC), as well as major roadways and other features.

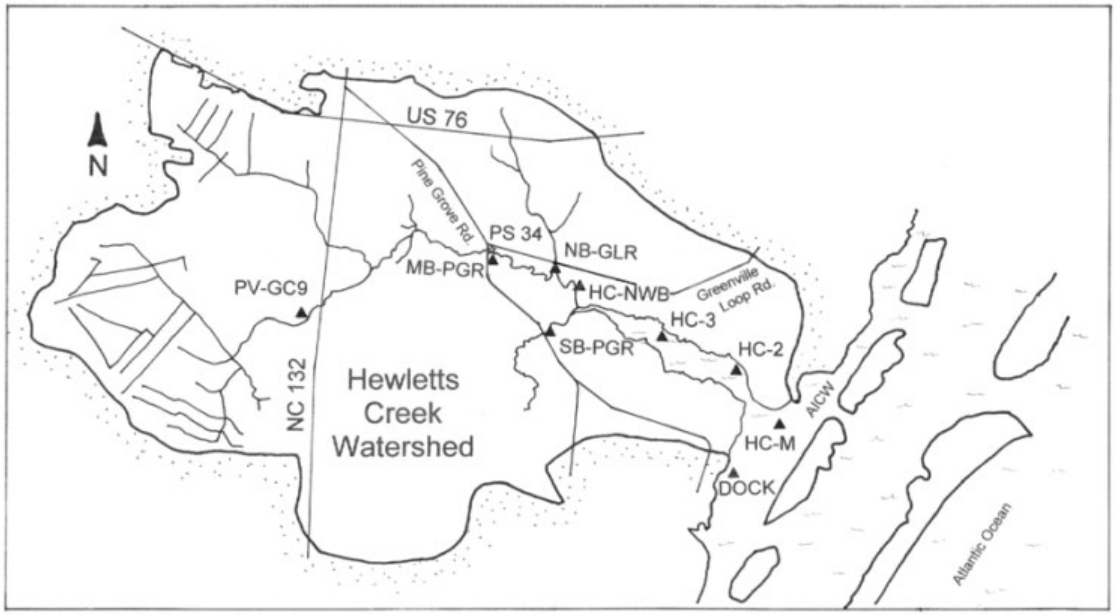

Fig. 6. Map of Hewletts Creek watershed showing sites sampled in response to sewage spills at Pump Station 34 (PS34) as well as major roadways and other features.

Fecal Indicator Bacteria: Sediment samples for analyses of sediment fecal microbial concentrations were collected in triplicate using sterile 2.3 cm diameter coring tubes. Sediment fecal coliforms, fecal streptococci, and fecal enterococci were analyzed using a suspension, dilution, and filtration procedure. Individual cores were suspended with stirring for two minutes using a magnetic stirrer in one liter of phosphate buffer solution (0.25M KH_2PO_4, pH adjusted to 7.2 with 0.1N NaOH). Triplicate aliquots (usually 10 ml or one ml) were withdrawn from stirred suspensions using sterile volumetric pipets and immediately filtered onto sterile membrane filters (0.45 μm pore size, 47 mm diam), after which standard incubation and plate count techniques were used for each group of fecal indicator bacteria. Triplicate filters for determination of fecal coliform bacteria were incubated for 24 hours at 44.5 ^{o}C in a Fisher Isotemp digital water bath on mFC agar (Fisher/Difco), following method 9222D (APHA 2001). Similarly, triplicate filters for determination of fecal streptococci were incubated for 48 hours at 35 ^{o}C in a Fisher Isotemp digital water bath on mEnterococcus agar (Fisher/Difco), following method 9230C.3.b (APHA 2001). Finally, triplicate filters for determination of fecal enterococcus bacteria were incubated for 48 hours at 41 ^{o}C in a Fisher Isotemp digital water bath on mE agar (Fisher/Difco), following method 9230C.3.a (APHA 2001). Bacterial colonies satisfying the respective criteria for each method were counted after incubations using an Olympus SZ-III stereo-microscope. Colony counts were expressed as Colony-Forming Units (CFU)/100 ml of suspension and calculated as CFU g^{-1} and CFU cm^{-2} of cored sediment.

Sediment Parameters: Sediment samples for analyses of chemical parameters were taken in triplicate by the same coring methods as for bacterial analyses. Triplicate cores for the respective sets of analyses were selected randomly from each set of six cores taken at each sampling location and time. Sediment cores destined for chemical analyses were iced immediately, frozen initially at -20 ^{o}C, then stored at -85 ^{o}C for 24 hours prior to lyophilization using a Virtis Benchtop 3.3 Vacu-Freeze lyophilizer. Lyophilized sediment samples were homogenized and stored in sealed containers at room temperature prior to sub-sampling for chemical analyses.

Sediment P analyses were conducted by two methods. Valderrama's (1981) boric acid-persulfate digestion method employs boric acid-persulfate digestion of a pre-weighed, dry sediment sample by autoclaving, followed by analysis of orthophosphate using the standard molybdenum-blue method (Parsons et al. 1984). Reagent blanks were prepared for each batch of samples, and blank values subtracted from sample values. Standardization with sodium glycero-phosphate yielded approximately 98% recovery. [Sediment P] was expressed as *u*g P (g sediment)$^{-1}$ and as *u*g P cm^{-2}. This method was used for samples from the LCFRP and Bradley Creek sites for consistency with the longer sediment P data base from those collections, dating back to 2002, and for comparison with the results of Rowland (2002), whose findings prompted this study. We think the persulfate-P method more accurately extracts and represents bio-available P than more vigorous digestion techniques commonly used for total sediment-P analyses, i.e., perchloric acid-nitric acid digestions (Strickland and Parsons 1972; Cahoon et al. 1990).

We also adapted the modified SEDEX sequential sediment P extraction technique of Anderson and Delaney (2000) to estimate the different fractions of P in sediments. Lengthy experimentation with the specific technique published by Anderson and Delaney (2000) identified several problems with its chemistry for our sample matrices, however. This led us to employ a combination of the standard Mehlich III soil testing technique (Mehlich, 1984) and modified SEDEX techniques to distinguish and quantify sediment P fractions. A four-part sequential extraction and measurement procedure was used to partition soil P into fractions. Step one was extraction of 2.0 g dried, homogenized sediment by shaking for five min with 20 ml of the Mehlich III reagent (0.2N acetic acid, 0.25N ammonium nitrate, 0.013N nitric acid, 0.015N ammonium fluoride, 0.001M EDTA). Following centrifugation, five ml of supernatant was analyzed directly for soluble reactive phosphorus (SRP) by the molybdenum-blue spectrophotometric technique (Parsons et al. 1984). This fraction was designated M3-SRP. Another nine ml sub-sample of the extract was digested by autoclaving with one ml boric acid - potassium persulfate reagent (Valderrama, 1981) to convert organic phosphates to ortho-phosphate, and then analyzed for SRP as above to yield Mehlich-III extractable total phosphorus, designated M3-TP. Mehlich III-extractable organic phosphorus, calculated by subtraction of M3-SRP from M3-TP, was designated M3-OP. Standardization of the molybdenum-blue analysis was performed with each batch of samples using 3.0 *u*M KH_2PO_4. Step two was based on the modified SEDEX procedure for analyses of authigenic P and particulate organic P, respectively (Anderson and Delaney 2000). The solid residue from the Mehlich III extraction was digested in 13 ml 0.01 N H_2SO_4 for 16 hr with stirring, after which 10 ml of the supernatant was used for molybdenum-blue spectrophotometry, as above, and the calculated results designated as authigenic P. Finally, the remaining residue was treated with one ml of 50% $MgNO_3$, dried, combusted for two hr at 550^oC, digested in 13 ml 0.01 N H_2SO_4 for 24 hr, then analyzed by molybdenum-blue spectrophotometry and designated as particulate organic phosphorus (POP). Soil P fractions were calculated as *u*g P g sediment^{-1}. The average of three replicate values from each sample site was calculated along with standard deviation for each P fraction. This method was employed for samples collected at boat ramp sites and drainage feature sites in Duplin and Pender counties.

The carbohydrate content of sediment samples was analyzed by the phenol-sulfuric acid method of Underwood et al. (1995). Approximately five mg of lyophilized, homogenized sediment was suspended in 1.0 ml of distilled water, to which 0.5 ml of 5% aqueous phenol solution and 2.5 ml of conc sulfuric acid were added while stirring vigorously. Resulting absorbance was measured at 485 nm on a Milton-Roy Spectronic 401 spectrophotometer in a one cm cuvet against a reagent blank. Standard curves were established each time the assay was performed using a dilution series of dextrose ($C_6H_{12}O_6$) and carbohydrate contents were expressed as μg C (g sediment)$^{-1}$. The average of three replicate values from each sample site was calculated along with standard deviation.

Water Quality Parameters: Water column data were collected as part of the City of Wilmington Watersheds/New Hanover County Tidal Creeks Program, with supplemental

funding from the City of Wilmington to collect and analyze post-sewage spill samples in Hewletts Creek. Water column field parameters (water temperature, pH, dissolved oxygen, turbidity, and conductivity) were measured at each site using a YSI 6920 Multi-parameter Water Quality Probe (sonde) linked to a YSI 610 display unit and calibrated prior to each sampling event. At other locations and times water temperature, conductivity, salinity, and dissolved oxygen data were collected using a hand-held YSI 85 Multi-Parameter water quality meter, calibrated prior to each use. Nitrate+nitrite (= 'nitrate') and orthophosphate were measured in triplicate following flitration using standard wet chemistry techniques (Parsons et al. 1984). Samples for total nitrogen (TN) and total phosphorus (TP) were collected in duplicate on site as well and were digested using a persulfate/boric acid agent following Valderrama (1981). Nitrate, orthophosphate, TN and TP were analyzed at the UNCW Center for Marine Science Nutrient Laboratory by a Bran & Luebbe AutoAnalyzer 3. Samples for ammonium were collected in duplicate, field-preserved with phenol, stored on ice, and analyzed in the laboratory according to Parsons et al. (1984). Fecal coliform samples were collected and determined using the mFC method (9222D, APHA 2001). Chlorophyll *a* samples were analyzed in triplicate for filtered samples extracted in acetone according to Welschmeyer (1994) and US EPA (1997, using a Turner AU-10 fluorometer calibrated with chlorophyll *a* (Sigma). Rainfall data for southeastern North Carolina were obtained from USWS rain gaging station at the Wilmington International Airport, and were reported as cumulative totals for each 24-hr period beginning at 0700 hrs local time.

Experimental Protocols: Sediment cores were collected as above at sampling locations in the Bradley Creek watershed (Fig. 3) and in several other locations for experimental determinations of responses of fecal indicator bacteria to added P and organic carbon. A randomly selected triplicate set of sediment cores was analyzed for initial concentrations of fecal coliform and fecal enterococcus bacteria as above. Four treatments were used in a 2 x 2 design, executed in triplicate: three sediment cores were incubated with one liter of incubation medium (0.4% NaCl buffered to pH 8.0 with sodium borate) +100 μg P/liter (as KH_2PO_4), three sediment cores were incubated with one liter of incubation medium + 1000 μg dextrose C/liter, three sediment cores were incubated with one liter of incubation medium + 100 μg P/liter + 1000 μg dextrose C/liter, and three sediment cores were incubated with one liter of incubation medium only. Incubations lasted 24 hours at 37^{o}C, after which sediment cores were processed for analyses of fecal coliform and fecal enterococcus bacteria as above.

Hewletts Creek Sewage Spills: Regulators from the N.C. D.W.Q. sampled the area on Saturday, July 2, then again on July 4 and 6. Researchers from the UNCW Aquatic Ecology Laboratory sampled the water column in the area on July 3, 5, 7, 15, 21, and on August 8. Researchers from the UNCW Department of Biology and Marine Biology collected sediment samples for fecal coliform bacteria and enterococci on July 6, 8, 11, 13, 15, 18, 20, 22, 26, 29, August 2 and August 11. Stations sampled included MB-PGR (spill site), SB-PGR (south branch at Pine Grove Rd.), NB-GLR (north branch at Greenville Loop Rd.), HC-M (creek mouth), HC-3 (at a dock on the north shore of the creek), HC-NWB (the northwest branch of the creek between HC-3 and the tributary stations), the Masonboro Channel on the ICW south of the creek mouth, and the Shinn

Creek channel on the ICW north of the creek mouth (Fig. 6). Stations sampled for sediment bacteria included MB-PGR, SB-PGR, NB-GLR and DOCK, a control site located near the junction of Hewletts Creek and the ICW (Fig. 6). A second sewage spill occurred in the Hewletts Creek watershed on Sept. 15, 2005, when a force main burst near Shipyard Blvd., apparently owing to flooding caused by Hurricane Ophelia's rains, discharging approximately 750,000 gallons (~2840 m^3) of sewage, much of which flowed through storm drains into the middle branch of Hewletts Creek. Sampling efforts similar to the previous scheme were conducted by UNCW researchers on Sept. 19 and 23.

Statistical Analyses: Data sets were examined for normality using the Shapiro-Wilk test. Non-normal data sets were transformed as appropriate; bacteria concentration data were typically log-transformed (Log[counts+1]). When transformation could not yield a normal distribution, non-parametric statistical tests were used to analyze original data. The effects of sediment P, carbohydrate, and other variables on bacterial concentrations were analyzed by linear regression. Correlations between bacterial indicators of fecal contamination were determined using Pearson's product-moment correlation. Comparisons of bacterial or sediment phosphorus concentrations among sites or treatments were performed using Analysis of Variance for normal data sets. All statistical analyses were performed on SAS Institute's JMP version 4.0, except as noted.

Soil P fraction concentrations (M3-SRP, authigenic P and total P) from drainage feature locations were compared within 11 paired sites using one-way analysis of variance. The relationship between Mehlich-III P (SRP, OP and total) and authigenic P concentrations for all sites was examined using Pearson's product-moment correlation analysis. One-way analysis of variance and Tukey's HSD *a posteriori* tests were used to compare mean sample soil P concentrations among sites categorized by different adjacent land covers, after confirming that distributions within each category and class of soil P were "normally" distributed using the Shapiro-Wilk test of goodness of fit to a "normal" distribution. Log transformations were used to address issues of non-normality, particularly skewness.

Results of experimental incubations employing a 2 x 2 design were tested for normality using the Shapiro Wilk test, and found to be non-normal. Results of experimental incubations employing a 2 x 2 design were log-transformed and analyzed by two-way ANOVA (Sokal and Rohlf, 1995); pooled experimental results were analyzed by a Kruskal-Wallis test.

Results

Sediment Fecal Indicator Bacteria: Sediment samples for analyses of fecal indicator bacteria concentrations were collected at 108 locations and times in the lower Cape Fear River basin, aquatic locations in and around Wilmington, and in New Hanover County tidal creeks (Figs. 2-5) between July, 2004 and June, 2005. Fecal coliform and enterococcus were analyzed in all these sediment samples and fecal streptococcus were analyzed as well until April, 2005, when analysis of results indicated that resources could be conserved by focusing on coliforms and enterococcus analyses exclusively. All three fecal contamination indicator groups were significantly correlated with each other (Table 1).

Table 1. Correlation statistics for comparisons of sediment concentrations (log [CFU+1]) of three fecal indicator bacteria types. Significant relationships indicated in bold.

Comparison	r^2	F	df	p
Streptococcus vs. Coliforms	**0.44**	**67.7**	**1,87**	**<0.0001**
Enterococcus vs. Coliforms	**0.31**	**47.2**	**1,107**	**<0.0001**
Streptococcus vs. Enterococcus	**0.30**	**38.5**	**1,88**	**<0.0001**

Sediment Fecal Indicators vs. Sediment Phosphorus: Comparisons of the concentrations of fecal indicator bacteria groups to sediment P concentrations analyzed as persulfate P revealed no statistically significant relationships between sediment P levels, which averaged 29 and ranged from 4 to 100 μg P (g sediment)$^{-1}$, at the 108 sampling locations and times surveyed in the lower Cape Fear River basin and in New Hanover County (Figs. 2-5) between July, 2004 and June, 2005 (Table 2).

Table 2. Regression statistics for relationships between fecal indicator bacteria types and sediment P (all non-significant).

Comparison	r^2	F	df	P
Coliforms vs. persulfate P	3×10^{-6}	0.0004	1,107	0.985
Streptococcus vs. persulfate P	0.014	1.26	1,87	0.26
Enterococcus vs. persulfate P	0.0015	0.16	1,107	0.69

Sediment P fractions: Samples were collected at 48 sites in Duplin and Pender counties, which were categorized according to observations *in situ* of the adjacent land cover (Table 3; Fig. 1). These categories were “Agricultural-CAFO”, “Agricultural-Row Crop”, “Agriculture-Proximity”, “Forest”, “Stream” and “Swale. The Agricultural-CAFO category included any sampling site directly adjacent to a CAFO, including planted fields contiguous with a CAFO and potentially receiving applications of animal wastes. Most of these were turkey CAFOs, which land apply dry litter without pretreatment in a lagoon, as typically occurs with liquid waste from swine CAFOs. The Agricultural-Row Crop category included sampling sites directly adjacent to any area actively managed

for row crop production, as evidenced by crop residues or tillage. The Agriculture-Proximity category included sites not directly adjacent to agricultural land but close enough to be potentially influenced by drainage from agriculture up-slope from the site or by wind-blown dust from agricultural fields. The Forest category included sites directly adjacent to locations with mature stands of trees and no other land cover type within 100 m, including one site that had recently been logged. The Stream category included perennial streams, in which stream channel sediments were sampled. The Swale category included sampling sites adjacent to grassed areas, including highway medians and residential lawns.

Table 3. Sampling dates, land cover designations and sample location number identification for drainage feature sediment samples collected in Duplin and Pender counties (locations shown in Fig. 2).

	Sampling Date 2005		
Land Cover Category	28 Jan.	4 Mar.	24 Mar.
Agriculture-CAFO	3	14, 16, 17, 24	7, 28, 29, 30, 33, 41, 46
Agriculture- Row Crop	1, 4, 8	9, 10, 12, 18, 19, 22	34, 35, 36, 37, 43, 47
Agriculture Proximity	2	11, 13, 23	32, 38, 39, 40
Forest	5, 6	20, 21	42
Stream		15	31, 44, 45, 48
Swale	7		25, 26

Table 4. Concentrations of sediment phosphorus fractions (μg P (g sediment)$^{-1}$, mean + std. dev.) by land cover category

	P Fraction				
Land Cover Category	Mehlich III SRP	Mehlich III OP	Authigenic P	Particulate OP	Total P
Agricultural-CAFO	12.3 ± 3.1	6.2 ± 2.7	22.3 ± 5.5	1.5 ± 1.6	42.3 ± 8.9
Agricultural-Crop	13.1 ± 4.4	7.1 ± 3.7	27.5 ± 11.9	2.3 ± 3.9	46.7 ± 21
Agriculture-Proximity	9.8 ± 3.2	5.7 ± 4.2	20.9 ± 12.3	1.7 ± 2.0	39.8 ± 10
Forest	7.3 ± 3.6	4.4 ± 1.9	16.3 ± 4.7	1.1 ± 0.9	29.0 ± 6.7
Stream	11.2 ± 2.0	6.0 ± 1.2	21.6 ± 7.4	1.9 ± 2.9	40.8 ± 9.8
Swale	7.6 ± 5.8	6.5 ± 7.1	24.9 ± 7.9	1.1 ± 1.1	40.2 ± 12

Table 5. Results of one-way analysis of variance (ANOVA) comparing sediment P concentrations (site means, μg P (g sediment)$^{-1}$) at paired sites. Asterisked F values indicate df = 1,3; for all other comparisons df = 1,4. Significant differences indicated in bold.

Site Pair (Land Cover Category)	Means [Mehlich III-SRP]; ANOVA	Means [Authigenic P]; ANOVA	Means [Total P}; ANOVA
1 (Ag Crop) vs. 2 (Ag Prox)	10.0 vs. 9.8; n.s.	19.6 vs. 15.8; n.s.	34.4 vs. 32.6; n.s.
3 (Ag CAFO) vs. 4 (Ag Crop)	**15.2 vs. 19.9; F=12.9, p=0.023**	23.9 vs. 53.5; n.s.	**45.3 vs. 78.6; F=28.7*, p=0.013**
5 (Forest) vs. 6 (Forest)	**1.3 vs. 10.1; F=107*, p=0.002**	**14 vs. 19.2; F=12.7, p=0.023**	**21.0 vs. 33.3; F=41.7, p=0.023**
7 (Swale) vs. 8 (Ag Crop)	**8.0 vs. 16.4; F=17.6, p=0.014**	**16.1 vs. 37.2; F=148, p=0.0003**	**26.2 vs. 61.1; F=119, p=0.0004**
11 (Ag Crop) vs. 12 (Ag Prox)	15.6 vs. 14.2; n.s.	**25.5 vs. 34.3; F=9.3, p=0.038**	**46.9 vs. 59.3; F=70.1, p=0.001**
13 (Ag Prox) vs. 14 (Ag CAFO)	**5.9 vs. 8.8; F=15.2, p=0.017**	20.3 vs. 18.7; n.s.	30.4 vs. 33.9; n.s.
22 (Ag Crop) vs. 23 (Ag Prox)	**7.0 vs. 10.7; F=11.1; p=0.029**	**5.5 vs. 9.4; F=83, p=0.0008**	**15.5 vs. 35.4; F=64.9*,p=0.004**
25 (Swale) vs. 26 (Swale)	**1.7 vs. 13.2; F=843, p=0.0001**	31.4 vs. 27.1; n.s.	50.3 vs. 44.1; n.s.
29 (Ag CAFO) vs. 30 (Ag CAFO)	8.0 vs. 8.9; n.s.	22.3 vs. 15.0; n.s.	37.6 vs. 27.1; n.s.
37 (Ag Crop) vs. 38 (Ag Prox)	**11.0 vs. 5.9; F=7.9, p=0.048**	25.2 vs. 26.6; n.s.	46.3 vs. 48.3; n.s.
42 (Forest) vs. 43 (Ag Crop)	9.8 vs. 10.9; n.s.	13.5 vs. 20.4; n.s.	26.5 vs. 33.6; n.s.

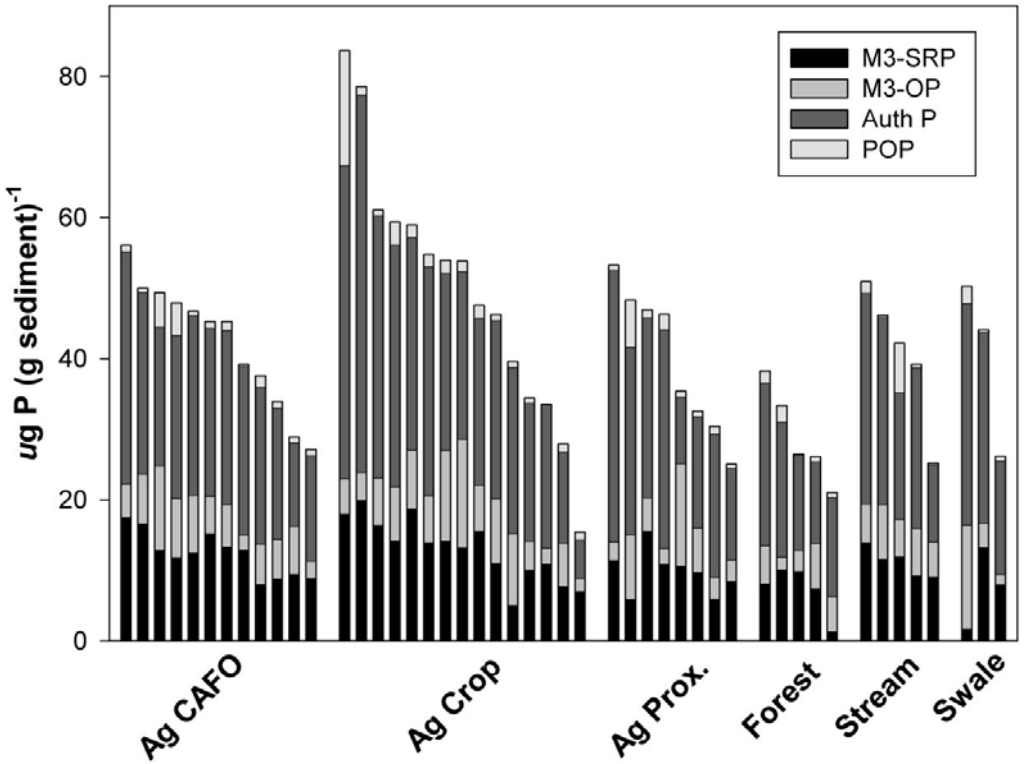

Fig. 7. Amounts of each P fraction (Mehlich-III extractable soluble reactive phosphorus, “M3-SRP”, Mehlich-III extractable organic phosphorus, “M3-OP”, authigenic phosphorus, “Auth P”, and particulate organic phosphorus, “POP”, in sediment samples, grouped by corresponding land cover type in descending order of total P (Ag-CAFO: sites 16, 17, 24, 33, 41, 3, 28, 46, 29, 14, 27, 30; Ag-Crop: sites 47, 4, 8, 12, 10, 36, 18, 19, 9, 37, 35, 1, 43, 34, 22; Ag-Prox: sites 32, 38, 11, 39, 23, 2, 13, 40; Forest: sites 21, 6, 42, 20, 5; Stream: sites 31, 15, 48, 44, 45; Swale: sites 25, 26, 7.

The range of total P values varied about 5.5-fold, from 15.5 to 83.1 *u*g P (g sediment)$^{-1}$ (Fig. 7). Authigenic P was usually the largest of the P fractions, averaging 54.2% of total P overall (Table 4). Mehlich-III extractable P (SRP + OP) was the next most important P fraction, averaging 41.2% of total P overall. An average of 64% of the Mehlich III-extractable P was soluble reactive P and the remainder organic P. The proportions of these two fractions in samples varied considerably among and within land cover categories. Authigenic P was positively but not strongly correlated with M3P-SRP ($F=25.4$, df= 1,46, $p < 0.0001$, $r^2 = 0.36$) and with M3-TP ($F=15.1$, df=1,46, $p= 0.0003$, $r^2 = 0.25$) but not with M3P-OP. Particulate organic P averaged only 3.8% of total P but with values as high as 19.4% in one sample.

The Agricultural-Row Crop category had the highest single sample values and largest range of values for TP readings, 15.5 to 83.7 μg P (g sediment)$^{-1}$ (Fig. 7). The Agricultural-CAFO category had the second highest maximum value of TP and values ranging from 27.1 to 56.1 μg P (g sediment)$^{-1}$. The Agricultural-Proximity category had the third highest maximum value and ranged from 25.1 to 53.3 μg P (g sediment)$^{-1}$. The Forest and Swale categories had similar maximum values and ranges of TP, 21 to 38.2 and 26.2 to 50.3 μg P (g sediment)$^{-1}$, respectively. The Stream category had TP values ranging from 25.2 to 51 μg P (g sediment)$^{-1}$.

Differences in P-fraction concentrations among land cover categories were examined by comparing values of Mehlich III-extractable SRP and organic P (M3-SRP and M3-OP, respectively), authigenic P, and total P (Table 4). Particulate organic P was a very small and invariant fraction of the total, so this fraction was not considered in these analyses. Comparisons among all land cover categories by one-way ANOVA revealed a significant difference for M3-SRP ($F=2.81$, df=5,42, $p=0.028$). *A posteriori* comparisons using Tukey's HSD @ $p<0.05$ produced no significant differences within any pairs of land cover categories, but pair-wise Student *t*-tests @ $p<0.05$ noted that mean M3-RP for the Forest land use category was significantly less than for the Agriculture-CAFO and Agriculture-Row Crop categories and that mean M3-SRP for the Swale category was significantly less than for the Agriculture-Row Crop category. Comparisons among all land cover categories for Mehlich III organic P (M3-TP), Mehlich III total P (M3-TP), authigenic P, and total P revealed no significant differences.

Elevation was also evaluated as a factor that might affect sediment P concentrations. Elevations of the 48 sampling locations varied from approximately 3 m above sea level (#48) to ~30 m above sea level (#25, 26, 37, 38, 39). Regression analyses revealed no significant relationships between elevation and concentrations of any of the P fractions.

Comparisons of paired sites for M3-SRP, authigenic P, and total P using one-way ANOVA revealed no consistent pattern of differences or similarities within paired sites for the different P fractions or between land cover categories (Table 5). There were sometimes differences and sometimes none in pairs with the same land use category. Differences were more frequent between paired sites for M3-SRP than for authigenic P and total P, likely reflecting the higher variances associated with the latter two measures. The overall pattern demonstrated the heterogeneity of the sampling sites

and the difficulty of differentiating them consistently based only on adjacent land cover category.

An additional 77 sets of triplicate samples were collected for analysis of sediment P fractions in triplicate samples at 11 boat ramp and five control locations along shorelines with public access in New Hanover (Fig. 3) at up to seven times between September, 2004 and July 2005, and comparison with sediment concentrations of fecal coliform and enterococcus bacteria. Results showed that Mehlich-III extractable P and authigenic P were the most important fractions of total sediment P in these samples (Table 6).

Table 6. Sediment P fractions (μg P (g sediment)$^{-1}$) at boat ramp and control sites in New Hanover County (Fig. 3); n= 231.

	M3-SRP	M3-TP	M3-OP	Auth P	POP
Mean	14.8	17.8	2.38	11.15	0.97
Std. Dev.	8.05	8.08	4.97	6.86	1.93

Sediment concentrations of fecal coliforms and enterococcus spanned over three orders of magnitude in concentration. Arithmetic mean, standard deviation, and range values were 880 $\pm$ 2,815 (0-17,756) CFU /cm^2 for fecal coliforms and 207 $\pm$ 501 (0-3,200) CFU/cm^2 for enterococcus, respectively. Regression of mean (site x time) log-transformed coliform and enterococcus concentration data against mean (site x time) sediment P fraction concentrations showed only very weak relationships (Table 7). There were a few statistically significant regressions, but with very low regression coefficients (r^2 all < 0.10), there appears to be no strong explanation of sediment bacteria concentrations attributable to sediment P fraction concentrations.

Table 7. Regression statistics for relationships between fecal indicator bacteria types (fecal coliforms = FC, fecal enterococcus = FE) and sediment P fractions, as in Table 6. Statistically significant relationships and slope (+/-) in bold.

Comparison	r2	F	df	p
FC x M3-SRP	0.006	0.38	1,57	0.54
FC x M3-TP	**0.08**	**4.70**	**1.57**	**0.03 (-)**
FC x M3-OP	**0.07**	**4.21**	**1,57**	**0.045 (-)**
FC x Auth P	0.025	1.78	1,57	0.23
FC x POP	**0.075**	**4.61**	**1,57**	**0.036 (+)**
FE x M3-SRP	0.01	0.80	1,60	0.37
FE x M3TP	0.0005	0.03	1,60	0.86
FE x M3-OP	0.04	2.57	1,60	0.11
FE x Auth P	0.002	0.10	1,60	0.75
FE x POP	0.002	0.15	1,60	0.70

Bradley Creek studies: Fecal Bacteria vs. Environmental Parameters: The concentrations of fecal indicator bacteria in sediments of Bradley Creek were highly variable, spanning over three orders of magnitude. Fecal coliform concentrations had a geometric mean of 179 CFU/ cm^2 (std. dev. = 411, range = 0 – 3230) in a total of 154 samples (sites x times) collected between January, 2003 and March, 2005. This geometric mean value corresponded to a value of 179 CFU/100 ml if all these bacteria were suspended in a water column one meter deep, a value just below that required to close the water to human body contact (200 CFU/100 ml). The regulatory standard for shellfishing is much lower, 14 CFU/100 ml; 113 of the 154 samples exceeded this value using analogous assumptions. Mean values for the respective sampling sites were highly variable (Table 8), but all sites had at least one value exceeding 200 CFU cm^{-2}. Fecal enterococcus concentrations had a geometric mean value of 285 CFU/cm^2 (std. dev. = 433, range = 0-1,726) in a total of 45 samples (sites x times) collected between July, 2004 and March, 2005. This geometric mean value corresponded to a value of 285 CFU/100 ml if all these bacteria were suspended in a water column one meter deep, a value well above that required to close the water to human body contact (33 CFU/100 ml). Thus, the levels of fecal indicator bacteria measured in Bradley Creek's sediments frequently represent serious potential problems for human uses of these waters.

Table 8. Concentrations of fecal indicator bacteria in sediments at sampling sites within the Bradley Creek drainage, CFU cm^{-2}. Fecal coliforms= FC, fecal enterococcus = FE. site designations as in Fig. 5.

	Site					
	A	CR	E	M	S	W
FC Mean	340	33	186	257	125	132
Std. Dev.	697	69	274	550	301	152
FE Mean	332	203	365	65	251	528
Std. Dev.	587	294	494	90	448	732

Analysis of correlations between fecal indicator bacteria concentrations and other parameters revealed mostly non-significant relationships. Neither fecal coliform nor fecal enterococcus concentrations in sediments were related to sediment phosphorus concentration, sediment carbohydrate content, or rainfall in the 24 or 48 hour periods prior to sampling (Table 9). There was a significant relationship between salinity and fecal coliform concentration in sediments (Fig. 8), but not between salinity and fecal enterococcus concentrations in sediments (Table 9), and between temperature and sediment fecal coliform concentrations (Fig. 9) but not between temperature and fecal enterococcus concentrations in sediments (Table 9). However, both significant relationships had very low correlation coefficients (r^2 values), indicating poor explanatory power.

Multiple regression was used to evaluate the possibility that interactions among the environmental parameters may have obscured relationships between concentrations of fecal indicator bacteria and environmental parameters (using 72 hr rainfall instead of 48 hr rainfall). Results of this analysis are shown in Table 10, and demonstrate a significant effect of 24 hr rainfall on sediment fecal coliform bacteria concentrations in sediments.

Table 9. Results of regression analyses between sediment fecal indicator bacteria concentrations (log[CFU cm^{-2}]) and environmental parameters. Significant effects in bold.

Parameter	Coliforms				Enterococcus			
	r^2	F	df	p	r^2	F	df	p
Salinity	**0.04**	**6.77**	**1,150**	**0.01**	0.003	0.11	1,43	0.74
Temperature	**0.05**	**7.9**	**1,150**	**0.006**	0.07	3.05	1,43	0.08
Sediment P	$3x10^{-7}$	0.00	1,151	>0.99	0.001	0.07	1,41	0.80
Sed. Carbohydrate	0.02	2.38	1,139	0.13	0.06	2.55	1,43	0.12
24 hr Rainfall	0.006	0.86	1,147	0.35	0.09	3.73	1,37	0.06
48 hr Rainfall	0.001	0.21	1,147	0.65	0.10	3.94	1,37	0.054

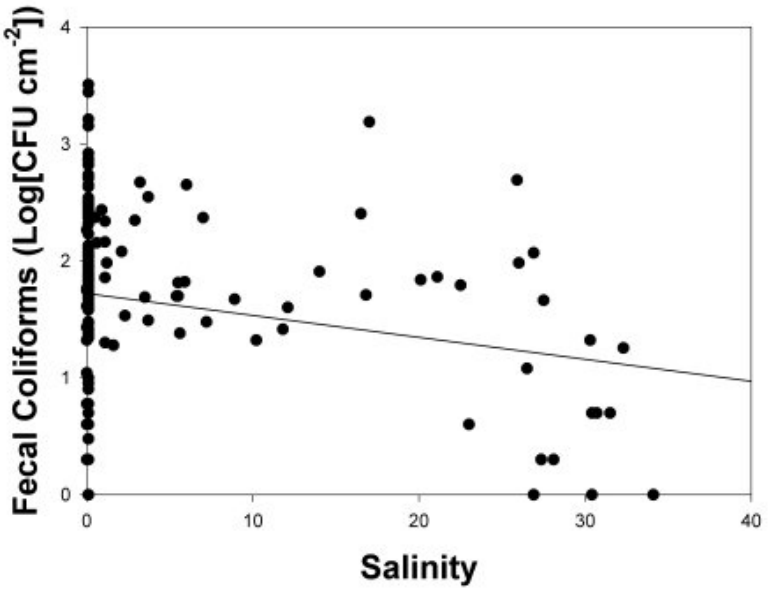

Fig. 8. Effects of salinity on sediment fecal coliforms in Bradley Creek.

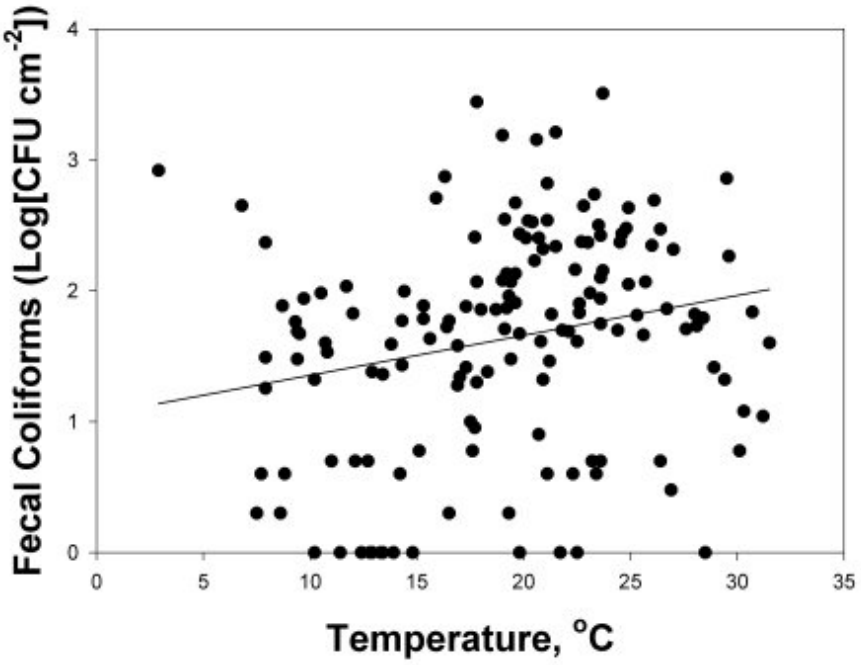

Fig. 9. Effects of temperature on sediment fecal coliforms in Bradley Creek.

The significant pair-wise relationships between fecal coliforms and temperature and salinity are not significant in this analysis, suggesting that interactions among parameters alias responses of fecal coliforms to individual parameters. Identification of a significant rainfall effect agrees with observations from the July 1 sewage spill at Hewletts Creek and subsequent spike in fecal coliform and enterococcus concentrations after a heavy rain on July 14, 2005, discussed elsewhere in this report.

Table 10. Results of multiple regression analysis of the effects of temperature, salinity, carbohydrate, phosphorus, and 24 hr and 72 hr rainfall on fecal coliform concentrations in sediments at Bradley Creek sites, Jan. 2003 – March, 2005. Significant effects in bold. Overall model r^2 value = 0.16.

Source	df	SS	MS	F	p
Model	**6**	**4022134**	**670356**	**3.91**	**0.0013**
Temperature	1	112	112	0.0007	0.9796
Salinity	1	4021.5	4021.5	0.0235	0.8784
Carbohydrate	1	458146	458146	2.675	0.1044
Phosphorus	1	135988	135988	0.7941	0.3746
24 hr rainfall	**1**	**2994796**	**2994796**	**17.49**	**<0.001**
72 hr rainfall	1	143975	143975	0.84	0.3609
Error	125	21404399	171235		
Total	131	25426533			

Experimental Studies: Fecal Bacteria vs. Phosphorus and Carbon: Experimental manipulations of the availability of phosphorus and carbohydrate evaluated the responses of fecal coliforms and enterococcus in sediment samples from several locations with varying natural sediment P and carbohydrate levels. Results of six experiments at five locations were analyzed by two-way ANOVA and are shown in Table 11. Added phosphorus supported significant growth of fecal enterococcus bacteria when initial sediment P levels were relatively low, but fecal coliforms never responded to added P. This latter response was consistent with the lack of any correlation between sediment P and sediment fecal coliforms described earlier (Tables 2 and 7). However, fecal coliform bacteria responded significantly to added dextrose, a form of bio-available carbon, in three of six individual experiments and in the overall analysis. Fecal enterococcus did not respond as frequently to added dextrose, but also showed a significant response in the overall analysis, suggesting that both groups of fecal indicator bacteria are more frequently limited by bio-available organic substrate than by phosphorus.

Analysis of storm water runoff in a rain event in the Bradley Creek drainage revealed that soluble carbohydrates (a measure of bio-available carbon) increased significantly, but temporarily, over background levels (Table 12). Thus, storm water runoff may

provide fresh bio-available carbon in addition to its role as a source mechanism for fecal bacteria that contaminate sediments.

Table 11. Effects of added phosphorus (P) and dextrose (C) and interactions of P and C (I) on changes in fecal coliform (FC) and fecal enterococcus (FE) concentrations in sediment samples from locations in New Hanover County. All treatment combinations run in triplicate and bacteria concentrations log-transformed prior to analysis by two-way ANOVA or Kruskal-Wallis test. Significant effects in bold**.** Sites as in Fig. 5, Site 210 in Fig. 3.

		Initial P	Initial C		FC		FE	
Site	Date	μg P / g sed	μg C / g sed	Effect	F	p	F	p
E	8/19	19.6	420	**C**	**29.9**	**0.001**	**11.9**	**0.009**
				P	0.65	0.44	**7.00**	**0.029**
				I	0.89	0.37	0.01	0.92
E	7/22	31.2	628	C	0.02	0.89	2.38	0.16
				P	3.53	0.09	**5.71**	**0.04**
				I	**57.6**	**0.001**	0.00	1.00
BWP	8/29	47.0	290	C	1.71	0.23	4.5	0.07
				P	1.05	0.34	0.50	0.49
				I	0.39	0.55	0.50	0.49
210	9/1	74.4	290	C	2.60	0.15	0.08	0.78
				P	3.36	0.10	0.50	0.49
				I	**8.04**	**0.02**	0.50	0.49
REC	8/24	102	2990	**C**	**7.71**	**0.02**	4.91	0.06
				P	0.22	0.65	0.48	0.51
				I	0.47	0.51	0.78	0.40
CRT	9/6	176	3600	**C**	**17.8**	**0.003**	0.08	0.60
				P	0.91	0.37	0.50	0.19
				I	3.06	0.12	0.50	0.19
Combined Data				**C**	**32.1**	**0.001**	**6.93**	**0.02**
				P	0.73	0.40	0.09	0.77
				I	0.73	0.40	1.24	0.28

Table12. Response of soluble carbohydrates to rain event in a pond on the UNCW campus.

Date	Condition	[Soluble Carbohydrates], μg C/L	Std. Dev.
8/3/05	Dry	525	462
8/9/05	Rain	1330	811
8/12/05	Dry	525	349
8/13/05	Dry	553	131

Hewletts Creek Sewage Spill: On July 2, the day after the spill, fecal coliform counts were high; at HC-3 they were 270,000 CFU/100 ml, and in the creek mouth they ranged from 2,000 to 3,200 CFU/100 ml (Table 7.5). However, counts were all below 100 CFU/100 ml in the ICW. Fecal coliform bacteria counts in the water column of the creek were high (15,000-21,000 CFU/ml) on July 3, and then decreased to 2,000 CFU/100 mL in the channel on July 4. After July 4 main channel fecal coliform bacteria counts generally stayed below 100 CFU/100 ml. In contrast the tributaries (SB-PGR and NB-GLR) had counts of approximately 3,000 CFU/100 ml until July 6, then a brief decrease, then an increase again on July 15 to 2,900 CFU/100 ml following a rain event (Table 13). From then on tributary fecal coliform counts decreased to normal levels. In the main channel during the first few days, loss of fecal coliforms from the water column followed a roughly logarithmic decrease. Loss of fecal coliforms from the water column can occur from predation by protozoans, mortality from sunlight (UV radiation), dilution by incoming tides and sedimentation. Sedimentation of fecal bacteria was a critically important issue following this sewage spill.

Table 13. Water column fecal coliform bacteria counts by date and station following the July 1, 2005 Hewletts creek sewage spill (as CFU/100 ml).

Station	7/2	7/3	7/4	7/6	7/7	7/15	7/21	8/8
HC-M	3,200	176		1	9	5	46	1
HC-3	270,000	21,000	220	69	21	24	96	2
HC-NWB		15,800			242			
SB-PGR			3,000	358	211	312	362	30
MB-PGR		2100	780		224	900	291	128
NB-GLR				3,200	546	2,900	655	180

Post-spill sediment bacteria sampling was initiated on July 6. Reference samples were available for Hewletts Creek as a WRRI-sponsored project regarding sediment fecal bacteria had been ongoing from 2004. Results (Tables 14a, 14b) showed that post spill samples were an order of magnitude higher than pre-spill counts. Counts appeared to decrease after a few days, but the rain event (noted above) caused high sediment

Table 14a. Sediment fecal coliform bacteria counts by date and station following the July 1, 2005 Hewletts Creek sewage spill (as CFU cm^{-2}). Samples collected 10/31/04 and 1/28/05 are shown as control (non-spill) counts for comparison.

Station	10/31/04	1/28/05	7/6	7/11	7/15	7/22	8/2	8/11
DOCK		0		0	23	0	0	11
SB-PGR	488	358	2,740	526	5,330	396	732	1,890
MB-PGR		5,110	1,150	1,450	777	457	80	
NB-GLR	53	579	3,510	442	991	663	1,310	914

Table 14b. Sediment fecal enterococcus bacteria counts by date and station following the July 1, 2005 Hewletts Creek sewage spill (as CFU cm^{-2}).

Station	7/6	7/8	7/11	7/13	7/15	7/18	7/22	7/26
DOCK	0	0	0	0	229	0	0	0
SB-PGR	152	229	0	381	10,300	76	686	114
MB-PGR	4,570	5,030	457	0	7,770	152	229	305
NB-GLR	533	76	152	610	9,370	457	76	229

counts again (7/15). Sampling was continued until early August. The latter dates showed a general decrease to background levels, with high counts periodically occurring. Fecal coliform and fecal enterococcus bacteria showed very similar patterns of increases and decreases in response to the original spill and the rain event of 7/15. The fecal bacteria in the sediments form a reservoir of viable fecal microbes that is available to enter the water column following a mixing/stirring event such as a rainstorm or people or pets wading or otherwise disturbing the sediments. As an on-site test, on 7/7 researchers for the Aquatic Ecology Laboratory collected a fecal coliform sample from the water at HC-3, and then proceeded to pass the motor over the site, stirring the water and sediments below. Counts taken from before the stirring were 21 CFU/100 ml while counts taken after the stirring were nearly three times greater, 60 CFU/100 ml. The relatively high fecal indicator bacteria levels in water and sediment samples within Hewletts Creek and very much lower concentrations in water and sediment samples taken outside the creek suggest that for the most part these contaminants were retained within the creek itself. Studies of the flushing rate of Hewletts Creek showed that about 50% of the water in the creek is exchanged with each tidal cycle (Hales 2001), so high retention of fecal contaminants indicates that sedimentation and other processes were very important in preventing broader dispersal of the contaminants generated by this spill.

The presence and persistence of the sediment fecal bacteria demonstrate that water column sampling of fecal bacteria is insufficient when analyzing an area for human contact safety after a pollution event; sediment sampling also produces necessary data. Rapid sedimentation of fecal indicator bacteria from the water column may falsely indicate a return to safe conditions, when contact with contaminated sediments is actually hazardous. Dr. Cahoon had inquiries from individuals working in direct contact

with Hewletts Creek sediments immediately after the spill and while fecal indicator bacteria counts in sediments were well above 200 CFU/cm^2. Although no regulatory standard for sediment-associated fecal pathogen indicators exists *per se*, a useful comparison is to consider that 200 CFU cm^{-2} of fecal coliform bacteria suspended in a one meter deep water column corresponds to the 200 CFU/100 ml standard for human body contact. Similarly, fecal enterococcus counts of 33 CFU cm^{-2} suspended in a one meter deep water column correspond to the human body contact standard of 33 CFU/100 ml. Persistence of relatively high concentrations of fecal indicator bacteria in sediments for several weeks after the spill, and long after water column values had declined, suggests that sediment sampling should be included in response to future major sewage spills, especially in situations in which direct human exposure is possible.

That was not the only pollution incident to affect Hewletts Creek in 2005, however. Another sewage spill occurred in the Hewletts Creek watershed September 15 when a 24-inch line ruptured and spilled an unknown volume of sewage onto Shipyard Drive and ditches and yards along Pine Valley Drive. Some of the waste entered storm drains, and from there entered Hewletts Creek. Repairs were completed the next morning. Samples collected in Hewletts Creek by the UNCW Aquatic Ecology Laboratory found elevated fecal coliform bacteria counts in the upper tributary stations (PV-GC9 – 2,487 CFU/100 mL; MB-PGR – 2,790 CFU/100 ml; SB-PGR – 2195 CFU/100 ml; NB-GLR – 840 CFU/100 ml). Station MB-PGR is located downstream from PV-GC9 (Fig. 6). Subsequent sampling on September 19 showed a considerable water-column decrease in fecal coliform bacteria (PV-GC9 – 380 CFU/100 ml; MB-PGR – 700 CFU/100 ml; SB-PGR – 160 CFU/100 ml; NB-GLR – 400 CFU/100 ml) to levels commonly found at these locations (Table 14a), although the main channel sites were still elevated (HC-3 – 60 CFU/100 ml; HC-2 – 100 CFU/100 ml). Sediment samples collected after this spill on Sept. 19 also showed high levels of fecal coliform and fecal enterococcus bacteria persisting for at least a week after the spill (Table 15).

Table 15. Sediment fecal coliform (FC) and enterococcus (FE) bacteria counts (as CFU/cm^2) by date and station following the Sept. 15, 2005 Hewletts Creek sewage spill. Sites as in Fig. 6.

		Date	
Station	Indicator	9/19	9/23
CONTROL	FC	0	0
	FE	0	0
SB-PGR	FC	1670	1600
	FE	1220	152
MB-PGR	FC	2360	6020
	FE	76	305
NB-GLR	FC	380	1300
	FE	229	381

Discussion

Fecal Indicator Bacteria: Concentrations of fecal indicator bacteria were frequently high enough to represent a potential for serious impairment of the overlying waters if those populations were to be wholly or even partially re-suspended by disturbance of the sediments. Informal experiments showed that this re-suspension was not unlikely under reasonable circumstances. Significant positive correlations among the three types of fecal indicator bacteria give confidence in the utility of these measures of fecal contamination in diverse aquatic habitats. Other studies have demonstrated the frequent and widespread occurrence in marine and freshwater sediments of high concentrations of total coliforms, fecal coliforms, fecal streptococci, *Clostridium perfringens*, enteric viruses, and thermotolerant protozoa, all indicators of fecal contamination from human and animal sources (Rittenberg et al. 1958; Van Donsel and Geldreich 1971; Grimes 1980; Sawyer 1980; O'Malley et al. 1982; Izzo et al. 1983; Valiela et al. 1991; Doyle et al. 1992; Burkholder et al. 1997; Sawyer et al. 1998; Palmer 2000; Lipp et al. 2001). Our study is the first of which we are aware quantifying fecal enterococcus in aquatic sediments. Our results show that levels of this indicator are relatively higher than those of fecal coliforms, and the correlation between the two indicators gives confidence that enterococcus concentrations are similarly useful in indicating the presence of actual pathogens.

The widespread occurrence of problematic levels of fecal contamination indicator bacteria argue that regulatory agencies should consider adopting sediment sampling protocols and rules for protection of public health and safety based on sampling data. Fecal coliform bacteria could be used as a standard in fresh water and fecal enterococcus in those salt water environments where the enterococcus standard is applied for determination of swimming closures. We note that boat ramps, where people are often in direct contact with sediments, were often problematic with regard to fecal contamination indicators, but that these places are not often tested for health and safety purposes.

Sediment P fractions: Drainage features exhibited a wide range of sediment P levels that included some higher values associated with agricultural land covers. Sediment M3-SRP levels associated with forested areas were significantly lower than sediment M3-SRP levels associated with adjacent row crop and CAFO land covers, suggesting that agricultural applications of commercial fertilizers and animal wastes contribute to the difference and that movement of phosphorus from agricultural lands into adjacent drainage features is occurring with some frequency. Cahoon (2002) reported that residential use of fertilizer was apparently responsible for elevated P levels in streams in coastal New Hanover County, so the effect of human activities on P movement into drainage systems is not surprising. Cahoon and Ensign (2004) noted that excessive soil P levels, defined by the state of North Carolina as phosphorus index (P-I) levels > 100 (exceeding all crop plant P requirements, equivalent to 120 mg P/kg soil by Mehlich III test; N.C. Cooperative Extension Service 1997) were increasingly frequent in eastern North Carolina's predominantly agricultural counties. For example, more than 80% of all 13,790 soil samples from Duplin County in 2000-2001 exceeded a P-I value of 100.

Agricultural research has shown that high soil P levels drive higher export of P, particularly during rain events, e.g., Pote et al. 1996; McDowell et al. 2001b. Thus it is not surprising to find higher levels of sediment P in some drainage features near agricultural fields. However, we note that sediment P levels were relatively low in some drainage features adjacent to agricultural fields, and that some, but not all fields were graded to limit direct runoff into ditches (one of several Best Management Practices that limit nutrient and sediment losses). Moreover, when the values of M3-TP were converted to P-I index values (120 μg P/g sediment = PI of 100), all values were well below levels of concern (P-I=50) and levels defined as excessive (P-I >100) (mean = 15, range = 5 to 24). These values do not include authigenic P, however, the largest fraction of sediment P in most samples, or particulate organic P, a less important fraction in most samples. Consequently, this indicates that agricultural management practices can make an important difference in the process of P transport off-site, but that export is occurring in some fraction of the landscape.

Authigenic P was the dominant portion of sediment P in most samples, and was positively correlated with M3-SRP values, which suggested an interaction of anthropogenically added P with natural or added soil minerals, especially calcium, which may be added to soils as lime or land plaster (gypsum). This fraction is not part of the soil test P evaluated and reported by the N.C. Division of Agronomy. The general predominance of authigenic P in these sediment samples indicated that conventional soil test procedures fail to measure most of the P in this landscape much of the time. If the correlation between M3-SRP and authigenic P reflects a chemical transformation of M3-SRP to authigenic P by reaction with soil minerals, then actual export of added P from agricultural fields might also be underestimated by use of conventional soil test P measures. Microbial action can liberate P from mineral phases (Cerezine et al. 1998; Reyes et al. 2001), so this fraction, although bound more tightly to soils than M3-SRP and M3-OP, less mobile, and refractory to conventional soil analyses, must be considered as bio-available in the longer term, and therefore an important soil P fraction.

Our selection of winter and early spring as a sampling time must be considered in evaluating these results. Most application of fertilizers and animal wastes takes place during the growing season, so our sampling followed the most recent likely applications by three to six months and appeared, from our observations of activity in agricultural areas, to precede fresh applications as well. Thus, P measured in ditch and stream sediments most likely resulted from longer-term transport processes. Note also that the period, January-March 2005 was an unusually dry period in southeastern North Carolina (rainfall totals were 4.7 cm in Jan., 6.3 cm in Feb., and 11.3 cm in Mar. at Burgaw in Pender County (National Climate Data Center)), with few of the heavy rains that would have moved sediment and nutrients through the landscape, nor did this region receive unusually heavy rains from tropical systems in the preceding year. Finally, by sampling before the growing season we likely sampled sediment P that might otherwise have been taken up by plant growth in the warmer months of the year. Phosphorus in vegetation is certainly present in the ecosystem, but not in a form detectable by simple soil analysis procedures. Thus, our sampling was reasonably

representative of the actual concentrations of and variability in sediment P in these landscape features.

The significance of sediment-associated phosphorus is not well appreciated in analyses of the interactions of terrestrial and aquatic ecosystems, although the importance of phosphorus in aquatic ecosystems is indisputable. Transport mechanisms are more complicated than was originally thought (Carlile and Phillips 1976; Pote et al. 1996; McDowell et al. 2001a, b). The most comprehensive data sets on soil P are derived from agricultural soil test results, but these data do not include important P fractions that are likely bio-available. As agricultural activities in particular drive up conventional soil test P values (Cahoon and Ensign 2004) the transport of all soil P fractions is likely to accelerate. Recent, more insightful evaluations of the bacterial processes stimulated by P loading in aquatic ecosystems (Sundareshwar et al. 2003; Mallin et al. 2004) suggest that much more than primary production is affected, and that we have yet to appreciate the full magnitude or importance of sediment P and coupling between terrestrial and aquatic ecosystems.

Fecal Indicator Bacteria vs. Environmental Parameters: The main hypothesis of our project, that fecal indicator bacteria were limited by the availability of the macronutrient phosphorus, was supported to a very limited degree by our analyses and experiments. The few experimental responses to added P we detected occurred only at relatively low P concentrations. The observed concentrations of phosphorus as persulfate-P or in the various fractions in most field samples were typically higher than these levels or the levels Rowland (2002) found to be limiting fecal coliform populations. Moreover, previous research has established that aquatic sediments serve as sinks for phosphorus, accumulating phosphorus in various forms, including the poorly quantified authigenic form, from the surrounding watershed (Cahoon 2002). In a rapidly urbanizing area such as New Hanover County, where storm water runoff is a significant issue (Mallin et al. 2002b) or in rural areas with extensive agricultural throughputs of P-containing fertilizers and animal wastes (Mallin et al. 2004), excessive P availability is clearly widespread. High concentrations of fecal indicator bacteria in sediments may be one consequence of this, but it is mostly too late to manage the fecal contaminants by managing P loading independently in these situations. In pristine habitats where sediment P levels are very low, fecal bacteria may be P-limited, and this beneficial condition may be protected by more effective measures to limit P-loading, as well as fecal contamination. The present survey showed that forested areas had soil P levels that were limiting to enterococcus growth (Table 4). Thus, conversion of these areas to agriculture or urban developments will likely lead to P stimulation of sediment fecal bacteria in formerly pristine forest area water bodies (ponds, streams, ditches, etc.).

The effect of fecal bacteria recruitment to sediments from storm water runoff and sewage spills demonstrated by our studies was a significant result, but not surprising, as many other studies have identified human sewage (Rittenberg et al. 1958; Sawyer 1980; O'Malley et al. 1982; Volterra et al. 1985; Lewis et al. 1986; Orozco Borbon and Delgadillo Hinojosa 1989; Palmer 2000), direct deposition by animals (Sherer et al. 1992; Buckley et al. 1998; Coyne et al. 1998), and storm water runoff as sources

(Struck 1988; Kolb and LaBudde 1993; Ferguson et al. 1996; Coelho et al. 1999; DeLuca- Abbott et al. 2000; Lipp et al. 2001).The relatively quick disappearance of fecal indicator bacteria from the water column may now be interpreted as partially a function of recruitment to the sediment by various mechanisms, including settling of suspended matter and direct filtration of particulates through porous sediments (Rusch and Huettel 2000; Fries and Trowbridge 2003). This reinforces the point made earlier about development of regulatory sampling protocols and rules for considering sediment contamination in addition to water column parameters.

Experimental results identifying bio-available organic carbon as a factor more frequently limiting fecal bacterial indicators than sediment phosphorus is a tantalizing finding. Most measures of organic carbon in aquatic ecosystems measures measure total organic carbon (TOC) or dissolved organic carbon (DOC). Much of that material, however, is relatively refractory, and would not readily support bacterial growth. Bio-available organic carbon is much less frequently measured (Avery et al. 2003, 2004). Our investigations of the factors limiting sediment fecal indicator bacteria have shown that carbohydrates (a form of BDOC) stimulate growth of sediment-associated fecal coliform and enterococcus bacteria, and that storm water runoff is enriched in soluble carbohydrates. A small number of other studies have shown that storm water runoff is enriched in BDOC (Buffam et al. 2001; Seitzinger et al. 2005), especially in urbanized watersheds (Goonetilleke et al. 2003), and that BDOC stimulates bacterial growth (Soendergaard et al. 2003). We hope to pursue this hypothesis in future studies.

Hewletts Creek Sewage Spill: Regulatory authorities lifted the ban on swimming in the ICW after a two-week period. However, due to the persistence of the fecal bacteria in the sediments and the increases noted after rain events, the ban on swimming in Hewletts Creek remained in effect for the remainder of the summer of 2005. Anecdotally, several individuals who were swimming or otherwise recreating in the ICW during the Fourth of July weekend came down with infections. The City made permanent repairs on the break Tuesday August 9 with a heavy-duty coupler made of cast iron. As an additional complimentary measure a low flow alarm was installed at the Southside Wastewater Treatment Plant to detect low flow from the pump station on Hewlett's Creek. The City was fined $50,000 (plus $1,450 or so in costs) by the North Carolina Division of Water Quality as a result of the spill. The City of Wilmington is currently studying the engineering aspects and costs of a large-scale sewage collection system upgrade project.

The July 1 sewage spill demonstrated two important points. First, following a major pollution incident where human or animal waste is involved, sampling the water column for fecal bacteria is not sufficient to obtain a complete picture of the system in terms of human health issues. Large quantities of the polluting bacteria settled to the sediments and remained viable for several weeks, and were clearly subject to resuspension in the water column after a mixing event. This has been demonstrated previously following a large swine waste lagoon spill that entered the New River (Burkholder et al. 1997). There significant quantities of fecal bacteria remained in the sediments for nearly three months. Fecal bacteria on or in the sediments are largely protected from UV radiation,

a principal means of death or deactivation in the water column. Also, the sediments contain carbon, nitrogen, and phosphorus, key nutrient for survival and growth. We recommend that regulatory authorities devise sampling and assessment plans for pollution incidents that consider sediment-associated fecal bacteria.

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Mallin, M.A. L.B. Cahoon, B.R. Toothman, D.C. Parsons, M.R. McIver, M.L. Ortwine, R.N. Harrington. Impacts of a Raw Sewage Spill on Water and Sediment Quality in an Urbanized Estuary. In prep. for submission to Marine Pollution Bulletin.

Toothman, B.R., M.L. Ortwine, and L.B. Cahoon. Fecal contamination of tidal creek sediments: Factors controlling indicator bacteria concentrations. In press, in Mallin, M.A. et al., Environmental Quality of Wilmington and New Hanover County Watersheds 2004-2005. CMS Report 06-0X

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