

## INTRODUCTION

The water quality status of many estuaries, rivers, and coastal areas is influenced by the presence of fecal coliform bacteria. Fecal coliform bacteria originate from the intestinal tract of warm-blooded mammals and may enter bodies of water from a number of sources, including agricultural fields, urban runoff, septic tanks, and storm water runoff (Palmateer et al., 1993; Davies et al., 1995; Lipp et al., 2001a). Although these bacteria are not pathogenic, they are considered as useful indicators of elevated concentrations of harmful microorganisms originating from fecal wastes that are present in a water body. Significant concentrations of fecal coliforms in the water can lead to the closure of shellfish beds as well as limit the recreational use of water bodies.

The survival of fecal coliforms in the water column depends upon a number of factors. The most important factor seems to be the adsorption of the bacteria to particulate matter (Palmateer et al., 1993), which in aquatic environments, appears to be a common occurrence (Paerl, 1975). In studies of stream sediments, the highest bacterial concentrations have been found associated with the clay particles ranging from 1 to 5  $\mu\text{m}$  in diameter, especially the mineral montmorillonite (Palmateer et al., 1993).

The availability of nutrients on the surface of particulate matter increases the survival time of bacteria attached to it, thereby allowing living bacteria to be transported greater distances (Gerba and MacLeod, 1976; Palmateer et al., 1993). Some studies indicate that bacteria not associated with particulate matter survive about five days in the water column, whereas bacteria associated with particles within agricultural run-off streams may survive for as long as 85 days (Palmateer et al., 1993).

Because fecal coliform bacteria adhere to suspended sediments, hydrodynamic processes that control sediment transport also influence distributions of bacterial concentrations in surface waters. Fecal coliform bacteria distribution in a river-lake contact zone declined in bacterial abundance in surface waters through sedimentation of suspended particulate matter in low flow velocities at the river mouth (Bergstein Bendan et al., 2001). As sediments to which the fecal coliform bacteria are attached settle out of the water column, concentrations in bottom substrates are enhanced and may ultimately contain 100 to 1000 times more bacteria than the overlying water (Davies et al., 1995; Gerba and McLeod, 1976).

The attachment of fecal coliforms to particulate matter may occur by different methods. Residual electrical charges on the clay and organic matter, as determined by the cation exchange capacity, may have an influence on the bacterial adsorption to sediments (Palmer et al., 1993). The microorganism itself also may bind to the sediment using a matrix of extracellular polymeric secretions (EPS), also called “microbial biofilms” (Decho, 2000). These biofilms create sticky surfaces on sediment and detrital particulate surfaces that promote the survival and, possibly, the propagation of bacteria. Additional methods of attachment include adhesive stalk formation, capsular secretion, and fibrillar appendages (Paerl, 1975).

The salinity of the water, amount of ultraviolet radiation, and the temperature of the water also influence the survival of fecal coliform bacteria. In a recent tidal creek dredging study conducted by Mallin et al. (2000b), the concentration of fecal coliforms decreased as higher salinity waters more readily flushed the creek. Bacteria are not tolerant of ultraviolet radiation exposure, but some protection from UV radiation is

gained by attaching to suspended particles (Kocasoy, 1989). Most studies have indicated that the concentration of fecal coliform bacteria is inversely correlated with water temperature (McFeters and Stuart, 1972; Howell et al., 1996; Lipp et al., 2001b)

The source, survival, and transport of fecal coliform bacteria within the aquatic environment have been well documented (Gerba and McLeod, 1976; Palmateer et al., 1993; Davies et al., 1995; Lipp et al., 2001a), but there is limited information regarding the mechanisms associated with trapping these bacteria in depositional regimes.

Wetlands are recognized as depositional sinks for nutrients, metals, and pollutants from upland environments (Mitsch and Gosselink, 2000). In many coastal fluvial systems, wetlands comprise the riparian corridors; those areas of land that follow the course of flowing water between the aquatic and the upland systems. These corridors are classified as riparian buffers if surface and subsurface waters from upland areas improve in quality before reaching the aquatic ecosystem (NCDEHNR, 1993). The extent to which pollutants are removed from upland runoff depends upon topographic, vegetative, hydrologic and soil properties. Forested riparian buffers tend to trap sediment efficiently due to the composition of the forest floor, which can be made up of woody debris, and extensive root and litter systems (NCDEHNR, 1993).

A study conducted by Ensign and Mallin (2001) documented an instance where a wetland environment proved to be an effective buffer for fecal coliforms originating from an upland source. While monitoring the coliform levels downstream from a clear-cut swamp in southeastern North Carolina, Ensign and Mallin (2001) observed that fecal coliform concentrations dramatically increased after logging and concluded that the loss of the riparian forest resulted in less retention of the bacteria. Another study conducted in

the non-tidal riparian swamps within the Cape Fear River watershed demonstrated that watersheds with 13.8% or greater wetland coverage tended to be efficiently buffered against fecal coliform bacteria and turbidity runoff from upland sources after rain events (Mallin et al., 2001). Thus, while riparian wetlands are touted as efficient buffers for contaminants from upland sources, the extent to which fecal coliform bacteria are trapped on a swamp surface from river waters has been poorly documented.

The mechanisms of suspended sediment deposition, and consequently the deposition of fecal coliform bacteria, on the tidal swamp surface are assumed to be similar to the sedimentation processes that occur on tidal salt marsh surfaces. Sediment deposition in tidal marsh environments depends upon a number of factors including source concentration, distance from the source, and duration of inundation (Friedrichs and Perry, 2001). Sediment deposition on the marsh surface tends to decrease with increasing distance from the source (Leonard et al., 1995b; Reed et al., 1999; Christiansen et al., 2000). During a flooding event, sedimentation occurs continuously, reducing the suspended sediment concentrations in the water column with time at a given sampling site (Leonard et al., 1995b, Reed et al., 1999). Few studies have investigated sedimentation in forested tidal swamps, but processes similar to those in tidal marshes likely occur along the tidally influenced riparian wetlands. Since fecal coliform concentrations tend to be associated with suspended sediments, patterns of fecal coliform deposition are expected to be similar to those of the suspended sediments.

In order to better understand the transport and accumulation of fecal coliform bacteria in the aquatic environment, the potential environments of coliform deposition need to be examined in greater detail. By comparing a tidal freshwater riparian swamp

located in a brownwater alluvial system, which is characterized by high suspended sediment loads, to a riparian swamp located in a blackwater alluvial system, which is characterized by low suspended sediment loads, the role that suspended sediments play in the deposition of fecal coliform can be determined. Since fecal coliform bacteria are not abundant in saline conditions, tidal freshwater wetlands located in both types of alluvial systems are ideal to study the deposition of fecal coliform from the water column and to evaluate the extent to which tidally influenced riparian wetlands serve as efficient sinks for fecal coliform bacteria from river water.

## OBJECTIVES

This study evaluated the extent to which tidally influenced freshwater wetland environments serve as sinks for fecal coliforms. Temporal and spatial gradients of fecal coliform concentrations across swamp surfaces of two sites over tidal inundation events were determined and compared to background concentrations in the river. Additionally, the relationship between total suspended solids concentration and the concentrations of fecal coliforms were examined by comparing two study sites with different sediment loads; one blackwater system and one brownwater system. The following hypotheses were tested using these data:

1. The brownwater riparian wetland environment serves as a sink for fecal coliform bacteria. As particulate matter is carried over the swamp surface during tidal flooding events, the concentration of total suspended solids and the concentration of fecal coliform bacteria in the water column will decrease over time.

2. Over an inundation event in the blackwater system, there will be no change in the fecal coliform concentration over time.
3. In comparison of the two study sites, the brownwater site will exhibit a higher concentration of fecal coliform bacteria because of higher TSS concentrations.

## SITE DESCRIPTIONS

Two sites in different swamp environments were chosen within the Cape Fear River (CFR) watershed, located in southeastern North Carolina (Figure 1). Elevated fecal coliform concentrations have been periodically documented in the river surface waters adjacent to these sites since June 1995 (Mallin et al., 2002). Additionally, these sites were selected because of an existing data set associated with an ongoing river monitoring study funded by the U.S. Army Corps of Engineers. This data set includes information on hydroperiod, water levels, site elevation, vegetation type, and soil geochemistry.

The first site, Dollisons Landing (P8), is located along the Cape Fear River (CFR). The CFR originates in the North Carolina Piedmont region and is characterized as a brownwater river system, which has high levels of total suspended solids (TSS) (Mallin et al., 2000c). The sampling site is located approximately 55 km upstream from the mouth of the CFR, where the river is mostly freshwater (average salinity 0.4 ppt, range 0 to 5.7 ppt). This site has an elevation of 1.66 m relative to NAVD88. At this location, semi-diurnal tidal pulses are the primary mechanism by which water moves onto and off of the swamp surface. Additionally, water also inundates the swamp surface when periods of heavy precipitation occur over large portions of the upstream watershed

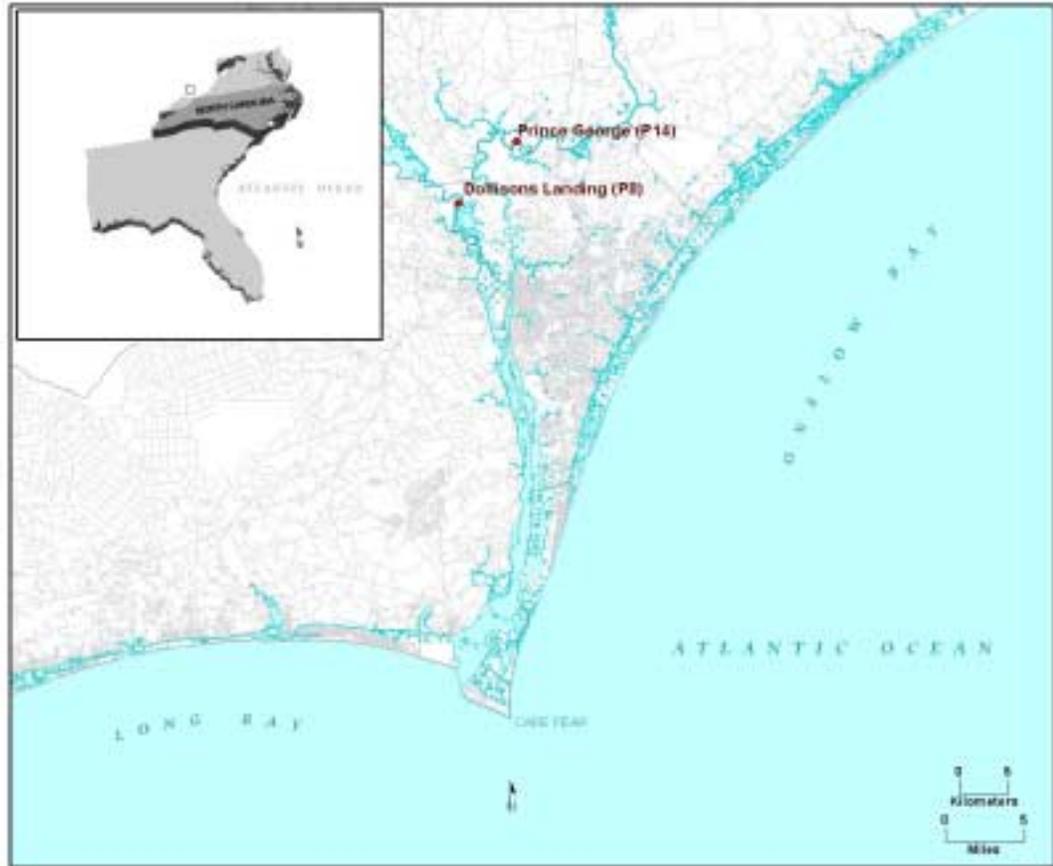


Figure 1. Location of Dollisons Landing on the Cape Fear River (brownwater) and Prince George on the Northeast Cape Fear River (blackwater).

and the river reaches flood stage. This site has a mean tidal range of 1.06 m and a mean water temperature of 16.3 °C.

The second site, Prince George (P14), is located along the Northeast Cape Fear River (NCFR), a tributary of the CFR. In contrast to the CFR, the NCFR drains the North Carolina coastal plain and is classified as a blackwater river system. This site is located approximately 68 km from the mouth of the CFR where the river is mainly freshwater (average salinity 0.2 ppt, range 0 to 5.1 ppt). The elevation of the swamp surface at the location of a concrete benchmark is 1.56 m NAVD88. The water movement onto and off of the swamp surface at this site is also primarily attributed to semi-diurnal tidal pulses. Water also inundates the swamp surface when the river reaches flood stage. The mean tidal range at this site is 0.72 m and the mean water temperature is 16.3 °C. The NCFR exhibits less turbid conditions and lower inorganic nutrient content, but is highly colored due to organic content (Mallin et al., 2000c). The CFR has a mean total suspended solids concentration of 13.2 mg L<sup>-1</sup>, indicating a large suspended sediment load, whereas the NCFR has lower mean TSS levels, 5 mg L<sup>-1</sup>, suggesting the transport of far fewer particulates (Mallin et al., 2000c).

Dollisons Landing (Figure 2) is characterized as a swamp forest, with lizard's tail (*Saururus cernuus*) and halberd-leaf tearthumb (*Polygonum arifolium*) as the dominant vegetation throughout the site. Vegetation near the river includes southern bald cypress (*Taxodium distichum*), green ash (*Fraxinus pennsylvanica*), and scattered pumpkin ash (*Fraxinus profunda*) (CZR Incorporated, 2001). The Prince George study site (Figure 3) is also characterized as a swamp forest, with lizard's tail (*Saururus cernuus*), halberd-leaf tearthumb (*Polygonum arifolium*), mild water-pepper (*Polygonum hydropiper*), and green



Figure 2. Brownwater swamp at Dollisons Landing along the Cape Fear River. Note the tubing extending into the swamp for collection of fecal coliform bacteria samples, and the ISCO in the background for the collection of TSS samples.



Figure 3. Blackwater swamp at Prince George along the Northeast Cape Fear River. Note the ISCO in the background of the picture for the collection of TSS samples.

arrow-arum grass (*Peltandra virginica*) growing near the river edge. Pumpkin ash (*Fraxinus profunda*), red maple (*Acer rubrum*), pond cypress (*Taxodium ascendens*), woodvamp (*Decumaria barbara*), eastern poison ivy (*Toxicodendron radicans*), and horsebrier (*Smilax rotundifolia*) also are commonly found throughout the site (CZR Incorporated, 2001).

## METHODOLOGY

This study examined the fecal coliform budget of tidal riparian wetlands. River water parameters, including salinity, temperature, and water level, were downloaded weekly by telemetry from data collection platforms (DCP) located adjacent to each swamp site. The DCPs house UNIDATA water level, conductivity, and temperature sensors and house dataloggers that measure and record these parameters every three minutes.

Prior to sampling, the direction of the tidal flow within each swamp site was determined by use of dye tracing. Fluorescent dye was injected into the water flowing across the swamp surface over an inundation event. The direction of the dye movement was observed and recorded. The locations of the interior and edge sampling sites were determined from the flow patterns within each swamp surface, to ensure to the extent possible that the same parcel of water was sampled at each site.

Two fixed sampling locations were delineated along the line of tidal flow at each site. The first sampling location was at the river edge (as determined by the tree line). The second location was approximately six to eight meters into the swamp, depending on the inundation period at each site. At the river edge, water samples were collected at an

approximate depth of 10 cm below the water surface. These samples consisted of the water mass that was moving onto the swamp. In the swamp interior, samples were collected at approximately 5 cm above the sediment surface. This strategy allowed for a maximum number of samples to be collected during inundation. The elevation at the sampling locations at the brownwater swamp was slightly lower than the sampling locations at the blackwater swamp, resulting in a longer inundation period at the brownwater site by about 90 minutes. This difference in hydroperiod resulted in the collection of more samples from the brownwater site during a single inundation event as compared to the number of samples collected from the blackwater site.

During an inundation event, a 500 ml sample was taken for fecal coliform bacteria analysis at the river edge and in the interior swamp site once every hour. Sampling continued every hour until the water receded from the swamp surface. These samples were used to evaluate changes in fecal coliform bacteria concentrations over time. Additionally, five replicate samples were taken at the river edge and five at the interior site at the midpoint of the rising water phase as well as at the midpoint of the falling water phase. These samples were used for statistical comparisons of mean rising water versus mean falling water fecal coliform bacteria concentrations. This replicate sampling allowed the analysis of several parameters (Table 1) that might affect the fecal coliform concentrations. The river edge samples were collected by hand, and a peristaltic pump outfitted with tubing was used to collect interior samples.

Three one-liter samples were taken at half hour intervals over the inundation event to determine total suspended solids (TSS). Swamp interior TSS samples were taken using an ISCO automated water sampler and the river edge TSS samples were

Table 1. Evaluated Parameters, Sampling Configuration, and Number of Samples

Parameter	Samples per Experiment	Total Samples
Tidal Stage (rising vs. falling water)	N = 20; 5 replicates at each location during mid-rise and mid-fall for each river independently	Daily samples (N=20) X 6 days = Total N = 120
Location (interior vs. exterior)	N = 20; 5 replicates at mid-rise and mid-fall for both locations for each river independently	Daily samples (N=20) X 6 days = Total N = 120
River System (brownwater vs. blackwater)	N= 40; 5 replicates at each location for each stage in each river system	Daily samples (N=40) X 6 days = Total N = 240

taken by hand. Each site was sampled a minimum of three inundation events during each season, summer and winter, for a total of at least 12 events between the two systems.

Processing the samples for fecal coliform bacteria followed the procedures described in the Standard Methods for the Examination of Water and Wastewater (APHA, 1995). The bacteria were counted as colony forming units and were reported as the number of colony forming units per 100 ml of water. After counting, digital photographs of each sample were taken and archived. Total suspended solids were determined by filtration through a one-micron pore-size glass fiber filter, drying the filtered samples, and reweighing to give concentration in  $\text{mg L}^{-1}$ .

To evaluate flow characteristics that may affect particulate and bacteria transport, flow conditions on the swamp surface were measured using a SonTek Flow Tracker Acoustic Doppler Velocimeter (ADV). This instrument is capable of measuring all 3 components of velocity (u, v, and w) and resolving very low flow speeds ( $<1 \text{ cm s}^{-1}$ ) thus making it ideal for use in the swamp environment. Flow data were used to account for differences in total suspended solids and transport of bacteria between sites potentially associated with differences in hydrodynamic regime.

Fecal coliform and TSS concentrations were log-transformed before statistical analyses were performed to approximate normal distributions. A one-way Analysis of Variance (ANOVA) was used to compare fecal coliform bacteria means between the five replicate stage samples. Since the variability among each group of the five tidal stage replicates was minimal, the time series data also were used to evaluate general trends of fecal coliform bacteria concentration over time. Comparisons between group means were tested using a one-way ANOVA when the variance between groups was equal. When the

variance was unequal, a Welsh ANOVA was used. Correlation analyses were conducted between the fecal coliform and TSS concentrations. Significant differences were determined at  $p < 0.05$ . All statistical analyses were conducted using JMP (SAS, 2001).

## RESULTS

### System Characteristics

#### Intersite Variability

The results of one-way ANOVA using all the hourly data collected from both sampling locations within each system indicated that brownwater fecal coliform bacteria concentrations were significantly greater than concentrations in the blackwater system ( $p=0.0080$ ,  $F=7.3424$ ,  $df=1$ ). Fecal coliform concentrations in the brownwater system ranged from 13 to 114 CFU 100 mL<sup>-1</sup> with a mean value of 44 CFU 100 mL<sup>-1</sup> at the swamp edge sampling location. Bacteria concentrations observed at the swamp edge in the blackwater system ranged from 15 to 99 CFU 100 mL<sup>-1</sup> with a mean of 37 CFU 100 mL<sup>-1</sup>. Mean blackwater concentrations were not significantly different ( $p=0.3105$ ,  $F=1.0501$ ,  $df=1$ ) from the brownwater concentrations at the edge location. At the interior location, brownwater fecal coliform concentrations were significantly higher ( $p=0.0101$ ,  $F=7.2084$ ,  $df=1$ ) than blackwater concentrations. The concentrations in the brownwater interior location ranged from 18 to 175 CFU 100 mL<sup>-1</sup> with a mean of 57 CFU 100 mL<sup>-1</sup>. In the blackwater interior, the fecal coliform concentrations ranged from 5 to 81 CFU 100 mL<sup>-1</sup> with a mean of 37 CFU 100 mL<sup>-1</sup>.

Overall, the TSS concentrations did not differ between the brownwater and blackwater swamp sites ( $p=0.3265$ ,  $F=0.9730$ ,  $df=1$ ). TSS concentrations at the swamp

edge sampling sites did not significantly differ ( $p=0.6871$ ,  $F=0.1642$ ,  $df=1$ ) between the two river systems. The brownwater edge TSS concentrations ranged from 2.9 to 39.3 mg L<sup>-1</sup> with a mean of 13.6 mg L<sup>-1</sup>. The TSS concentrations at the blackwater edge location ranged from 0.8 to 43.0 mg L<sup>-1</sup> with a mean of 14.5 mg L<sup>-1</sup>. In the interior of the brownwater system, the TSS concentrations ranged from 2.6 to 40.0 mg L<sup>-1</sup> with a mean of 14.5 mg L<sup>-1</sup>. The TSS concentrations in the interior of the blackwater system ranged from 0.2 to 52.2 mg L<sup>-1</sup> with a mean of 11.4 mg L<sup>-1</sup>. TSS concentrations at the interior sampling sites also were not significantly different ( $p=0.1051$ ,  $F=2.7436$ ,  $df=1$ ) between the brownwater and blackwater system.

#### Intrasite Variability

Within the brownwater swamp system, fecal coliform concentrations at the interior sampling location were not significantly different from concentrations at the edge sampling location ( $p=0.1114$ ,  $F=2.6303$ ,  $df=1$ ). The TSS concentrations also did not significantly differ between the interior and edge sampling location on the brownwater swamp surface ( $p=0.7136$ ,  $F=0.1365$ ,  $df=1$ ). Similarly, the hourly fecal coliform concentrations measured during rising water phase were not significantly different from the hourly concentrations measured during falling water ( $p=0.9535$ ,  $F=0.0034$ ,  $df=1$ ) with a mean of 51 CFU 100 mL<sup>-1</sup> for the rising water and a mean of 49 CFU 100 mL<sup>-1</sup> for the falling water phase. The mean TSS concentration for the hourly samples during rising water phase on the brownwater swamp surface was 13.3 mg L<sup>-1</sup> and the mean for the hourly samples during the falling water phase was 15.0 mg L<sup>-1</sup>. These differences also were not significant ( $p=0.8078$ ,  $F=0.0602$ ,  $df=1$ ).

In the blackwater swamp system, the fecal coliform bacteria concentrations did not significantly differ between the interior and edge sampling locations ( $p=0.5618$ ,  $F=0.3414$ ,  $df=1$ ). TSS concentrations also did not differ significantly between the two sampling locations on the blackwater swamp surface ( $p=0.0856$ ,  $F=3.0862$ ,  $df=1$ ). The mean fecal coliform concentration for hourly samples collected during the rising water phase was 27 CFU 100 mL<sup>-1</sup> and the mean concentration for hourly samples collected during the falling water phase was 41 CFU 100 mL<sup>-1</sup>. These differences, however, were not significant ( $p=0.3905$ ,  $F=0.7512$ ,  $df=1$ ). The mean hourly TSS concentrations on the blackwater swamp surface during rising water (16.2 mg L<sup>-1</sup>) were significantly higher than during falling water (8.9 mg L<sup>-1</sup>;  $p=0.0268$ ,  $F=5.2311$ ,  $df=1$ ).

#### Temporal Changes in Fecal Coliform and TSS

In general, fecal coliform concentrations (Figure 4) showed an increasing trend during swamp inundation in the blackwater system at both the interior and edge sampling locations. Hourly fecal coliform concentrations at the interior site were usually slightly greater than concentrations at the edge sites, but these differences were not significant. In the brownwater system (Figure 5), mean hourly fecal coliform concentrations decreased during rising water and then increased as waters receded from the swamp surface at the interior sampling location. At the swamp edge location, the mean hourly fecal coliform concentration remained relatively constant during rising water and then increased during the falling water phase of the inundation event. Once again, the mean hourly fecal coliform concentrations at the interior site usually exceeded concentrations at the edge site, but not significantly.

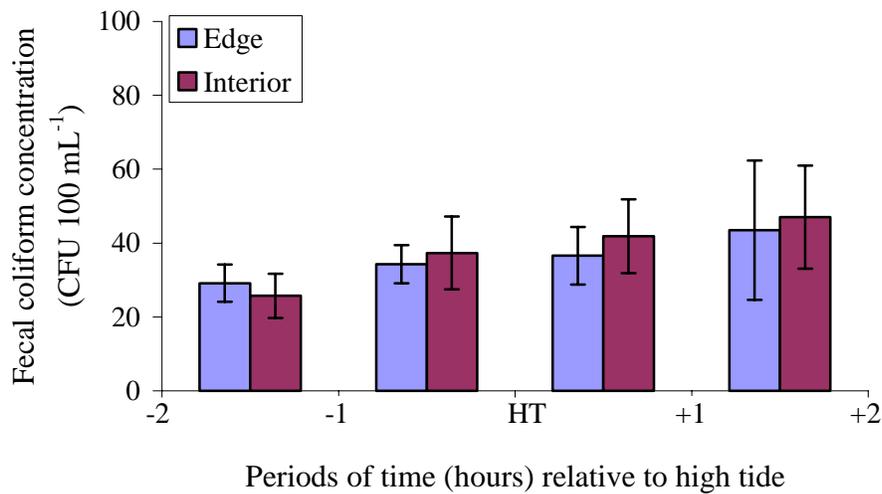


Figure 4. Blackwater swamp fecal coliform concentrations over time. Each bar represents the mean of all fecal coliform concentrations measured in that time interval relative to high tide. The error bars indicate the standard error of each mean.

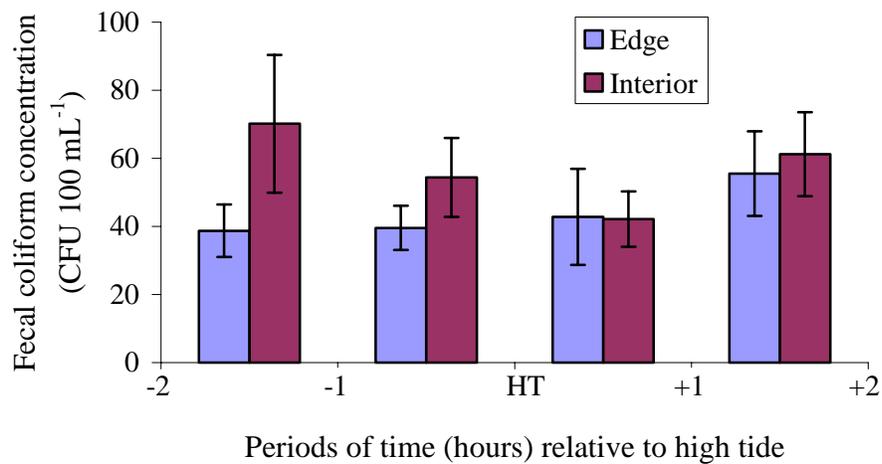


Figure 5. Brownwater swamp fecal coliform concentrations over time. Each bar represents the mean of all fecal coliform concentrations measured in that time interval relative to high tide. The error bars indicate the standard error of each mean.

In the blackwater system (Figure 6), mean hourly TSS concentrations decreased over time at the edge location. At the swamp interior site, the mean hourly TSS concentrations increased, but not significantly, during the rising water phase, then decreased and remained constant during the falling water phase. In the brownwater system (Figure 7), the mean hourly TSS concentrations at the interior location initially decreased during the rising water and increased slightly, but not significantly, during the falling water phase, similar to the trend observed in the fecal coliform time series. At the brownwater edge location, mean hourly TSS concentrations increased slightly during the rising water phase. During falling water phase, concentrations increased then decreased.

#### Rising vs. Falling Water

The statistical results of the replicate sampling focusing on the possible differences between the fecal coliform concentrations during the rising water phase and the falling water phase are presented in Table 2 and Table 3. For both the brownwater and blackwater systems, fecal coliform bacteria concentrations at the interior location during the falling water phase almost always exceeded the concentrations during the rising water phase of inundation. When these differences were significant ( $p < 0.05$ ) they always constituted increases in the blackwater system. In the brownwater system, falling water concentrations were significantly higher than rising water concentrations for 2 out of 3 sampling events. For the third event, the difference in concentration was a significant decrease (Table 3). At the edge sites, rising and falling water bacteria concentrations showed significant differences two out of three times in the brownwater system and one out of three times in the blackwater system. For both river systems, when

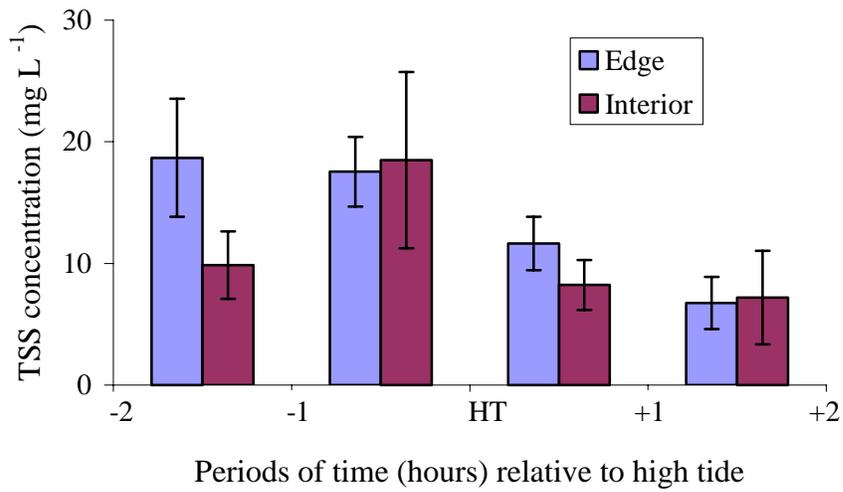


Figure 6. Blackwater swamp TSS concentrations over time. Each bar represents the mean of all TSS concentrations measured in that interval relative to high tide. The error bars indicate the standard error of each mean.

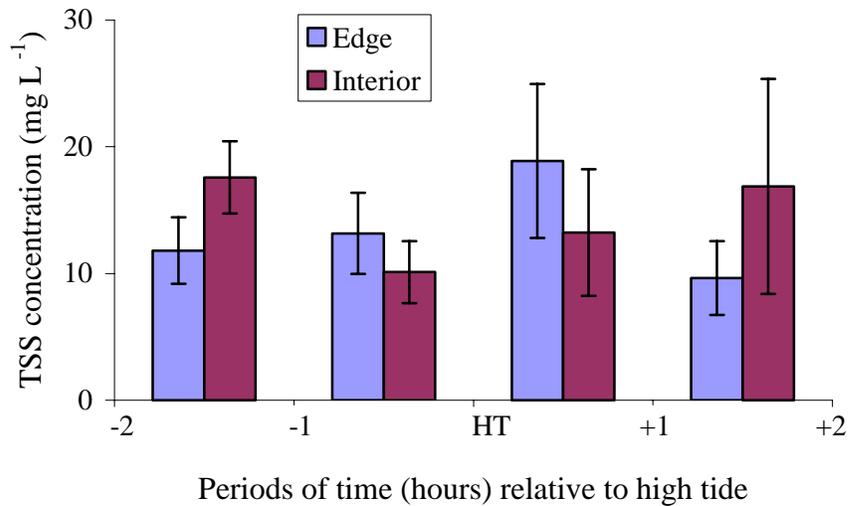


Figure 7. Brownwater swamp TSS concentrations over time. Each bar represents the mean of all TSS concentrations measured in that interval relative to high tide. The error bars indicate the standard error of each mean.

Table 2. Results of One Way ANOVA for Blackwater System  
Fecal Coliform Concentrations

Date	Edge samples	Change from rising to falling	Inner samples	Change from rising to falling
Nov. 18	p= 0.0722 F= 4.2868 df= 1	decrease	p= 0.0439* F= 5.7087 df= 1	increase
Dec. 6	p= 0.9483 F= 0.0045 df= 1	no change	p= 0.0002* F= 43.8356 df= 1	increase
Dec. 19	p= 0.0003* F= 35.5864 df= 1	increase	p= 0.2216 F= 1.7566 df= 1	increase

\* denotes a significant difference in rising water vs. falling water concentrations

Table 3. Results of One Way ANOVA for Brownwater System  
Fecal Coliform Concentrations

Date	Edge samples	Change from rising to falling	Inner samples	Change from rising to falling
Nov. 19	p = 0.0348* F= 6.4458 df= 1	increase	p <.0001* F= 133.9518 df= 1	increase
Nov. 22	p <.0001* F= 124.0927 df= 1	increase	p <.0001* F= 102.0834 df= 1	increase
Dec. 9	p = 0.8284 F= 0.0512 df= 1	no change	p = 0.0143* F= 11.6452 df= 1	decrease

\* denotes a significant difference in rising water vs. falling water concentrations

significant concentration differences occurred between rising and falling phases at the interior, they were almost always increases.

#### Fecal Coliform and TSS Correlation

All available data from all sites were used to determine if fecal coliform concentrations were correlated with TSS concentration. In the blackwater system, there was no significant correlation between the fecal coliform bacteria concentrations and TSS concentrations ( $r=0.04$ ,  $p=0.1727$ ; Figure 8), nor did any correlation exist when data from interior ( $r=0.0582$ ,  $p=0.2673$ ) and edge ( $r=0.0157$ ,  $p=0.5512$ ) sampling locations were examined independently. In the brownwater system, the fecal coliform concentrations were also not significantly correlated with TSS concentrations ( $r=0.0033$ ,  $p=0.6981$ ; Figure 9). Further, no significant correlations existed between fecal coliform bacteria and TSS for data collected at the brownwater interior sampling location ( $r=0.0319$ ,  $p=0.4260$ ) or at the brownwater edge sampling location ( $r=0.0764$ ,  $p=0.1716$ ).

#### Seasonal Variability

The coliform concentrations measured at the edge and interior locations during the growing season (April – September 15<sup>th</sup>) were significantly higher ( $p=0.0012$ ,  $F=11.1012$ ,  $df=1$ ) than the remainder of the sampling period. In the blackwater system, this difference was highly significant ( $p<0.0001$ ,  $F=30.6348$ ,  $df=1$ ) even though the means were  $52 \text{ CFU } 100 \text{ mL}^{-1}$  and  $27 \text{ CFU } 100 \text{ mL}^{-1}$  for the growing and non-growing season, respectively. These mean concentrations are well below the North Carolina state standard of  $200 \text{ CFU } 100 \text{ mL}^{-1}$  for recreational use waters, and slightly above the state standard of  $14 \text{ CFU } 100 \text{ mL}^{-1}$  for shellfishing waters. Therefore, although these

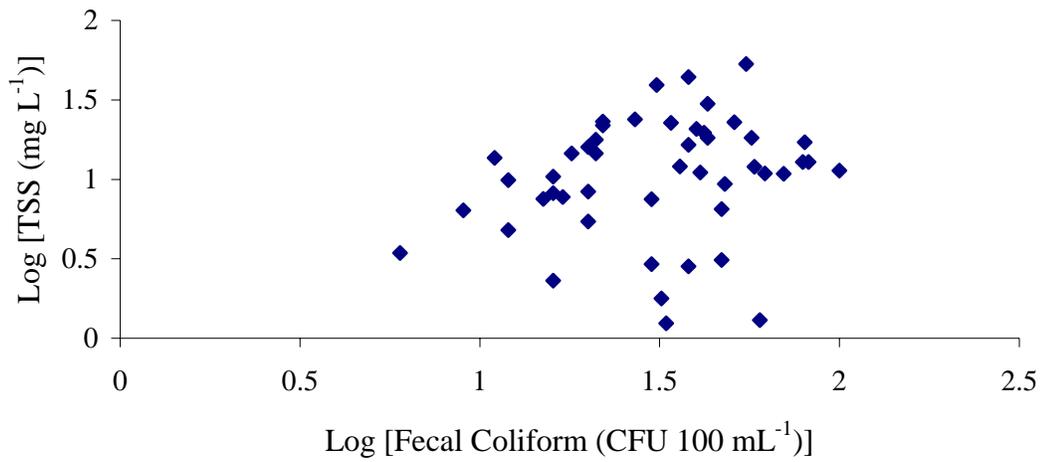


Figure 8. Blackwater site fecal coliform and TSS scatter plot combining interior and edge sample measurements ( $r=0.04$ ;  $p=0.1727$ ).

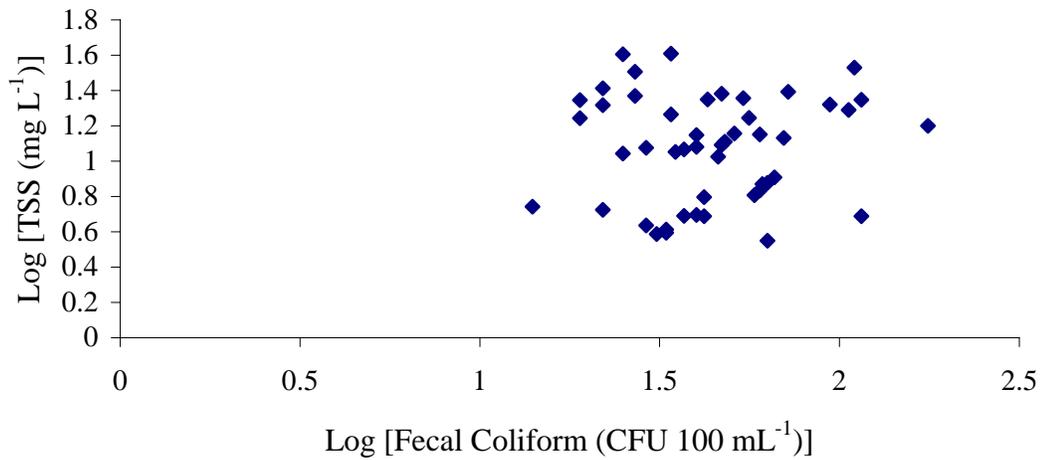


Figure 9. Brownwater site fecal coliform and TSS scatter plot with the interior and edge sampling location measurements ( $r=0.0033$ ;  $p=0.6981$ )

differences in concentration were statistically significant, these results may not constitute a meaningful biological difference in the concentrations observed.

#### Flow Conditions

The flow velocity at the blackwater site ranged from 0.99 to 3.32 cm s<sup>-1</sup>, with a mean velocity of 2.43 cm s<sup>-1</sup>. The corresponding Reynolds numbers for these velocities ranged from 12325 to 41334 and indicate that turbulent flow conditions exist on the surface of these swamps that may preclude particle settling. Turbulence intensities on the surface were approximately 0.0058 m s<sup>-1</sup>, therefore particles with a settling velocity greater than this are able to settle out. Using Stokes settling law and assuming a particle density of 2.65 g cm<sup>-3</sup>, only particles with a diameter larger than 72 microns would be able to settle under these conditions. Thus, extremely fine sands, silts, clays and most organic aggregates would not be settleable at this site. At the brownwater site, the flow velocity was generally lower than at the blackwater site and ranged from 0.10 to 2.94 cm s<sup>-1</sup> with a mean velocity of 1.33 cm s<sup>-1</sup>. For the range of velocities measured, the corresponding Reynolds numbers (1245 to 36603) indicate that both laminar and turbulent flow conditions are possible at this site during an inundation event. The mean turbulence intensity associated with flows in this system was 0.0038 m s<sup>-1</sup> and, again, using Stokes settling law and assuming a particle density of 2.65 g cm<sup>-3</sup>, only particles larger than 58 microns in diameter (very coarse silt and larger) are capable of settling from the water column. Thus, at the brownwater site a wider range of particle sizes could have settled under ambient flow conditions.

## DISCUSSION

The results of this study suggest that the tidal riparian swamps examined during this study, regardless of the river system that they are located in, are sources of fecal coliform bacteria and export the bacteria to the river over the course of an inundation event. At both the blackwater and brownwater sites, fecal coliform concentrations increase throughout the inundation events, suggesting that more bacteria were moved off of the swamp surface than were imported onto the swamp surface. Further, the results suggest that suspended particulates have little effect on fecal coliform bacteria trapping in the wetlands examined during this study. The lack of correlation between the coliform bacteria concentration and TSS concentration at both sites further suggests that bacteria present in the waters flooding the swamp surface are not strongly associated with particles. One possible explanation for this lack of correlation may be the low TSS levels measured on the swamp surfaces during this study. Under these conditions, less substrate is available in the water column for the bacteria to attach to and therefore the importance of sediment dynamics as a control over the distribution of bacteria is reduced. Thus, the conditions and relationships identified by this study probably constitute the baseline conditions of fecal coliform and TSS concentrations that exist in the two study sites.

### Blackwater System

In the blackwater swamp, the inverse relationship between fecal coliform bacteria and TSS during inundation supports the hypothesis that in this particular system, the bacteria are essentially free floating and are not attached to the suspended particles. At the same time the surface also appears to be acting as a source of bacteria, which are exported to the river. The replicate sampling that specifically examined fecal coliform

concentrations before and after a high tide event showed increases in concentration at the interior sampling location for all three events, two of which were significant increases. Bacteria concentrations at the interior sampling location were usually higher than at the edge further suggesting that the swamp surface was a source of bacteria. Although the swamp surface at the edge location could also be a source of bacteria, the water that was being sampled at this location always contained some river water.

An anecdotal observation made during a sampling trip also suggests that the surface of the blackwater swamp is the source of coliform bacteria. Increases in fecal coliform concentrations were seen immediately following the disturbance of the swamp surface by the wake of a passing boat. During an inundation event in October 2002, the highest concentration of coliform bacteria measured at the interior location was 19 CFU 100 mL<sup>-1</sup>. The final sample was collected at the interior location as a boat passed and its wake reached the swamp surface. The fecal coliform bacteria in this sample was measured at 57 CFU 100 mL<sup>-1</sup>, more than double the coliform concentration measured during this particular inundation event, suggesting that the disturbance of the sediment released fecal coliforms into suspension.

#### Brownwater System

In the brownwater system, the fecal coliform and TSS concentrations, particularly at the interior sampling location exhibited an initial, but insignificant, decrease followed by an increase in concentration over the remainder of the inundation event. These patterns raise the possibility that bacteria concentrations were somehow influenced by TSS, however, no significant correlation between fecal coliform bacteria and TSS at the interior location was observed ( $r=0.0162$ ;  $p=0.5721$ ). Thus, similar to the blackwater

system, the brownwater swamp surface appears to be exporting fecal coliform bacteria to the river with suspended particulates playing a negligible role over the duration of this study. The results of replicate sampling that focused on the fecal coliform concentrations during rising and falling water support this argument as significant increases in fecal coliform concentrations were observed at the interior location in each of the three inundation events sampled. During two of the events, the concentration of fecal coliform bacteria in the falling water was at least 143% greater than the concentration measured in the rising water. The coliform bacteria concentration also was significantly higher during falling water phase than during the rising water phase at the edge sampling location during the same two inundation events examined.

The lack of significant differences in TSS concentration between edge and interior sampling locations and over time suggests that particle settling, as identified in tidal salt marshes with similar tidal regimes and TSS concentrations, was not occurring in the brownwater swamp during this study. Numerous studies in tidal marshes have shown that the concentration of suspended sediments in the water moving onto the marsh surface decreases with time and with distance into the marsh (Leonard et al., 1995b; Leonard, 1997; Reed et al., 1999) and that during rising tide, sedimentation on the marsh surface tends to be higher at the marsh edge than in the interior (Christiansen et al., 2000). During this study, TSS concentrations changed little with distance or time on the surface of the swamp. Even though the flow velocities measured during this study were less than those measured in most tidal marsh systems, the associated Reynolds numbers and turbulence intensities suggest that turbulent conditions usually existed on the swamp surface and precluded the settling of the particles that were in suspension.

## Conceptual Model

A conceptual model, based upon observed flow patterns on the swamp surface, was developed to explain the export of fecal coliform bacteria from the swamp surface during an inundation event. This model is based upon differences in fecal coliform concentrations, flow direction and the relative contribution of river water and swamp water. As water first moves onto the swamp surface during the rising water phase of inundation, the primary source of fecal coliforms would be the river water from the river channel. Eventually, at some point in the rising water phase, however, the water on the swamp surface consists of a combination of river water and swamp water. Swamp water is classified as river water that has traversed some distance of the swamp before reaching the sampling location. The ratio of river water to swamp water decreases until just before high tide. After this point, the water moving off of the swamp surface during the falling water phase is increasingly comprised of swamp water.

The first sampling time in the blackwater fecal coliform time series reflects the initial concentrations of fecal coliform bacteria as the water first floods the swamp surface (Figure 10a). The next sampling time during mid-tide shows an increase in the bacterial concentrations (Figure 10b). As water moved over the swamp surface, the volume of swamp water covering the swamp surface increased, and fecal coliform bacteria, most likely from the sediment surface, became suspended in the water column. During the falling water phase, fecal coliform concentrations increase again (Figure 10c). At the interior location, the water that was sampled is most likely made up entirely of swamp water and the concentration of fecal coliform bacteria at this location increases during the falling water phase because the water moving from the back of the swamp,

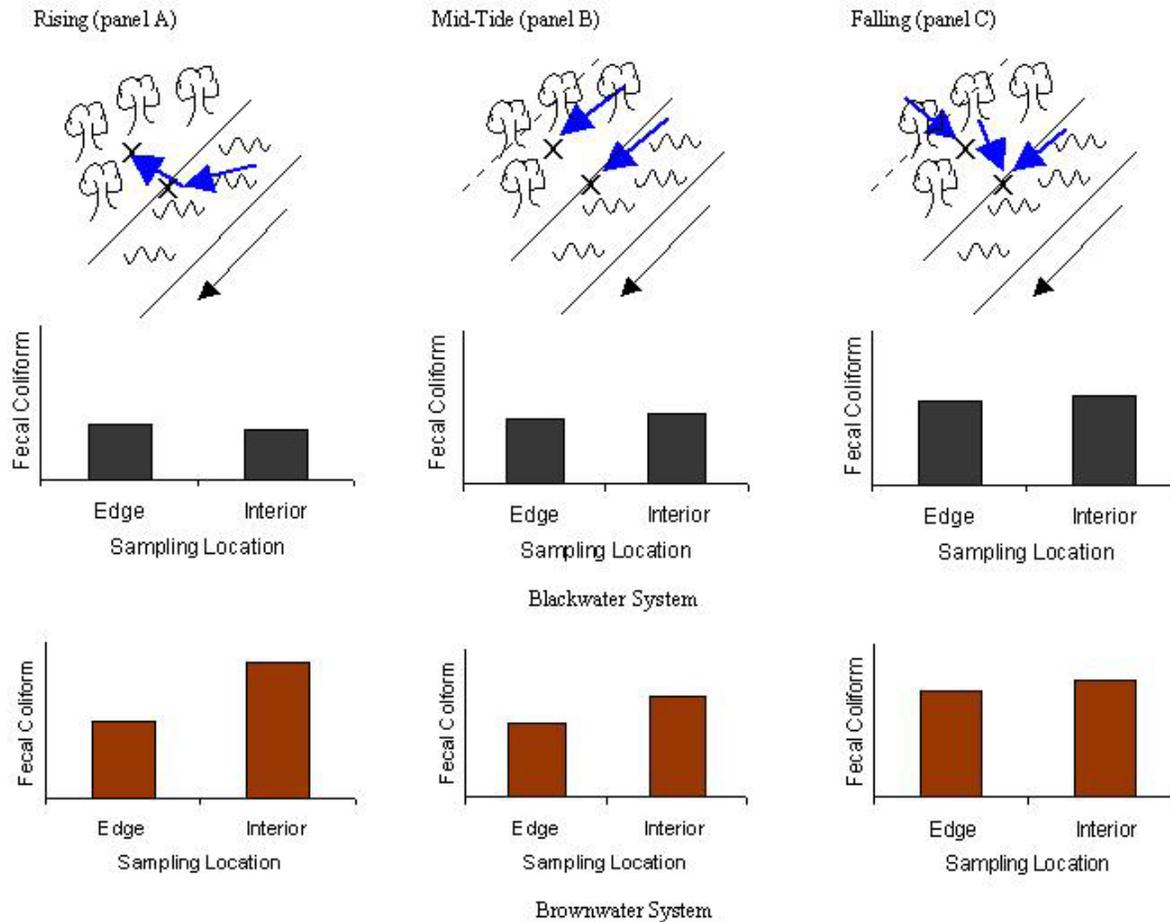


Figure 10. Conceptual model demonstrating flow patterns on and off the swamp surface and the resulting changes in fecal coliform bacteria concentrations during the rising water, mid-tide and falling water phases of an inundation period. The concentration of fecal coliform bacteria increases with greater input of swamp water with the exception of initial flooding in the brownwater system. At this site, a higher abundance of debris on the swamp surface may have contributed to the elevated concentrations.

past the sampling location to the edge of the swamp continues to pick up bacteria from the surface. The observed increase in fecal coliform concentrations at the edge sampling location may occur because the water moving past this location consists of a greater proportion of swamp water to river water until the water is completely off the surface.

The conceptual model also applies to the changes in fecal coliform bacteria concentrations observed at the brownwater site, except for the initially elevated concentrations measured in the interior sampling location. At this site, the higher concentrations may reflect the release of the fecal coliform bacteria from the organic matter lying on the swamp surface as water first comes into contact with it. Significant numbers of fecal coliform bacteria can be eluted from decaying aquatic vegetation (wrack) deposits on intertidal shorelines by repeated tidal flooding (Weiskel et al., 1996). The brownwater site was covered with organic litter, whereas in comparison, the blackwater site had relatively little organic debris on the swamp surface. The fecal coliform concentrations at both the edge and interior sampling locations remained relatively constant throughout the rising water phase, suggesting that there is neither a net gain nor net loss of bacteria in the overlying water during this portion of the tidal cycle. Following high slack water, the fecal coliform concentrations increased at both the edge and interior sites. The increase in fecal coliform concentrations measured at the interior location, in particular, is most likely due to flow direction as the water comes from further in the swamp interior, picking up bacteria from the swamp surface. These results are consistent with the conceptual model proposed for the blackwater system (Figure 10) and suggest that the water moving off the swamp surface is exporting bacteria.

## Drought Effects

In general, wetland environments are considered to be sinks for particulate matter, bacteria, and other suspended contaminants. The results of this study, however, suggest that fecal coliform bacteria may be exported from the riparian wetland surface when TSS concentrations are low ( $14.5 \text{ mg L}^{-1}$ ). This study was conducted during a period of severe drought conditions that existed in the Cape Fear River watershed from the spring of 2000 to the fall of 2002. While the drought condition does not reflect the “normal” state of the fluvial system, it did allow for the identification and evaluation of the background/baseline fecal coliform and TSS level in the brownwater system.

Reduced precipitation and lower flow conditions (Figure 11; Surface Water Data for North Carolina, 2003), especially in the headwater region of the mainstem Cape Fear (i.e. brownwater system), resulted in lower TSS concentrations in the river (Figure 12) than prior to the drought. In the Cape Fear River, the concentration of fecal coliform bacteria were significantly correlated with turbidity (Mallin et al., 2000b), suggesting that with lower TSS concentrations in the river water, fecal coliform bacteria concentrations would also be reduced, possibly because there would be less material available for the bacteria to attach to. Additionally, with lower TSS in the river, there would be less material available to settle out onto the surface of the swamp. As a result, bacteria were not as readily trapped in the brownwater swamps as originally anticipated at the beginning of the study. In the blackwater system, as expected, the fecal coliform concentrations did not decrease throughout the inundation event due to the low particulate load, approximately  $5 \text{ mg L}^{-1}$  (Mallin et al., 2000c). However, the bacterial concentrations increased, which was an unexpected result but a result apparently

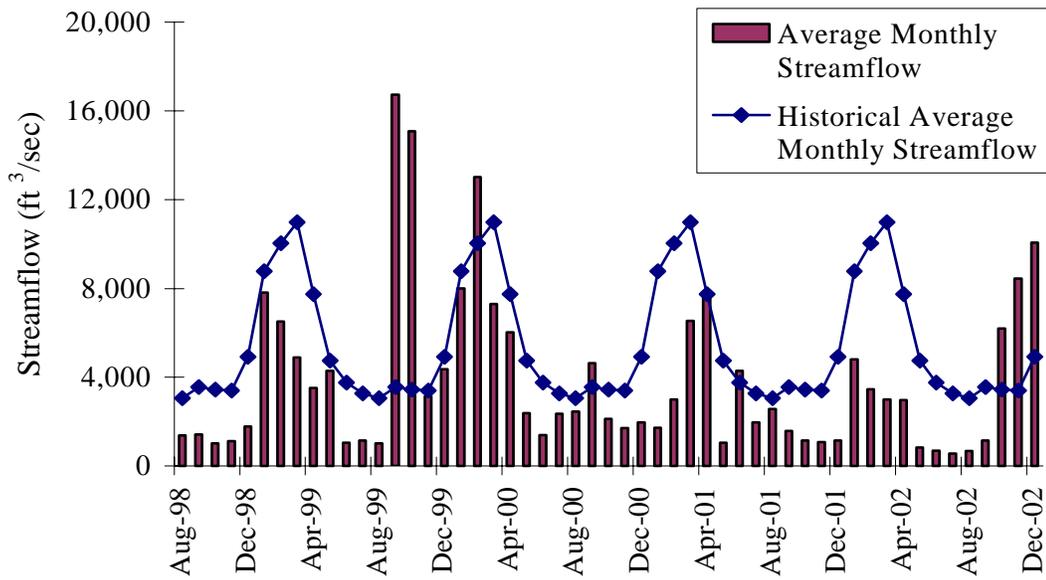


Figure 21. Historical and monthly average of streamflow in the Cape Fear River from August 1998 to December 2002. Data from the USGS gauging station at Lock #1.

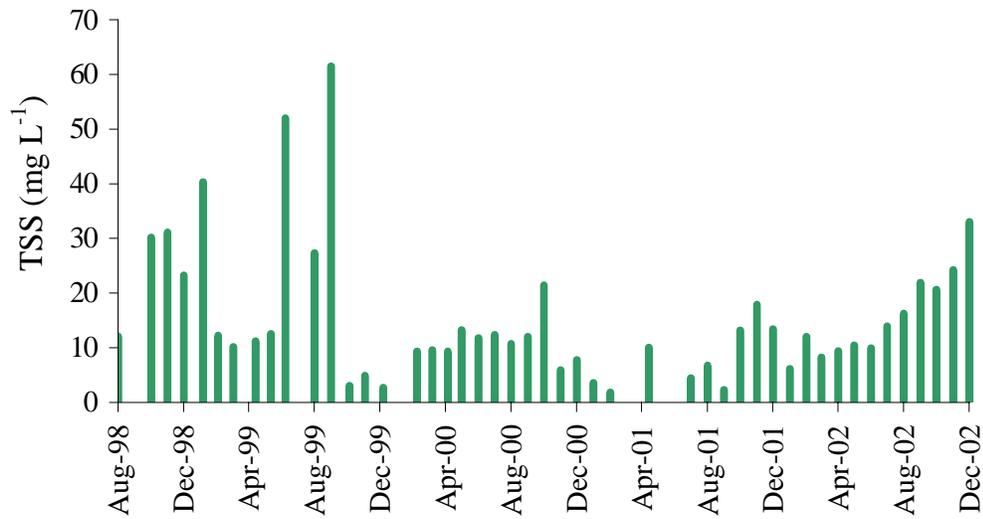


Figure 12. Cape Fear River TSS from August 1998 to December 2002. Data from Dr. Lynn Leonard (unpublished).

consistent with observations made in non-tidal blackwater systems. Previous work has shown that swamp and marsh environments contain several potential sources of bacteria, including for example, degrading organic matter (Meyer, 1990) and wildlife excrement (Mallin et al., 2000a). Headwater and riparian swamps, in particular, have been identified as a major source of bacterial biomass in non-tidal blackwater systems (Edwards and Meyer, 1986; Meyer, 1990).

Although this study was conducted during drought conditions, the blackwater system was not as strongly impacted by the drought as the brownwater system because the headwaters of blackwater system lie in the coastal plain area of the watershed where the drought was not as severe. Thus, the brownwater results are less consistent with the literature and the blackwater results are more consistent. It appears that during drought conditions, when runoff and sediment loading were reduced, that the brownwater system behaves more like a blackwater system than originally expected.

There are two potential direct effects of the drought that could have affected the results obtained during this study. The first mitigating factor may have been the location of the brownwater swamp sampling site. This site was located below the confluence of the brownwater mainstem Cape Fear River and a significant blackwater tributary (Black River) and therefore, waters sampled at that site consisted of a mixture of both brownwater and blackwater inputs. During the drought portion of sampling, the discharge from the Black River constituted approximately 13% of the flow below the confluence (Daily Streamflow for North Carolina, 2003a). Following the drought, Black River inputs accounted for approximately 6% of the total discharge near the brownwater sampling site (Daily Streamflow for North Carolina, 2003b). Even though fecal coliform

concentrations, measured by the Aquatic Ecology Lab at UNCW (unpublished data), in the Black River exceeded those measured in the mainstem above the confluence, the actual flux of bacteria from the Black River was less than one-half of the bacteria flux in the brownwater mainstem (Figure 13). Thus, it is unlikely that input from the Black River significantly impacted fecal coliform levels at the sampling site.

The second drought effect is an overall reduction in "availability" of bacteria in the river. Due to a combination of reduced runoff and associated particle and bacteria loading in the upper watershed of the Cape Fear mainstem, and an overall lower discharge, the fluxes of both fecal coliform bacteria and TSS were lessened in the river channel adjacent to the sampling site during the drought (Figure 13 and Figure 14). As a result, the potential for bacteria and TSS advection onto the swamp surface also was reduced. Thus, during the first half of this study, the potential for the river to supply TSS and bacteria to the surface of the brownwater swamp was limited. When discharge and runoff began to increase in October 2002, supply potential also increased, especially during the flood conditions of March 2003 when river discharge, TSS, and bacteria concentrations were at a maximum. However, on the brownwater swamp surface, the fecal coliform concentrations did not reflect the increases in fecal coliform availability noted in the river following the drought (Figure 15). Since the availability increased in the river following the drought and increased fecal coliform concentrations were not observed on the swamp surface, the possibility exists that the fecal coliform bacteria are not able to reach the swamp surface from the river channel. If this is the case, then this provides additional support that these systems are not acting as a sink for fecal coliform bacteria coming from the river channel.

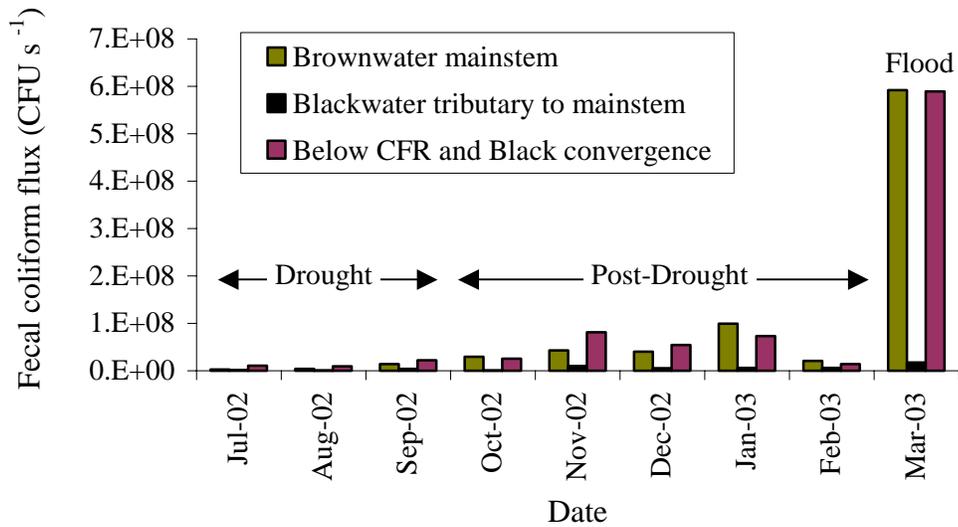


Figure 13. Flux of fecal coliform bacteria in the mainstem Cape Fear River, Black River, and below the convergence of the Black and Cape Fear Rivers. Data from the Aquatic Ecology Lab at UNCW (unpublished).

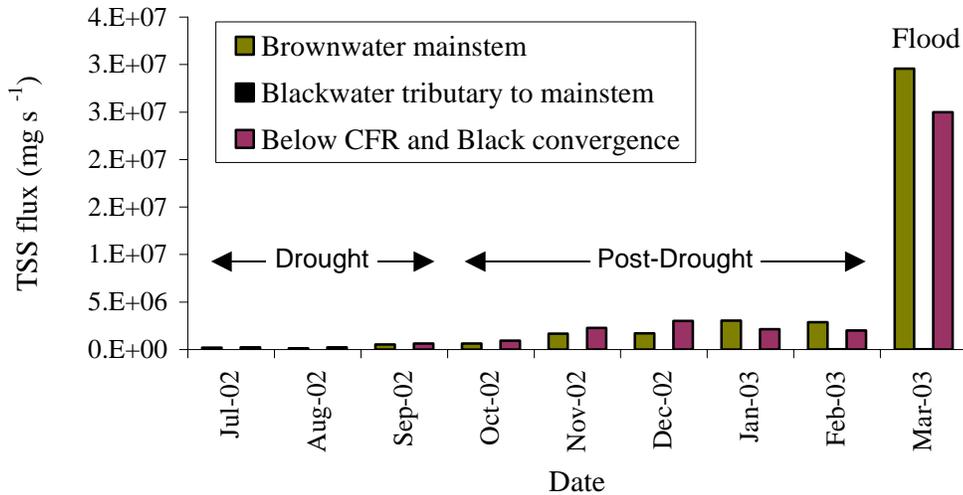


Figure 14. Flux of TSS in the mainstem Cape Fear River, Black River, and below the convergence of the Black and Cape Fear Rivers. Data from the Aquatic Ecology Lab at UNCW (unpublished).

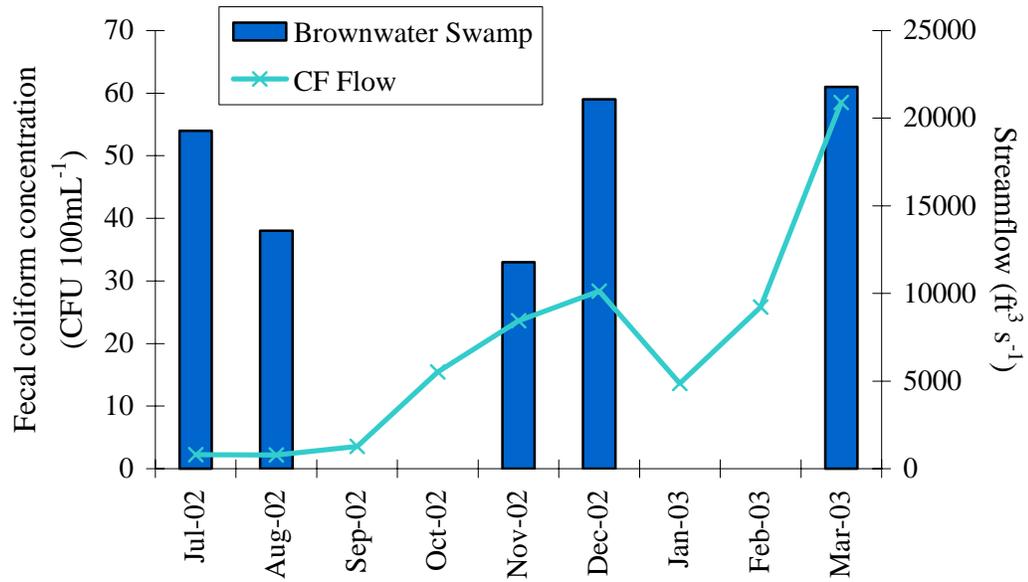


Figure 15. Fecal coliform concentrations measured on the brownwater swamp surface and changes in Cape Fear River streamflow during the study period. Streamflow data from the USGS gauging station at Lock #1 on the CFR.

It is interesting to note that in samples collected from the surface waters of the river proper by the Aquatic Ecology Lab (unpublished) between June 2002 to March 2003 the TSS concentrations were significantly and positively correlated with bacteria in the brownwater mainstem both above the Black River confluence and below the confluence (Figure 16 and Figure 17). These results are inconsistent with those obtained from examination of waters collected on the brownwater swamp surface (Figure 18) over the same period; most likely due to lower TSS levels in the swamp and inputs of bacteria from the swamp surface. In contrast, there was no correlation between TSS and fecal coliform concentration for samples collected in the Black River; a result consistent with those obtained during this study in the blackwater swamp. These data suggest that bacteria are somehow associated with TSS, most likely the clays from the Piedmont, in the brownwater mainstem, especially when TSS levels are elevated to levels higher than those measured on the swamp surface during this study. These data further suggest that the brownwater swamp surface is not receiving the TSS and associated bacteria from the river channel, and therefore this swamp surface does not have an opportunity to behave as a sink. Given a return to normal flow conditions and increased TSS concentrations, it appears that the brownwater swamp site may not be able to significantly reduce TSS concentrations and the associated fecal coliform bacteria when inundated, as it appears that the suspended sediment and associated bacteria are not reaching the swamp surface.

These brownwater results may be different if this study was conducted in a swamp site that was closer to a point source of TSS and/or fecal coliform bacteria, as significant particulate removal, especially of higher density clays and silts typically found

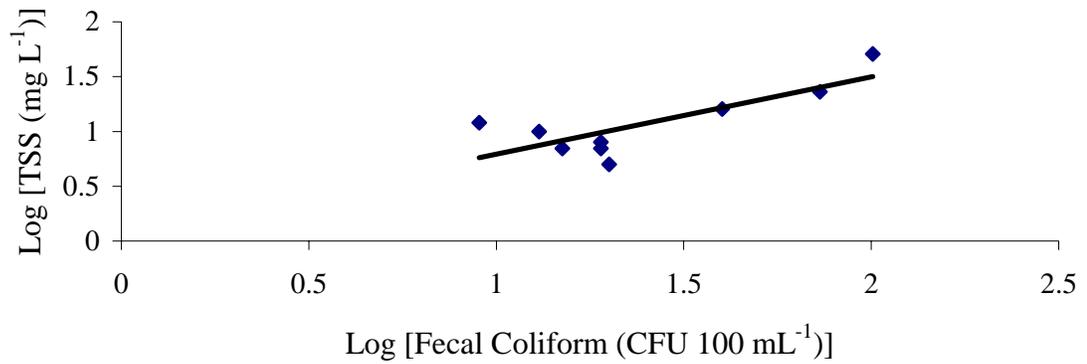


Figure 16. Fecal coliform and TSS correlation in the mainstem CFR above the confluence from July 2002 to March 2003 ( $r=0.6299$ ,  $p=0.0107$ ,  $n=9$ ).

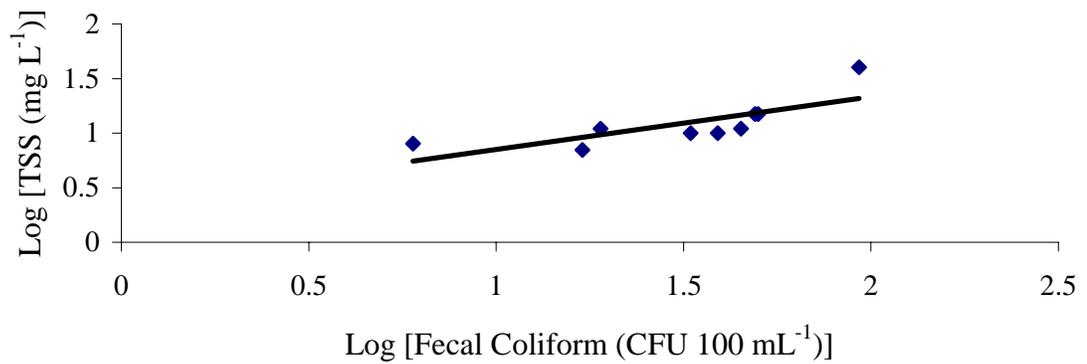


Figure 17. Fecal coliform and TSS correlation in the CFR below the confluence of the mainstem CFR and Black River tributary from July 2002 to March 2003 ( $r=0.5742$ ,  $p=0.0180$ ,  $n=9$ ).

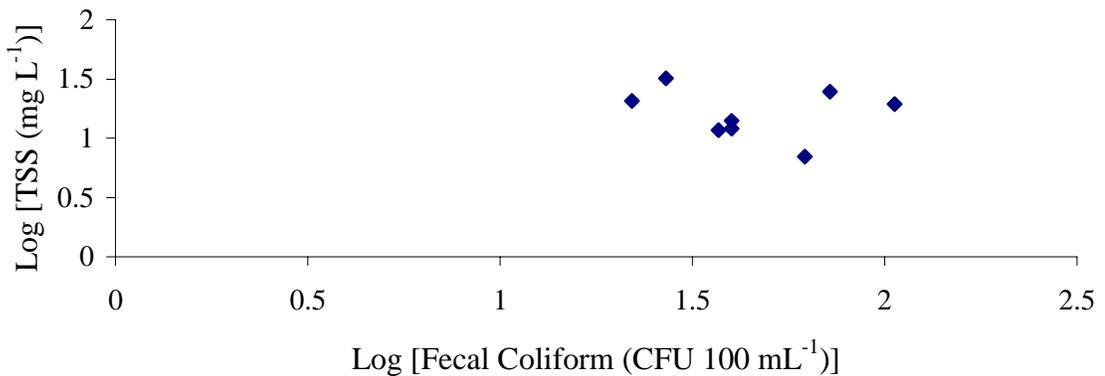


Figure 18. Fecal coliform and TSS correlation on the brownwater swamp surface from July, August, November, December 2002 and March 2003 ( $r=0.0330$ ,  $p=0.6668$ ,  $n=8$ ).

in runoff, is likely given the low flow conditions ( $0.10$  to  $2.94 \text{ cm s}^{-1}$ ) that exist on the swamp surface during an inundation event. The blackwater swamp site, on the other hand, is expected to continue to export fecal coliform bacteria even under non-drought conditions since low particulate loads consisting of lighter organic aggregates (Mallin et al., 2000c) will limit bacteria attachment to the more settleable fraction and flow characteristics ( $0.99$  to  $3.32 \text{ cm s}^{-1}$ ) may be sufficiently strong to retain unattached bacteria in suspension.

## CONCLUSIONS

This study examined the relationship between fecal coliform bacteria concentrations and total suspended solids at two riparian swamp sites, one brownwater and one blackwater, in southeastern North Carolina. Prior to this study, it was anticipated that fecal coliform bacteria concentrations would be correlated with TSS and that, as a result, the brownwater swamp would serve as a more effective trap for fecal coliform bacteria than the blackwater swamp. Instead, the results of this study suggest that fecal coliform bacteria concentrations were not significantly correlated with TSS in either swamp type and that both swamp sites were exporting bacteria from their surfaces to the river. The most likely explanation for these results was the presence of drought conditions in the watershed during the first half of the study period. These conditions resulted in a lower availability of suspended particulates and fecal coliform bacteria in the brownwater mainstem. Thus, the water flooding the surface of the brownwater swamp, characterized by lower levels of fecal coliform bacteria and TSS availability,

particularly during the first few months of this study, may the reason the brownwater swamp behaved differently than expected.

The TSS concentrations on both of the swamp surfaces were low, which reduced the availability of substrates for fecal coliform bacteria to attach to. Because of the low availability of both TSS and fecal coliform bacteria during this study, the results of this study most likely represent baseline conditions (i.e. negligible upland or upstream input) for both sites. During the spring of 2003, however, the river level was in flood stage and the TSS and fecal coliform availability increased in the brownwater river channel. The concentrations of these parameters measured on the brownwater swamp surface, however, did not increase, which raises the question of whether or not TSS and fecal coliform bacteria are actually reaching the swamp surface from the river channel even when availability is high.

Although it appears that these riparian wetlands are not trapping the fecal coliform bacteria coming from the river, these environments are still important in the role of maintaining water quality. From the anecdotal observations made during this study, as well as from studies conducted within the Cape Fear River watershed (Ensign and Mallin, 2001; Mallin et al., 2001), riparian wetlands retain significant levels of fecal coliform bacteria on and within the sediment. Thus, even though the role of these systems as buffers of fecal coliform bacteria and TSS in water moving on to and off of the swamp surface by tidal pulses are questioned during this study, their role in trapping fecal coliform bacteria and TSS from upland sources (Ensign and Mallin, 2001; Mallin et al., 2001) and preventing the release of the bacteria in sediment into the aquatic environment necessitates their preservation in coastal plain systems.

While the results of this study are limited by the duration of the study, the presence of drought conditions during sampling, and the location of the sampling sites in undeveloped areas, the available data suggest that:

- On the brownwater swamp site surface, fecal coliform bacteria were not strongly associated with the suspended particulates.
- The blackwater swamp site exported fecal coliform bacteria during an inundation event.
- The brownwater swamp site behaved similarly to the blackwater swamp site in terms of being a source of fecal coliform bacteria to the river.
- During this study, in which baseline conditions were examined, the swamp surfaces mainly exported bacteria. Under higher TSS, fecal coliform bacteria and flow conditions, the swamp surfaces (particularly the brownwater swamp) may still have an export component of bacteria, which may be undetectable when the TSS conditions are much higher than were documented during this study.
- The brownwater swamp site may not have an opportunity to act as a sink for fecal coliform bacteria, as it appears that the suspended particulates and associated bacteria are not reaching the swamp surface, even during non-drought periods.

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