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Effects of changing land use on the microbial water quality of tidal creeks

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ABSTRACT

Population growth along the southeastern United States coast has precipitated the conversion of forested watersheds to suburban and urban ones. This study sampled creeks representing forested, suburban, and urban watersheds along a longitudinal gradient for indicators of water quality, including traditional indicator bacteria (fecal coliforms and enterococci) and alternative viral indicators (male-specific and somatic coliphages). Tested microorganisms were generally distributed with highest concentrations in creek headwaters and in more developed watersheds. The headwaters also showed the strongest predictive relationship between indicator concentrations and urbanization as measured by impervious cover. A seasonal pattern was observed for indicator bacteria but not for indicator viruses. Coliphage typing indicated the likely source of contamination was nonhuman. Results suggest that headwater creeks can serve as sentinel habitat, signaling early warning of public health concerns from land-based anthropogenic activities. This study also implies the potential to eventually forecast indicator concentrations under land use change scenarios.

1. Introduction

We benefit enormously from our coastal resources, which provide seafood, commerce, and recreational opportunities, as well as many free ecological services such as waste processing, shoreline protection, and biodiversity conservation. In fact, over half of the US population lives in coastal counties, and more people are relocating to the coast every day. In South Carolina (SC), for example, the population of the six coastal counties has increased over 30% since 1990 (US Census Bureau, http://quickfacts.census.gov/qfd/maps/south_carolina_map.html, accessed on Feb 26, 2008). Current coastal development practices are consuming land at a rate 3–6 times faster than the population is growing (Beach, 2002; Allen and Lu, 2003). For instance, between 1974 and 1994 the developed area near Charleston, SC increased 250% while the human population only increased 40% (Allen and Lu, 2003). The urban sprawl associated with coastal development, as indicated by unprecedented increases in impervious cover (e.g., roofs, parking lots, roads), is a threat to the health of coastal resources (Schueler, 1994; Arnold and Gibbons, 1996). Small changes in impervious cover are associated with large changes in ecological properties (Holland et al., 2004).

In the southeastern US, the coastal uplands adjacent to tidal creeks and salt marshes are increasingly popular targets for building homes, resorts, retirement destinations, and recreational facilities. These tidal creek networks are also critical feeding grounds, spawning areas, and nursery or primary habitats for many species of fish, shellfish, birds, and mammals. Tidal creeks also form the primary hydrologic link between estuaries and land-based activities and, as such, reflect the impacts of coastal development earlier than larger coastal waterbodies (Holland et al., 2004). Nonpoint source pollution (e.g., stormwater runoff) carries sediments, chemicals, bacteria, viruses, and other pollutants into tidal creeks and salt marshes and degrades water quality. There is an emerging consensus that increasing coastal development is associated with increasing fecal pollution in tidal creeks, estuaries, and bathing beaches (Mallin et al., 2000b; Holland et al., 2004; Mallin, 2006). From a human health perspective, the accumulation of pathogens in the water, sediments, and organisms may render seafood products unsafe to eat and water unsafe for body contact recreation (Stewart et al., in press). Diseases that could result include gastroenteritis, ear and respiratory infections, hepatitis, and skin rashes.

Despite the human health implications, spatial distributions and population dynamics of fecal microorganisms in aquatic environments are not well understood. Measurements of indicator

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bacteria, such as fecal coliforms and enterococci, are typically used to measure contamination. However, constraints in sample processing capacity typically lead research and monitoring programs to resort to little more than ad hoc sampling designs. The overall result is that concentrations of these indicator bacteria in aquatic systems appear highly variable, influenced by a dynamic interplay of habitat characteristics such as temperature, sunlight, tidal stage, and availability of particulates and nutrients; and biological factors including cell integrity and viability (Rhodes et al., 1983; Fleisher, 1985; Anon., 1995). Furthermore, bacteria are inadequate indicators for all microbial contaminants that may be associated with fecal pollution, particularly enteric viruses. Coliphages, viruses that infect *Escherichia coli*, have been proposed as a more appropriate indicator for enteric viruses in water (IAWPRC, 1991; Havelaar et al., 1993).

Spatial and temporal variability in conditions along tidal creek networks must be better understood to enable effective monitoring, assessment, and prediction of the effects of coastal urbanization on tidal creeks and estuaries. Stratification of tidal creek networks into units that represent relatively homogenous environments or creek classes is one tool for characterizing and understanding the variability within tidal creek networks. Classifying watersheds that drain into specific creek networks based on the degree and type of development that has occurred is another tool for understanding variability among creek networks. This study integrated both approaches to evaluate spatial and temporal variability of water quality indicators in tidal creek systems. The specific objectives of this study were to: (1) determine if concentrations of indicator bacteria or indicator viruses in tidal creeks were associated with the degree of anthropogenic development in the watershed, (2) quantify the concentrations of indicator bacteria and indicator viruses within different classes of the tidal creek network, (3) examine seasonal variation in indicator concentrations, and (4) evaluate indicator viruses as a supplement to indicator bacteria for inclusion in future monitoring studies.

2. Materials and methods

2.1. Study sites

Eleven tidal creek ecosystems located along the coast of South Carolina were sampled during January–March and again in June–August, 2005 (hereafter referred to as "winter" and "summer," respectively). One additional system was added during the summer period. Watershed details are given in Table 1, and locations of study creeks are shown in Fig. 1. Tidal creeks in South Carolina have a 1–3 m tidal range, depending on latitude, with semi-diurnal tides. Surrounding salt marshes are dominated by *Spartina alterniflora* and *Juncus roemarianus*.

In order to characterize indicator variability along the entire creek length (i.e., within the creek network), a tidal creek classification model, analogous to the freshwater stream model (Horton, 1945; Strahler, 1957), was employed. The first order, or headwaters, of each creek directly drained coastal uplands and was characterized by its narrow width and predominately intertidal habitat. The second order of each creek was formed by the confluence of two or more first order creeks. Second order systems were wider and had subtidally dominated habitats. The third order of each creek was formed by the confluence of two or more second order creeks. Third order systems were large creeks with very little intertidal habitat. Along the SC coast, third order creeks usually empty into the larger tidal rivers and coastal estuaries, although occasionally a fourth order creek formed by the confluence of two or more third order creeks can occur. Not every tidal creek system has all orders and not every order could be sampled in each creek for this study. Therefore, the 12 creeks varied in regards to the number of orders (1–3) that were sampled and also in respect to the surrounding land use (Table 1). To make sure collected data were comparable for this study, each creek order was divided into three equidistant reaches using ArcGIS 9 (ESRI, Redlands, CA), and one station was randomly located within the second reach (i.e., the middle section) of each creek order for sample collection. Samples from other reaches were collected for other variables but are beyond the scope of the research presented here.

2.2. Land use and watershed determinations

Creek watersheds were classified into the following land use categories based on impervious cover as modified from Holland et al. (2004): (1) forested (<10% impervious cover); (2) suburban ($\geqslant$10% but <30% impervious cover) and (3) urban ($\geqslant$30% impervious cover). There was one exception to this classification. The Orange Grove watershed was estimated to have 37.3% impervious cover; however, since this was primarily light residential development and a small amount of upland (127 ha) relative to the total watershed size (322 ha), we considered this a suburban watershed.

Impervious cover data were determined for each creek network (watershed) and each creek order (sub-watershed). To do this, watersheds and their sub-watersheds were delineated based on elevation contours defined by 1:24,000 United States Geological Survey topographic maps. The outline of each sub-watershed was then digitized into ArcGIS 9. The impervious cover within each watershed was calculated using the 2001 National Land Cover Database (NLCD) impervious layers (Homer et al., 2004). Only upland

Table 1
Tidal creek and watershed attributes for systems sampled for microbial indicators in winter and summer, 2005

Creek land use type	Creek system	Latitude (°N)	Longitude (°W)	Area (ha)	Imp cov[a] (%)	Orders sampled
Forested	Guerin Creek	32.943	79.771	3427	3.04	3
	North Inlet Creek	33.338	79.186	1860	2.93	3
	Village Creek	32.422	80.519	2016	3.98	3
Suburban	Albergottie Creek	32.450	80.716	2096	23.94	3
	James Island Creek	32.744	79.958	1820	29.45	5[b]
	Okatee Creek	32.304	80.922	5501	13.33	3
	Orange Grove Creek	32.812	79.978	322	37.33	2
	Parrot Creek	32.734	79.907	501	17.71	3
Urban	Bulls Creek	32.826	80.026	510	38.11	3
	Murrells Inlet Creek	33.569	79.016	1297	40.34	2
	New Market Creek	32.806	79.940	199	70.38	1
	Shem Creek[c]	32.801	79.870	1269	47.66	2

[a] Imp Cov = impervious cover.
[b] Five creek segments were sampled in the James Island Creek system: two first order creeks, two second order creeks, and one third order creek.
[c] Shem Creek was sampled only during summer; all other creeks were sampled in both winter and summer.

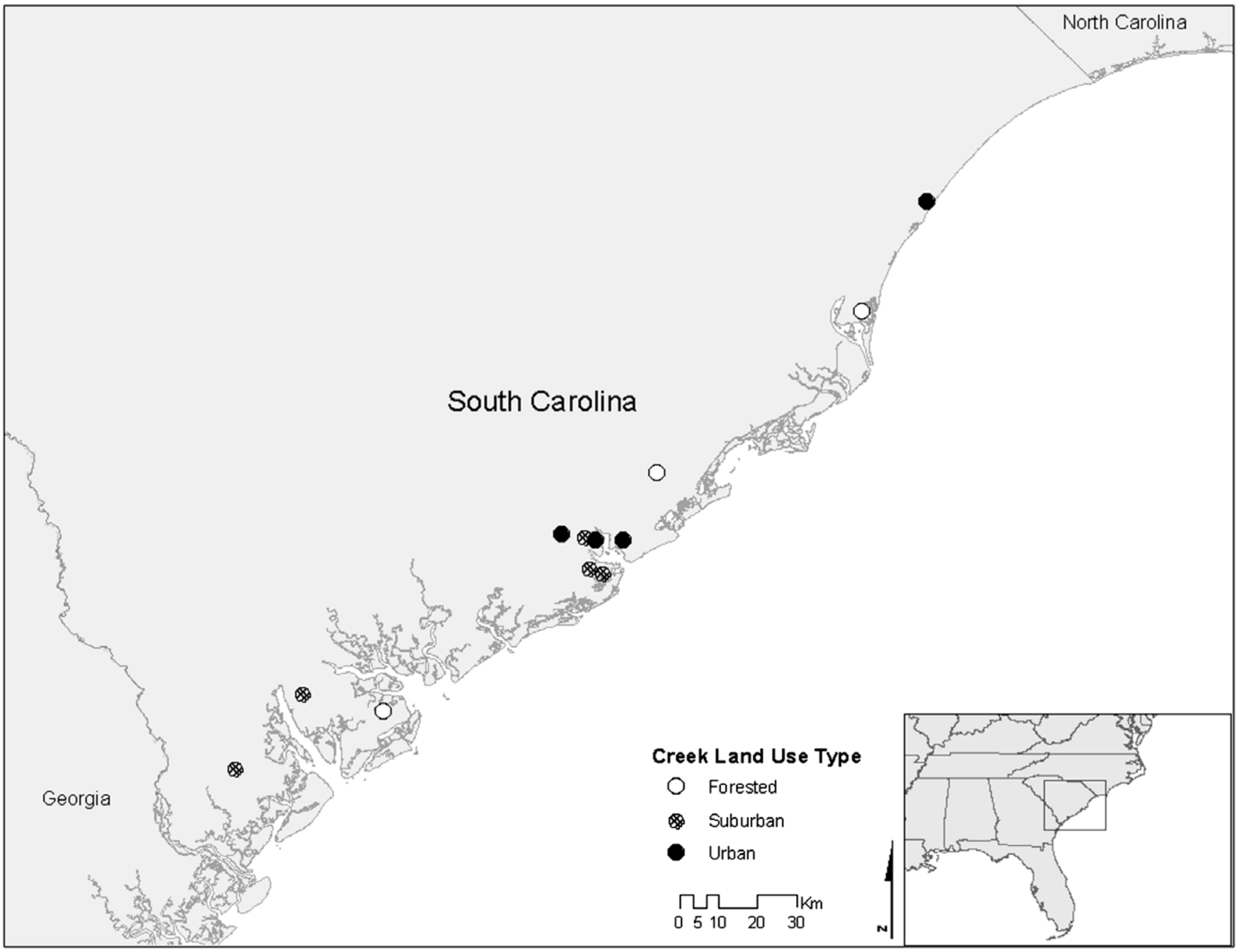

Fig. 1. The South Carolina (U.S.A.) coastal region and the locations of creeks sampled in 2005. Symbols indicate land use type in the surrounding watershed (open circle, forested; hatched circle, suburban; filled circle, urban).

habitat was considered for calculating impervious cover for this study; that is, all estuarine water and salt marsh areas were excluded before clipping the NLCD data by each sub-watershed or watershed boundary. Impervious cover levels were then calculated directly from the NLCD for all sub-watersheds and watersheds. These NLCD-derived impervious cover estimates were compared to values published in Holland et al. (2004). Both of these data sets used aerial photography from similar time frames to estimate impervious cover, but the NLCD impervious cover values underestimated the ground-truthed data reported in Holland et al. (2004). A similar underestimation was reported by Jarnagin et al. (2006). A quadratic equation ($y = 2.9301 + 2.16789x - 0.01611x^2$) was developed to adjust the NLCD-derived estimates. Adjusted values are reported and used here.

2.3. Sampling design

Creeks were sampled on the ebb tide, approximately 2–3 h prior to low tide, in order to capture the upland influence on water quality. The first order was sampled by wading into the creek, while the second and third orders were sampled by boat. Sampling was conducted in an upstream direction to minimize resuspension of sediments (and associated microbes). Water samples were collected in sterile 2 L polypropylene bottles at approximately 0.3 m below surface or mid-water column in water less than 0.3 m in depth. Samples were held on ice until processed which always occurred within 24 h of sample collection. Following sample collection, a YSI 6600 data logger was deployed at each site to record water temperature, salinity, and dissolved oxygen over two tidal cycles. Data loggers remained in the creek overnight and were collected the next day. Water quality data (temperature, salinity, dissolved oxygen) were downloaded and averaged over the 24 h for each creek order. These were further averaged to get creek system values for both winter and summer.

2.4. Detection of indicator bacteria and viruses

Fecal coliforms (FC) and enterococci (Ent) were enumerated by membrane filtration according to standard methods (APHA, 1998). Coliphages were enumerated and characterized as described in Stewart-Pullaro et al. (2006). Both male-specific and somatic coliphages were enumerated by the single agar layer method, adapted from US EPA Method 1602 (USEPA, 2001b). Male-specific coliphages were further detected by an enrichment presence/absence method, adapted from US EPA Method 1601 (USEPA, 2001a). Up to 100 plaques representing male-specific coliphages were picked per sample and differentiated as F^+ RNA or F^+ DNA based on their ability to propagate in the presence of RNAse A. Confirmed F^+ RNA coliphage isolates were genetically typed into one of four groups to distinguish human (types II and III) from nonhuman (types I and IV) sources of pollution. This genetic typing was performed by hybridization with nonradioactive oligonucleide probes

(Hsu et al., 1995). Use of coliphage typing to differentiate sources of pollution is based on ecology studies (Havelaar et al., 1986; Furuse, 1987). While the associations appear to be significant (Schaper et al., 2002), this approach appears useful, although not absolute, for distinguishing human from nonhuman sources of pollution (Brion et al., 2002; Cole et al., 2003; Stewart-Pullaro et al., 2006).

2.5. Statistical analyses

Each of the four indicators [fecal coliforms (FC), enterococci (Ent), somatic coliphages (F− col), and male-specific coliphages (F+ col)] was analyzed separately. All data were $\log 10(x+1)$ transformed prior to analysis to obtain normality and homoscedasticity. Analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC).

The first analysis examined the seasonal data separately and evaluated the effect of land use (forested, suburban, urban) and creek order (first, second, third) on indicator concentrations using a two-way ANOVA (Proc GLM), with land use type and creek order as fixed factors. A second analysis examined the indicator concentrations across season (winter, summer) and land use type using a two-way ANOVA (Proc GLM), with season and land use type as fixed factors. Analyses were performed for each creek order separately. The focus of this latter analysis was to determine if indicator concentrations differed across summer and winter study periods. Land use type was included in the model to account for this variability before testing for a seasonal difference. In all analyses of variance, the first order interaction term was evaluated and excluded from the final model if determined to be non-significant ($p > 0.05$). When a factor was determined to be significant ($p \leqslant 0.05$), specific pairwise differences were examined by comparing least square means. A third analysis used regression (Proc REG) to evaluate the relationship between impervious cover and indicator concentration for each creek order.

3. Results

Creek water temperature ranged from 6 to 14 °C in winter and 25 to 32 °C in summer. Dissolved oxygen (expressed as % saturation) was higher in the winter (82–107%) than summer (44–91%). From the headwaters to larger subtidal creek classes, salinity generally increased by 41% in winter and 44% in summer. The average salinity across all creeks was approximately 24 ppt in winter and 19 ppt in summer. The maximum salinity range observed in either winter or summer was 24 ppt (Okatee) and the minimum was 0.7 ppt (Parrot). Most of the creeks had an average salinity of 18–35 ppt; however, Guerin Creek and Bulls Creek were lower in salinity than the others (4–6 ppt in summer; 10–15 ppt in winter). In summer, Okatee Creek had a salinity of approximately 11 ppt.

Concentrations of bacterial indicators, FC and Ent, tended to fall within an order of magnitude of each other for a given sample. FC concentrations ranged from <1 to 4.1×10^4 colony forming units (CFU)/100 mL while Ent concentrations ranged from 2 to 2.1×10^4 CFU/100 mL. Levels of measured viruses tended to be lower than those of the bacteria, ranging from <0.1 to 2.2×10^2 plaque forming units (PFU)/100 mL for F+ coliphages and from <1 to 2.6×10^3 PFU/100 mL for F− coliphages. All tested indicators were positively correlated to each other ($p < 0.05$) using a Spearman correlation test.

3.1. Land use comparison

During winter, a general land use pattern emerged, with first order creek segments draining forested watersheds having lower

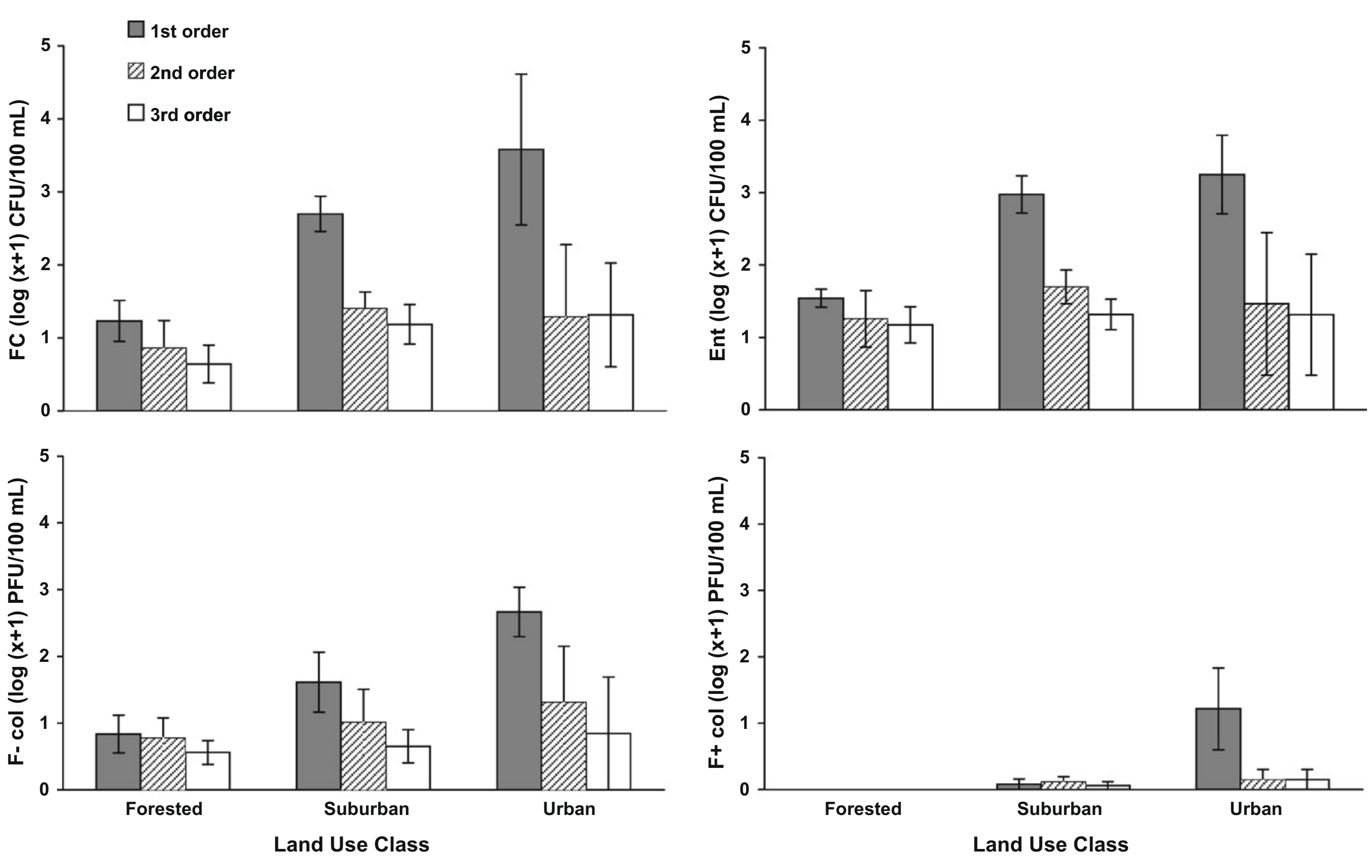

Fig. 2. Results for sampled bacterial and viral indicators from Winter, 2005. Bars represent creek order means, and error bars are +1 standard error. FC = fecal coliform, Ent = enterococcus, col = coliphage, CFU = colony forming unit, PFU = plaque forming unit.

Table 2
Results of 2-way ANOVAs performed on four microbial indicators sampled in winter and summer, 2005

	Parameter	Model *p*	Land use *p*	Order *p*	R^2	Land use	Order
Winter	Enterococcus	0.0003	0.052	0.0002	0.548	For[a] Urb[a,b] Sub[b]	Ord3[a] Ord2[a] Ord1[b]
	Fecal coliform	0.0001	0.0092	0.0002	0.575	For[a] Sub[b] Urb[b]	Ord3[a] Ord2[a] Ord1[b]
	F− coliphage	0.054	ns	0.051	0.292		Ord3[a] Ord2[a,b] Ord1[b]
	F+ coliphage[1]	0.001	0.001	0.006	0.677		
Summer	Enterococcus	0.0033	ns	0.0016	0.420		Ord3[a] Ord2[a] Ord1[b]
	Fecal coliform	<0.0001	0.0104	<0.0001	0.582	For[a] Sub[b] Urb[b]	Ord3[a] Ord2[a] Ord1[b]
	F− coliphage	0.0053	ns	0.0075	0.399		Ord3[a] Ord2[a] Ord1[b]
	F+ coliphage[1]	0.0084	0.0055	ns	0.377	For[a] Sub[a] Urb[b]	

Post hoc multiple comparisons were performed by comparing least squared means; model factors (arranged from low to high) with same superscripts are not statistically different.
ns = not significant; For = forested; Sub = suburban; Urb = urban; Ord = order.
[1] There was a significant ($p = 0.003$) interaction term between land use and order; pairwise comparisons not completed.

indicator concentrations than more urbanized creeks (Fig. 2). While some of the indicators in second and third order creeks showed slightly higher concentrations in the more developed creeks, the differences were not as consistent or as marked when all orders were considered collectively. FC concentrations were significantly lower in forested creeks compared to suburban and urban creeks, which were not different from each other (Table 2). Ent concentrations showed a non-significant land use effect in the 2-way ANOVA ($p = 0.052$), but the forested watersheds were significantly lower in Ent concentration compared to suburban watersheds. F− col concentrations did not show a significant land use effect, but F+ col concentrations showed a highly significant land use effect ($p = 0.001$), although there was a significant interaction ($p = 0.003$) between land use and creek order (Table 2). This significant interaction term was driven by high concentrations of F+ col in urban first order creeks (Fig. 2).

During summer, first order creek segments again demonstrated the highest indicator concentration, and this effect was more marked in urbanized watersheds (Fig. 3). Across all creek orders, both FC and F+ col concentrations showed a significant effect of land use (Table 2). FC concentrations were significantly higher in urban and suburban creeks compared to forested creeks, while F+ col concentrations were significantly higher in urban creeks compared to suburban and forested creeks. Land use effects were not significant for F− col and Ent; however, both indicators showed an increasing trend in concentrations in the developed systems compared to the undeveloped systems (Fig. 3).

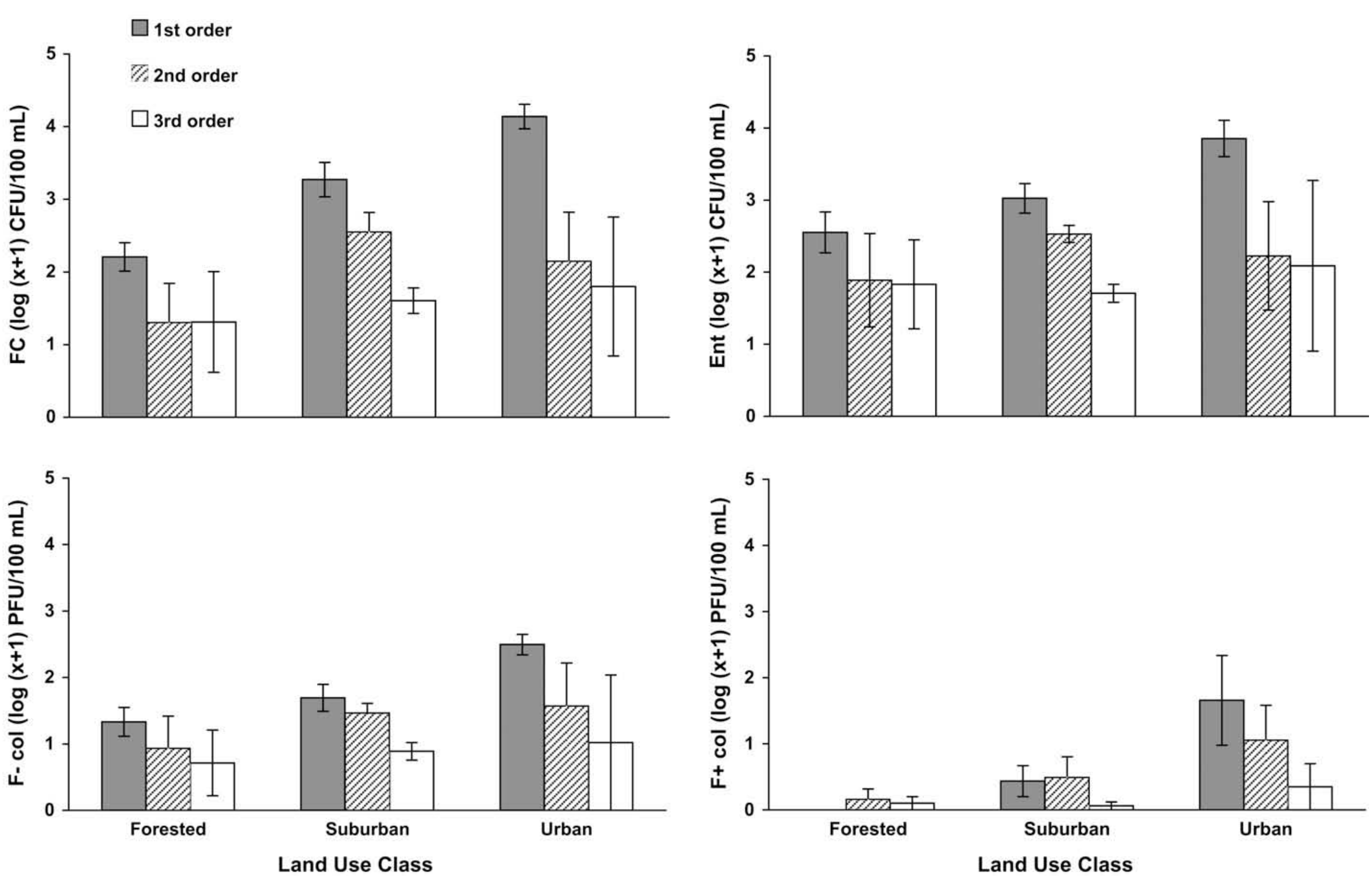

Fig. 3. Results for sampled bacterial and viral indicators from Summer, 2005. Bars represent creek order means, and error bars are +1 standard error. FC = fecal coliform, Ent = enterococcus, col = coliphage, CFU = colony forming unit, PFU = plaque forming unit.

Table 3
Results of 2-way ANOVAs performed on four microbial indicators sampled in winter and summer, 2005

Parameter	Order	Model p	Season p	Land use p	R^2	Season	Land use
Enterococcus	1	0.0007	ns	0.0006	0.583		For^a Sub^b Urb^b
	2	ns	0.0392	ns	0.287	Win^a Sum^b	
	3	ns	ns	ns	0.164		
Fecal coliform	1	<0.0001	0.013	<0.0001	0.710	Win^a Sum^b	For^a Sub^{a,b} Urb^b
	2	0.0258	0.019	ns	0.412	Win^a Sum^b	
	3	ns	ns	ns	0.216		
F− coliphage	1	0.0163	ns	0.0078	0.410		For^a Sub^a Urb^b
	2	ns	ns	ns	0.126		
	3	ns	ns	ns	0.057		
F+ coliphage	1	0.0008	ns	0.0005	0.574		For^a Sub^a Urb^b
	2	ns	ns	ns	0.319		
	3	ns	ns	ns	0.216		

Post hoc multiple comparisons were performed by comparing least squared means; model factors (arranged from low to high) with same superscripts are not statistically different.
ns = not significant; For = forested; Sub = suburban; Urb = urban; Ord = order; Win = Winter; Sum = Summer.

3.2. Creek order comparison

During the winter, both Ent and FC concentrations exhibited similar spatial gradients, with first order creeks having significantly higher concentrations of bacterial indicators than second and third order creeks (Table 2, Fig. 2). F− col indicator concentrations were not significantly affected by creek order (p = 0.051); F+ col showed a strong land use by creek order interaction.

During summer, first order creek segments often had higher indicator concentrations than second and third order creek segments (Table 2, Fig. 3) across all land use classes. Both Ent and FC concentrations were significantly lower in second and third order, compared to first order creeks, and the F− col concentration longitudinal gradient was similar to the bacterial indicators. The F+ col concentrations did not demonstrate a significant spatial pattern.

3.3. Seasonal comparison

Seasonal differences were only observed for Ent and FC concentrations in certain creek orders (Table 3). The Ent concentrations in winter were significantly lower than during summer, but only in the second order. On the other hand, FC concentrations were

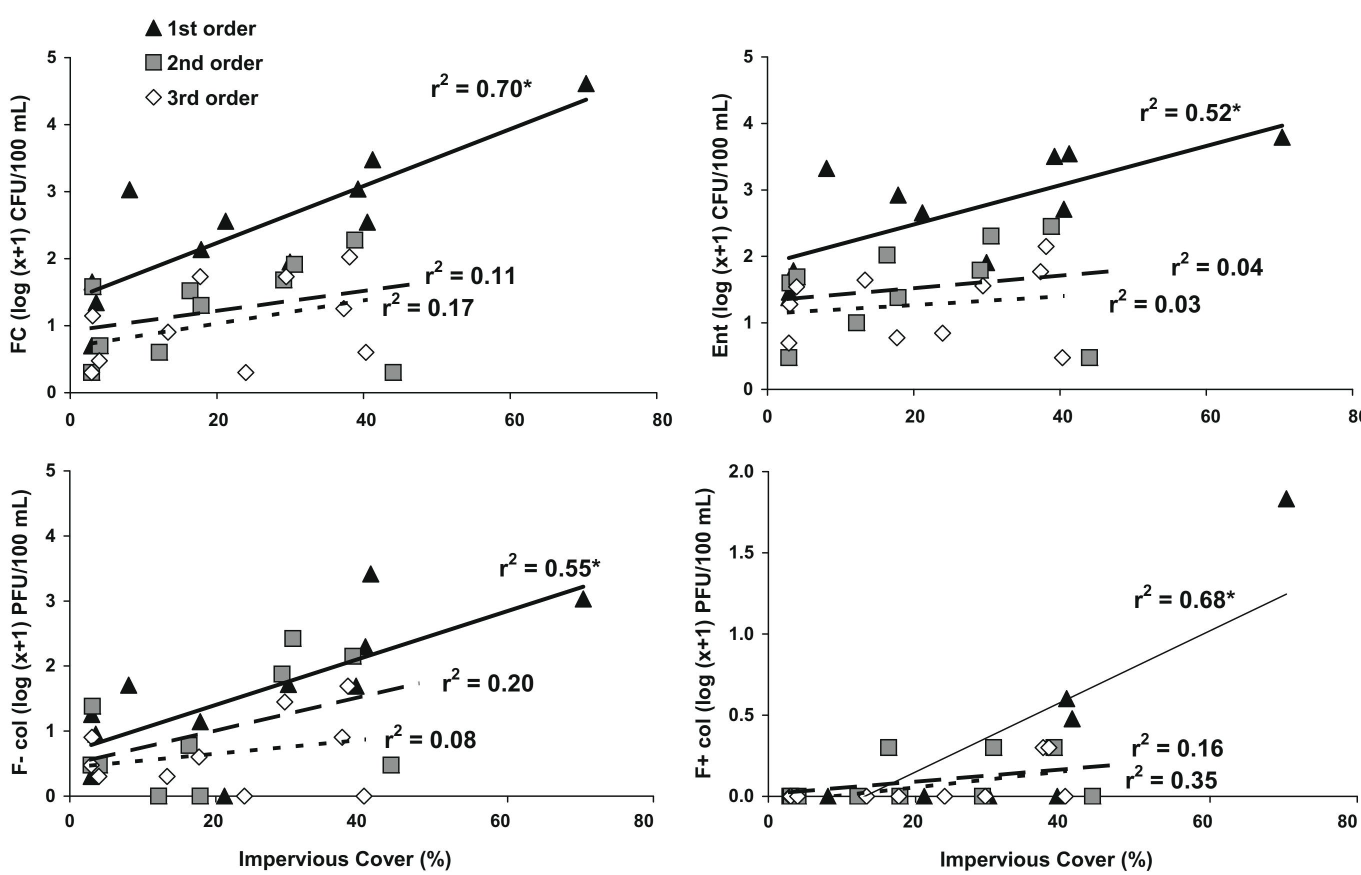

Fig. 4. Regression results for sampled bacterial and viral indicators from Winter, 2005. Solid, dashed, and dotted lines indicate the least squares regression line for first order, second order, and third order creeks, respectively. All r^2 are shown for each regression, with asterisks (*) indicating significant (p < 0.05) regressions. FC = fecal coliform, Ent = enterococcus, col = coliphage, CFU = colony forming unit, PFU = plaque forming unit.

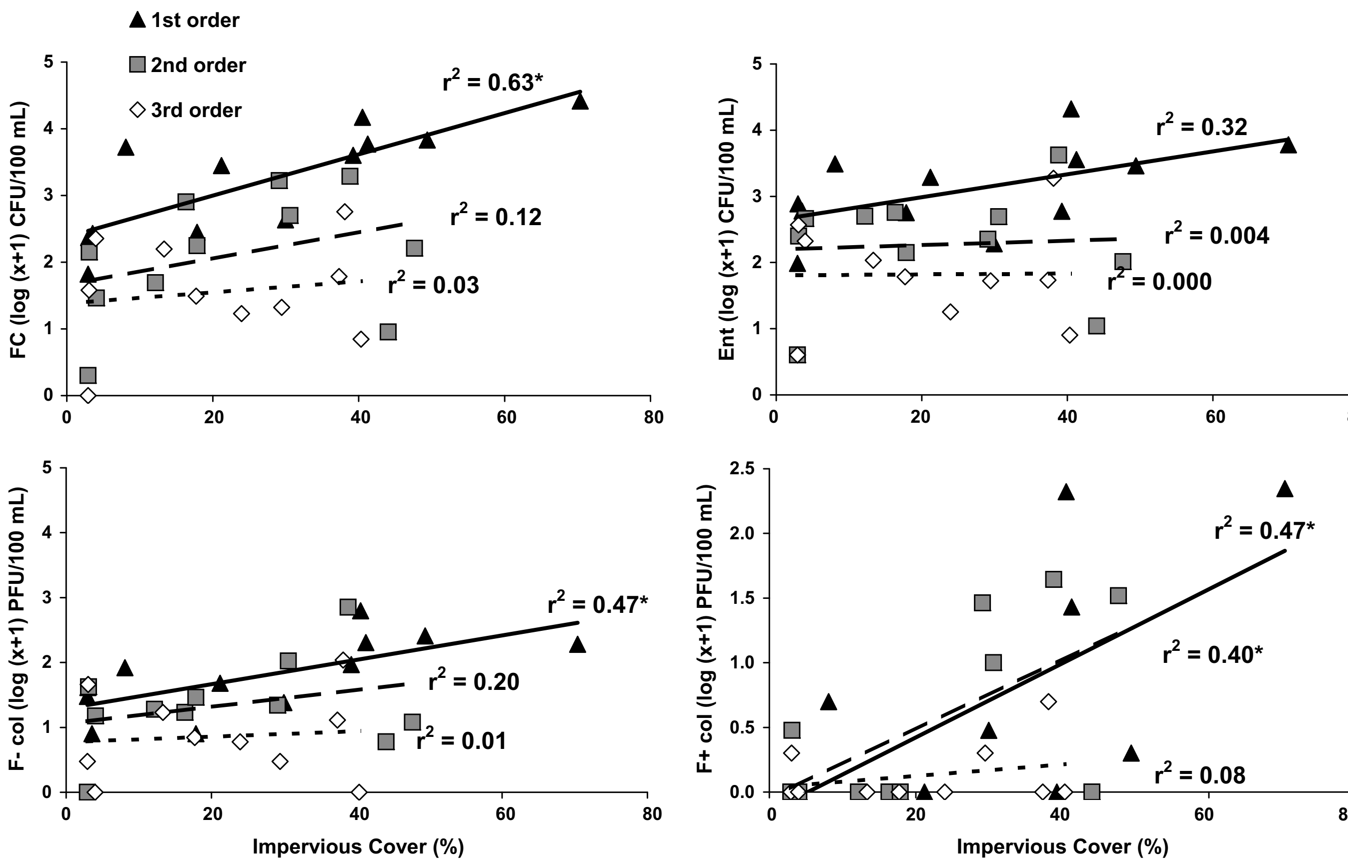

Fig. 5. Regression results for sampled bacterial and viral indicators from Summer, 2005. Solid, dashed, and dotted lines indicate the least squares regression line for first order, second order, and third order creeks, respectively. All r^2 are shown for each regression, with asterisks (*) indicating significant ($p < 0.05$) regressions. FC = fecal coliform, Ent = enterococcus, col = coliphage, CFU = colony forming unit, PFU = plaque forming unit.

significantly lower in winter relative to summer in both first and second order creeks. No other statistically significant seasonal differences were observed (Table 3).

3.4. Impervious cover analysis

In general, concentrations of indicators tended to increase with increasing levels of watershed impervious cover in first order creeks. Impervious cover was a significant predictor of winter indicator concentration in first order creek segments for all tested indicators (FC, $p = 0.0012$; Ent, $p = 0.0122$; F− col, $p = 0.0093$; F+ col, $p = 0.0018$; Fig. 4). The data also suggest ($p = 0.07$) a positive relationship between F+ col concentrations and impervious cover in third order creek segments during winter. However, all of the other creek orders showed much weaker, and not statistically significant, relationships between the measured indicators and impervious surface. In summer, impervious cover was a significant predictor of FC concentrations ($p = 0.0019$) in first order creek segments, but not of Ent concentrations ($p = 0.055$). We observed a significant positive relationship between impervious cover and coliphage concentrations in first order creeks (F− col, $p = 0.0138$; F+ col, $p = 0.0142$). There was a significant relationship between impervious cover and F+ col concentrations ($p = 0.0367$) in second order creeks as well (Fig. 5). The relationships between indicator concentrations and impervious cover in other creek orders were much weaker, if they showed any relationship at all.

3.5. Coliphage typing

Confirmed F^+ RNA coliphages were isolated from all positive samples, including 24 (77%) of 31 winter samples and 4 (12%) of 33 summer samples. The majority (93%) of positive samples contained group I coliphages, indicative of animal-source contamination. First order samples collected from Bulls Creek in the summer and Guerin Creek in the winter were found to further harbor group IV F^+ RNA coliphages, also indicative of animal-source contamination. The only human source signal detected during the study was from an urban creek. The positive sample was collected in the summer from the second order of Bulls Creek and was found to harbor a strain of group II F^+ RNA coliphage.

4. Discussion

Use of the tidal creek classification system described herein allowed overall trends in the spatial and temporal distribution of water quality indicators to become apparent. First, these data show a typical increase in the concentration of bacterial and viral indicators with increasing urbanization as well as a general spatial distribution with highest indicator concentrations in the tidal creek headwaters. Second, the headwater creek segments are the locus of the strongest predictive relationships between microbial indicators and impervious cover, an important indicator of development. Third, bacterial indicators showed a seasonal pattern in some but not all creek orders, while no seasonal patterns were discerned for the tested viral indicators.

Observed increases in fecal indicator microorganisms in more urbanized watersheds suggest that land use changes associated with coastal development, particularly increases in watershed impervious cover, affect water quality. These results are consistent with previous studies that showed increases in fecal bacteria concentrations along gradients of coastal urbanization. Karn and Harada (2001) using secondary data collected from various agencies in South Asia documented elevated coliform concentrations with increased urbanization. Similarly, linear regression indicated that the amount of impervious cover in southeastern US watersheds explained 95% of the variability in average estuarine fecal coliform concentrations (Mallin et al., 2000b). Holland et al. (2004) also found a significant relationship between watershed impervious cover and fecal coliform concentrations in headwater (first order) tidal creeks.

The relationship between watershed development and the environmental and ecological condition of headwater tidal creeks in SC is fairly well understood (Sanger et al., 1999a,b; Lerberg et al., 2000). For headwater tidal creeks, Holland et al. (2004) proposed a conceptual model linking human population growth, changes in the physiochemical environment, and ultimately, ecosystem responses. Adverse changes were observed in the physical and chemical environment, including increased fecal coliform abundance, when impervious cover reached 10–20% of the adjacent land surface. Ecological responses (i.e., changes in benthic faunal composition, nekton abundances) occurred when impervious cover exceeded 20–30%. This model was developed with limited microbial indicator data, but the bacterial indicator data from our study support that initial model. There is a significant increase in both FC and Ent concentrations in first order creeks during winter between forested and suburban/urban systems. During summer, the increase in concentration across land use classes is more gradual. With respect to the viral indicators, urban systems show the highest F+ coliphage concentrations in first order creeks, suggesting viral indicators do not respond until impervious surface reaches $\sim$30% in first order watersheds.

These data also demonstrate the value of dividing creek networks into orders. The creek classification revealed a consistent and significant spatial pattern for bacterial indicators in winter and summer and one viral indicator in summer (Table 2) as well as the impact of urbanization at the sub-watershed scale. The greatest measurable signals from land use impacts occurred in first order tidal creeks. Similarly, Reeves et al. (2004) observed the highest concentrations of microbial indicators in inland areas of California, which have the closest association with the upland and development activities, and decreasing concentrations towards the coastal ocean. Therefore, the spatial scale at which research and monitoring activities occur and proximity to pollution sources are very important for identifying the impacts of land use changes on ecosystems. Because they are close to land-based activities and because they are hydrologically linked to upland, first order tidal creek segments serve as sentinel habitats, providing early warning of water quality and ecosystem impairment along the coast. Second order creeks may also have sentinel characteristics (F+ col, Fig. 5), but further work is needed to validate this.

Consistent detection of microbial indicators during this study suggests that the tested tidal creek systems harbor fecal pollution, with pollution highest in more developed watersheds and in the headwaters. The state regulatory agencies, however, routinely monitor larger creek systems (i.e., 2nd and 3rd orders) and larger water bodies (e.g., tidal rivers, bays, sounds), regions of high human use, to determine if a waterbody should be closed or restricted. Our study findings indicate that headwaters have significantly reduced water quality compared to the commonly monitored deeper water habitats, suggest that regulatory agencies may not detect bacterial problems where they first arise, and imply that increased attention to headwaters may better quantify potential risks. For example, comparing the US EPA criteria for recreational water use (<200 CFU/100 mL FC or <35 CFU/100 mL Ent) to our survey data, 23 of 64 samples (36%) collected over winter and summer exceeded the threshold for FC while 46 of 64 (72%) exceeded the EPA threshold for Ent. Of the 23 exceeding the FC standard, 17 of the samples (74%) were collected in headwater areas. Samples violating the Ent standard were more evenly distributed across the creek gradient; however, 21 of 46 (46%) were collected in first order creeks. The US EPA guideline for shellfishing waters is even stricter (median FC concentration <14 MPN/100 mL), and 77% (49 of 64) of our samples across both seasons would have exceeded those guidelines. The majority, but not all, of these samples were from first order creeks. Indeed, urbanization has led to the closure of shellfish beds in many North and South Carolina estuaries (DeVoe et al., 1992; Mallin et al., 2000a). Both residents and visitors may underestimate the risk of exposure to enteric pathogens when using headwater creeks for recreation and fisheries. Additional study is merited to better quantify these risks.

Coliphage typing suggests that the majority of sites are impacted by animal (nonhuman) sources of pollution. These results are not unexpected since most of the areas sampled are not on septic systems and because development tends to push wildlife into corridors along water boundaries (Siewicki et al., 2007). Further, there are likely to be increases in pets and pet wastes associated with suburban development (Kelsey et al., 2004). Risks to humans may vary depending on pollution sources. Human waste is more likely to carry human pathogens; however, animal waste has the potential for zoonotic transmission of disease (Fayer et al., 2004; Hill et al., 2005). An estimated 75% of emerging infectious diseases are zoonotic (Chomel et al., 2007), and anthropogenic influence on ecosystems appears to be a common factor in the emergence and reemergence of zoonotic pathogens (Daszak et al., 2001). Comprehensive epidemiological studies have yet to adequately differentiate risk levels associated with contamination sources, but differences in the frequency or severity of illnesses are likely. Beyond direct human health concerns, animal-source fecal pollution can add nutrients to waterbodies leading to algae blooms and hypoxia (Whitall et al., 2004).

Different seasonal and land use patterns were observed for the bacterial indicators than for the viral indicators. A more pronounced gradient was observed for the bacterial indicators, suggesting that viruses survive longer downstream and that die-off, not just dilution and other factors, probably accounts for decreases in bacteria. Bacterial die-off is likely attributable to an interplay of factors, including salinity, temperature, UV irradiation, and protistan grazing (Gonzalez et al., 1992; Burkhardt et al., 2000; Noble et al., 2004). Our results further demonstrate that not all bacteria die-off at the same rate. Enterococci appeared to survive longer downstream relative to fecal coliforms, most likely due to differential survival under the varying salinity regimes (Hanes and Fragala, 1967; Noble et al., 2003). Regardless, standard bacterial indicators do not appear to adequately model viruses in these systems; indicator coliphages appear to add valuable information to water quality assessments.

The decrease in bacterial indicators along the tested longitudinal gradient may also be attributed, in part, to sedimentation. Particles can play a major role in the survival and distribution of bacteria in coastal environments. Upon introduction to the water column, microbes can adsorb to particulates and settle to the bottom (Krometis et al., 2007). Although the settling process removes microbes from the water column, it does not cause their inactivation and may even allow their proliferation (Anderson et al., 2005). Through this process, fecal organisms can accumulate in sediments and become concentrated relative to water. As a consequence, sediments represent an important reservoir for enteric pathogens and likely contribute to their survival and transport. Recontamination of the water column can then occur via bioturbation or during stormwater flows, tidal currents, dredging or recreational use of the overlying water (Gardner et al., 1989; Grimes, 1980; Muirhead et al., 2004).

In summary, headwater creeks are sensitive indicators of impacts from land use activities and urbanization. Since the scale of our tidal creek study watersheds (100s–1000s ha) is also the spatial scale at which coastal land use decisions and remediation actions typically occur, managers or land use planners are afforded a valuable tool to understand the impacts of coastal development on microbial water quality and incorporate that information in to the decision-making process. Future research should aim to develop forecasting models quantifying microbial contamination in tidal creeks and estuarine ecosystems resulting from human land use changes. Spatial distribution is also an important factor, and separate models may need to be developed at different spatial scales. Additional factors, such as microbial die-off and sedimentation rates, still need to be studied for inclusion in forecast models.

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