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PRIMARY RESEARCH PAPER

Phosphorus and carbohydrate limitation of fecal coliform and fecal enterococcus within tidal creek sediments

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Abstract Aquatic sediments can be a significant reservoir of bacterial indicators of fecal contamination at levels higher than the waters above them. Several environmental factors have been identified that can enhance the role of sediments as a reservoir for enteric pathogens, including carbon and/or phosphorus availability. In order to investigate the influence of these and other environmental factors on sediment fecal bacteria populations, sediment samples were collected from a coastal watershed in southeastern North Carolina and analyzed for fecal coliform and fecal enterococcus using a modified membrane filtration technique. Measurements of sediment phosphorus, sediment carbohydrate, and environmental factors were made and relationships with bacteria concentrations were assessed. These observations were accompanied by an experimental laboratory manipulation of phosphorus and carbohydrate and their effects on sediment-associated fecal coliform and enterococcus. Field results suggested that sediment-associated indicator bacteria were not limited by sediment phosphorus or carbohydrate. Experimental results suggested that sediment-associated fecal bacteria were more frequently limited by bioavailable carbohydrate. Sediment phosphorus was limiting for fecal enterococcus only where sediment P was initially low (<31 μg P g^{-1}). A strong positive response by sediment fecal coliform concentrations to recent (24 h) precipitation was evidence that stormwater runoff delivers fecal bacteria loadings that are only partly measurable by conventional water sampling schemes, and by driving sediment and sediment P-loading plays a significant role in enhancing aquatic sediments as reservoirs for fecal microbes.

Keywords Fecal bacteria · Phosphorus · Carbohydrate · Microbial contamination · Sediment · Water quality

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Introduction

The microbial quality of coastal water bodies is important from both an economic and public health standpoint and is often the cause of shellfishing and recreational water closures. Aquatic sediments have been identified as a significant reservoir for fecal

indicator bacteria and may host concentrations up to one to three orders of magnitude higher than the overlying waters, sufficient to cause non-attainment of use standards were they to be suspended in the water column by storms or other disturbances (Van Donsel & Geldreich, 1971; Grimes, 1975, 1980; LaLiberte & Grimes, 1982; Doyle et al., 1984; Tunnicliff & Brickler, 1984; Doyle et al., 1992; Buckley et al., 1998). Several environmental factors, including anthropogenic effects, have been hypothesized to enhance the role of sediments as a reservoir for fecal bacteria and enteric pathogens.

Carlsson & Caron (2001) and Mallin et al. (2004) found that phosphorus (P) rather than nitrogen could limit bacteria production in freshwater systems. Bacteria typically exhibit low N:P ratios, suggesting a stronger tendency to phosphorus limitation than microalgae (Carlsson & Caron, 2001). Sundareshwar et al. (2003) and Castillo et al. (2003) both concluded that sediment bacterial production is limited by P in coastal ecosystems and postulated that this is also the case in many other P deficient ecosystems. Sundareshwar et al. (2003) found experimentally that increases in phosphate concentrations resulted in bottom-up ecological changes affecting the nitrogen cycle, carbon cycle, and primary production. Fecal bacteria are among the bacteria that may be enhanced by increased P concentrations. Burkholder et al. (1997) and Mallin et al. (2000) suggested that a positive correlation between increased fecal bacteria and increased nutrient concentrations could be due to either a common source or the coincidental arrival of both to a common location. Sheheta & Marr (1971) found that in areas of low nutrient concentrations *E. coli* growth rates were highly dependent on PO_4^{3-} (<1.0 mM).

Heterotrophic organisms, including enteric bacteria, rely on some external source of carbon. Potential co-limitation of bacterial production by P and carbon has been identified where the organic carbon supporting bacterial production is derived primarily from algal carbon fixation (Castillo et al., 2003). Evidence exists that bacteria are capable of out-competing algal communities for inorganic nutrients and that bacterial production is effectively limited by algal production of fixed carbon (Currie & Kalff, 1984; Kirchman, 1994). By this proposed mechanism, bacteria may be indirectly limited by P or by another limiting factor affecting algal carbon production.

Fecal bacteria originate as gut flora primarily from warm-blooded animals, although aquatic reptiles have also been identified as sources (Harwood et al., 1999). Fecal coliform (FC) bacteria have been the most commonly used indicators of the presence of fecal material, sewage contamination, and pathogenic enteric bacteria in coastal waters (Dadswell, 1993; NCDEHNR, 1996; Rees et al., 1998; APHA, 2001; Mallin et al., 2000, 2007; Benson, 2002). However, some studies have found that FC are not always reliable as an indicator of bacterial or viral pathogens in the water column or sediments, underscoring the need for additional testing methods, such as the use of fecal enterococcus (FE) as a fecal pollution indicator (Burton et al., 1987; Ferguson et al., 1996; Davis et al., 1977; LaBelle et al., 1980; Sayler et al., 1975). These bacteria are more likely to persist in the water column than FC (APHA, 2001), particularly in saline waters (Hanes & Fragala, 1967). *Enterococcus faecalis* and *Enterococcus faecium* in particular are associated with human sewage and may provide a more reliable distinction between human wastes and that of avian and domesticated/wild macrofauna (Farrow et al., 1983; Laukova & Juris, 1997; Pinto et al., 1999).

FC persistence in the water column is relatively short due to high susceptibility to solar irradiance (Chamberlain & Mitchel, 1978; Solic & Krstulovic, 1992; Gerba & McLeod, 1976), high salinity (Cabelli, 1978; Lessard & Sieburth, 1983), and predation (Davies et al., 1995). FC bacteria as well as many of the pathogenic enteric microflora with which they are associated have been observed to persist at much higher concentrations in the sediments just below the water/sediment interface than in the overlying waters (Sherer et al., 1992; Burton et al., 1987). There is also a growing body of evidence that sediment-bound FC are capable of growth within the sediments (Chan et al., 1979; Gerba & McLeod, 1976; Hood & Ness, 1982; LaLiberte & Grimes, 1982; Sheheta & Marr, 1971).

Bacteria are thought to enter sediments through the sedimentation of particles to which they are adsorbed (Gannon et al., 1983). Research has shown that sediment-associated bacteria may benefit from the relationship. Sediment particles provide shelter from solar irradiance, may ease pressures from predation, and can enhance bacterial persistence (Davies & Bavor, 2000). Concentrations of sediment-bound

bacteria are typically inversely proportional to sediment grain size (Chan et al., 1979; Erkenbrecher, 1981). Bacteria also preferentially adsorb to smaller (≤2 μm) sediments, which settle more slowly and provide enhanced mobility and importation of bacteria including FC bacteria (Dale, 1974). Smaller sediment grain sizes provide a higher ratio of surface area to volume and are more often associated with organic and inorganic nutrients (Dale, 1974; Chan et al., 1979). Others have also concluded that association with fine grained silts and clays may enhance the survival of FC (Burton et al., 1987; Hood & Ness, 1982; Howell et al., 1996).

Fecal bacteria contamination is pervasive in developed watersheds in coastal areas (Mallin et al., 2000; Holland et al., 2004; Mallin et al., 2009). We performed combined field and laboratory studies to test hypotheses about several factors potentially limiting (or enhancing) the growth and survival of sediment-associated fecal bacteria in freshwater and estuarine areas of an urbanized coastal watershed known to be impacted by fecal bacteria contamination. We hypothesized that sediment fecal bacteria growth in this system was primarily limited by phosphorus and secondarily by carbon.

Site description

The Bradley Creek watershed (Fig. 1) is 72% residential and has been identified as having the poorest overall microbial water quality among the tidal creeks in New Hanover County, NC (Mallin et al., 2000). Researchers from the University of North Carolina Wilmington sampled at five of this study's sampling locations for the previous 11 years http://www.uncwil.edu/cmsr/aquaticecology/laboratory/index.htm. During 2001–2002 FC concentrations at five stations in Bradley Creek exceeded the North Carolina standard for human contact (200 CFU $(100\ ml)^{-1}$) more than a third of the times sampled and as often as 83% of the times sampled for one station (Mallin et al., 2003). Mallin et al. (2000) identified urban stormwater runoff as a major contributor of FC bacteria to the watershed.

Sediments from six sample sites in the Bradley Creek watershed were sampled during this study (Fig. 1). Sample Sites BC-SBU, BC-CR, BC-D, and BC-NBU are in freshwater tributaries to Bradley

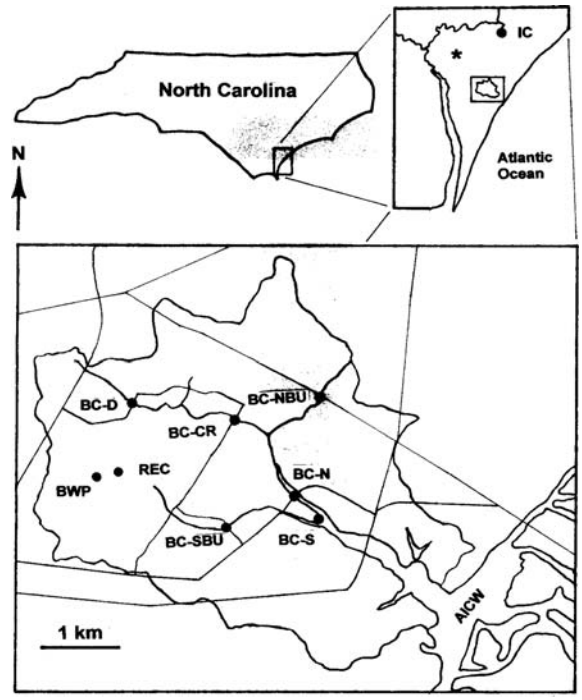

Fig. 1 Bradley Creek Watershed and field sampling locations. "*" denotes rain gage location. Station identities as in text

Creek. BC-D is a residential storm water detention pond that flows into the watershed, and was newly sampled for this study. These sites have typically small-grained (<250 μm) sands mixed with some organic matter. Sites BC-N and BC-S are in the Bradley Creek estuarine zone. Water depths (@mean high tide at tidal sites) at each site were: BC-SBU: 0.75 m, BC-D: 0.5 m, BC-NBU: 0.4 m, BC-CR: 0.25 m, BC-S: 1.0 m, BC-N: 1.0 m.

Materials and methods

Field sampling

Sediment samples were collected from each site once monthly between January, 2003 and March, 2005. Tidally influenced sites (BC-N and BC-S) were sampled within an hour of low tide. At each site temperature and salinity of the water were recorded using a YSI 85 multi-parameter water quality meter. Total precipitation data for the preceding 24, 48, and 72 h of all sampling events were acquired from NOAA Weather Station KILM, 34°16′14″N 077°54′09″W;

http://weather.noaa.gov/weather/current/KILM.html. This location was within 9 km of all sampling locations in the Bradley Creek watershed. Six sediment cores approximately 2 cm deep and 2.36 cm diam. were collected using sterile, acid washed PVC coring tubes and placed into pre-weighed, acid washed, sterile polypropylene 50 ml centrifuge tubes. Three tubes were randomly designated for bacterial counts by membrane filtration within 4 h of collection and the remaining three tubes were designated for total phosphorus (TP) and total carbohydrate (TC) analyses. The tubes were cooled on ice until they could be processed. The supernatant was then decanted from each core and discarded. The three cores designated for sediment TP and TC analysis were placed into an ultra cold freezer (−80°C) for 24 h before lyophilization using a Virtis Benchtop 3.3 Vacu-Freeze lyophilizer.

Cores selected for bacterial analyses were mechanically suspended in 1 l of phosphate buffered (0.25 M KH_2PO_4, pH adjusted to 7.2 with 0.1 N NaOH) rinse water. Sterile magnetic stir bars were used to stir the suspension for 2 min to homogenize and release the sediment-associated bacteria into the buffer solution. FC and (beginning later in the field sampling campaign) FE were enumerated from 10 ml of this suspension using membrane filtration methods outlined by APHA (2001 method 9222 D, method 9230 C, respectively). Following incubation individual dark blue colonies (FC) and red or dark colonies (FE) were assumed to represent colony forming units (CFU). The numbers of CFU cm^{-2} were then derived by the following equation:

$$\text{CFU cm}^{-2} = \frac{(\text{Colonies} \times 1{,}000\ \text{ml})/4.37\ \text{cm}^2}{10\ \text{ml}} \quad (1)$$

where 4.37 cm^2 is the area of the sediment/water interface sampled by the core. Bacterial concentrations were expressed as CFU cm^{-2} in order to assess potential for bacterial contamination throughout the overlying water column. If 1 cm^2 of the top layer of stream sediment containing fecal bacteria is suspended into a 100 cm deep water column the resulting volume of the suspension is 100 ml. This facilitates comparable measurements of bacteria in the sediment and the water column, which are commonly expressed as CFU $(100\ \text{ml})^{-1}$. As noted above, average depths at our sampling sites were generally 100 cm or less. Fecal bacteria tend to be located in the top layer of sediments at the sediment/water interface (Burton et al., 1987; Sherer et al., 1992). The results of the three membrane filtrations for each site were then averaged for a site mean.

The freeze dried cores were analyzed for total phosphate (TP) and total carbohydrate (TC) concentrations. TP was quantified using a persulfate digestion method outlined by Valderrama (1981). TC quantification was performed according to the phenol–sulfuric acid method outlined by Underwood et al. (1995). TP per unit dry weight of sediment was calculated as follows:

$$\mu\text{g TP g}^{-1} = (\mu\text{MP} \times 0.04\ \text{l} \times 31)/\text{Dry weight of sediment (g)} \quad (2)$$

where 0.04 l is the sample volume with oxidation reagent and distilled water, and 31 is the molecular weight of P. The concentration of TC was calculated as follows:

$$\mu\text{g TC g}^{-1} = \underline{(\text{Abs} - \text{Constant})/(\text{Coefficient})}\Big/\text{Dry weight of sediment(g)} \quad (3)$$

where constant (c) and coefficient (m) were obtained from a linear calibration curve derived from glucose standards, where $y = mx + c$.

Laboratory experiments

In order to test experimentally the effects of varying P and organic C concentrations on sediment bacteria, sediment cores were taken from field sites and incubated with solutions varying in P and C concentrations in a 2 × 2 design with two levels of each. Sediment cores were procured during six sampling events at five separate locations (Fig. 1) by the same methods described for field samples. Cores for the first two trials (BC-NBU-7/12, BC-NBU-8/19) were procured from a site also sampled during the field data collection, BC-NBU, as was site BC-CR. Site REC is a storm water detention pond located within the Bradley Creek watershed. Site IC (Fig. 1) is 17 km north of the Bradley Creek watershed in a tributary, Island Creek, of the Northeast Cape Fear River (34°22′01.47″N, 77°48′54.01″W), a relatively pristine and undeveloped area of northern New Hanover County, NC. Site BWP is a small natural

pond within the UNCW Bluthenthal Wildflower Preserve, also located within the Bradley Creek watershed.

Initial TP, TC, FC, and FE were determined using the same methods outlined for the field data analysis for each sampling event. To determine effects of phosphorus (P) and carbohydrate (C) concentrations, 12 cores were randomly distributed into four triplicate sets and each set was incubated with one of the possible treatment combinations for 24 h at 20°C. Treatment solutions consisted of 500 ml of sodium borate/boric acid buffer (pH = 8) with one of the four possible treatment combinations of P or C addition/deletion (P + C, P only, C only, and deionized water only). Monobasic potassium phosphate (KH_2PO_4) and dextrose ($C_6H_{12}O_6 \cdot H_2O$) were used as sources of readily available P and C, respectively. In order to reflect the relative ratios of nutrient uptake amendments of C (dextrose) at 1,000 mg l^{-1} and P (potassium phosphate monobasic) at 439 mg l^{-1} (molar ratio carbon: $P = 10.3$) were made. All sediment cores were suspended and incubated in sterile, acid washed 1 l beakers containing the incubation solution with gentle aeration. At the start and end of the 24 h incubation the triplicate samples from each treatment were subsampled for FC and FE enumeration according to the methods used for the field samples. Effects were recorded as changes in FC and FE compared to initial pretreatment values.

Statistical analysis

Statistical analyses were performed using SAS Institute's JMP Version 4.0.2 (Academic) except where indicated. Both FC and FE data were non-normal as determined by the Shapiro–Wilk test and were normalized through log transformation when possible. Microbial field data were analyzed as a whole set as well as by site. Correlations for transformed data were determined by calculation of Pearson Product–Moment correlation coefficients. Non-normally distributed or bimodal data that could not be reasonably transformed were analyzed for possible correlations using Spearman's Rho correlation coefficient, a rank-order method. A Simple Logistic Regression was performed to determine if any significant relationships existed between microbial data and TP and TC concentrations. A 2-factor multiple regression analysis was also employed to determine covariations between microbial and nutrient concentrations. A multiple regression was also used to determine relationships among all measured parameters (temperature, salinity, FC, sediment TP, sediment TC, and precipitation for 24 and 72 h).

Data collected from the experimental manipulation of P and C concentrations were non-normally distributed by the Shapiro–Wilk test and log transformed. Data were analyzed using a Two-Way ANOVA to identify differences in treatment effects and possible interactions between treatments. In order to investigate differences among treatments using pooled data from all trials, net change from initial pre-treatment values was analyzed using the Kruskal–Wallis Two-Way ANOVA for non-parametric data (Sokal & Rohlf, 1995) in Microsoft Excel.

Results

Field sampling

Sediment fecal bacteria counts and nutrient concentrations varied considerably for the entire field sampling data set as well as on a site by site basis (Table 1). Coefficients of variation (standard deviation/mean) exceeded 50% for all sample sites and usually exceeded 100%, reflecting substantial variability among the sampling times at each site (26 FC sampling events at each location except for 25 at BC-N; 8 FE sampling events at each location except for 7 at BC-NBU, BC-N and BC-S). High and low geometric means for FC counts occurred at Station BC-SBU (340 CFU cm^{-2}) and Station BC-S (125 CFU cm^{-2}), respectively. The high and low mean FE counts occurred at Station BC-N (528 CFU cm^{-2}) and station BC-CR (65 CFU cm^{-2}), respectively. A significant positive correlation (Pearson's Product-Moment) occurred between sediment (log)FC and (log)FE for all sites overall ($F = 15.3$, $P = 0.0003$, $r^2 = 0.262$, df = 45), while significant correlations between FC and FE were also found at stations BC-SBU ($F = 11.6$, $P = 0.014$, $r^2 = 0.660$, df = 7) and BC-S ($F = 62.3$, $P < 0.001$, $r^2 = 0.912$, df = 7) when data were analyzed by site.

Mean sediment TP values ranged from 101 μg g^{-1} at station BC-CR to 316 μg g^{-1} at station BC-S (Table 1). No significant regression was found between sediment TP and either bacterial indicator

in combined field data or for any individual site. Mean sediment total carbohydrate (TC) ranged from 714 μg g^{-1} at Station BC-CR to 23,500 μg g^{-1} at Station BC-S (Table 1). FC counts regressed negatively but weakly with sediment total carbohydrate concentrations for combined field data: LogFC = 2.45 − 0.23 LogTC, $F = 4.54$, $P = 0.04$, df = 139, $r^2 = 0.031$. FC counts did not regress significantly with sediment total carbohydrate at any individual site, but there was a significant negative regression between FE and sediment total carbohydrate at BC-NBU: LogFE = 5.25 − 1.01 LogTC, $F = 9.62$, $P = 0.03$, df = 5, $r^2 = 0.658$. Multi-way ANOVA showed that there was no significant combined effect from sediment P and sediment C on FC or FE for any or all sites (Table 2).

The effects of physical factors (salinity, temperature, and rainfall) on sediment FC and FE were also considered. Sites BC-S and BC-N were located at the edges of a salt marsh and subject to tidal exchange. Data from both sites were combined to look for significant effects. Salinities ranged from 0.5 to 34.1 ppt. FC counts decreased significantly with increasing salinity (LogFC = 2.14 − 0.04 Sal,

Table 2 Multi-way ANOVA of effects of temperature, salinity, carbohydrate, phosphorus, and precipitation for the previous 24 and 72 h on sediment-associated FC concentrations for all field sites

Source	d.f.	*F* value	*P* value
Model	6	**3.91**	**0.0013**
Temp	1	0.00	0.98
Salinity	1	0.02	0.88
Carbohydrate	1	2.67	0.10
Phosphorus	1	0.79	0.37
24 h precip	1	**17.48**	**<0.0001**
72 h precip	1	0.84	0.36
Error	125		
C. total	131		

F values and *P* values from ANOVA are shown with significant values in *bold*

Table 1 Sediment bacteria and nutrient data for all sites and individual sites

Site	Coliform	Enterococcus	Total phosphorus	Total carbohydrate
All	322 (411)	285 (473)	179 (144)	7,630 (10,100)
	0–3,230	0–1,730	6.1–671	202–45,000
	$n = 155$	$n = 45$	$n = 155$	$n = 141$
BC-SBU	340 (696)	332 (587)	137 (111)	1,300 (659)
	2–3,230	29–1,730	39.8–464	114–2,940
	$n = 26$	$n = 8$	$n = 26$	$n = 24$
BC-D	32 (68)	202 (293)	145 (98)	9,210 (9,190)
	0–295	0–868	15.8–354	1,780–45,400
	$n = 26$	$n = 8$	$n = 26$	$n = 24$
BC-NBU	186 (274)	365 (493)	119 (136)	2,640 (3,520)
	0–1,430	22–1,370	6.1–465	202–14,800
	$n = 26$	$n = 7$	$n = 26$	$n = 23$
BC-CR	257 (550)	65 (90)	101 (76)	714 (655)
	3–2,770	0–234	6.1–465	202–14,800
	$n = 26$	$n = 8$	$n = 26$	$n = 24$
BC-S	125 (301)	251 (448)	316 (153)	23,500 (11,700)
	0–1,550	4–1,230	12.4–671	2,070–45,200
	$n = 26$	$n = 7$	$n = 26$	$n = 23$
BC-N	132 (152)	528 (733)	261 (141)	8,910 (4,440)
	0–492	37–1,670	65.2–654	2,430–18,300
	$n = 25$	$n = 7$	$n = 25$	$n = 23$

Data as mean (SD)/range, bacteria in CFU cm^{-2} and nutrients in μg g^{-1} for field sites as in Fig. 1

$F = 23.7$, $P < 0.0001$, df = 49, $r^2 = 0.326$). When analyzed by individual site rising salinity had a significant negative effect on FC counts at both Station BC-S (LogFC = 2.1 − 0.03 Sal, $F = 10.2$, $P = 0.004$, df = 24, $r^2 = 0.30$) and Station BC-N (LogFC = 2.17 − 0.04 Sal, $F = 11.6$, $P = 0.002$, df = 23, $r^2 = 0.335$). No significant relationship with salinity was found for FE at either site or in combination. Water temperatures ranged from 2.5 to 31.5°C. Water temperature had no significant effect on either FC or FE for the combined field data. When data were examined by site there were significant positive effects of temperature on FC counts at stations BC-SBU (LogFC = 0.07 Temp + 0.44, $F = 5.38$, $P = 0.03$, df = 23, $r^2 = 0.19$), BC-CR (LogFC = 0.06 Temp − 0.5, $F = 8.54$, $P = 0.008$, df = 23, $r^2 = 0.27$), and BC-NBU (LogFC = 0.08 Temp + 0.54, $F = 10.04$, $P = 0.004$, df = 24, $r^2 = 0.29$), as well as a significant temperature effect on FE at site BC-SBU (LogFE = 0.19 Temp − 1.78, $F = 6.17$, $P = 0.048$, df = 6, $r^2 = 0.51$). Sites BC-SBU, BC-CR, and BC-NBU are relatively shallow freshwater creeks and are likely subject to greater temperature ranges than some of the deeper sampling locations. When rainfall, temperature, and salinity effects were considered together with sediment TP and TC, multiway analysis of variance revealed a very strong ($P < 0.0001$) 24 h rainfall effect on FC (Table 2), indicating that recent rain drove sediment-associated FC values. Sediment C, sediment P, salinity, temperature, and rainfall for the previous 72 h had no significant effects on FC in this analysis. The FE data were insufficient for this analysis.

Data for P as orthophosphate and FC in the water column in Bradley Creek were obtained from concurrent monitoring studies (NHTCP/WWP, Mallin et al., 2003, 2004, 2005). FC in the water column at a tributary adjacent to station BC-D exceeded NC standards (200 CFU (100 ml)$^{-1}$) 82% of times sampled in 2003–2004 (geometric mean = 807 CFU (100 ml)$^{-1}$) and 86% of times sampled in 2004–2005 (geometric mean = 1,207 CFU (100 ml)$^{-1}$). Orthophosphate in the water column at all NHTCP/WWP sites ranged from 0.001 to 0.052 mg P l^{-1} (=μg P cm^{-3}) with all means ≤0.02 mg P l^{-1} for 2002–2003, and for 2003–2004 P ranged from 0.002 to 0.044 with means for each site ≤0.019 mg P l^{-1}.

Experimental results

Differences between initial and post-incubation bacterial counts ranged from −440 to +8,990 CFU cm^{-2} with a mean of +1,830 CFU cm^{-2} for FC and −5 to +1,640 CFU cm^{-2} with a mean of +84 CFU cm^{-2} for FE. Dextrose treatments produced strong significant effects for both FC and FE overall, but treatments with P produced no significant overall effect on FC or FE (Table 3). Significant effects on FC occurred in dextrose treatments during three of six individual trials (BC-NBU-8/19, REC-8/24 and CR-9/6) and on FE in one trial (BC-NBU-8/19). Significant responses to added dextrose occurred for samples from sites with both high and relatively low initial C concentrations. Significant effects for P on FE were observed during two trials (BC-NBU-7/22 and BC-NBU-8/19). This location had relatively low initial TP concentrations (Table 1). There was also a significant interaction between dextrose and P treatments during one trial (BC-NBU-7/22). One other trial showed nearly significant ($0.05 < P < 0.1$) responses by FC to P treatment (BC-NBU-7/22) and, similarly, two trials by FE to C treatments (BWP and REC; Table 3).

Discussion

Results of the field component of this study demonstrated no significant positive relationships between sediment-associated FC or FE bacterial indicators and sediment TP, sediment total carbohydrate, salinity or temperature. Recent rain events were the only significant predictor of sediment fecal indicator bacteria levels (Table 2). This result suggests tight coupling between sources (humans and animals) of fecal indicator bacteria and aquatic sediment habitats via storm water runoff and deposition processes. Numerous studies have documented the importance of storm water runoff as a source of fecal contamination to surface waters, particularly in developed watersheds in coastal areas (Mallin et al., 2000, 2009), but this study links storm water runoff and sediment fecal contamination as well, as have others elsewhere (Jing et al., 2004; Jeng et al., 2005; Krometis et al., 2007). Several mechanisms may be hypothesized to drive loading of fecal indicator bacteria to sediments: prior association of fecal

Table 3 Effects of dextrose (C) and phosphorus (P) treatments and interactions (I) between dextrose and phosphorus treatments on fecal coliforms (FC) and enterococcus (FE) in cores for experimental trials

Site	Initial [P] µg/g sed	Initial [C] µg/g sed	Treatment	FC		FE	
				F	*P*	*F*	*P*
BC-NBU	20	420	C	**29.9**	**0.001**	**11.9**	**0.009**
(8/19)			P	0.65	0.44	**7.00**	**0.029**
			I	0.89	0.37	0.01	0.92
BC-NBU	31	628	C	0.02	0.89	2.37	0.16
(7/22)			P	3.53	0.1	**5.71**	**0.044**
			I	**57.6**	**<0.0001**	<0.001	1.00
BWP	47	290	C	1.71	0.22	4.50	0.07
			P	1.05	0.34	0.50	0.49
			I	0.39	0.55	0.50	0.49
210	74	290	C	2.60	0.15	0.08	0.78
			P	3.36	0.10	0.50	0.49
			I	8.04	0.22	0.50	0.50
REC	102	2,990	C	**7.71**	**0.024**	4.91	0.06
			P	0.22	0.65	0.47	0.51
			I	0.47	0.41	0.78	0.40
CR	176	3,600	C	**17.8**	**0.003**	0.08	0.59
			P	0.91	0.37	0.50	0.19
			I	3.06	0.12	0.50	0.19
All*			C	**11.6**		**5.59**	
			P	0.96		0.14	
			I	0.00		3.34	

Data were log transformed and analyzed by Two-Way ANOVA (df = 11). Data that could not be transformed to normality were analyzed using Kruskal–Wallis ranked Two-Way ANOVA (df = 23) and are indicated by "*"; *H* statistic is given in lieu of an *F* value and critical $X^2 \geq 3.84$. Significant effects are in *bold*

bacteria with particles that deposit in aquatic habitats and settle after transport in storm water runoff (Jeng et al., 2005), scavenging of suspended bacteria by flocs or particulates that subsequently settle, and pumping of bacteria-laden water through sediment matrices by wave action and other pressure differential-inducing mechanisms (Precht & Huettel, 2003). Moreover, rapid loading of indicator bacteria (and potentially other problematic microbes) derived from storm water runoff into sediments may mask the full impact of such non-point pollution as measured conventionally by water column sampling alone.

Experimental results confirmed the inference from field studies that sediment TP levels were generally not limiting for sediment-associated FC and FE bacteria, indicating that sediment P levels in this urbanized coastal watershed were higher than concentrations likely to be limiting. More frequent stimulation of FE and FC growth by added carbohydrate than by added P in these 2-factor experiments strengthens the inference that these bacteria were not limited by P availability. P sources within this developed watershed include residential use of fertilizers on well drained sandy soils (Cahoon, 2002) and deposition of animal wastes (Mallin et al., 2000). Mechanisms of P partitioning to sediments, including particle adsorption, sedimentation, precipitation, and biological uptake, can support accumulation of sediment P to concentrations well above background levels found in pristine watersheds. Sediment TP concentrations exceeded water column P concentrations (Mallin et al., 2004, 2005) at these sampling sites by 4–5 orders of magnitude (Table 4). Measurements of P in the water column alone therefore seriously underestimate the presence and availability of TP in this urbanized watershed. Thus, sediment bacterial populations are not likely to be

P limited in more developed watersheds, despite the relatively greater need for P by bacteria than by microalgae (Goldman et al., 1987; Caron, 1991; Carlsson & Caron, 2001). We speculate that such high levels of sediment-associated TP could help maintain both high concentrations of sediment microbiota as well as episodic enhancement of water column populations via sediment resuspension and P recycling across the sediment–water interface.

Experimental stimulation of sediment-associated fecal indicator bacteria by added carbohydrate indicates potential coupling with allochthonous and autochthonous organic carbon sources. Storm water runoff has been identified as a significant source of bio-available C (Buffam et al., 2001; Soendergaard et al., 2003; Seitzinger et al., 2005). Concentrations of 5-day biochemical oxygen demand (BOD5) in tidal creek watersheds in this same area have been positively correlated with rain events and measures of urban development (Mallin et al., 2009). Thus, increases in relative concentrations of bio-available C are correlated with increased urbanization, which may help explain the significant effects of recent precipitation identified in this study. We hypothesize that bio-available C release from extensive vegetated residential landscapes in developed watersheds may complement fertilizer loadings from these landscape features.

Sediment-associated bacteria populations did not always follow well established physical patterns for indicator bacteria in the water column. In other studies FC and FE concentrations have been inversely related to salinity (Hanes & Fragala, 1967; Evison, 1988; Solic & Krstulovic, 1992; Mallin et al., 2000) and positively with temperature in the water column (Struck, 1988; Solic & Krstulovic, 1992; Howell, et al., 1996), but sediment-associated bacteria exhibited attenuated responses to these factors at best in this study. Lack of a significant FE response to salinity altogether may have been due to smaller sample size. Significant temperature effects were only seen at selected locations rather than for the data as a whole. This does not conform well to patterns of positive responses to temperature by sediment-associated bacteria documented by others (Van Donsel et al., 1967; Edmonds, 1976; Davies et al., 1995). Combinations of other factors may be favorable enough within the sediments to overcome the hindrance of growth and persistence associated with higher salinities at a few sites and cooler water temperatures in general.

Table 4 Comparisons of phosphorus concentrations to a core depth of 1 cm at sediment sampling stations and at adjacent water column sampling locations in Bradley Creek, 2002–2004 (water column data from Mallin et al., 2004, 2005)

Site	Sediment μg TP cm^{-3}	Water column μg P cm^{-3}
BC-SBU	61.5	0.010
BC-D	37.7	0.020[a]
BC-NBU	79.0	0.004
BC-CR	70.8	0.006
BN-N	75.3	0.010

[a] Measurements recorded from a stream adjacent to the stormwater detention pond where site BC-D is located

Aquatic sediments can be a significant reservoir of fecal indicator bacteria, and potentially a source of contamination to the overlying water column. Results of this study show that sediments in a coastal, developed watershed can be heavily contaminated on an almost continuous basis. Broad correlation between concentrations of the two fecal indicator bacteria types strengthens the inference that fecal pathogens co-occur in the sediments, although these relationships clearly require further direct examination. Sediments containing these microorganisms may easily be suspended by a variety of disturbances. Dredging, wave action, changes in stream flow associated with precipitation and/or tidal stage, boating, or other physical disturbances may all resuspend bacteria from within the sediment bed (Grimes, 1980; Tunnicliff & Brickler, 1984; Doyle et al., 1992; Mallin et al., 2007). Fecal indicator bacteria concentrations in sediments of 32–528 CFU cm^{-2} (Table 1) correspond to populations of 320,000–5,280,000 m^{-2} in these shallow habitats, predicting significant risk of water quality impairments by suspension processes. Previous studies by the New Hanover County Tidal Creeks Project (Mallin et al., 2003) of FC concentrations in Bradley Creek headwaters yielded a geometric mean of 807 CFU $(100\ ml)^{-1}$ and range of 60–6,000 CFU $(100\ ml)^{-1}$, consistent with bacterial contributions from suspended sediments, among other sources. Such contributions may be significant in triggering more frequent regulatory closures of these waters to designated uses.

The implications of large concentrations of fecal indicator microbes associated with tidal watershed sediments are clearly profound. Results of this study indicate that environmental factors potentially controlling persistence and growth in situ are less important overall than the storm water sources of these indicator bacteria in urbanized watersheds. Relatively high concentrations of nutrients, organic carbon, and bacteria populations in sediments versus the water column signify the real magnitude of the challenge to water quality management. The likely covariance of storm water runoff with nutrient and organic carbon sources in addition to bacterial transport processes in developed watersheds strongly implies that management of microbial water quality requires better management of runoff and nutrient inputs (Mallin et al., 2000; Buffam et al., 2001; Soendergaard et al., 2003; Sundareshwar et al., 2003; Seitzinger et al., 2005). These findings amplify the need for more insightful and effective management of human effects on coastal watersheds.

Conclusion

Fecal bacteria are commonly a cause of water quality impairment in developed areas where tidal watersheds are valuable recreational and commercial resources. FC and FE have been shown to indicate the potential presence of other fecal pathogens in surface waters (APHA, 2001; Lipp et al., 2001), and are likely to do so in sediment habitats as well. Concentrations of sediment-associated fecal indicator bacteria occur at levels comparable to if not greater than in the overlying water column and are strongly linked to precipitation, suggesting that storm runoff is a controlling factor for this microflora. Field and experimental evidence suggest that availabilities of P and (usually) organic C in sediments of developed watersheds exceed limiting thresholds for fecal indicator bacteria. Temperature and salinity variation appear less predictive of fecal indicator bacteria concentrations in sediments than in the water column. There is much evidence that management of sediment-bound fecal bacteria may be aided by appropriate management of stormwater inputs as has been demonstrated in surface waters. Given that recreational and commercial use of coastal waters often accompanies exposure to waterway sediments initiatives to monitor sediment indicator bacteria should be undertaken.

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