ENVIRONMENTAL QUALITY OF WILMINGTON AND NEW HANOVER COUNTY WATERSHEDS, 2008

by

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Executive Summary

This report represents combined results of Year 10 of the Wilmington Watersheds Project. Water quality data are presented from a watershed perspective, regardless of political boundaries. The program involved 9 watersheds and 28 sampling stations. In this summary we first present brief water quality overviews for each watershed from data collected between January and December 2008.

Barnards Creek – Barnards Creek drains into the Cape Fear River Estuary. It drains a 2,944 acre watershed that consists of about 17% impervious surface coverage, and a population of 12,547. There was one station sampled in this watershed during 2008, lower Barnard's Creek at River Road. Based on limited data (three samplings) this site was sampled only in cool weather, November-December and there were no algal bloom or turbidity problems, and dissolved oxygen was above standard on all three occasions. However, fecal coliform bacteria exceeded the NC standard of 200 CFU / 100 mL on all three trips, and the geometric mean of the counts was 476 (Table 3.1).

Bradley Creek – Bradley Creek drains the largest tidal creek watershed in the area (6,016 acres), including much of the UNCW campus, into the Atlantic Intracoastal Waterway (ICW). The watershed contains about 23% impervious surface coverage. Four sites were sampled, all from shore. In 2008 there were no problems with turbidity exceeding the state standard, but total suspended solids (TSS) were high at BC-NB and BC-SB in September (25-33 mg/L). Station BC-SB had three significant algal blooms (34-40 µg-chlorophyll a/L) in May, July and September, and averaged nearly 24 µg/L as chlorophyll a. Average dissolved oxygen was good to fair at three sites, but poor at BC-76, the marina. The three upper sites sampled were rated poor for fecal coliform bacteria, with BC-SB having especially high counts; the downstream location BC-76 was rated fair. We note that construction activity has been ongoing upstream of BC-NB.

Burnt Mill Creek – Burnt Mill Creek drains a 4,288 acre watershed which is extensively urbanized (36% impervious surface coverage) into Smith Creek. Six locations were sampled from 2005-2008. This creek has very poor water quality, with large algal blooms characterizing the lower creek, substandard dissolved oxygen at several sites, and major issues with high fecal coliform counts, with all six sites exceeding the human contact standard > 25% of occasions sampled. These levels of pollution have characterized the system for the past several years. Restoration efforts are continuing in a joint effort by the City, NCSU, and UNCW funded through the US EPA. Sediment metals concentrations were mostly below harmful levels except for lead at the Princess Place and Wallace Park sites. However, sediment polychlorinated aromatic hydrocarbon (PAH) concentrations have regularly exceeded levels known as harmful to aquatic biota at five of the six sampling sites. *Within this report we provide a summary of Burnt Mill Creek water quality for the four-year period 2004-2008*.

The effectiveness of Ann McCrary wet detention pond and the Kerr Avenue wetland as pollution control devices was decidedly mixed over the past several years. Comparing inflows to outflows, the Kerr Avenue wetland showed statistically significant decreases in nitrate, ammonium and chlorophyll *a* over the four year period, with no significant increases in other parameters. Further downstream, in creek water passing through

Ann McCrary Pond there were significant decreases in conductivity and fecal coliform bacteria, and a significant increase in dissolved oxygen. However, there was a significant increase in nitrate and chlorophyll *a*. Several water quality parameters showed a worsening in pollutant levels along the creek from where it exited the detention pond to the downstream Wallace Park and Princess Place sampling stations, including dissolved oxygen, fecal coliform bacteria, nitrogen and phosphorus.

Futch Creek – Futch Creek is situated on the New Hanover-Pender County line and drains a 3,106 acre watershed into the ICW. UNC Wilmington was not funded to regularly sample this creek in 2008. The County employed a consulting firm to sample this creek and data are available on the County website.

Greenfield Lake – This lake drains a watershed of 2,560 acres, covered by about 36% impervious surface area. This urban lake was sampled for physical parameters at three tributary sites and for all parameters at three in-lake sites. The three tributaries of Greenfield Lake (near Lake Branch Drive, Jumping Run Branch, and Lakeshore Commons Apartments) all suffered from severe low dissolved oxygen problems, particularly the Lake Branch station GL-LB.

Algal blooms are periodically problematic in Greenfield Lake, and have occurred during all seasons, but are primarily a problem in spring and summer. In 2008 algal blooms exceeding the North Carolina water quality standard occurred on two of six sampling occasions at Stations GL-2340 and GL-YD, and three of six occasions at GL-P (at the park). This represents a general increase form 2007 (which was a drought year and likely had less stormwater runoff and lower nutrient inputs as a result of the drought). Low dissolved oxygen was found only at the uppermost lake station GL-2340. High biochemical oxygen demand (BOD5 > 3.0 mg/l) continues to occur at the in-lake stations, and is in part a result of the algal blooms. High fecal coliform counts continue to impact the lake, particularly Station GL-2340.

From 2005 to 2008 several steps were taken by the City of Wilmington to restore viability to the lake. Sterile grass carp were introduced to the lake to control (by grazing) the overabundant aquatic macrophytes and four SolarBee water circulation systems were installed in the lake to improve circulation and force dissolved oxygen from the surface downward toward the bottom. Also, on several occasions a contract firm and City staff applied herbicides to further reduce the amount of aquatic macrophytes. These actions led to a major reduction in aquatic macrophytes lake wide. In 2008 there was good dissolved oxygen at two of the stations (especially nearest the SolarBees), but low dissolved oxygen concentrations were common at GL-2340, in the upper lake. In 2007 and 2008 there was a statistically significant relationship within the lake between chlorophyll *a* and BOD5, meaning that the algal blooms are likely an important cause of low dissolved oxygen in this lake. Thus, a challenge for Greenfield Lake is to continue to reduce the frequency and magnitude of the algal blooms, which will lead to continuing dissolved oxygen improvements.

Hewletts Creek – Hewletts Creek drains a large (5,952 acre) watershed into the Intracoastal Waterway. This watershed has about 19% impervious surface coverage. In recent years this system has been plagued by a number of sewage spills. In 2008

the creek was sampled at four tidal sites and one non-tidal freshwater site. There were several incidents of low dissolved oxygen seen in our sampling in July and September; two each at NB-GLR (the north branch at Greenville Loop Rd.), SB-PGR (the south branch at Pine Grove Rd.), HC-3 (in the upper main creek), and PVGC-9 (drainage from Pine Valley Golf Course); although none were severe (below 3.3 mg/L). No major algal blooms were seen at these stations in 2008.

Fecal coliform bacterial pollution continued to impact Hewletts Creek in 2008, with all stations with the exception of HC-3 exceeding the North Carolina standard of 200 CFU/100 mL 50% of the time or more.

Howe Creek – Howe Creek drains a 3,264 acre watershed into the ICW. This watershed hosts a population of 4,224 with about 19% impervious surface coverage. Three stations were sampled in Howe Creek in 2008. Two major algal blooms were seen at the uppermost station HW-DT in May and July, and a minor bloom occurred at HW-GP in July. The uppermost station sampled was rated poor for fecal coliform bacteria, while HW-GP and HW-FP were fair and good, respectively. Dissolved oxygen concentrations were good to fair in Howe Creek in 2008. Since wetland enhancement was performed in 1998 above Graham Pond the creek below the pond at Station HW-GP has had fewer and smaller algal blooms than before the enhancement.

Motts Creek – Motts Creek drains into the Cape Fear River Estuary. This creek was sampled three times at one station at River Road, only in November and December following an influx of funding from the private sector. Dissolved oxygen concentrations were below the state standard of 5.0 mg/L on one of the three sampling occasions in 2008. Neither turbidity nor suspended solids were problematic in 2008, and there were no algal blooms encountered in the limited sampling. However, fecal coliform bacteria contamination was a problem in Motts Creek, with the State standard of 200 CFU/100 mL exceeded on two of three occasions. Thus, in November and December of 2008 this creek showed mixed water quality, with no algal bloom or turbidity problems, but minor dissolved oxygen issues and major fecal coliform problems.

Pages Creek – Pages Creek drains a 3,039 acre watershed into the ICW. UNC Wilmington was not funded to regularly sample this creek in 2008. The County employed a consulting firm to sample this creek and data are available on the County website.

Smith Creek – Smith Creek drains into the lower Northeast Cape Fear River just upstream of where it merges with the Cape Fear River. It has a watershed of 2,880 acres that has about 28% impervious surface coverage, with a population of about 26,000. One estuarine site on Smith Creek proper, SC-CH, was sampled by UNCW under the auspices of the Lower Cape Fear River Program (LCFRP) 2008. Overall the water quality can be described as poor due to low dissolved oxygen concentrations and high turbidity, although fecal coliform concentrations were not a problem in 2008.

Whiskey Creek – Whiskey Creek is the southernmost large tidal creek in New Hanover County that drains into the ICW. It has a watershed of 1,344 acres, a population of about 7,100, and is covered by approximately 17% impervious surface area. One

station, on Masonboro Loop Road, was sampled from shore along this creek in 2008. This site had low to moderate nutrient concentrations and one minor algal bloom. Dissolved oxygen was substandard (3.2-3.4 mg/L) in July and September. Fecal coliform bacteria counts were generally good at this site in 2008.

Water Quality Station Ratings – The UNC Wilmington Aquatic Ecology Laboratory utilizes a quantitative system with four parameters (dissolved oxygen, chlorophyll *a*, turbidity, and fecal coliform bacteria) to rate water quality at our sampling sites. If a site exceeds the North Carolina water quality standard for a parameter less that 10% of the time sampled, it is rated Good; if it exceeds the standard 10-25% of the time it is rated Fair, and if it exceeds the standard > 25% of the time it is rated Poor for that parameter. We applied these numerical standards to the water bodies described in this report, based on 2008 data, and have designated each station as good, fair, and poor accordingly (Appendix B).

Fecal coliform bacterial conditions for the entire Wilmington City and New Hanover County Watersheds system (25 sites sampled for fecal coliforms) showed 16% to be in good condition, 8% in fair condition, but **76%** in poor condition. Dissolved oxygen conditions system-wide (28 sites) showed 25% of the sites were in good condition, 29% were in fair condition, and 46% were in poor condition. For chlorophyll *a*, 61% of the stations were rated as good, 13% as fair and 26% as poor. In terms of turbidity 93% of the sites were rated as good, 4% as fair and 4% as poor. It is important to note that the four water bodies with the worst water quality in the system also have the most developed watersheds with the highest impervious surface coverage (Burnt Mill Creek – 36% impervious coverage; Greenfield Lake – 36% impervious coverage; Smith Creek – 28% impervious coverage; Bradley Creek 23%).

Clearly, the number one pollutant impacting the tidal creeks and other waterways of New Hanover County is fecal bacteria, which has lead to posted warnings for human contact and extensive closures of shellfish beds to harvest. In order to take appropriate remedial action it is important to determine the sources of the fecal contamination; i.e. human, avian, canine, etc. The standard method for fecal coliform pollution measurement enumerates, but does not distinguish between sources. Between December 2005 and June 2007 we conducted a study to determine sources of fecal bacteria in Futch, Pages, Howe, Bradley, Hewletts and Whiskey Creeks. We used standard methods for fecal coliform bacteria enumeration as well as the molecular methods of polymerase chain reaction (PCR) and terminal restriction fragment length polymorphism (T-RFLP) for bacterial source tracking using the genera *Bacteroides* as a target. As such we were able to identify areas with high levels of fecal coliform bacteria pollution as well as distinguish between human, canine and ruminant sources. Of the 54 samples collected during this project, about 23% were positive for canine fecal contamination by PCR; these canine-positive samples were mostly associated with rainfall and would thus be brought to the creek during stormwater runoff. Ruminant sources were found in 12 of the 54 PCR samples (22%) collected during this study, mainly in the upstream sampling areas (deer are likely an important source) and also near a known horse farm. Human fecal contamination was found in 18% of the PCR samples, indicating human waste treatment and conveyance problems. The second molecular process, T-RFLP, produced 40 peaks, each corresponding to a bacterial

taxon. Using a Web-available phylogenetic assessment tool, it was possible to identify 13 of the 40 peaks, 11 of which were human-specific. The presence of human-specific fecal contamination is of particular concern, because New Hanover County has been plagued with sewer-system failures. Human fecal contamination in these tidal creeks is indicative of either continued sewer-line problems, septic system failures, or a general persistence in the bacteria itself in sediments from earlier pollution episodes.

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1.0 Introduction

In 1993 scientists at the UNC Wilmington Center for Marine Science Research began studying five tidal creeks in New Hanover County. This project, funded by New Hanover County, the Northeast New Hanover Conservancy, and UNCW, yielded a comprehensive report detailing important findings from 1993-1997, and produced a set of management recommendations for improving creek water quality (Mallin et al. 1998a). Data from that report were later published in the peer-reviewed literature (Mallin et al. 2000a; Mallin et al. 2001) and were used 2006-2008 by the N.C. General Assembly (Senate Bill 1967) as the scientific basis to redefine low density coastal areas as 12% impervious surface coverage instead of the previously used 25% impervious cover. In 1999-2000 Whiskey Creek was added to the matrix of tidal creek watersheds analyzed in our program.

In October 1997 the Center for Marine Science began a project (funded by the City of Wilmington Engineering Department) with the goal of assessing water quality in Wilmington City watersheds under base flow conditions. Also, certain sites were analyzed for sediment heavy metals concentrations (EPA Priority Pollutants). In the past ten years we have produced combined Tidal Creeks – Wilmington City Watersheds reports (Mallin et al. 1998b; 1999; 2000b; 2002a; 2003; 2004; 2006a; 2007; 2008). In fall 2007 New Hanover County decided to stop funding UNCW sampling on the tidal creeks. In the present report we present results of sampling conducting during 2008, with principal funding by the City of Wilmington. The UNCW Aquatic Ecology Laboratory is also involved with a project headed up by North Carolina State University (NCSU) and funded through the EPA 319 Grant program that is designed to provide stream restoration to Burnt Mill Creek. This report includes a summary of four years of data from that project. In fall 2008 we were pleased to obtain funding from a private company dedicated to environmentally sound development, the Newland Corporation. The Newland Corporation is designing and building a large residential project called River Lights along River Road between Barnards and Motts Creeks. Through this funding we have reinitiated sampling of Motts and Barnards Creeks along River Road. There has been no construction near either creek as of yet related to this project, thus water quality at our Barnards and Motts Creek stations reflect current upstream development and construction activities.

Water quality parameters analyzed in these nine watersheds include water temperature, pH, dissolved oxygen, salinity/conductivity, turbidity, total suspended solids (TSS), nitrate, ammonium, total Kjeldahl nitrogen (TKN), total nitrogen (TN), orthophosphate, total phosphorus (TP), chlorophyll *a* and fecal coliform bacteria. Biochemical oxygen demand (BOD5) is measured at selected sites.

1.1 Water Quality Methods

Samples were collected on six occasions within the Wilmington City watersheds from January through November 2008. Field parameters were measured at each site using a YSI 6920 Multiparameter Water Quality Probe (sonde) linked to a YSI 650 MDS display unit. Individual probes within the instruments measured water temperature, pH, dissolved oxygen, turbidity, salinity, and conductivity. YSI Model 85 and 55 dissolved

oxygen meters were also used on occasion. The instruments were calibrated prior to each sampling trip to ensure accurate measurements. The UNCW Aquatic Ecology laboratory is State-Certified for field measurements (temperature, conductivity, dissolved oxygen and pH) and for laboratory chlorophyll *a* measurements. Samples were collected on-site for analysis of ammonium, nitrate+nitrite (referred to within as nitrate), total Kjeldahl nitrogen (TKN), orthophosphate, total phosphorus, total suspended solids (TSS), fecal coliform bacteria, and chlorophyll *a*.

The analytical method used by the UNCW Aquatic Ecology Laboratory to measure chlorophyll *a* (EPA Method 445.0) is based on Welschmeyer (1994) and US EPA (1997). Chlorophyll *a* concentrations were determined from the 1.0 micrometer glass fiber filters used for filtering samples for nitrate+nitrite and orthophosphate analyses. All filters were wrapped individually in aluminum foil, placed in an airtight container and stored in a freezer. During the analytical process, the glass filters were separately immersed in 10 ml of a 90% acetone solution and allowed to extract the chlorophyll from the material for three hours; filters were ground using a Teflon grinder prior to extraction. The solution containing the extracted chlorophyll was then analyzed for chlorophyll *a* concentration using a Turner AU-10 fluorometer. This method uses an optimal combination of excitation and emission bandwidths that reduces errors in the acidification technique.

Nutrients (nitrate, ammonium, total Kjeldahl nitrogen, total nitrogen, orthophosphate, and total phosphorus) and total suspended solids (TSS) were analyzed by a state-certified contract laboratory using EPA and APHA techniques. We also computed inorganic nitrogen to phosphorus molar ratios for relevant sites (N/P). Fecal coliform concentrations were determined using a membrane filtration (mFC) method (APHA 1995).

For a large wet detention pond (Ann McCrary Pond on Burnt Mill Creek) and for a constructed wetland on Kerr Avenue (at the headwaters area of Burnt Mill Creek) we collected data from input and outfall stations. We used these data to test for statistically significant differences in pollutant concentrations between pond input and output stations. The data were first tested for normality using the Shapiro-Wilk test. Normally distributed data parameters were tested using the paired-difference t-test, and non-normally distributed data parameters were tested using the Wilcoxon Signed Rank test. Statistical analyses were conducted using SAS (Schlotzhauer and Littell 1987).

For comparative purposes, North Carolina water quality standards are listed in Appendix A.

2.0 Barnards Creek

Snapshot
Watershed area: 2,944 acres (1,191 ha) Impervious surface coverage: 17%
Watershed population: 12,547
Overall water quality: Fair
Problematic pollutants: High fecal bacteria counts

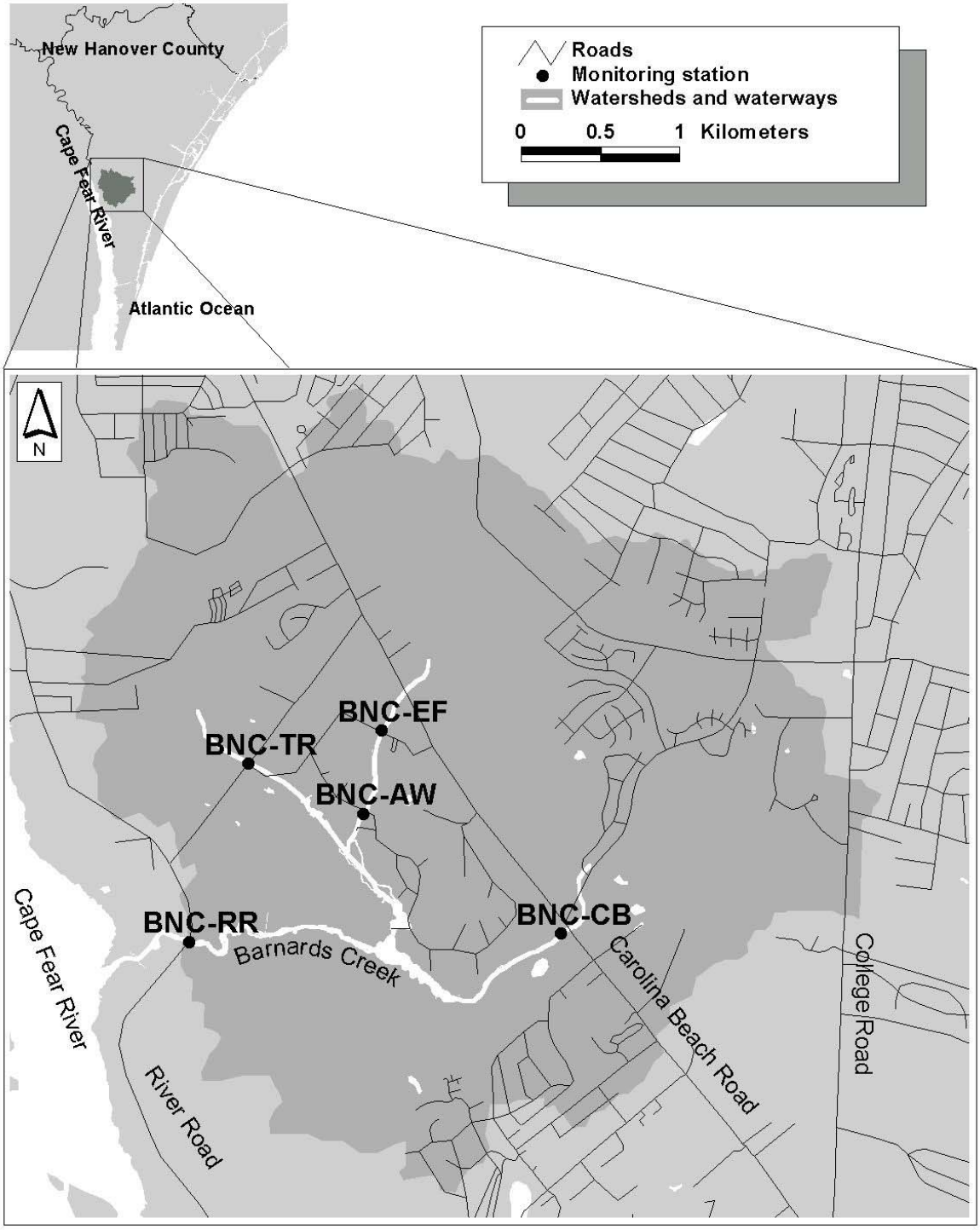
The water quality of lower Barnard's Creek is an important issue as single family and multifamily housing construction has occurred upstream of Carolina Beach Rd. in the St. Andrews Dr. area and along Independence Boulevard near the Cape Fear River. Another major housing development (River Lights) is breaking ground for the area east of River Road and between Barnards and Motts Creeks, although no project construction has occurred near Barnards Creek. In 2008 we collected data at a station located on Barnards Creek at River Road (BNC-RR) that drains part of this area (Fig. 2.1); limited data are available (three samples) for 2008 as sample collection began in November 2008. We do have extensive data on this site under a previous funding arrangement from 1999 – 2007 (see the following website for reports on-line: http://www.uncwil.edu/cmsr/aquaticecology/TidalCreeks/Index.htm.

Based on limited data we present the following summary information: BNC-RR had an average salinity of 9.2 ppt with a range of 5.3-14.5 ppt. This station had dissolved oxygen levels ranging from 7.5-10.1, but since this was only cold-weather data the results are artificially skewed toward high concentrations. Concentrations of nitrate, ammonium and orthophosphate were among the highest in streams and tidal creeks in the Wilmington area (Table 2.1). Turbidity on average was low (6.6 NTU), and did not exceed the state standard for estuarine waters of 25 NTU. Average total suspended solids concentrations were likewise low during this period, and no algal blooms occurred during this period (Table 2.1). Average BOD5 was comparatively low for urban streams with mean of 1.1 mg/L (Mallin et al. 2006a; 2007; 2008). However, fecal coliform counts exceeded the state standard on all three sampling occasions, and had a geometric mean over twice the state standard (476 CFU/100 mL).

Table 2.1. Mean and standard deviation of water quality parameters in Barnards Creek watershed, November – December 2008. Fecal coliforms as geometric mean; N/P ratio as mean (n = 3 for all parameters).

Parameter BNC-RR	mean (st. deviation)	range
Salinity (ppt)	9.2 (4.8)	5.3-14.5
DO (mg/L)	8.8 (1.3)	7.5-10.1
Turbidity (NTU)	6.6 (3.0)	3.2-9.0
TSS (mg/L)	3.7 (2.5)	1.0-6.0
Nitrate (mg/L)	0.28 (0.16)	0.11-0.42
Ammonium (mg/L)	0.19 (0.05)	0.14-0.22
TN (mg/L)	0.88 (0.06)	0.81-0.92
Phosphate (mg/L)	0.07 (0.01)	0.07-0.08
TP (mg/L)	0.08 (0.01)	0.07-0.08
N/P molar ratio	14.9	
Chlorophyll *a* (μg/L)	11.3 (4.2)	8.0-16.0
BOD5	1.1 (0.3)	0.8-1.4
Fecal coliform bacteria (/100 mL)	476	220-1,091

Figure 2.1 Barnards Creek watershed



3.0 Bradley Creek

Snapshot
Watershed area: 6,016 acres (2,435 ha) Impervious surface coverage: 23%
Watershed population: 16,719
Overall water quality: poor
Problematic pollutants: fecal bacteria, occasional low dissolved oxygen, occasional algal blooms

The Bradley Creek watershed has been a principal location for Clean Water Trust Fund mitigation activities, including the purchase and renovation of Airlie Gardens by the County. The ongoing development of the former Duck Haven property bordering Eastwood Road is of great concern in terms of its potential water quality impacts to the creek. This creek is one of the most polluted in New Hanover County, particularly by fecal coliform bacteria (Mallin et al. 2000a). Three upstream stations (BC-SB, BC-NB and BC-CA) were sampled in the past year, both fresh and brackish (Fig. 3.1). An additional downstream location, BC-76, was sampled as part of another project and these data are presented here as well.

Turbidity was not a major problem during 2008 (Table 3.1). The standard of 25 NTU was not exceeded, but it was approached on two occasions during our sampling. TSS was elevated on two occasions in September when it was 33 mg/L at BC-NB and 25 mg/L at BC-SB (there are no NC ambient standards for TSS). There were minor issues with low dissolved oxygen (hypoxia) upstream, with BC-NB and BC-SB having DO < 5.0 mg/L on only one occasion each during the six sampling occasions (Appendix B). However, BC-76 was substandard on three of seven occasions, April through August.

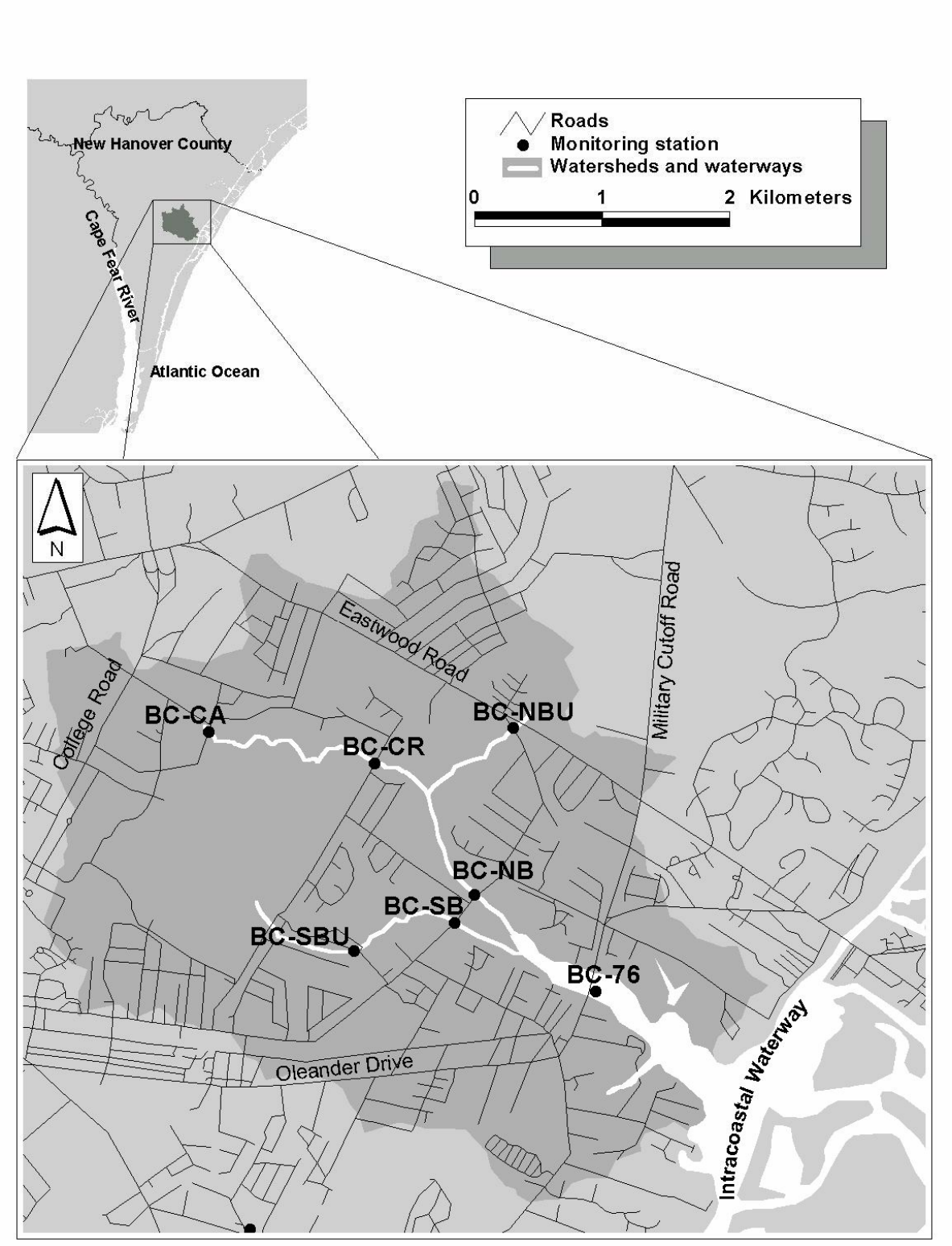
Nitrate concentrations were highest at station BC-CA, but there was little difference in concentrations of the other nutrient parameters among the three stations (Table 3.1). Bradley Creek did not host excessive algal blooms in 2008 except at BC-SB, which had a bloom of 40 μg/L as chlorophyll *a* in July and blooms of 34 μg/L in May and September (Table 3.1).

Fecal coliform bacteria counts were excessive at all three upstream stations during all seasons, with the NC standard being exceeded on at least 67% of occasions sampled at all sites. The geometric means of the fecal coliform counts ranged from just over the standard at BC-NB to 6X the standard at BC-SB (Table 3.1). Samples collected from Station BC-76 (Fig. 3.1) showed a geometric mean of 49 and one pulse of fecal coliform bacteria of 1,000 CFU/100 mL (during a December 2008 rain event). Disturbingly, preliminary information based on DNA source tracking showed human source signals at BC-76 in both February and April of 2008, although on both dates counts were below the NC contact standard.

Table 3.1 Water quality parameter concentrations at Bradley Creek sampling stations, 2008. Data as mean (SD) / range, N/P ratio as mean/median, fecal coliform bacteria as geometric mean / range, n = 6 months). BC-76 data are available from another project for 7 months.

Station	BC-CA	BC-NB	BC-SB	BC-76
Salinity (ppt)	0.1 (0.0) 0.1-0.1	11.4 (6.1) 1.6-18.2	3.2 (2.0) 1.1-5.1	28.3 (8.1) 10.2-33.0
Dissolved Oxygen (mg/L)	7.5 (0.9) 6.4-8.7	7.5 (2.2) 3.6-9.5	8.4 (2.2) 4.8-11.5	6.0 (1.9) 3.5-8.9
Turbidity (NTU)	2.3 (2.9) 0.0-8.0	8.7 (3.5) 5.0-14.0	12.7 (7.9) 3.0-23.0	5.0 (4.2) 1.0-12.3
TSS (mg/L)	1.5 (0.8) 1.0-3.0	12.2 (10.5) 5.0-33.0	12.7 (8.1) 2.0-25.0	
Nitrate (mg/L)	0.22 (0.09) 0.12-0.30	0.03 (0.02) 0.01-0.05	0.03 (0.02) 0.01-0.05	
Ammonium (mg/L)	0.026 (0.014) 0.005-0.040	0.029 (0.020) 0.005-0.050	0.051 (0.037) 0.005-0.110	
TN (mg/L)	0.59 (0.41) 0.05-1.30	0.44 (0.26) 0.05-0.80	0.67 (0.35) 0.26-1.14	
Orthophosphate (mg/L)	0.03 (0.04) 0.01-0.10	0.03 (0.03) 0.01-0.10	0.04 (0.03) 0.01-0.10	
TP (mg/L)	0.05 (0.04) 0.01-0.10	0.05 (0.03) 0.03-0.10	0.08 (0.04) 0.03-0.14	
N/P	50.7 60.3	7.1 7.2	7.8 7.8	
Chlorophyll *a* (μg/L)	1.3 (1.4 0.1-4.0	8.1 (4.3) 2.0-14.5	23.7 (15.2) 4.0-40.0	
Fecal coliforms (CFU/100 mL)	534 43-4,500	314 28-2,000	1,246 500-9,500	49 4 -1,000

Figure 3.1. Bradley Creek watershed and sampling sites.



4.0 Burnt Mill Creek Summary Report, 2005-2008

Snapshot
Watershed area: 4,288 acres (1,735 ha) Impervious surface coverage: 36%
Watershed population: 26,511
Overall water quality: poor
Problematic pollutants: Fecal bacteria, algal blooms, low dissolved oxygen, high sediment PAH and lead concentrations

Introduction

In 1997 the City of Wilmington contracted with the Aquatic Ecology Laboratory at the UNC Wilmington Center for Marine Sciences to begin citywide water quality sampling. Since then the Burnt Mill Creek watershed (Fig. 1) has been sampled just upstream of Ann McCrary Pond on Randall Parkway (BMC-AP1), and about 40 m downstream of the pond outfall (BMC-AP3). Ann McCrary Pond is a large (28.8 acres) regional wet detention pond draining 1,785 acres, with an apartment complex at the upper end near BMC-AP1. The pond itself periodically hosts thick growths of submersed aquatic vegetation, with *Hydrilla verticillata, Egeria densa, Alternanthera philoxeroides, Ceratophyllum demersum* and *Valliseneria americana* having been common at times. There have been efforts to control this growth, including addition of triploid grass carp as grazers. The ability of this detention pond to reduce suspended sediments and fecal coliform bacteria, and its failure to reduce nutrient concentrations, was detailed in a scientific journal article (Mallin et al. 2002b). Numerous waterfowl utilize this pond as well.

In 2005 sampling began on the inflow (BMC-KA1) and outflow (BMC-KA3) channels of the Kerr Avenue constructed wetland (Fig. 1). This sampling began as a part of a larger project (through North Carolina State University funded by the EPA 319 Program) to provide stream restoration to Burnt Mill Creek. Construction of the 0.7 acre Kerr Avenue Wetland was funded by the N.C. Wetlands Restoration Program, now known as the Ecosystem Enhancement Program. Wetland construction was completed in November 2000 and the first aquatic macrophyte planting (sponsored by Cape Fear River Watch) occurred later that month (various rushes, sedge, pickerelweed, lizard's tail, water tupelo, wax myrtle, black gum, pond pine, bald cypress, etc.). Since then there have been many supplemental plantings as well as tree donations. The vegetation coverage is presently so dense that macrophytes from this site have been transplanted into other wetland restoration sites. This Best Management Practice (BMP) lies in the headwaters of Burnt Mill Creek, which is on the State 303(d) list for poor biological condition. The wetland has a forebay to collect sediment, and the system is designed to retain and treat the first 1.3 cm (0.5 inches) of a rainfall event before an overflow channel is utilized. The wetland drains a 6.1 ha (15 acre) area that is about 87% developed, mainly by businesses including large and small retail operations, several restaurants, a car wash and two auto repair businesses. A portion of the University of North Carolina Wilmington drains into Burnt Mill Creek here as well, with some nearby multifamily residential dwellings. Further downstream, a fifth station is located along the main stem of the creek in the Wallace Park area (BMC-WP) and a sixth station is also on the creek at the bridge at Princess Place (BMC-PP - Fig. 1).

Recent water quality results of these continuing studies have been published previously (Mallin et al. 2006a; Mallin et al. 2007; Mallin et al. 2008).

Methods

Sampling Sites: Stream sampling was conducted on 26 dates from January 2005 through August 2008. Samples were collected from six stations on the main body of the creek (Table 1). Nearest the headwaters, the two uppermost stations were the inflow (BMC-KA1) and outflow (BMC-KA3) channels of the Kerr Avenue constructed wetland (Table 1; Fig. 1).

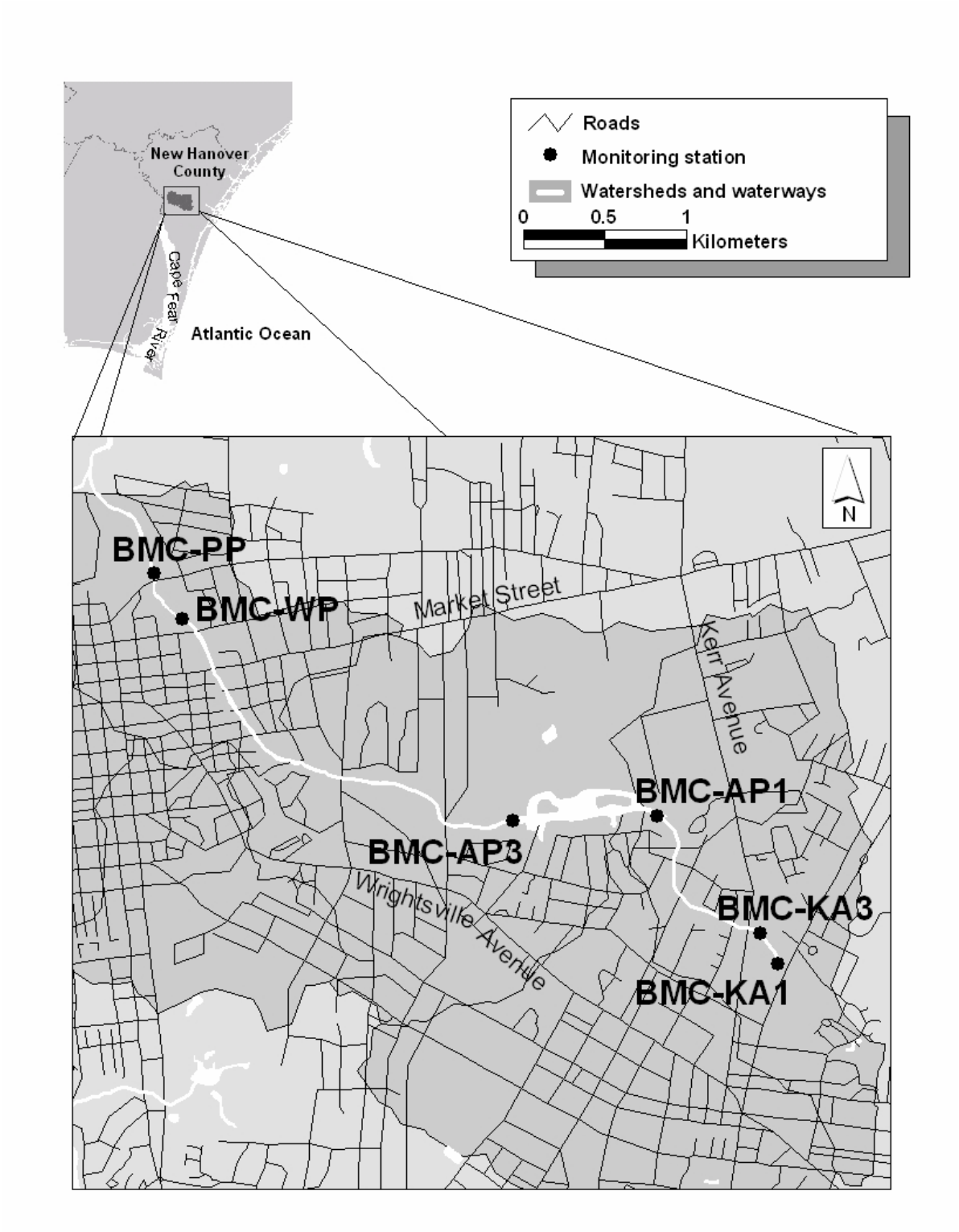
Table 1. GPS coordinates for Burnt Mill Creek water quality sampling stations.

BMC-KA1	N 34.22207	W 77.88506
BMC-KA3	N 34.22280	W 77.88601
BMC-AP1	N 34.22927	W 77.86658
BMC-AP2	N 34.22927	W 77.89792
BMC-AP3	N 34.22927	W 77.90143
BMC-WP	N 34.24083	W 77.92419
BMC-PP	N 34.24252	W 77.92510

About one km downstream of that wetland is the aforementioned Ann McCrary Pond, a large regional wet detention pond on Randall Parkway which was sampled just upstream (BMC-AP1) and about 40 m downstream (BMC-AP3) of the pond (Table 1; Fig. 1).

Several km downstream of Ann McCrary Pond are the final two stream stations, BMC-WP and BMC-PP, located in Wallace Park and at the Princess Place bridge over the creek, respectively (Table 1; Fig. 1). These are main stem stations in what is considered to be the mid-to-lower portion of Burnt Mill Creek, in a mixed residential and retail area. The cost for sampling three of the sites (BMC-KA1, BMC-KA2, and BMC-WP) has been funded by the US EPA 319 Program through North Carolina State University. The cost of sampling BMC-AP1, BMC-AP3 and BMC-PP has been funded by the City of Wilmington, through its Stormwater Services section.

Figure 1. Burnt Mill Creek watershed and water quality sampling sites.



Sample Parameters and Analyses: Data were collected at all stations for water temperature, conductivity, salinity, dissolved oxygen, pH and turbidity using a YSI 6920 Multiparameter Water Quality Probe (sonde) linked to a YSI 650 MDS display unit. While on-site, samples were collected for total suspended sediments, total nitrogen, nitrate-N, ammonium-N, total phosphorus, orthophosphate-P, fecal coliform bacteria, and chlorophyll *a*. Samples for five-day biochemical oxygen demand (BOD5) were collected at three stations; KA1, KA2 and BMC-WP (Fig. 1). Samples were immediately stored on ice, and returned to the laboratory. Nutrients, BOD5 and fecal coliform bacteria analyses were conducted at a state-certified laboratory using protocols described in Standard Methods (APHA 1995). These included nitrate (Method 4500-NO3 F), ammonium (Method 4500-NH3 H), total Kjeldahl nitrogen (TKN-Method 4500-Norg B), orthophosphate (Method 4500-P E), and total phosphorus (TP-Method 4500-P E with persulfate digestion), and total suspended solids (Method 2540-D). Total nitrogen (TN) was computed as TKN plus nitrate. Fecal coliform bacteria concentrations were determined using a membrane filtration (mFC) method that utilizes a 24 h incubation time at 44.5 ^{o}C and an enriched lactose medium (Method 9222-D - APHA 1995), and reported as colony-forming units (CFU) 100 ml^{-1}. The analytical method used to measure chlorophyll *a* is EPA 445.0 , based on Welschmeyer (1994); an acetone extraction analyzed for chlorophyll *a* concentration using a Turner AU-10 fluorometer. Chlorophyll *a* analysis was performed at the UNC Wilmington Aquatic Ecology Laboratory, which is state-certified for chlorophyll *a* measurements as well as field data collections (conductivity, pH, dissolved oxygen and temperature).

For the Kerr Avenue Wetland and Ann McCrary Pond we used these data to test for statistically significant differences in pollutant concentrations between wetland and pond input and output stations. The data were first tested for normality using the Shapiro-Wilk test. Normally distributed data parameters were tested using the paired-difference t-test, and non-normally distributed data parameters were tested using the Wilcoxon Signed Rank test. Statistical analyses were conducted using SAS (Schlotzhauer and Littell 1997).

Results and Discussion

The Upper Creek

The most obvious feature of the upper creek is the Kerr Avenue Wetland. BMC-KA1 is the input station to this wetland, and Station BMC-KA3 is located at the drainage from the wetland just before the creek passes under Kerr Avenue (Fig. 1). Inputs to the wetland were not unusually high in terms of particulate matter. Total suspended solids exceeded 25 mg/L on only one occasion, and turbidity concentrations were below 25 NTU except for one unusual peak value of 385 NTU in May 2006. Incoming waters were frequently low in dissolved oxygen, however, with 50% of the samples below the NC freshwater standard of 5.0 mg/L (Table 2).

We were able to utilize data from 26 sampling trips within the period 2005-2008 to assess the efficacy of this wetland as a pollutant removal device. In terms of particulate matter, results showed that neither turbidity nor suspended solids showed a significant change between entering and leaving the wetland (Table 2). However, both ammonium and nitrate showed significant decreases in passing through the wetland, with

ammonium decreasing on average 63% and nitrate decreasing on average 40% (Table 2). There were no significant differences in total nitrogen, orthophosphate, or total phosphorus entering and leaving the wetland, and there was no significant difference in BOD5 entering or leaving the wetland. Fecal coliform bacteria maintained high concentrations entering and exiting the wetland, with no statistical difference entering or leaving the pond. The presence of a number of dumpsters surrounding the site, and consequent small mammals foraging and defecating, may be a localized source of fecal coliform bacteria, BOD and organic nutrients. Additionally, there is a stormwater drainage pipe that enters the wetland within 20 m of the outfall at BMC-KA3 that essentially circumvents the wetland during rain events. In addition to the two above factors, the size of the wetland itself (0.7 acres) is considered too small to properly treat the 15 acre drainage area, thus the lack of significant decreases in many of the water quality pollutant parameters (Table 2).

About one km downstream from Kerr Avenue along Randall Parkway is the large regional wet detention pond known as Ann McCrary Pond. Data were collected at the input (BMC-AP1) and outflow (BMC-AP3) stations from 2005-2008. Turbidity and suspended solids concentrations entering and leaving this large regional pond were low to moderate, with no statistical difference between inflow and outflow (Table 2). Fecal coliform concentrations entering Ann McCrary Pond at BMC-AP1 were high (Table 2), possibly a result of pet waste runoff from the apartment complex and runoff from urban upstream areas (including the Kerr Avenue wetland). Over the sampling period 84% of the samples collected at BMC-AP1 had counts exceeding 200 CFU/100 mL, and 48% of the samples from BMC-AP3 exceeded the standard. On average there was a statistically significant, 77% reduction in fecal coliform abundance through the pond.

There was only one major algal bloom at BMC-AP1 that exceeded the North Carolina water quality standard of 40 µg/L during the study, whereas at BMC AP-3 there were six major algal blooms that exceeded the State standard. This resulted in significantly higher chlorophyll *a* concentrations exiting the pond compared with entering the pond (Table 2). The efficacy of Ann McCrary Pond as a nutrient removal device was poor throughout the study; in fact, nitrate showed a significant increase from inflow to outflow (Table 2). None of the other nutrient parameters showed a significant change passing through the pond. It is likely that inputs of nutrients enter the pond from a suburban drainage stream midway down the pond, short circuiting the ability of the pond to remove nutrients. Also, intensive waterfowl use of the pond, particularly at a tributary near the outfall, may have contributed to nitrogen and phosphorus loading in the lower pond and along its shoreline. However, the concentrations of nutrients entering the pond were not high to begin with. There was a significant decrease in conductivity through the pond. Dissolved oxygen significantly increased through the pond (by 46% on average), probably because of in-pond photosynthesis and aeration by passage over the final dam at the outfall. There was a significant increase in pH, probably due to utilization of CO_2 during photosynthesis in the pond.

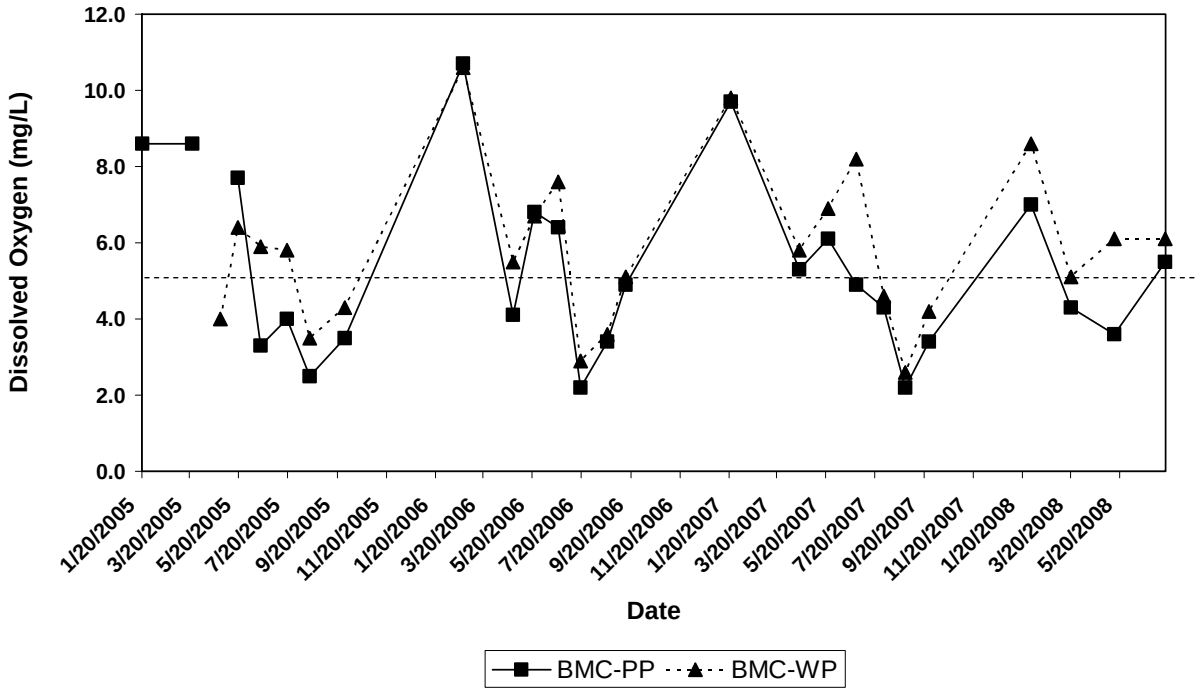
Table 2. Water quality data in upper Burnt Mill Creek, January 2005 – July 2008, as mean (standard deviation)/range. Fecal coliforms as geometric mean; N/P as median.

Parameter	BMC-KA1	BMC-KA3	BMC-AP1	BMC-AP3
DO (mg/L)	5.5 (2.5) 1.8-10.6	5.7 (2.0) 2.9-10.6	6.8 (1.7) 3.9-10.6	9.9 (1.4)** 7.9-12.5
Cond. (μS/cm)	306 (117) 19-483	307 (97) 71-400	245 (62) 84-297	226 (39)* 144-275
pH	7.1 (0.5) 6.0-7.9	7.1 (0.3) 6.5-7.8	7.2 (0.3) 84-297	8.0 (0.4)** 144-275
Turbidity (NTU)	22 (77) 0-385	12 (11) 0-42	10(11) 1-54	8 (5) 0-24
TSS (mg/L)	7 (6) 2-26	15 (20) 1-83	18 (28) 1-101	10 (7) 4-29
Nitrate (mg/L)	0.086 (0.084) 0.010-0.370	0.052 (0.077)* 0.010-0.300	0.059 (0.064) 0.010-0.030	0.076 (0.070)** 0.010-0.350
Ammonium (mg/L)	0.113 (0.085) 0.010-0.260	0.042 (0.059)* 0.005-0.260	0.045 (0.028) 0.005-0.130	0.046 (0.057) 0.005-0.290
TN (mg/L)	0.825 (0.350) 0.250-1.930	0.754 (0.791) 0.035-3.900	0.825 (0.435) 0.260-2.150	0.846 (0.306) 0.380-1.670
OrthoPhos. (mg/L)	0.047 (0.135) 0.005-0.660	0.018 (0.028) 0.005-0.140	0.013 (0.011) 0.005-0.060	0.009 (0.002) 0.004-0.013
TP (mg/L)	0.110 (0.201) 0.020-1.010	0.127 (0.163) 0.030-0.640	0.079 (0.079) 0.010-0.350	0.068 (0.036) 0.040-0.190
N/P molar ratio	24.4	6.6	14.9	27.7
Chlor. *a* (μg/L)	8.7 (24.2) 0.2-121.0	7.7 (7.4)* 1.2-27.6	20.1 (56.2) 0.7-286.6	29.0 (30.2)** 1.3-104.9
FC (CFU/100 mL)	1473 45-60,000	1054 118-26,000	823 49-6,000	192** 3-3,000
BOD5	2.2 (1.9) 0.5-7.3	2.2 (1.9) 0.6-9.6	no data	no data

* Statistically significant difference between inflow and outflow at $p<0.05$; ** $p < 0.01$.

Lower Burnt Mill Creek: Both the Wallace Park (BMC-WP) and the Princess Place location (BMC-PP) experienced severe water quality problems during the sampling period. One parameter that is key to aquatic life health is dissolved oxygen. Dissolved oxygen was substandard (between 2.0 and 5.0 mg/L) on 33% of occasions sampled at BMC-WP and 52% of occasions sampled further downstream at BMC-PP (Fig. 2). The state standard for turbidity in freshwater is 50 NTU; there was only one exceedence of this value in the entire data set. Total suspended solids (TSS) concentrations have no ambient state standard. Based on our long term observances in the lower Cape Fear River basin, for the lower Coastal Plain a reasonable TSS "interest concentration" is 25 mg/L. This concentration was exceeded on only two occasions in the lower creek.

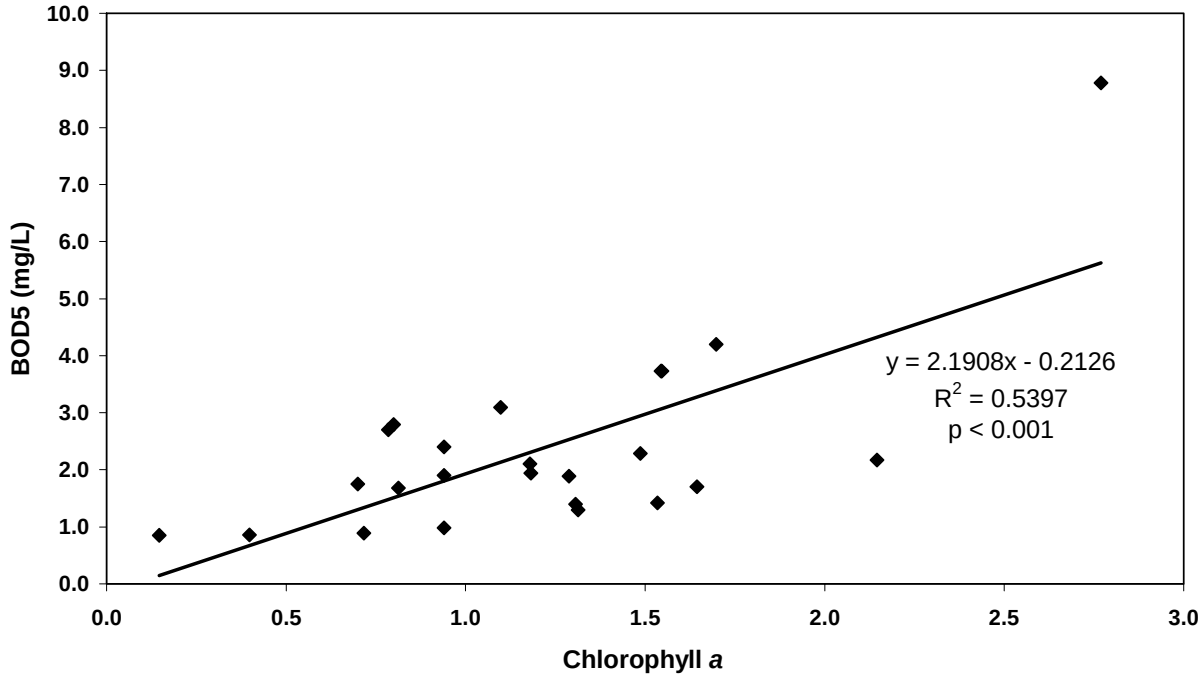
Figure 2. Dissolved oxygen concentrations in mainstem Burnt Mill Creek, 2005-2008.



However, the lower creek was prone to algal bloom formation, with chlorophyll *a* concentrations on several occasions exceeding 100 µg/L (Table 3; Fig. 3). As mentioned, the North Carolina water quality standard for chlorophyll *a* is 40 µg/L; this was exceeded on six occasions at BMC-PP and four occasions at BMC-WP. BMC-WP also hosted several blooms with chlorophyll *a* concentrations between 30 and 40 µg/L . Algal blooms can cause disruptions in the food web, depending upon the species present (Burkholder 2001). However, blooms can also play a direct role in lowering stream dissolved oxygen concentrations. This occurs when algal blooms die and decompose into labile organic matter, which exerts a biochemical oxygen demand (BOD). Concentrations of chlorophyll *a* have been strongly correlated with BOD5 in urban lakes, tidal creeks, and the Cape Fear River in southeastern North Carolina (Mallin et al. 2006). BOD5 analyses were performed at Wallace Park, with median concentrations (1.9 mg/L) higher than rural streams but typical of urban streams in the

Wilmington area (Mallin et al. 2006). However, on five occasions BOD5 exceeded 3.0 mg/L, once reaching 8.8 mg/L. In lower Burnt Mill Creek chlorophyll *a* and BOD5 were significantly correlated ($R = 0.73$, $p < 0.001$). Regression analysis shows that algal blooms as chlorophyll *a* accounted for approximately 54% of the variability in BOD5 (Fig. 3). While other particulate and dissolved materials that exert a BOD undoubtedly enter the creek through stormwater runoff, clearly algal blooms are one important part of the low dissolved oxygen problem.

Figure 3. BOD5 as a function of log chlorophyll *a* in lower Burnt Mill Creek, 2005-2008.



An important question is what drives algal bloom formation in Burnt Mill Creek? Nutrient concentrations were unremarkable at either site. Examination of inorganic nitrogen to phosphorus ratios (Tables 2 and 3) shows that in most areas of the creek mean N/P ratios are generally in the 20-34 range, while median N/P ratios are lower. In waters where the N/P ratio is well below 16 (the Redfield Ratio for algal nutrient composition) it is generally considered that algal production is limited by the availability of nitrogen (i.e. phosphorus levels are sufficient); where N/P ratios are well above 16, additions of phosphate should encourage algal blooms. In Burnt Mill Creek it appears that in most circumstances phosphate is the limiting factor (and needs to be controlled); however, periodically (particularly in summer) the N/P ratios are very low indicating control of nitrogen inputs is needed. Thus, there is a need for control of inputs of both N and P to help reduce algal blooms in Burnt Mill Creek.

Table 3. Water quality data in lower Burnt Mill Creek, January 2005 – July 2008, as mean (standard deviation)/range. Fecal coliforms as geometric mean; N/P as median.

Parameter	BMC-WP	BMC-PP
DO (mg/L)	5.8 (2.1) 2.6-10.6	5.3 (2.4) 2.2-10.7
Cond. (μS/cm)	1142 (2031) 241-8450	1573 (2863) 237-9987
pH	7.3 (0.2) 6.8-7.5	7.2 (0.2) 6.9-7.5
Turbidity (NTU)	8 (4) 0-20	8 (6) 0-29
TSS (mg/L)	8 (6) 3-26	7 (5) 2-30
Nitrate (mg/L)	0.121 (0.072) 0.010-0.240	0.116 (0.104) 0.005-0.490
Ammonium (mg/L)	0.076 (0.048) 0.010-0.160	0.079 (0.058) 0.010-0.220
TN (mg/L)	1.130 (0.833) 0.570-3.810	1.111 (1.206) 0.350-6.750
OrthoPhos. (mg/L)	0.023 (0.020) 0.005-0.080	0.028 (0.025) 0.005-0.100
TP (mg/L)	0.105 (0.086) 0.012-0.450	0.108 (0.050) 0.030-0.230
N/P molar ratio	19.2	16.6
Chlor. *a* (μg/L)	46.6 (118.8) 1.4-588.0	65.2 (150.2) 0.9-646.0
Fec. col. (CFU/100 mL)	924 75-29,000	559 22-15,500
BOD5	2.4 (1.7) 0.9-8.8	no data

Important from a public health perspective are the excessive fecal coliform bacteria counts, which maintained geometric means (924 CFU/100 mL at BMC-WP and 559 CFU/100 mL at BMC-PP) well in excess of the State standard for human contact waters (200 CFU/100 mL). Fecal coliform counts were greater than the State standard on 73% of occasions sampled at Wallace Park and 68% of occasions sampled at Princess Place. It is notable that fecal coliform bacteria counts increased along the passage from BMC-AP3 (geometric mean 192 CFU/100 mL) to the Wallace Park location (Fig. 4), while dissolved oxygen decreased (Tables 2 and 3). It is likewise notable that nutrient concentrations increased from the inflow to Ann McCrary Pond downstream to the two lower main stem stations (Fig. 5). Nutrients appear to peak at the Wallace Park station and decline slightly further downstream at the Princess Place location; fecal coliform counts also decline from BMC-WP to BMC-PP (Fig. 4).

Figure 4. Geometric mean fecal coliform bacteria abundance in Burnt Mill Creek, 2005-2008, moving downstream from the headwaters.

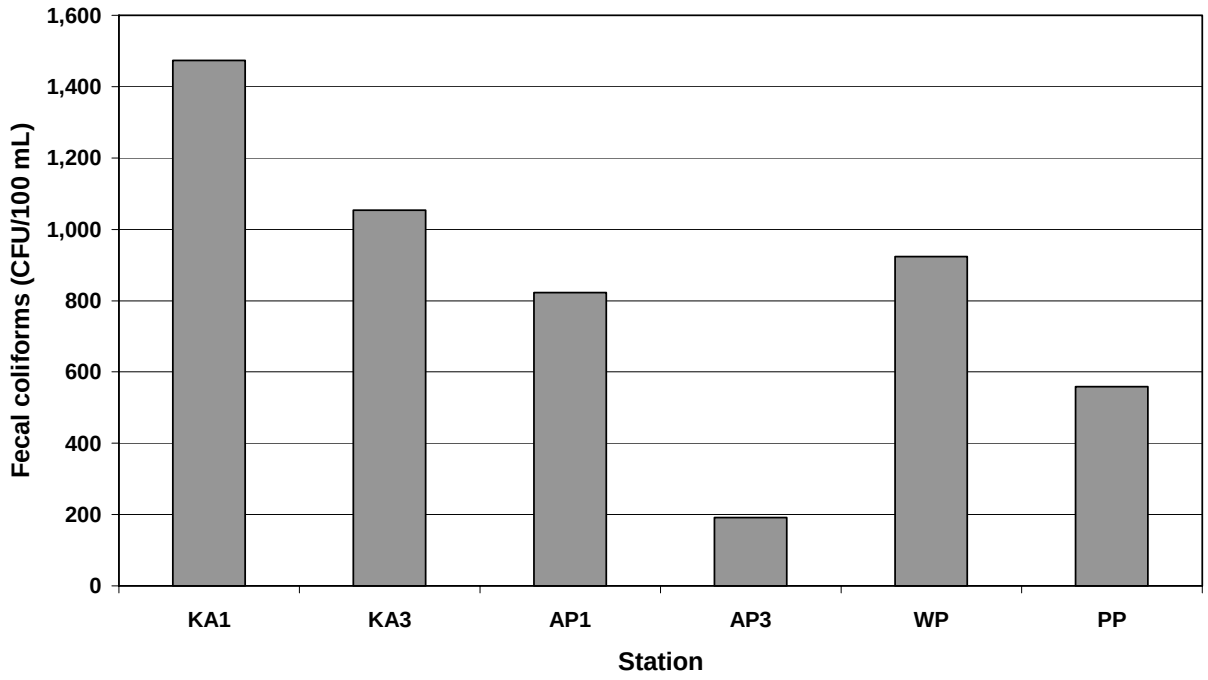
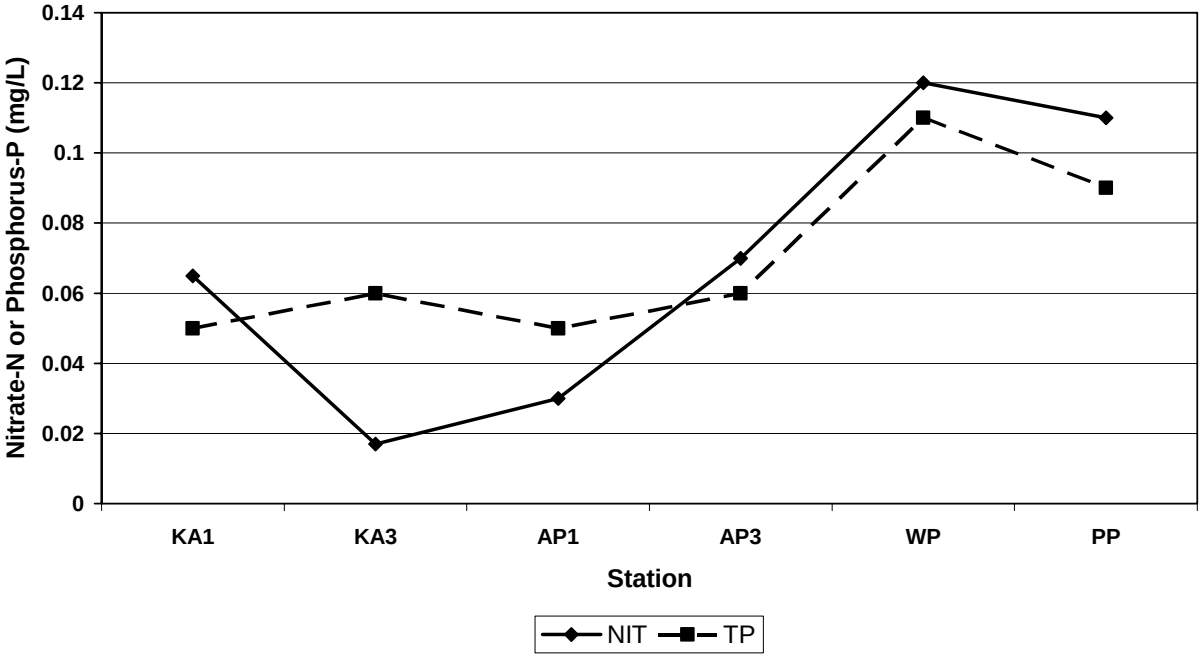


Figure 5. Median nitrate and total phosphorus concentrations in Burnt Mill Creek, 2005-2008, moving downstream from the headwaters.



To summarize, Burnt Mill Creek has problems with low dissolved oxygen (hypoxia) at some of the upper stations but most notably in the lower creek stations. Dissolved oxygen (DO) is elevated after the water passes through the large regional wet detention pond known as Ann McCrary Pond, but decreases considerably a few km downstream at Stations BMC-WP and BMC-PP. Part of the hypoxia problem is caused by elevated BOD caused by decaying algal blooms. These blooms occur with some frequency at BMC-WP and BMC-PP, and to a lesser extent in the detention pond upstream as measured at BMC-AP3. The N/P ratios in the creek indicate that inputs of either nitrogen or phosphorus are likely to stimulate algal bloom formation, depending upon season and inputs. It is notable that nutrient concentrations increase from the upper portion of the regional wet detention pond as one moves downstream toward the lower creek. An important human health issue is the high fecal bacteria counts found at most sampling stations, with the exception of BMC-AP3 below the detention pond. As NPDES point source discharges are not directed into this creek, the fecal bacteria (and nutrient) loading appears to be caused either by non-point source stormwater runoff, illegal discharges, or leakage from sanitary sewer lines.

Sediment Metals and PAH Concentrations

As part of the stream restoration effort funded through NCSU and the EPA 319 program, we collected sediment samples on one occasion annually throughout Burnt Mill Creek for analysis of sediment metals and polycyclic aromatic hydrocarbons (PAHs). The State of North Carolina has no official guidelines for sediment concentrations of metals and organic pollutants in reference to protection of invertebrates, fish and wildlife. However, academic researchers (Long et al. 1995) have produced guidelines (Table 4) based on extensive field and laboratory testing that are used by the US Environmental Protection Agency in their National Coastal Condition Report II (US EPA 2004).

Table 4. Guideline values for sediment metals and organic pollutant concentrations (ppm, or mg/kg, or μg/g, dry wt.) potentially harmful to aquatic life (Long et al. 1995; U.S. EPA 2004). ERL = (Effects range low). Concentrations below the ERL are those in which harmful effects on aquatic communities are rarely observed. ERM = (Effects range median). Concentrations above the ERM are those in which harmful effects would frequently occur. Concentrations between the ERL and ERM are those in which harmful effects occasionally occur.

Metal	ERL	ERM
Arsenic (As)	8.2	70.0
Cadmium (Cd)	1.2	9.6
Chromium (Cr)	81.0	370.0
Copper (Cu)	34.0	270.0
Lead (Pb)	46.7	218.0
Mercury (Hg)	0.15	0.71
Nickel (Ni)	20.9	51.6
Silver (Ag)	1.0	3.7
Zinc (Zn)	150.0	410.0
Total PCBs	0.0227	0.1800
Total PAHs	4.02	44.80
Total DDT	0.0016	0.0461

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds with a fused ring structure. PAHs with two to five rings are of considerable environmental concern. They are compounds of crude and refined petroleum products and coal and are also produced by incomplete combustion of organic materials. They are characteristic of urban runoff as they derive from tire wear, automobile oil and exhaust particles, and leaching of asphalt roads. Other sources include domestic and industrial waste discharge, atmospheric deposition, and spilled fossil fuels. They are carcinogenic to humans, and bioconcentrate in aquatic animals. In these organisms they form carcinogenic and mutagenic intermediaries and cause tumors in fish (US EPA 2000). All of the PAH sediment samples exceeded the ERM except for Station AP3, below Ann McCrary Pond, where PAHs were below the detection limit except for the 2008

collection (Table 5). The two Kerr Avenue wetland stations maintained the highest PAH concentrations on a consistent basis, year after year. The highest individual sample collected was of total PAHs exceeding 53,000 mg/kg at the Wallace Park station BMC-WP in 2008.

Most of the stations had sediment metals concentrations that were well below levels considered potentially toxic to benthic organisms. One exception was lead, in which both the mean and medians for the four annual samples exceeded the ERL at the Princess Place station (BMC-PP) and at the Wallace Park station BMC-WP (Table 5). Lead concentrations at times approached the ERL at BMC-KA1 as well. Mercury concentrations were low with the exception of BMC-PP, where Hg concentrations approached but did not exceed the ERL during 2005 and 2008. Of the other metals, Zinc exceeded the ERL at BMC-PP in 2008 but was not problematic on any other occasion. Thus, the Burnt Mill Creek sediments consistently contained excessive total PAH concentrations at five of six locations, and excessive lead concentrations consistently at the two lower main stem sites. Excursions exceeding or approaching harmful levels for other metals did occur but were rare. On a related note, Burnt Mill Creek has been listed as Impaired for aquatic life because of a Poor benthic community rating by the North Carolina Division of Water Quality (NCDENR 2005), with toxic impacts and habitat degradation listed as stressors.

Table 5. Median concentrations of sediment metals and polycyclic aromatic hydrocarbons (PAHs) in Burnt Mill Creek, 2005-2008 (as mg/kg = ppm). Medians are from four samples collected. Concentrations in bold type exceed the level at which harmful effects to benthic organisms may occur (see Table 4 for more detail).

Parameter	KA1	KA3	AP1	AP3	WP	PP
Antimony	0.073	0.059	0.059	0.024	0.054	0.119
Arsenic	0.059	0.066	0.067	0.060	0.070	0.072
Beryllium	0.058	0.038	0.012	0.028	0.181	0.135
Cadmium	0.171	0.086	0.026	0.068	0.596	0.400
Chromium	2.885	2.015	0.356	1.550	8.270	4.530
Copper	5.370	5.980	1.007	2.000	14.540	8.805
Lead	13.890	7.340	2.135	2.010	**69.200**	**56.450**
Mercury	0.003	0.003	0.003	0.004	0.037	0.077
Nickel	1.770	0.981	0.219	1.038	3.035	2.585
Selenium	0.085	0.070	0.070	0.091	0.080	0.080
Silver	0.060	0.063	0.070	0.060	0.070	0.080
Thallium	0.012	0.013	0.014	0.012	0.050	0.030
Zinc	48.800	14.000	5.380	28.30	74.200	60.50
Total PAH	**10,472**	**12,707**	**3,665**	BDL	**2,936**	**608**

BDL = below detection limit

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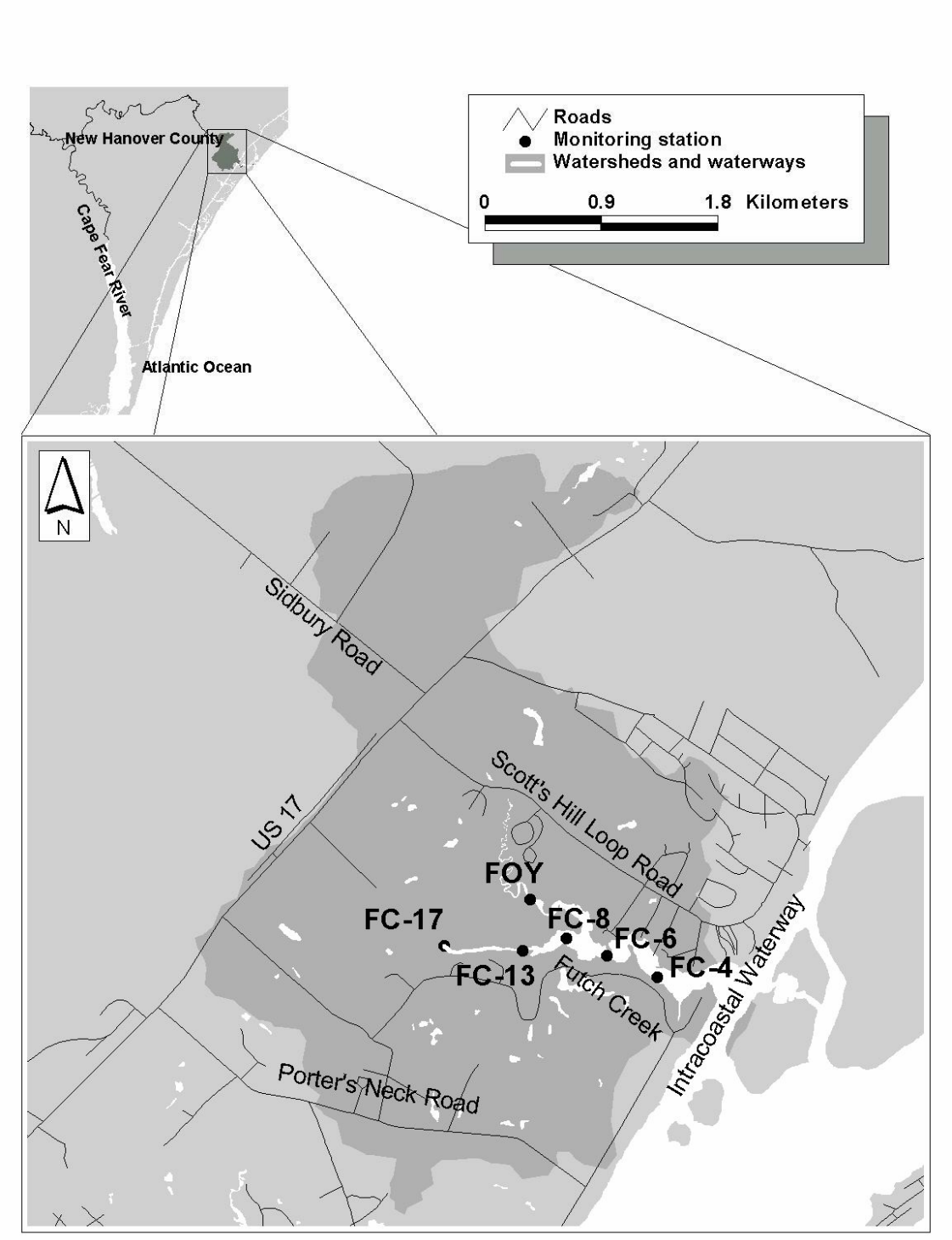
5.0 Futch Creek

Snapshot
Watershed area: 3,136 aces (1,269 ha) Impervious surface coverage: >11%
Watershed population: 1,720 (New Hanover County only)

Six stations have been sampled by the University of North Carolina Wilmington in Futch Creek from 1993 through 2007. UNCW was not funded by the County to sample Futch Creek in 2008. We present the above information and map below purely for informational purposes. Water quality information for 2008 is available on the County Planning Department website:
http://www.nhcgov.com/AgnAndDpt/PLNG/Pages/WaterQualityMonitoring.aspx.

Figure 5.1. Futch Creek watershed and sampling sites.



6.0 Greenfield Lake Water Quality

Snapshot

Watershed area: 2,560 acres (1,036 ha) Impervious surface coverage: 36%
Watershed population: 12,270 Overall water quality: Poor, improving
Problematic pollutants: Fecal bacteria, low dissolved oxygen in tributaries and the upper lake, algal blooms

Three tributaries of Greenfield Lake were sampled for physical field parameters only in 2008 (Table 6.1, Fig. 6.1). All three tributaries suffered from hypoxia, with GL-JRB (Jumping Run Branch), GL-LB (creek at Lake Branch Drive) and GL-LC (creek beside Lakeshore Commons) all showing average concentrations below the state standard (DO < 5.0 mg/L). Dissolved oxygen levels were 3.0 mg/L or less on four occasions at GL-LB, two occasions at GL-LC and once at GL-JRB (Table 6.1; Appendix B). Turbidity concentrations were generally low in the tributary stations (Table 6.1).

Table 6.1. Mean and (standard deviation) / range of selected field water quality parameters in tributary stations of Greenfield Lake, 2008. n = 6.

Parameter	GL-JRB	GL-LB	GL-LC
DO (mg/L)	4.6 (1.8) 1.9-7.3	2.2 (1.8) 0.5-5.0	4.1 (1.3) 2.7-6.1
Turbidity (NTU)	4.4 (2.4) 1.0-8.0	3.8 (3.0) 1.0-9.0	7.6 (5.8) 3.0-19.0

Three in-lake stations were sampled (Table 6.2). Station GL-2340 represents an area receiving a considerable influx of urban/suburban runoff, GL-YD is downstream and receives some outside impacts, and GL-P is at Greenfield Lake Park, away from inflowing streams but in a high-use waterfowl area (Fig. 6.1). Low dissolved oxygen was only a problem at GL-2340, with concentrations below the state standard of 5.0 mg/L on all six of six occasions (see Section 6.1). Turbidity and suspended solids were low to moderate at these three sites, except for high TSS (46 mg/L) in July at GL-YD. Fecal coliform concentrations were problematic at all three sites in the lake, exceeding the State standard on all six sampling occasions at GL-2340, three times at GL-YD, and twice at GL-P. During September 2008 fecal coliform counts exceeded 60,000 CFU/mL at GL-2340, from an unknown source.

Nitrogen concentrations were generally highest at GL-2340, with lower concentrations at GL-YD and GL-2340; total nitrogen was higher in 2008 than 2007, possibly reflecting the breaking of the drought and more runoff inputs. There was a pulse of ammonium in the lake in early 2008. Total phosphorus concentrations were generally similar among stations, and none of the phosphorus values were remarkable (Table 6.2). Inorganic N/P molar ratios can be computed from ammonium, nitrate, and orthophosphate data and can help determine what the potential limiting nutrient can be in a water body. Ratios well below 16 (the Redfield ratio) can indicate potential nitrogen limitation, and

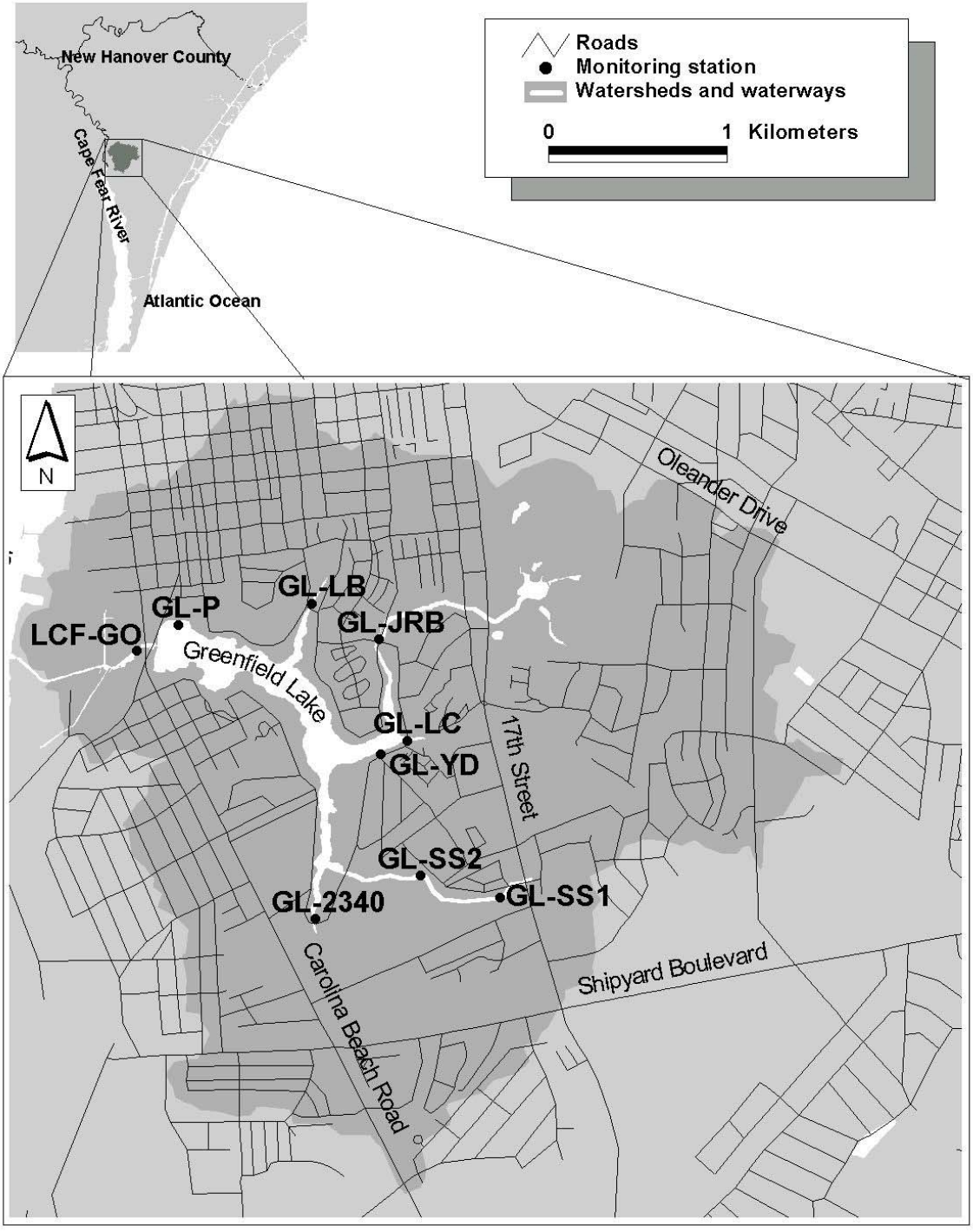
ratios well above 16 can indicate potential phosphorus limitation (Hecky and Kilham 1988). Based on the median N/P ratios (Table 6.2), phytoplankton growth in Greenfield Lake was mainly limited by nitrogen. Our previous bioassay experiments also indicated that nitrogen was usually the limiting nutrient in this lake (Mallin et al. 1999).

Phytoplankton blooms are periodically problematic in Greenfield Lake (Table 6.1), and usually consist of green or blue-green algal species, or both together. These blooms have occurred during all seasons, but are primarily a problem in spring and summer. Two blooms exceeding the North Carolina water quality standard of 40 μg/L of chlorophyll *a* occurred at both GL-YD and GL-2340 in 2008, with three such blooms occurring at GL-P; one of which was extremely high (303 μg/L of chlorophyll *a* in September). This represents a large increase in algal blooms over 2007 when there was only one in-lake bloom exceeding the state standard of 40 μg/L. We surmise that increased rainfall-driven stormwater runoff caused elevated nutrient loading in 2008 compared to the drought year 2007. Biochemical oxygen demand (BOD5) was generally high at all three stations in the lake (Table 6.1). The elevated BOD was in part driven by organic detritus created by the algal blooms (see subsequent section, Fig. 6.7) and by stormwater runoff inputs. Elevated BOD contributes directly to lower dissolved oxygen concentrations. Thus, during 2008 Greenfield Lake was impaired by large algal blooms, high fecal coliform counts and low dissolved oxygen concentrations, although the latter parameter continues to be better than the 2003-2004 pre-restoration period (see Section 6.1A). The tributary stations were also impaired by low dissolved oxygen. These same problems have occurred in the lake for several years (Mallin et al. 1999; 2000; 2002; 2003; 2004; 2005; 2006; 2007; 2008).

Table 6.2. Mean and (standard deviation) / range of water quality parameters in Greenfield Lake sampling stations, 2008. Fecal coliforms given as geometric mean, N/P ratio as median; n = 6 samples collected.

Parameter	GL-2340	GL-YD	GL-P
DO (mg/L)	3.7 (0.9) 2.7-4.9	7.5 (2.4) 4.3-11.4	8.6 (1.3) 7.0-10.4
Turbidity (NTU)	4.8 (2.0) 3.0-7.0	4.1 (1.9) 2.0-6.0	5.0 (2.8) 1.0-8.0
TSS (mg/L)	6.0 (6.9) 2.0-20.0	10.8 (17.4) 2.0-46.0	7.3 (5.6) 1.0-13.0
Nitrate (mg/L)	0.14 (0.09) 0.01-0.24	0.08 (0.07) 0.01-0.20	0.05 (0.08) 0.01-0.20
Ammonium (mg/L)	0.134 (0.110) 0.005-0.310	0.069 (0.063) 0.005-0.150	0.085 (0.106) 0.005-0.230
TN (mg/L)	1.17 (0.75) 0.62-2.66	0.99 (0.57) 0.43-2.10	0.98 (0.51) 0.30-1.84
OrthoPhosphate (mg/L)	0.03 (0.02) 0.01-0.05	0.04 (0.03) 0.01-0.10	0.04 (0.03) 0.01-0.08
TP (mg/L)	0.15 (0.14) 0.04-0.42	0.11 (0.06) 0.07-0.23	0.15 (0.07) 0.08-0.29
N/P molar ratio	17.8	6.1	7.9
Fec. col. (CFU/100 mL)	1,317 363-60,000	215 105-560	237 72-1,455
Chlor. *a* (μg/L)	21.8 (25.6) 2.0-55.0	25.9 (26.0) 5.0-68.8	80.8 (110.5) 5.2-303.0
BOD5	4.6 (3.2) 2.0-10.0	3.5 (1.6) 1.9-5.8	3.6 (1.6) 1.7-5.7

Figure 6.1 Greenfield Lake watershed



6.1 A Continuing Assessment of the Efficacy of the 2005-2008 Greenfield Lake Restoration Measures

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Introduction

Greenfield Lake is a 37 ha blackwater system located in the City of Wilmington, North Carolina. It was first dammed and filled as a millpond in 1750, and purchased for a city park in 1925. It has an average depth of 1.2-1.5 m, it is about 8,530 m around the shoreline, and its watershed drains approximately 1,036 ha (2,560 acres). The lake has one outfall, but is fed by six perennial inflowing streams (as well as intermittent ditches). The lake is surrounded by a watershed that is comprised mainly of residential, office, institutional and commercial areas, with an overall watershed impervious surface coverage of 36%.

In recent decades a number of water quality problems have become chronic within the lake, including high fecal coliform bacterial counts, low dissolved oxygen problems, nuisance aquatic macrophyte growths, algal blooms and fish kills. Some of these problems are typically related to eutrophication, a process driven by loading of excessive nutrients to a body of water. The State of North Carolina Division of Water Quality considers the lake to have a problem with aquatic weeds (NCDENR 2005). Periodic phytoplankton blooms have occurred in spring, summer and fall. Some of the most frequent bloom forming taxa are the cyanobacterium *Anabaena cylindrica* and the chlorophytes *Spirogyra* and *Mougeotia* spp. The free-floating macrophyte *Lemna* sp. (duckweed) is frequently observed on the surface, and below a massive *Lemna* bloom in summer 2004 dissolved oxygen concentrations at the park station were nearly anoxic. In-situ monitoring instruments have demonstrated that dissolved oxygen concentrations can decrease by as much as 45% at night compared with daytime DO measurements.

In 2005 several steps were taken by the City of Wilmington to restore viability to the lake (David Mayes, City of Wilmington Stormwater Services, personal communication). During February one thousand sterile grass carp were introduced to the lake to control (by grazing) the overabundant aquatic macrophytes. During that same month four SolarBee water circulation systems were installed in the lake to improve circulation and force dissolved oxygen from the surface downward toward the bottom. Finally, from April through June 2005 a contract firm applied the herbicide Sonar to further reduce the amount of aquatic macrophytes. On March 29-31 2006 City crews applied 35 gallons of K-Tea algaecide and on July 18 applied 0.63 gallons of habitat aquatic herbicide. A contract firm stocked the lake with 500 additional grass carp on April 4, 2006 and applied 40 gallons of Nautique aquatic herbicide on April 25, and treated the lake with Nautique again on July 31, 2007. The firm also added 200 more grass carp March 28, 2007, but no further fish were added in 2008. City crews added spot applications of

herbicide in April, September, October and November 2007 and April, May and June 2008.

Since 1998 the University of North Carolina Wilmington's Aquatic Ecology Laboratory, located at the Center for Marine Science, has been performing water quality sampling and associated experiments on Greenfield Lake. The City of Wilmington Engineering Department has funded this effort. Monitoring of various physical, chemical, and biological parameters has occurred monthly. These data allow us to perform a assessment of the effectiveness of the City's lake restoration efforts by comparing summer data from 2003 and 2004 (before restoration efforts) with data from the summers of 2005 through 2008 (after restoration efforts have been ongoing).

Results

To assess the results so far we have chosen several parameters to examine over time. One parameter that is not quantified is surface coverage by nuisance macrophyte vegetation. In the summers of 2003 and 2004 extensive mats of duckweed (*Lemna* sp.), mixed with algae and other vegetation covered large areas of the lake's surface, with visible estimates for some coves exceeding 95% coverage. In summer of 2005 surface coverage was minimal; with most lake areas 95% clear of surface mats. Some coverage returned in 2006 and minimal coverage was seen in 2007 and 2008.

Dissolved oxygen (DO): During 2003 and 2004 hypoxia (DO < 4.0 mg/L) was common in surface waters. Areas beneath thick *Lemna* mats were anoxic (DO of zero) or nearly so, especially at GL-P, the main Park area (Fig. 6.1). Following the onset of herbicide addition in April 2005, the May DO (mean of the three in-lake stations) showed a distinct decrease; however, it subsequently rose in June and remained at or above the State standard of 5 mg/L through the rest of the summer of 2005 (Fig. 6.2). In summer of 2006 the average lake DO levels decreased compared with 2005, but were still higher than in 2003 and 2004 (Fig. 6.2). This was because Station GL-2340 experienced low DO levels from 1.2 to 3.8 mg/L from July through September, although the other two in-lake stations (GL-P and GL-YD) maintained good DO levels. In 2007 GL-2340 continued to have poor dissolved oxygen problems but the other two in-lake stations had generally good dissolved oxygen (Table 6.2). This pattern continued through 2008 (with lake average DO concentrations acceptable but stressful conditions in the uppermost station (Fig. 6.2).

Turbidity: Turbidity was not excessive in the lake during the two years prior to restoration efforts (Mallin et al. 2006). It has remained low following these efforts throughout 2008 (Table 6.2).

Ammonium: Ammonia, or ammonium is a common degradation product of organic material, and is an excretory product of fish and other organisms. The addition of grass carp and the herbicide usage did not raise ammonium concentrations in the lake for several years (Fig. 6.3). However, in early 2008 there was a large increase in average ammonium lake-wide, which decreased in late spring (Fig. 6.3). There were no herbicide sprayings immediately before this pulse, and no fish kills, so the reason for this remains unknown.

Nitrate: Nitrate is an inorganic form of nitrogen that is known to enter the lake during rainfall and runoff periods (Mallin et al. 2002). The concentration of nitrate in the lake does not appear to have been influenced by the restoration efforts (Table 6.2). Nitrate concentrations are generally impacted by stormwater runoff, and the low rainfall in 2007 likely provided minimal nutrient inputs to the lake. During 2008 there was a sharp increase in nitrate concentrations, especially in the upper and middle lake stations, which we suspect was largely stormwater runoff-driven (Table 6.2).

Total nitrogen: Total nitrogen (TN) is a combination of all inorganic and organic forms of nitrogen. Mean concentrations and concentrations at individual stations appeared to be unaffected by the restoration efforts (Table 6.2; Fig. 6.4).

Orthophosphate: Orthophosphate is the most common inorganic form of phosphorus, and is utilized as a key nutrient by aquatic macrophytes and phytoplankton. Orthophosphate concentrations have not experienced any major changes in the water column either before (Mallin et al. 2006a) or after the restoration effort (Table 6.2). Earlier research found that a significant quantity of phosphorus is the lake is contributed by waterfowl through excretion.

Total phosphorus: Total phosphorus (TP) is a combination of all organic and inorganic forms of phosphorus in the water. Although pulses of TP occurred in summer 2005 and spring 2006, they were similar in magnitude to pulses of TP seen in 2003 and 2004 (Fig. 6.5). Pulses in 2007 were smaller than the previous years (Figure 6.5). In 2008 there was a jump in TP, which may in part by caused by high phytoplankton biomass and the phosphorus locked up as cell tissue (see next section). Another reason may include increased runoff of phosphorus into the lake with increased rainfall.

Chlorophyll *a*: Chlorophyll *a* is the principal measure used to estimate phytoplankton biomass (algal bloom strength) in water bodies. As mentioned above, algal blooms have been a common occurrence in this lake. They are generally patchy in space, usually occurring at one or two stations at a time. However, in summer 2005 extensive phytoplankton blooms occurred at all three in-lake stations, with levels well exceeding the State standard of 40 μg/L (Fig. 6.6). Blooms continued throughout 2006 as well (Table 6.2; Fig. 6.6). A positive signal was that blooms within the lake in 2007 were fewer than in previous years (Fig. 6.6), either because of continuing restoration efforts or lower stormwater driven inputs of nitrate to feed the blooms. Unfortunately the latter was the likely explanation, as in 2008 the blooms returned in force (Fig. 6.6; also see previous section).

Algal blooms are the result of nutrient inputs, either from outside the lake or from release from decaying material. Algal blooms, when they die, cause a BOD (biochemical oxygen demand) load (Mallin et al. 2006b). This is organic material that natural lake bacteria feed on and multiply, using up dissolved oxygen in the lake as they do so. We performed correlation analysis on our 2007 chlorophyll *a* concentrations with the corresponding BOD concentrations for the three in-lake stations, and found that, statistically speaking, approximately 40% of the variability in Greenfield Lake BOD is caused by algal blooms. We performed similar correlation analysis using our 2008

chlorophyll *a* and BOD data (Fig. 6.7). The results showed a significant positive correlation between the two parameters, although regression analysis indicated that only 26% of the variability in dissolved oxygen was accounted for by chlorophyll *a* in 2008. Thus, the algal blooms can lead to low dissolved oxygen in the lake, but there are other factors that contribute as well. Research conducted on Burnt Mill Creek, Smith Creek, and Prince Georges Creek (Mallin et al. 2009) showed that BOD was strongly correlated with watershed rainfall, indicating that runoff of oxygen-demanding materials (organic waste, debris, various chemicals) can make a significant contribution to reducing dissolved oxygen in aquatic systems.

Fecal coliform bacteria: Fecal coliform bacteria are commonly used to provide an estimate of the human or animal derived microbial pollution in a water body. Greenfield Lake is chronically polluted by high fecal coliform counts, well exceeding the state standard of 200 CFU/100 mL during many months (Table 6.2; Fig. 6.8). In summer 2005 there were particularly large fecal coliform counts at each in-lake station, though the individual stations did not have pulses during the same months. Excessive fecal coliform counts occurred to a lesser degree in 2006 in the lake, mainly at GL-2340 (Table 6.2). In 2007 high fecal coliform counts occurred within the lake on about 43% of the occasions sampled (Fig. 6.8). In 2008 the lake was highly polluted by fecal coliforms (Fig. 6.8), with stormwater runoff likely the principal source. These counts are not expected to be influenced by the type of restoration efforts currently ongoing in the lake. In September at the upper station, GL-2340, there was a high concentration (60,000 CFU/100 mL) of fecal coliform bacteria. City staff was unaware of any sewage spills in that area, so the source remains unknown.

Discussion

A risk that is taken when applying herbicides to lakes is the creation of biochemical oxygen demand (BOD) from decomposing organic matter that is a product of dead or dying plant material. As mentioned above, this would serve to drive the lake DO concentrations downward. DO levels in summer 2005 were nearly twice what they were during summers of 2003 and 2004, and DO levels in 2006 were also higher than 2003 and 2004. It is very likely that the use of the SolarBee circulation systems maintained elevated DO even when there was an obvious BOD source. The in-lake station with lowest DO levels in 2006 was GL-2340, which is located quite a distance from the SolarBees. This pattern continued into 2007 and 2008.

Water column nutrient concentrations did not appear to change notably after the introduction of grass carp or use of herbicide. Certainly ammonium, an excretory and decomposition product would be expected to rise following the consumption and death of large quantities of plant material. Likewise phosphorus did not increase, although it is a common excretory product. However, ammonium (like orthophosphate) is readily used as a primary nutrient by phytoplankton. Nutrient addition bioassay experiments have demonstrated that phytoplankton in this lake is limited by nitrogen (Mallin et al. 1999). It is likely that ammonium produced by fish excretion or dying plant material was utilized by phytoplankton to produce the excessive algal blooms that characterized the lake in 2005 and 2006. The phytoplankton blooms were dominated by blue green algae (cyanobacteria) including species containing heterocysts. These species have

the added ability to fix atmospheric nitrogen when phosphorus is replete. Thus, while large amounts of macrophyte material disappeared from the lake, some of the resultant nutrients were utilized by phytoplankton to produce the blooms. As mentioned, a problem with algal blooms is that when they die, they become labile forms of organic material, or BOD (Fig. 6.7). Published research has previously demonstrated that chlorophyll *a* in this lake is strongly correlated with BOD (Mallin et al. 2006b). However, the positive news from 2007 was that algal blooms were fewer than in previous years. This may have been due to the restoration efforts, less stormwater runoff during the drought, or some combination of the two. However, since the blooms returned in 2008 it is likely that nitrogen loading from stormwater runoff is the principal factor controlling their magnitude and frequency of occurrence.

The continuing problems with fecal coliform bacteria do not appear to be related to any of the restoration activities. Fecal coliform bacteria enter the environment from the feces of warm blooded animals, so it is possible that increases in waterfowl or dogs brought to the lake by their owners, or feral cats could lead to increased fecal coliform bacteria counts, but we have no data to support this speculation either way. Likewise on rare occasions large pulses of fecal bacteria have appeared in the lake or tributaries, potentially related to either sewage leaks or spills, or illicit connections.

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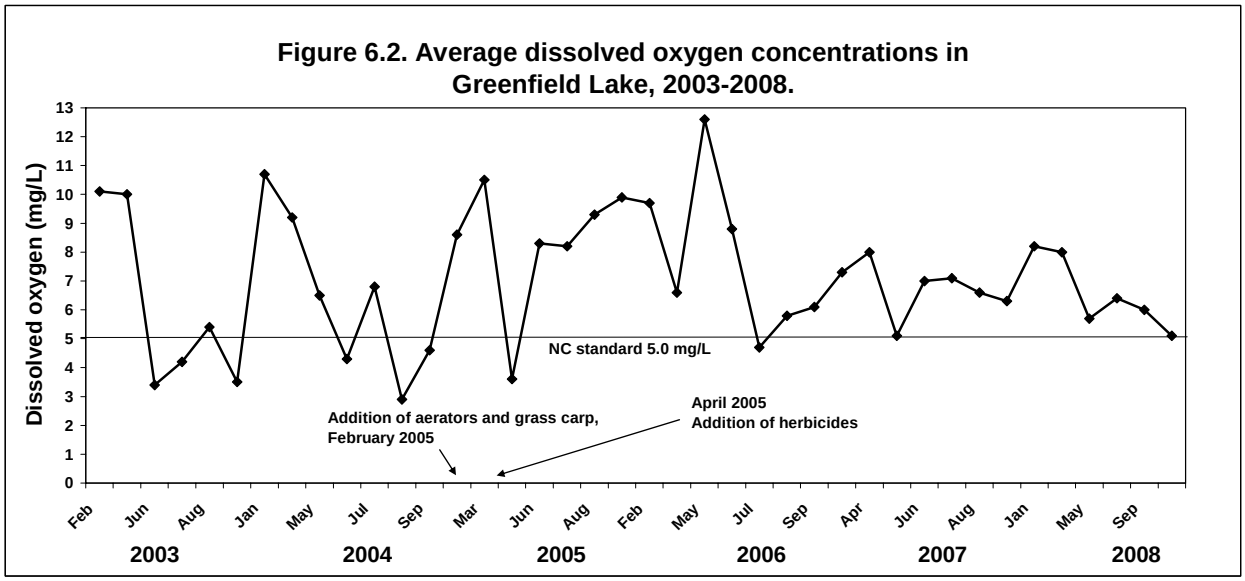


Figure 6.2. Average dissolved oxygen concentrations in Greenfield Lake, 2003-2008.

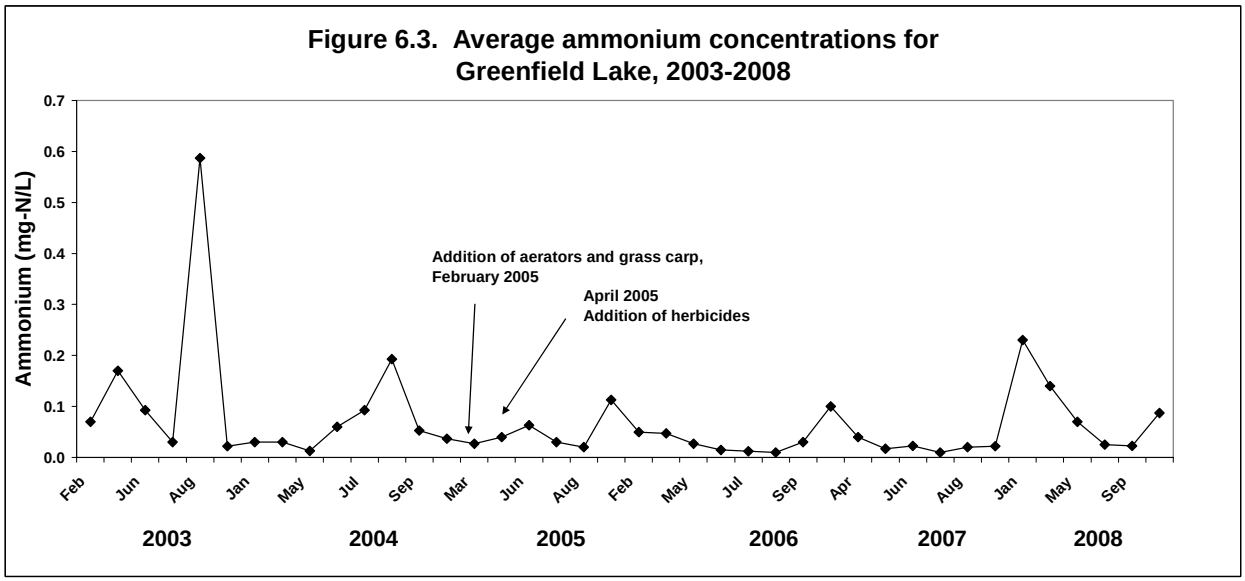


Figure 6.3. Average ammonium concentrations for Greenfield Lake, 2003-2008

Figure 6.4. Average total nitrogen (TN) concentrations in Greenfield Lake, 2003-2008

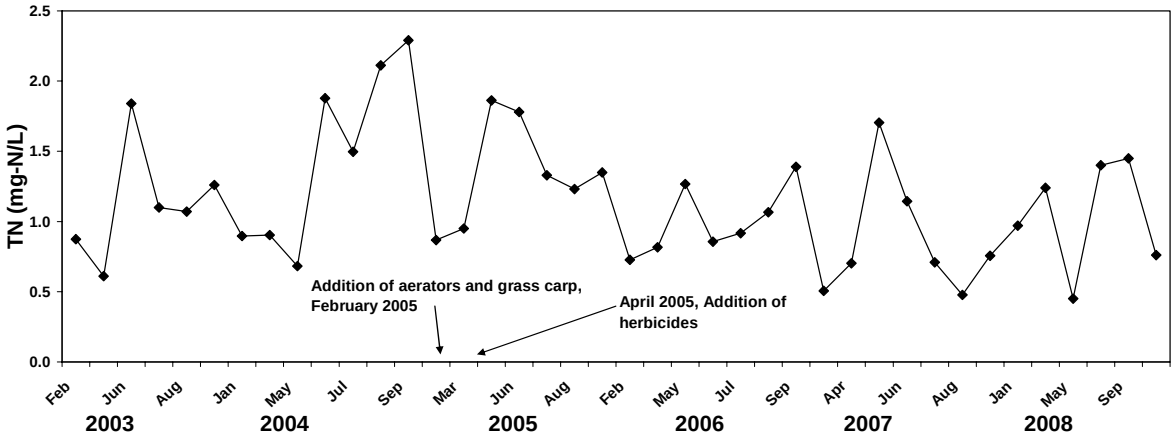


Figure 6.5. Average total phosphorus (TP) concentrations for Greenfield Lake, 2003-2008

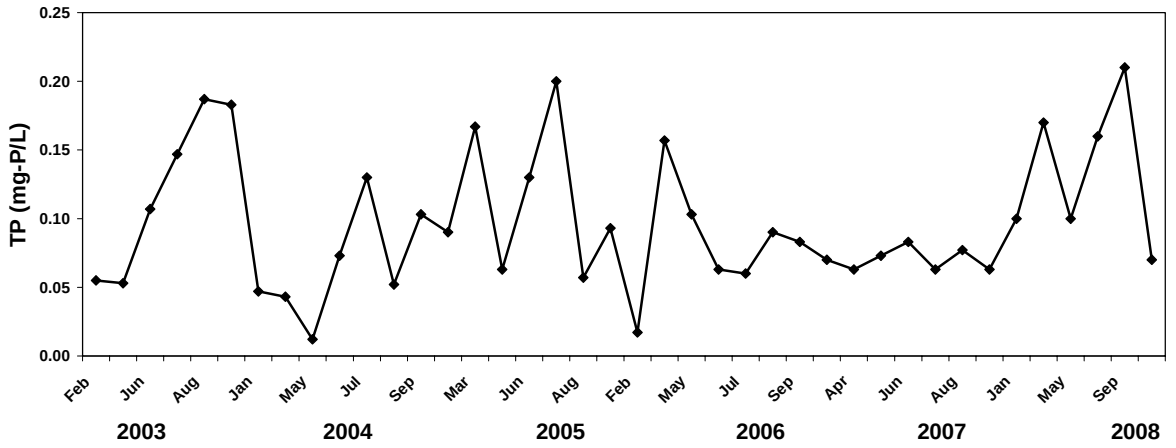


Figure 6.6. Average chlorophyll *a* values in Greenfield Lake, 2003-2008

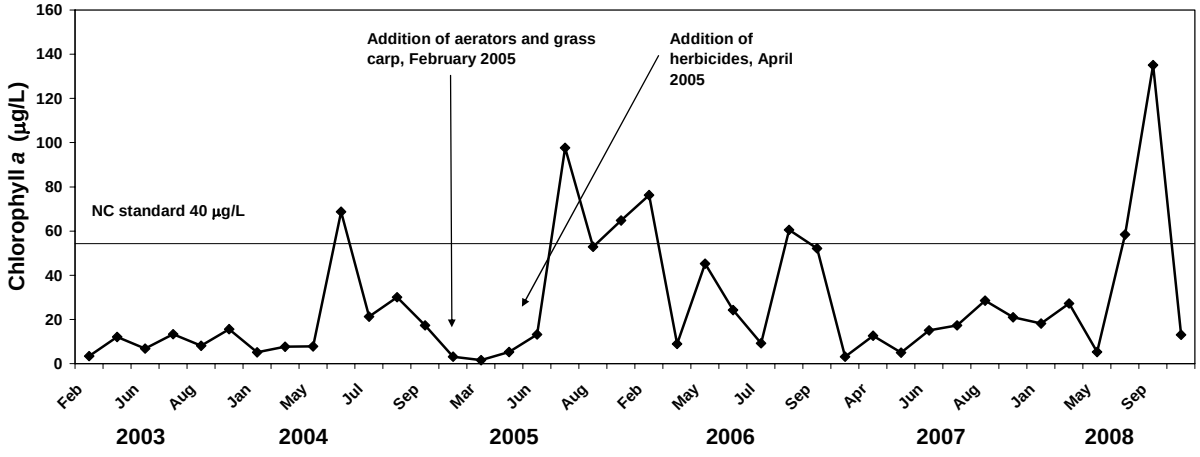


Figure 6.7. BOD5 as a function of chlorophyll *a* in Greenfield Lake, 2008 (data from all three in-lake stations combined).

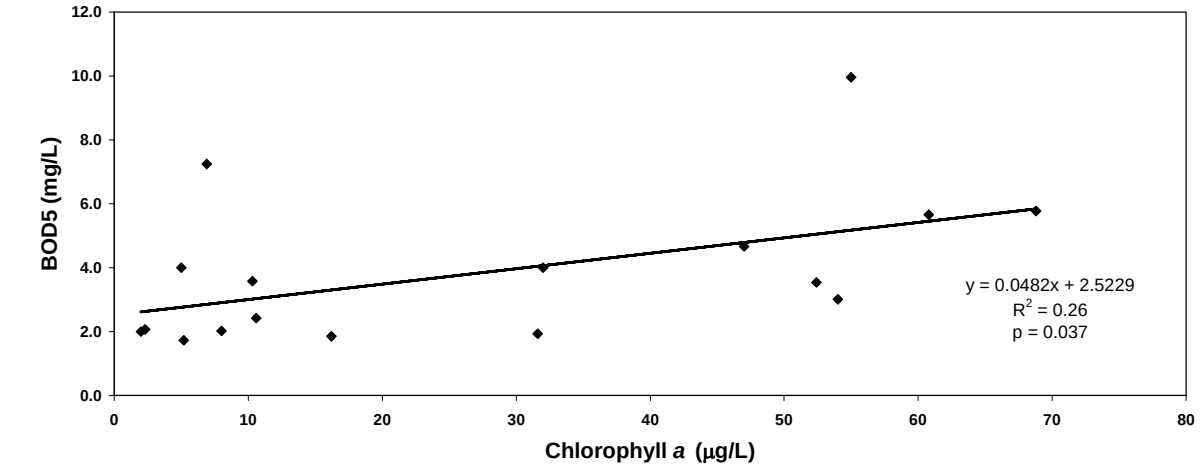
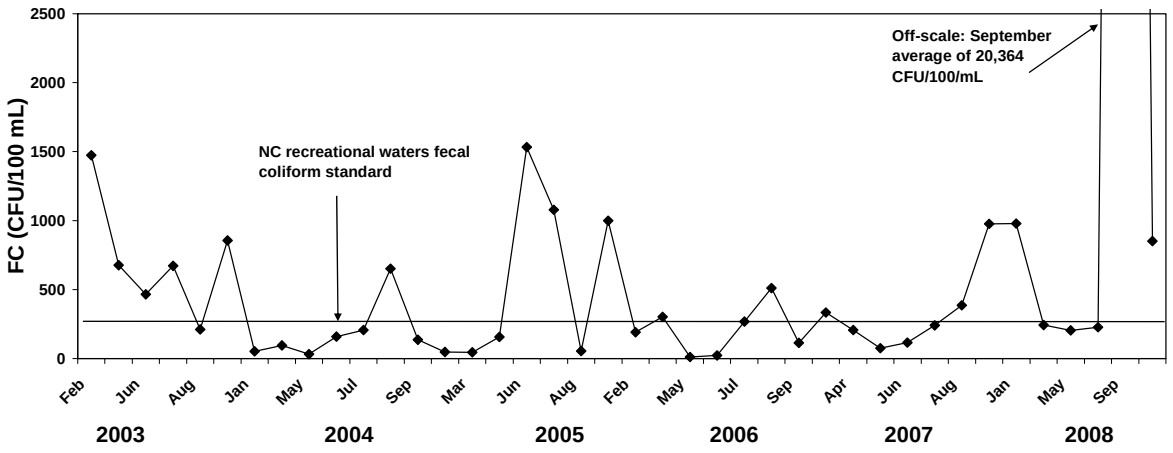


Figure 6.8. Average fecal coliform bacteria counts (FC) for Greenfield Lake, 2003-2007



7.0 Hewletts Creek

Snapshot
Watershed area: 5,952 acres (2,409 ha) Impervious surface coverage: 19%
Watershed population: 21,335
Overall water quality: Fair
Problematic pollutants: minor algal blooms, high fecal bacteria, minor dissolved oxygen issues

Hewletts Creek was sampled at four tidally-influenced areas (HC-3, NB-GLR, MB-PGR and SB-PGR) and a freshwater stream station draining Pine Valley Country Club (PVGC-9 - Fig. 7.1). At the tidal stations the physical data indicated that turbidity was well within State standards during this sampling period except for an incident of 30 NTU in May 2008 at NB-GLR (Table 7.2). There were two incidents of hypoxia seen at four of our five stations in July and September 2008; at NB-GLR, SB-PGR, HC-3 and PVGC-9, although none were below 3.3 mg/L. Nitrate concentrations were elevated leaving the golf course at PVGC-9 relative to the other stations (Tables 7.1 and 7.2). From there the next station is MB-GLR, which also receives inputs from the Wilmington Municipal Golf Courses (Fig. 7.1; Mallin and Wheeler 2000). Nitrate was still elevated there; however, none of the other stations had elevated nitrate concentrations. Nitrate showed increased concentrations from 2007, due to increased stormwater runoff. Phosphate concentrations decreased somewhat from the previous year. The chlorophyll *a* data (Tables 7.1 and 7.2) showed that Hewletts Creek was free of algal blooms in 2008; a surprising positive finding since algal blooms have been common in upper Hewletts Creek in the past (Mallin et al. 1998a; 1999; 2002a; 2004; 2005; 2006; 2008).

Fecal coliform bacteria counts exceeded State standards at least 50% of occasions sampled at all stations except HC-3 (Tables 7.1 and 7.2). The north and middle branches of the creek had the highest counts in general.

Table 7.1. Selected water quality parameters at upper and middle creek stations in Hewletts Creek watershed as mean (standard deviation) / range, 2008. Fecal coliform bacteria presented as geometric mean / range.

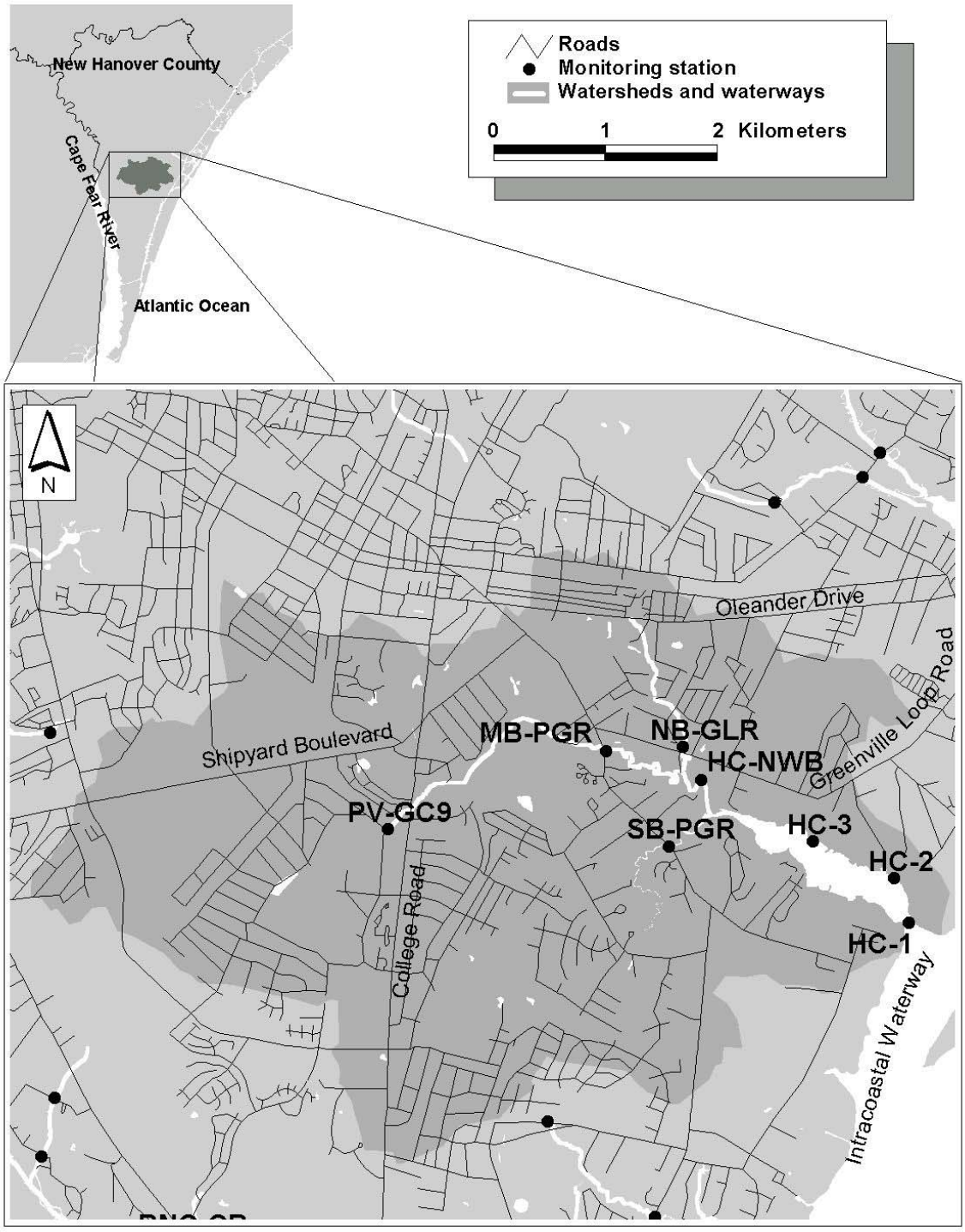
Parameter	PVGC-9	MB-PGR
Salinity (ppt)	0.1 (0) 0.1-0.1	0.1 (0) 0.1-0.2
Turbidity (NTU)	3.5 (2.7) 0-14.0	0.8 (0.8) 0-2.0
TSS (mg/L)	4.5 (5.6) 1.0-14.0	3.0 (3.9) 1.0-10.0
DO (mg/L)	7.0 (2.1) 4.6-9.3	7.5 (1.3) 5.7-8.9
Nitrate (mg/L)	0.88 (0.35) 0.42-1.19	0.36 (0.07) 0.26-0.44
Ammonium (mg/L)	0.037 (0.014) 0.020-0.050	0.029 (0.017) 0.005-0.050
TN (mg/L)	1.62 (0.40) 1.10-2.21	0.70 (0.21) 0.94-0.40
Orthophosphate (mg/L)	0.03 (0.04) 0.01-0.10	0.01 (0.01) 0.01-0.02
TP (mg/L)	0.04 (0.02) 0.02-0.07	0.03 (0.01) 0.01-0.05
Mean N/P Median	152.9 178.3	66.8 65.3
Chlorophyll *a* (μg/L)	6.6 (6.3) 2.0-18.9	2.4 (2.2) 0.5-6.1
Fecal col. (CFU/100 mL)	461 181-1,100	803 143-803

Table 7.2. Selected water quality parameters at stations in Hewletts Creek watershed, 2008, as mean (standard deviation) / range, fecal coliforms as geometric mean / range, n = 6 months.

Parameter	HC-3	NB-GLR	SB-PGR
Salinity (ppt)	32.1 (1.2) 30.2-34.0	8.8 (8.2) 1.6-23.4	17.5 (6.2) 11.0-28.3
Turbidity (NTU)	5.4 (4.1) 2.0-11.2	13.3 (9.0) 3.0-30.0	7.8 (4.3) 3.0-14.0
TSS (mg/L)	11.2 (5.7) 4.0-19.0	12.3 (5.3) 7.0-21.0	11.3 (3.4) 7.0-17.0
DO (mg/L)	7.2 (2.3) 4.1-9.1	7.5 (1.3) 3.5-11.1	7.2 (3.0) 3.3-9.8
Nitrate (mg/L)	0.03 (0.04) 0.01-0.10	0.08 (0.03) 0.04-0.13	0.05 (0.05) 0.01-0.14
Ammonium (mg/L)	0.018 (0.017) 0.005-0.050	0.041 (0.037) 0.005-0.100	0.020 (0.025) 0.010-0.050
TN (mg/L)	0.33 (0.15) 0.20-0.50	0.68 (0.37) 0.20-1.30	0.26 (0.19) 0.05-0.54
Orthophosphate (mg/L)	0.03 (0.04) 0.01-0.10	0.09 (0.11) 0.01-0.30	0.03 (0.04) 0.01-0.10
TP (mg/L)	0.03 (0.02) 0.01-0.06	0.08 (0.04) 0.04-0.14	0.05 (0.01) 0.03-0.07
Mean N/P ratio Median	6.1 3.9	9.8 5.7	12.3 14.9
Chlor *a* (μg/L)	4.3 (2.7) 0.9-7.6	10.0 (6.6) 1.0-18.0	7.6 (5.1) 2.0-13.8
Fecal coliforms (CFU/100 mL)	8 2-62	664 290-3,000	181 46-550

Dobo Property/Bethel Rd. wetland: The New Hanover County Tidal Creeks Advisory Board, using funds from the North Carolina Clean Water Management Trust Fund, purchased a former industrial area owned by the Dobo family in August 2002. This property was bought to be used as a passive treatment facility for the improvement of non-point source runoff drainage water before it enters Hewletts Creek. As such, the City of Wilmington contracted with outside consultants to create a wetland on the property for this purpose. Baseline data were needed to assess water quality conditions before and after the planned improvements. From January 2004 through late 2007 the UNCW Aquatic Ecology Laboratory sampled three inflowing creeks and the single outflowing creek. Construction of the wetland has been completed, so that sampling ceased in 2008. A series of rainfall events are to be sampled over the next two years to test the efficacy of the wetland, and this work is commencing.

Figure 7.1 Hewletts Creek watershed



8.0 Howe Creek Water Quality

Snapshot
Watershed area: 3,264 acres (1,321 ha) Impervious surface coverage: 19%
Watershed population: 4,224
Overall water quality: Fair
Problematic pollutants: Fecal coliform bacteria, some low dissolved oxygen

Howe Creek was sampled for physical parameters, nutrients, chlorophyll *a*, and fecal coliform bacteria at three locations during 2008 (HW-FP, HW-GP and HW-DT- Fig. 8.1). Turbidity was generally low and did not exceed the North Carolina water quality standard of 25 NTU at any site (Table 8.1; Appendix B). Dissolved oxygen concentrations were good to fair, with HW-GP and HW-DT each below the standard of 5.0 mg/L on only one occasion and the other station within standard on all occasions (Appendix B). Nitrate concentrations were generally low at all three sites in 2008 and ammonium was low as well although slightly elevated at HW-DT at times (Table 8.2). Orthophosphate was generally low at the three sites.

Median inorganic molar N/P ratios were low, indicating that nitrogen was probably the principal nutrient limiting phytoplankton growth at all stations. Previously Mallin et al. (2004) demonstrated that nitrogen was the primary limiting nutrient in Howe Creek. There was one major algal bloom of 76 μg/L as chlorophyll *a* at HW-DT, and a minor bloom of 29 μg/L as chlorophyll *a*. One minor bloom of 33 μg/L as chlorophyll *a* occurred at HW-GP. Since wetland enhancement was performed in 1998 above Graham Pond the creek below the pond at HW-GP has had fewer and smaller algal blooms than before the enhancement (Fig. 8.2). For fecal coliform bacteria, the creek ranged from no exceedences of the water contact standard of 200 CFU/100 mL at the lower station HW-FP to 100% exceedences (all six sampling occasions) at the upper station HW-DT, where the geometric mean was 3X the NC standard (Table 8.1; Fig. 3).

Table 8.1. Water quality summary statistics for Howe Creek, 2008, as mean (st. dev.) / range. Fecal coliform bacteria as geometric mean / range.

	Salinity (ppt)	Diss. oxygen (mg/L)	Turbidity (NTU)	TSS (mg/L)	Chlor *a* (μg/L)	Fecal coliforms (CFU/100 mL)
HW-FP	29.3 (14.0) 0.8-36.2	7.7 (1.2) 5.9-9.2	6.5 (6.0) 2.0-18.0	10.3 (5.6) 4.0-17.0	2.9 (2.8) 0.4-7.5	6 1-14
HW-GP	26.0 (8.3) 15.5-33.6	6.3 (1.7) 4.0-8.5	6.0 (5.8) 1.0-17.0	11.5 (3.9) 6.0-16.0	8.4 (12.4) 0.7-33.0	86 10-460
HW-DT	11.8 (15.1) 0.2-35.0	6.6 (1.8) 4.7-9.0	6.9 (2.4) 3.7-10.0	11.3 (6.0) 3.0-20.0	19.6 (29.5) 0.6-75.8	648 300-1,546

Figure 8.1. Howe Creek watershed and sampling sites.

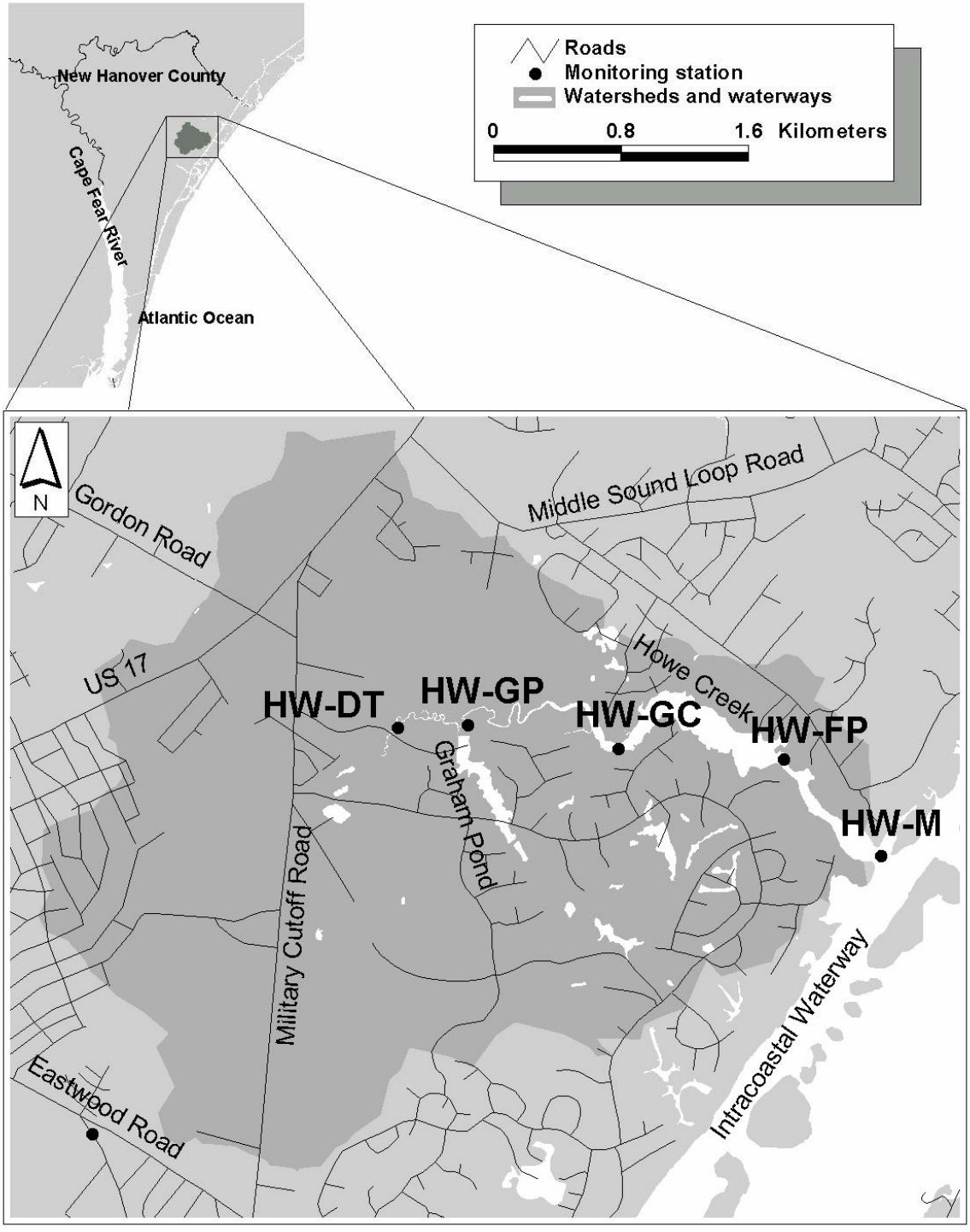


Figure 8.2. Chlorophyll *a* concentrations (algal blooms) in Howe Creek below Graham Pond before and after 1998 wetland enhancement in upper Graham Pond.

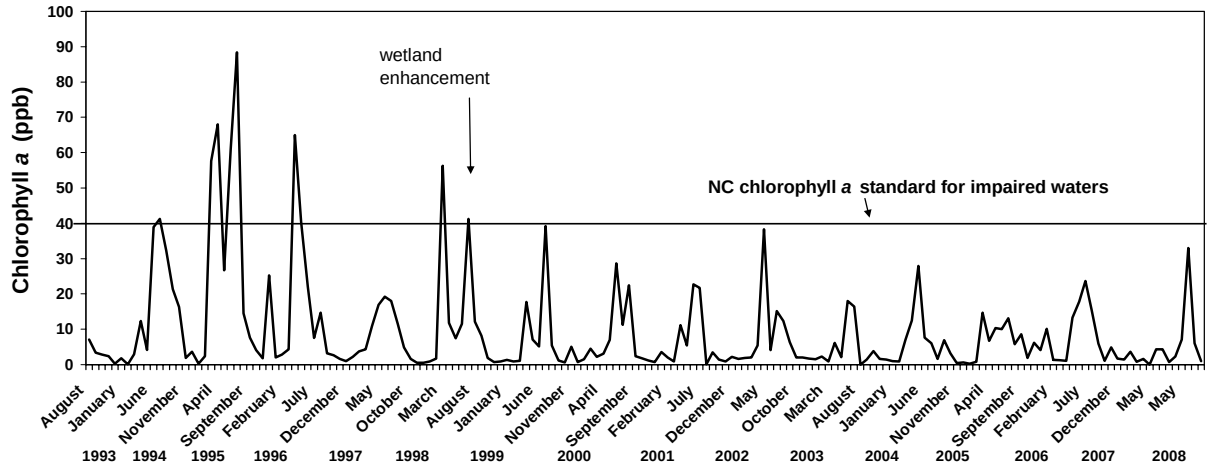
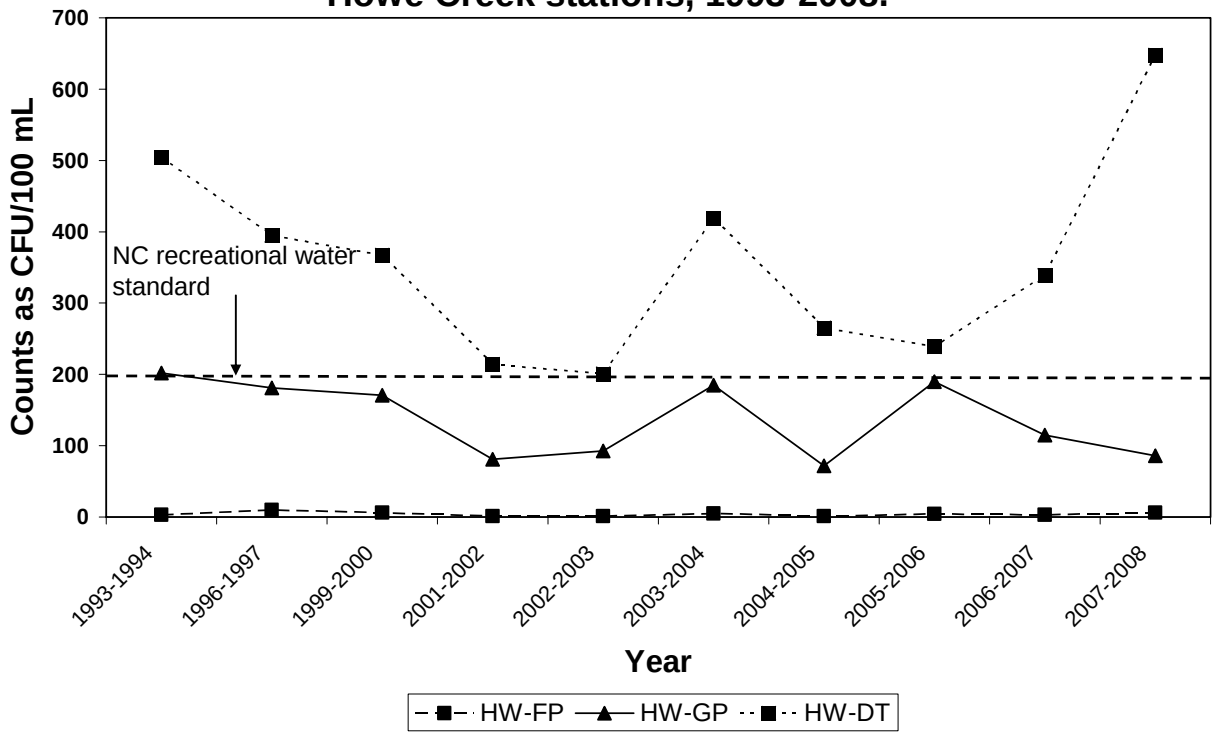


Table 8.2. Nutrient concentration summary statistics for Howe Creek, 2008, as mean (standard deviation) / range, N/P ratio as mean / median.

	Nitrate (mg/L)	Ammonium (mg/L)	Orthophosphate (mg/L)	Molar N/P ratio
HW-FP	0.03 (0.03) 0.01-0.07	0.017 (0.013) 0.005-0.040	0.03 (0.04) 0.01-0.10	7.0 5.0
HW-GP	0.02 (0.02) 0.01-0.05	0.017 (0.012) 0.005-0.030	0.03 (0.04) 0.01-0.10	6.0 3.9
HW-DT	0.03 (0.04) 0.01-0.12	0.027 (0.020) 0.010-0.060	0.03 (0.03) 0.01-0.10	8.3 4.4

Figure 8.3. Fecal coliform counts over time for three Howe Creek stations, 1993-2008.



9.0 Motts Creek

Snapshot
Watershed area: 2,389 acres (967 ha) Impervious surface coverage: 14%
Watershed population: 4,800
Overall water quality: fair
Problematic pollutants: fecal coliform bacteria

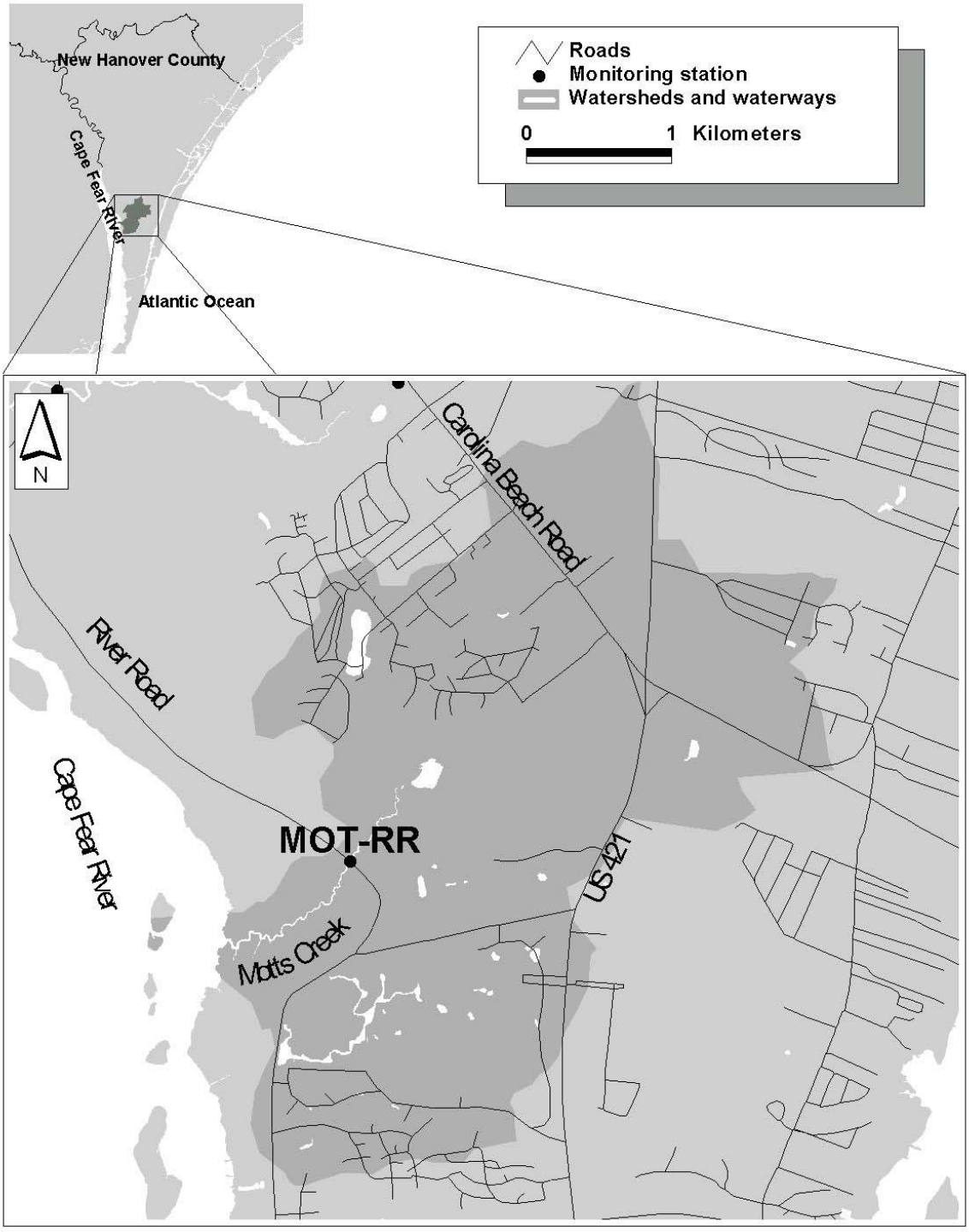
Motts Creek drains into the Cape Fear River Estuary (Fig. 9.1), and the creek area near River Road has been classified by the State of North Carolina as a Natural Heritage Site because of the area's biological attributes. These include the pure stand wetland communities, including a well-developed sawgrass community and unusually large flats dominated by *Lilaeopsis chinensis* and spider lily, with large cypress in the swamp forest. UNCW scientists sampled Motts Creek at the River Road bridge on three occasions in November and December 2008 (Fig. 9.1). A large residential development (River Lights) is under construction upstream of the sampling site between Motts and Barnards Creeks; however, this development has no construction activity ongoing within a half-mile of Motts Creek. In recent years extensive commercial development has been occurring along Carolina Beach Road near its junction with Highway 421.

Based on limited (and cold-weather) data, dissolved oxygen concentrations were generally good but slipped below the North Carolina brackish water standard of 5.0 mg/L on one occasion (to 4.6 mg/L in November). Neither turbidity nor suspended solids were problematic in this period in 2008. Total nitrogen, ammonium, and total phosphorus levels were low to moderate, and chlorophyll *a* concentrations were not a problem (Table 9.1). BOD5 was comparatively low, yielding a mean value of 1.1 mg/L. Fecal coliform contamination was a problem in Motts Creek, with the geometric mean of 507 CFU/100 mL more than twice the State standard of 200 CFU/100 mL; the standard was exceeded on two of the three sampling occasions in 2008.

Table 9.1. Selected water quality parameters at a station (MOT-RR) draining Motts Creek watershed before entering the Cape Fear Estuary, as mean (standard deviation) and range, November – December 2008. Fecal coliforms as geometric mean / range.

Parameter	MOT-RR	
	Mean (SD)	Range
Salinity (ppt)	0.6 (0.3)	0.4-1.0
TSS (mg/L)	9.7 (2.5)	7.0-12.0
Turbidity (NTU)	7.0 (3.5)	5.0-11.0
DO (mg/L)	6.9 (2.0)	4.6-8.5
Nitrate (mg/L)	0.18 (0.11)	0.12-0.31
Ammonium (mg/L)	0.07 (0.02)	0.05-0.09
Total nitrogen (mg/L)	0.48 (0.12)	0.41-0.62
Orthophosphate (mg/L)	0.01 90.01)	0.01-0.02
Total phosphorus (mg/L)	0.03 (0.02)	0.02-0.05
Mean N/P ratio	55.8	
Chlor *a* (μg/L)	8.3 (4.9)	5.0-14.0
BOD5 (mg/L)	1.1 (0.1)	1.0-1.2
Fecal coliforms (CFU/100 mL)	507	46-2,400

Figure 9.1 Motts Creeks watershed

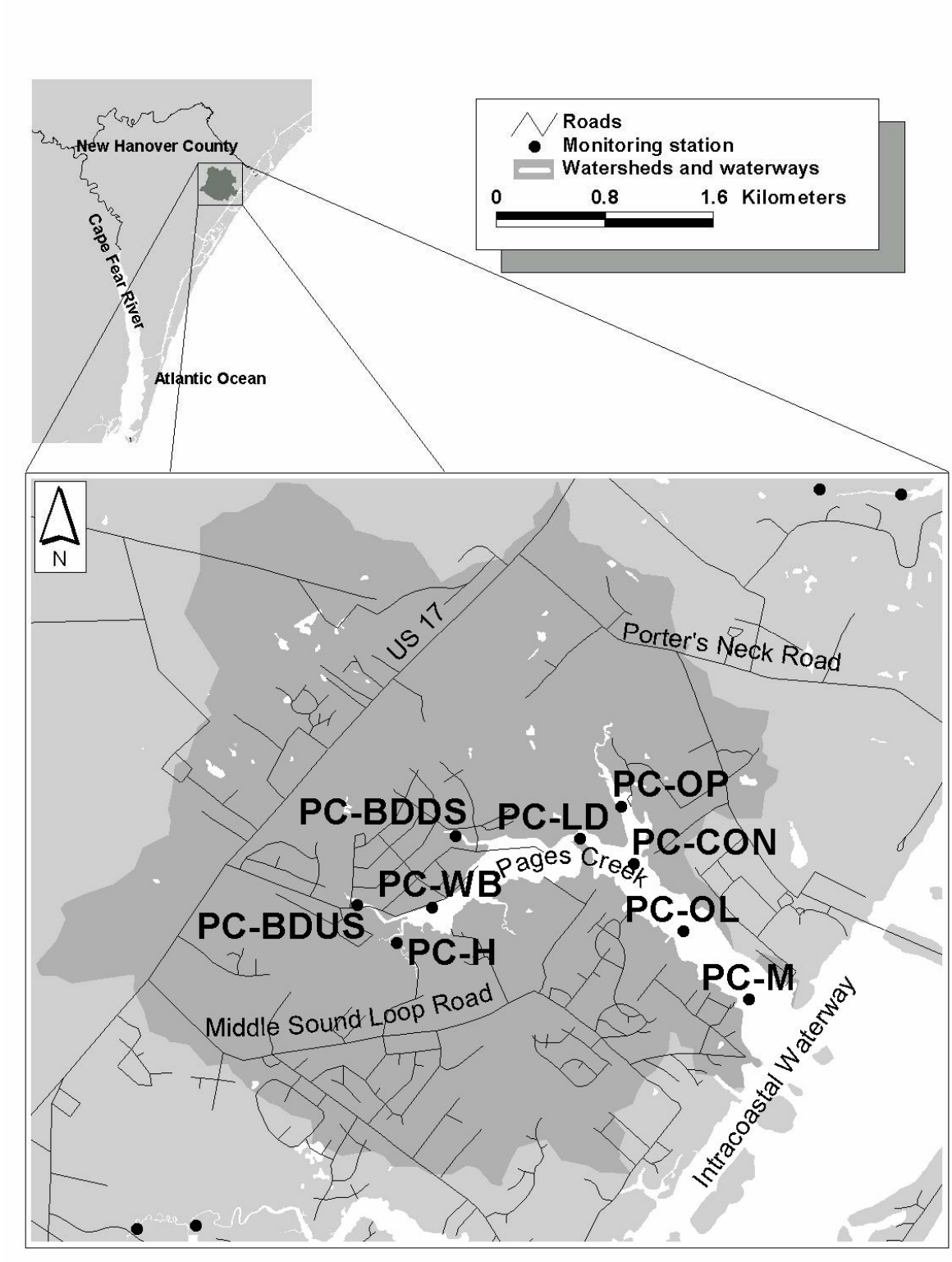


10.0 Pages Creek

Snapshot
Watershed area: 3,546 acres (1,435 ha) Impervious surface coverage: >13%
Watershed population: 4,600

The University of North Carolina Wilmington was not funded by the County in 2008 to sample Pages Creek. The information above and map below is supplied solely for informational purposes. Water quality information for this creek is available on the County Planning Department website: http://www.nhcgov.com/AgnAndDpt/PLNG/Pages/WaterQualityMonitoring.aspx.

Figure 10.1. Pages Creek watershed and sampling sites.



11.0 Smith Creek

Snapshot
Watershed area: 2,880 acres (1,166 ha) Impervious surface coverage: 28%
Watershed population: 25,904
Overall water quality: Poor
Problematic pollutants: High turbidity, low dissolved oxygen

Smith Creek drains into the lower Northeast Cape Fear River just before it joins with the mainstem Cape Fear River at Wilmington (Fig. 11.1). The University of North Carolina Wilmington was not funded by the County to sample Smith Creek during 2008. However, one location on Smith Creek, SC-CH (Fig. 11.1) is sampled monthly by UNCW under the auspices of by the Lower Cape Fear River Program for selected parameters (field physical parameters and fecal coliform bacteria) and these data are shown below (Table 11.1).

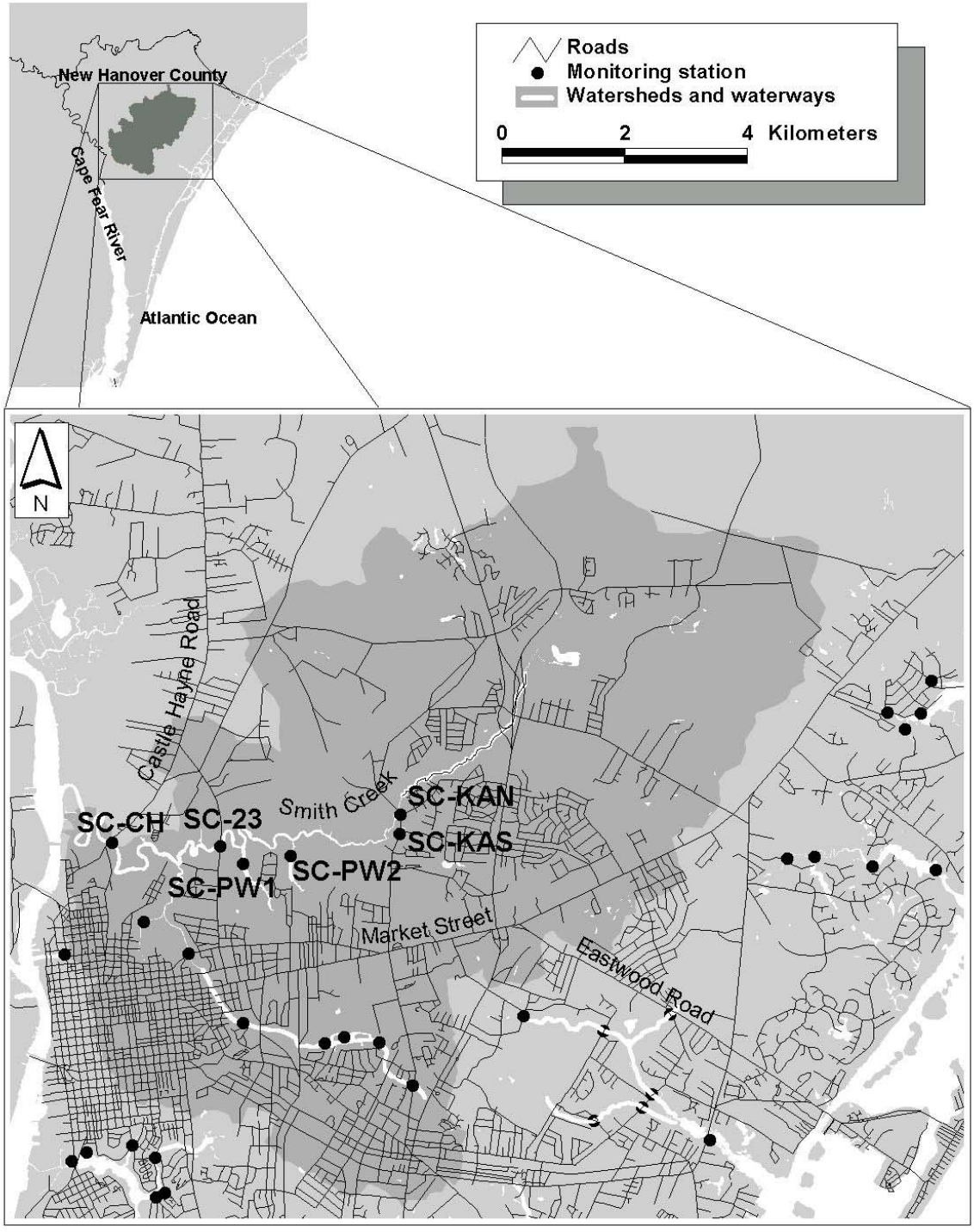
Dissolved oxygen concentrations were below 5.0 mg/L on four occasions at SC-CH between January and September 2008, with the lowest value 3.8 mg/L. The North Carolina turbidity standard for estuarine waters (25 NTU) was exceeded four times, with a maximum of 79 NTU in December 2008.

Fecal coliform bacteria concentrations were above 200 CFU/100 mL on only one occasion at SC-CH in 2008, for a Good rating. All months tested exceeded the shellfishing standard (14 CFU/100 mL) in this estuarine portion of the creek (Table 11.1).

Table 11.1. Selected water quality parameters in Smith Creek watershed as mean (standard deviation) / range, 2008, n = 12 months.

Parameter	SC-CH	
	Mean (SD)	Range
Salinity (ppt)	4.2 (4.3)	0.3-14.8
Dissolved oxygen (mg/L)	6.8 (2.2)	3.8-9.5
Turbidity (NTU)	24.0 (20.0)	4.0-79.0
Fecal col. /100 mL (geomean / range)	75	37-419

Figure 11.1 Smith Creek watershed



12.0 Whiskey Creek

Snapshot
Watershed area: 1,344 acres (544 ha) Impervious surface coverage: 19%
Watershed population: 7,107
Overall Water Quality: Fair
Problematic pollutants: Low dissolved oxygen on occasion

Whiskey Creek drains into the ICW. Sampling of this creek began in August 1999, at five stations. One station was dropped due to access issues in 2005; four stations were sampled until and including 2007; in 2008 this was reduced to one station, WC-MLR (from the bridge at Masonboro Loop Road – Fig. 12.1). Salinity at this station was relatively high, what scientists consider euhaline, ranging from 23 – 31 ppt and averaging about 29 ppt (Table 12.1).

Dissolved oxygen concentrations were below the State standard on two of six sampling occasions at WC-MLR (Table 12.1). Turbidity was within state standards for tidal waters on all sampling occasions (Table 12.1; Appendix B). Algal blooms are relatively rare in this creek; there was one bloom of 25 μg-chlorophyll *a*/L at WC-MLR in July 2008 (Table 12.1). Nutrient concentrations were unremarkable at this station, and averages were similar to those at this site in the previous year.

Fecal coliform bacteria were just acceptable for human contact at this site; on two occasions counts were 200 CFU/100 mL, but on no occasions was that North Carolina standard exceeded. Whiskey Creek is presently closed to shellfishing by the N.C. Division of Marine Fisheries.

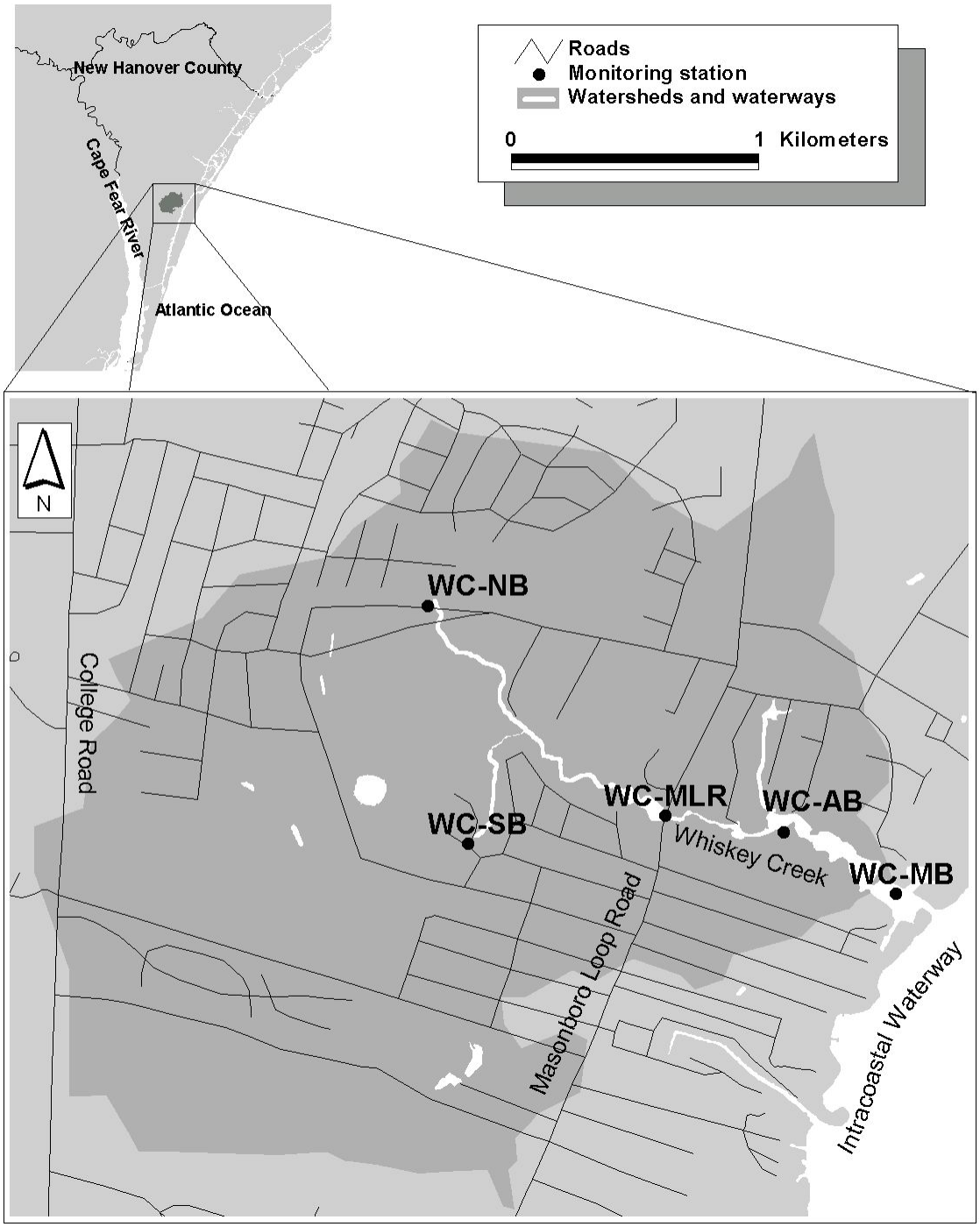
Table 12.1. Water quality summary statistics for Whiskey Creek, 2008, presented as mean (standard deviation) / range, fecal coliforms as geometric mean / range.

	Salinity (ppt)	DO (mg/L)	Turbidity (NTU)	TSS (mg/L)	Chlor *a* (μg/L)	FC CFU/100 mL
WC-MLR	28.1 (3.2)	6.9 (2.9)	7.0 (4.3)	13.2 (5.9)	9.2 (9.7)	89
	23.2-31.3	3.2-9.4	2.0-13.0	8.0-21.0	1.0-25.4	32-200

Table 12.2. Nutrient concentration summary statistics for Whiskey Creek, 2008, as mean (standard deviation) / range, N/P ratio as mean / median.

	Nitrate (mg/L)	Ammonium (mg/L)	TN (mg/L)	Phosphate (mg/L)	TP (mg/L)	N/P ratio
WC-MLR	0.06 (0.06)	0.03 (0.02)	0.30 (0.20)	0.03 (0.04)	0.05 (0.02)	8.7
	0.01-0.12	0.01-0.06	0.05-0.63	0.01-0.10	0.01-0.08	7.4

Figure 12.1. Whiskey Creek. Watershed and sampling sites.



13.0 Detection of Fecal Bacteria Sources in New Hanover County Tidal Creeks by Molecular-Based Methods

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ABSTRACT

The number one pollutant impacting the tidal creeks of New Hanover County, N.C. is fecal bacteria, which has lead to posted warnings for human contact and extensive closures of shellfish beds to harvest. In order to take appropriate remedial action it is important to determine the sources of the fecal contamination; i.e. human, avian, canine, etc. The standard method for fecal coliform pollution measurement enumerates, but does not distinguish between sources. Between December 2005 and June 2007 we conducted a study to determine the sources of fecal bacteria in Futch, Pages, Howe, Bradley, Hewletts and Whiskey Creeks in New Hanover County. We used standard methods for fecal coliform bacteria enumeration as well as the molecular methods of polymerase chain reaction (PCR) and terminal restriction fragment length polymorphism (T-RFLP) for bacterial source tracking using the genera *Bacteroides* as a target. As such we were able to identify areas with high levels of fecal coliform bacteria pollution as well as distinguish fecal contamination sources between human, canine and ruminant. Of the 54 samples collected during this project, less than 23% were positive for canine fecal contamination based on PCR detection; these canine-positive samples were mostly associated with rainfall and would thus be brought to the creek during stormwater runoff. Ruminant fecal contamination was found in 12 of the 54 samples collected during this study, mainly in the upstream sampling areas (deer are likely an important source) and also near a known horse farm. Human fecal contamination was found in 18% of the examined samples, indicating human waste treatment and conveyance problems. The positive samples were reevaluated with T-RFLP fingerprint analysis, specific for the *Bacteroides* group. The fingerprint analysis yielded a total of 40 fragments with different sizes, which correspond to different members of the *Bacteroides* group. Using a Web-based phylogenetic assessment tool (the MiCA T-RFLP PAT+), it was possible to identify 13 of the 40 fragments, 11 of which were found to be from human-specific *Bacteroides* species. The detection of human-specific *Bacteroides* in water samples is of particular concern, because New Hanover County has been plagued with sewer-system failures. The presence of human-borne *Bacteroides* is indicative of either continued sewer-line problems, septic system failures, or a general persistence in the bacteria itself in sediments from earlier pollution episodes.

INTRODUCTION

Tidal creek ecosystems are suffering more each day as a consequence of increased urbanization. Because tidal creek ecosystems are aesthetically beautiful and recreationally appealing, more and more people relocate to these areas every year. This rise in urbanization has intensified the anthropogenic impact on these ecosystems (Mallin et al. 2000). The clearcutting and development associated with urbanization decreases the density of vegetation adjacent to the tidal creeks, which increases both the velocity and the volume of stormwater entering the creek (Mallin and Lewitus 2004). This stormwater runoff often contains fecal bacteria and other pollutants.

In the Florida Keys, human waste has been linked to a high presence of enteric viruses in the waters and resulted in the documentation of a swimmers' risk in the area (Griffin et al. 1999, Nobles et al. 2000). In 2001, 13,410 recreational area closings and advisories were issued nationwide. Nearly 90% of these closings were due to the level of fecal coliform bacteria in the water (Olyphant 2003). Since humans are more likely to catch a human-borne illness, anthropogenic fecal coliform sources are of special concern (McLellan and Jensen 2003). For tidal creeks, the potential for both shellfish bed closures and human contact risk from fecal-polluted waters points toward the need for better understanding and management of these waters.

In order to understand and manage fecal bacteria pollution in any body of water, one must first be able to identify the source of the pollution (Kelsey et al. 2004). The most significant source of non-point source pollution in urban watersheds is runoff (Marsalek and Rochfort 2004). In order to meet set water quality goals in urban watersheds, advanced knowledge and understanding of non-point source pollution is necessary (Marsalek and Rochfort 2004).

There are some steps/actions that minimize the risk of water contamination, especially with respect to human pathogens. Since the testing for specific pathogens is difficult and expensive, the use of indicator organisms has become a popular solution (Jamieson et al. 2003). *Escherichia coli* (*E. coli*), fecal coliform bacteria, and *Enterococci* are commonly used in standard indicator organism tests to determine the presence of fecal contamination. While the standard methods for fecal coliform bacteria measurement (and other indicators) offer extensively-used estimates of the level of fecal pollution in a given watershed, they do not distinguish between the many possible warm-blooded sources of the pollution. A complete assessment of the human health risk and development of better watershed management practices requires a method that can pinpoint the sources of contamination (Field et al. 2003).

Bernhard and Field (2000a) developed molecular methods to use *Bacteroides* as indicators because of the abundance of these bacteria in warm-blooded intestinal tracts and because they are not sensitive to salinity like fecal coliforms. Two molecular techniques known as length heterogeneity polymerase chain reaction (LH-PCR) and terminal restriction fragment length polymorphism (T-RFLP) were used to determine host-specific patterns of Bacteroides communities as well as to pinpoint fingerprint fragment lengths that were unique to cows or humans. Bernhard and Field (2000a)

showed the detection of human and cow fecal contamination in freshwater and saltwater ecosystems based on the developed fingerprint methods. In addition, Bernhard and Field (2000b) were able to develop specific primers targeting Bacteroides members present in either human or ruminants. Recently, Recently, Dick et al. (2005) used a subtractive hybridization to design specific primers for *Bacteroidales* in dogs. These researchers subsequently tested the species-specific markers against other known samples to rule out any possibility that the DNA primers developed from their findings would amplify anything other than their target DNA segments. It is the primers that these researchers developed and published in Bernhard and Field (2000a and 2000b) and Dick et al. (2005) that were used in this study. The goal of this study was to identify sources of fecal bacteria pollution among human, canine and ruminant in New Hanover County tidal creeks using PCR amplification and T-RFLP fingerprint analyses.

MATERIALS AND METHODS

Sample Collection, Filtration, and Storage

Samples were collected from Futch Creek (FC-17 and FOY), Howe Creek (HW-GP and HW-DT), Bradley Creek (BC-SB and BC-NB0, Hewletts Creek (NB-GLR) and Whiskey Creek (WC-MB) during the months of December 2005 and January, February, June, July and August 2006. Some of the sampled creeks have experienced sewage spills (Mallin et al. 2007; Tavares et al. 2008). Monthly samples from Pages Creek (PC-BDDS) were collected from January to June, 2007. Samples were collected at each station for nutrients, chlorophyll *a* analysis, fecal coliform counts, and DNA extraction. Physical characteristics of the water at each station were measured using a YSI 6920 multi-parameter water quality sonde (i.e. water temperature, salinity, conductivity, pH, turbidity, and dissolved oxygen). The analytical method used to measure chlorophyll *a* was fluorometry as described in Welschmeyer (1994) and US EPA (1997). The fecal coliform samples were collected in autoclaved 500mL Pyrex glass bottles. The samples were transported on ice and were held for no longer than six hours before filtration. Fecal coliform samples were filtered and incubated per Standard Methods (APHA 1998).

The water samples for DNA analysis were collected in autoclaved 500mL Pyrex glass bottles. The samples were transported on ice and allowed to sit no longer than six hours before filtration. Upon returning to the lab, the samples were filtered using autoclaved glassware and sterile Whatman GF/F 47mm filters, with a nominal pore size of 0.7µm. After filtration, the filters were wrapped individually in aluminum foil and stored at $^{-}$20°C until DNA extraction from the filter could be performed.

DNA Extraction

The DNA was extracted using the PowerSoil™ DNA Isolation Kit from MO BIO Laboratories, with the protocol slightly modified for extraction from a filter instead of a soil sample. A portion of the filter was ground using a PowerBead Tube and tissue grinder, and then the extraction was completed per manufacturer's instructions. The MO BIO PowerSoil™ DNA Isolation Kit uses a detergent to lyse the cells and release the DNA, and then uses several solutions to help precipitate materials that may reduce

the purity of the DNA (such as non-DNA humics, cell debris, and proteins) and inhibit PCR reactions.

PCR Detection Methods

A list of all of the primers that were used in this study can be found in Table 1. The 27F and 1522R primers were paired together to amplify universal 16S rDNA, which is the DNA region used for identifying bacteria. All of the other forward primers were paired with the Bac708R reverse primer.

Table 1: Host-specific primers used in this study.

Primer	Sequence (5'-3')	Target	Specificity	Reference
27F	AGAGTTTGATCMTGGCTCAG	16s rRNA gene	Bacteria	Suzuki and Giovannoni (1996)
1522R	AAGGAGGTGATCCANCCRCA	16s rRNA gene	Bacteria	Suzuki and Giovannoni (1996)
DF475F	CGCTTGTATGTACCGGTACG	SHDogf	Canine	Dick et al (2005)
HF183F	ATCATGAGTTCACATGTCCG	HF8 cluster, HF74	Human	Bernhard and Field (2000b)
CF193F	TATGAAAGCTCCGGCC	CF151 cluster	Ruminant	Bernhard and Field (2000b)
Bac32F	AACGCTAGCTACAGGCTT	*Bacteroides-Prevotella*	General *Bacteroides*	Bernhard and Field (2000a)
Bac708R	CAATCGGAGTTCTTCGTG	*Bacteroides-Prevotella*	General *Bacteroides*	Bernhard and Field (2000a)

All of the samples were first amplified with the universal 16s primers. These PCR products were then used as template for all PCR reactions for each sample. This process is known as nested PCR. It reduces the potential contamination that can be caused by the primers binding to unspecified sites, as it is unlikely that non-target DNA would be amplified by two separate sets of primers.

PCR Mixtures and Conditions:

All of the mixtures and thermocycler parameters for each PCR reaction are outlined in Table 2. All of the PCR reactions were run with a positive and negative control.
able 2: PCR mixtures and thermocycler parameters for each PCR reaction.

Primer	Taq	$MgCl_2$	Buffer	Primers	Denaturation	Annealing	Extension
16s	1µL	2.5µL	2.5µL	1µL 27F, 1µL 1522R	94°C for 30 sec	55°C for 30 sec	72°C for 2 mins
Bac	1µL	2.5µL	2.5µL	1µL Bac32F, 1µL Bac708R	94°C for 30 sec	55°C for 30 sec	72°C for 2 mins
Canine	1µL	2.5µL	2.5µL	1µL DF475F, 1µL Bac708R	94°C for 30 sec	55°C for 30 sec	72°C for 2 mins
Human	1µL	2.5µL	2.5µL	1µL HF183F,	94°C for 30	55°C for	72°C for 2

				1µL Bac708R	sec	30 sec	mins
Ruminant	1µL	2.5µL	2.5µL	1µL CF193F, 1µL Bac708R	94°C for 30 sec	55°C for 30 sec	72°C for 2 mins

DNA fragments that have been amplified by PCR can be separated by gel electrophoresis, which allows us to separate fragments by size (Klug and Cummings, 1997). The PCR products are run on a 1% agarose gel to separate the fragments (see Figure 1). Gels are run with a DNA ladder, which provides a reference against which the resultant bands can be measured for length (in base pairs). For the purposes of this study, any resultant band of the correct size in the agarose gel indicated that target DNA was obtained from the sample in question.

T-RFLP

In an effort to gain a more complete understanding of the bacterial communities present in each sample, and to confirm the results obtained by the direct PCR methods, PCR mixtures for select samples were also set up with a Bac32F/Bac708R primer pair, in which the forward primer was labeled with fluorescent tags (FAM labeled). The PCR mixture for these samples contained 12.5µL of Fisher Promega's GoTaq® Green Master Mix and 1µL of each primer, at a concentration of 0.4µm. The template was added at a volume of 1µL, and the reaction was then brought up to a 25µL volume using sterile deionized water. These PCR products were then run on a 1% agarose gel to determine the presence or absence of the target segment of DNA and to separate the positive bands and cut them from the gel. A GENECLEAN® Turbo Kit from Q-BIO gene was then used to purify these cut fragments.

In order to determine the concentration of DNA in these samples, a Quant-iT™ DNA HS Assay from Invitrogen was used. The samples were read using a Qubit fluorometer, which provides a fluorescence measurement used in determining the concentration of DNA in the sample. The resultant information about the concentration of DNA present in each sample was then used to determine the needed formula for enzyme digestion. These reactions were set up and incubated overnight at 37°C for digestion to occur.

The following day, these enzyme digestion mixtures were precipitated and a 10µL mixture of HiDi formamide and GS 500 Rox was added. DNA fingerprinting was conducted on an ABI PRISM® Genetic Analyzer, from Applied Biosystems.

Upon completion of fingerprinting, each sample was represented by a profile. The profiles have representative peaks for each bacterial taxon present in the samples. The size of each fragment present is indicated in base pairs, and these fragments can be matched to a database of known fragments of DNA to identify what is present. This analysis was completed using the Microbial Community Analysis 3 (MiCA) T-RFLP Analysis Phylogenetic Assessment Tool (PAT+) found at http://mica.ibest.uidaho.edu/pat.php.

Statistical analyses, including principal component analysis and correlation analysis, were conducted using Caneco for Windows® and Microsoft Excel.

RESULTS AND DISCUSSION

The physical parameters collected during field sampling via the YSI 6920 multi-parameter water quality sonde, as well as the nutrient and chlorophyll *a* data have been provided in two previous reports (Mallin et al. 2007 and 2008).

Fecal Coliform Bacteria Counts

The fecal coliform bacteria counts for each station and month of sampling are provided in Table 3. A blank entry in the table means that no samples were taken for that month. The counts are an average of two incubated filters and are reported as the number of colony-forming units (CFUs) per 100mL of water. Fecal coliform bacteria were found to be present in every sample taken, with the exception of WC-MB for the month of July 2006. State standard MFC guidelines require a reported value of <1 for samples that have a count of 0. This value is reported as 0.5 in Table 3 and rounded up to 1.

All stations sampled had a fecal coliform level that exceeded the state standard of 200 CFUs/100mL during at least one month of sampling, with the exception of WC-MB. Both Bradley Creek stations exceeded the standard during only one month of sampling (June 2006), along with FOY (August 2006), and PC-BDDS (January 2007).

The Howe Creek stations both exceeded the standard during the months of December 2005 and June and July 2006. HW-GP also exceeded the standard during the month of February 2006. In Futch Creek FC-17 had fecal coliform counts that exceeded the standard during four of the six months of sampling. The Hewletts Creek station of NB-GLR exceeded the MFC standard in five of the six months of sampling.

Table 3. Monthly fecal coliform bacteria counts at each station. Bolded entries are samples that were taken during rain events.

Month	BC-NBU	BC-SB	FC-17	FOY	HW-DT	HW-GP	NB-GLR	PC-BDDS	WC-MB
Dec-05	98	128	380	96	365	305	475		7
Jan-06	**48**	**56**	**91**	**11**	**41**	**86**	**113**		**7**
Feb-06	**60**	**127**	**123**	**35**	65	221	>300		1
Jun-06	**310**	**740**	1405	129	**213**	**201**	655		3
Jul-06	18	41	270	19	**3025**	**4075**	**272**		**1**
Aug-06	207	116	**1505**	**228**	173	87	615		13
Jan-07								**1040**	
Feb-07								29	
Mar-07								114	
Apr-07								**42**	
May-07								162	
Jun-07								72	
Min	18	41	91	11	41	86	113	29	1
Max	310	740	1505	228	3025	4075	655	1040	13
Geomean	83	121	367	52	218	273	358	109	4

PCR Results

The PCR results for each station and month are provided in Table 4. The presence or absence of each target segment of DNA for each station is indicated by a yes or a no in the corresponding cell in Table 4. The presence or absence of these target segments was determined by running the PCR results on an agarose gel like the one shown in Figure 1.

The bands corresponding to the 16S, *Bacteroides*, canine, and human target fragments are all labeled in Figure 1. The 16S fragment has a size of 1495 basepairs, the *Bacteroides* fragment is 676 basepairs, the canine fragment is 233 basepairs, the human fragment is 525 basepairs, and the ruminant fragment is 515 basepairs.

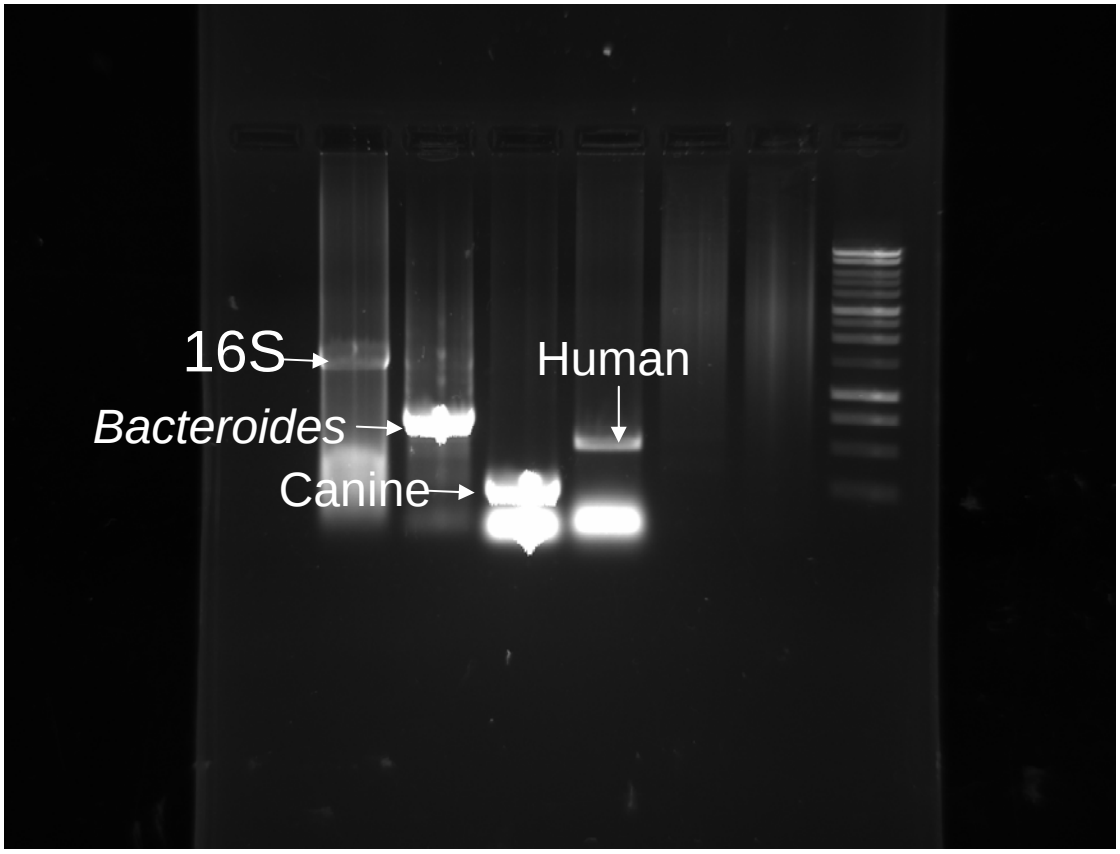

Figure 1: Agarose gel picture of PCR products.

The majority of the samples were positive for 16S rDNA, as well as *Bacteroides*. With many of the negative results for 16S and *Bacteroides* PCR occurring during the winter months, there are two likely explanations. The first reason could be failure in the extraction or PCR processes, or PCR inhibition. The second reason could be that bacteria grow better in the warmer months and warmer temperatures, since they are found in the guts of warm-blooded organisms. Fecal coliform bacteria were found to be present in all but one of the samples taken, but the levels were generally lower in winter than those found during the summer months. The negative results can most likely be attributed to a combination of both factors.

The presence of canine fecal contamination was found at seven of the nine stations via PCR. It was found sporadically, with the first positive being found in January 2006 at FC-17. During the month of February 2006, canine fecal contamination was found at BC-SB and also at NB-GLR, which is the station downstream of a pet boarding and grooming facility. Stations BC-NBU, BC-SB, FC-17, FOY, HW-DT, and HW-GP were all positive for canine sources during the month of June 2006. July 2006 had positives at FC-17, HW-GP, and NB-GLR. FC-17 had the only positive during August 2006. Of the twelve samples that were determined to be positive for canine fecal contamination, two-thirds of them (eight) coincided with rain events, showing the susceptibility of fecal bacteria from dog manure to rainfall-generated runoff.

Table 4. PCR detection results. Bolded entries are samples that were taken during rain events.

Station	Month	16s	*Bacteroides*	Canine	Ruminant	Human
BC-NBU	Dec-05	Yes	Yes	No	Yes	No
	Jan-06	Yes	Yes	No	No	No
	Feb-06	Yes	Yes	No	No	No
	Jun-06	Yes	Yes	Yes	Yes	No
	Jul-06	Yes	Yes	No	No	Yes
	Aug-06	Yes	Yes	No	No	Yes
BC-SB	Dec-05	No	No	No	No	No
	Jan-06	Yes	Yes	No	No	No
	Feb-06	Yes	Yes	Yes	No	No
	Jun-06	Yes	Yes	Yes	Yes	Yes
	Jul-06	Yes	Yes	No	No	No
	Aug-06	Yes	Yes	No	No	No
FC-17	Dec-05	Yes	Yes	No	Yes	No
	Jan-06	Yes	Yes	Yes	No	No
	Feb-06	Yes	Yes	No	No	No
	Jun-06	Yes	Yes	Yes	Yes	Yes
	Jul-06	Yes	Yes	Yes	No	No
	Aug-06	Yes	Yes	Yes	No	No
FOY	Dec-05	Yes	No	No	No	No
	Jan-06	Yes	Yes	No	No	No
	Feb-06	Yes	Yes	No	No	No
	Jun-06	Yes	Yes	Yes	Yes	No
	Jul-06	Yes	Yes	No	No	Yes
	Aug-06	Yes	Yes	No	No	No
HW-DT	Dec-05	Yes	Yes	No	Yes	No
	Jan-06	Yes	Yes	No	No	No
	Feb-06	Yes	Yes	No	No	No
	Jun-06	Yes	Yes	Yes	Yes	Yes
	Jul-06	Yes	Yes	No	No	No
	Aug-06	Yes	Yes	No	No	Yes
HW-GP	Dec-05	Yes	Yes	No	No	No
	Jan-06	Yes	Yes	No	No	No

	Feb-06	Yes	Yes	No	No	No
	Jun-06	Yes	Yes	No	Yes	No
	Jul-06	Yes	Yes	Yes	No	No
	Aug-06	Yes	Yes	No	No	No
NB-GLR	Dec-05	Yes	Yes	No	Yes	Yes
	Jan-06	Yes	No	No	No	No
	Feb-06	Yes	Yes	Yes	No	No
	Jun-06	Yes	Yes	No	Yes	No
	Jul-06	Yes	Yes	Yes	No	No
	Aug-06	Yes	Yes	No	No	No
PC-BDDS	**Jan-07**	Yes	Yes	No	No	Yes
	Feb-07	No	No	No	No	No
	Mar-07	Yes	Yes	No	No	No
	Apr-07	Yes	Yes	No	No	No
	May-07	No	No	No	No	No
	Jun-07	No	No	No	No	No
WC-MB	Dec-05	Yes	No	No	No	No
	Jan-06	Yes	Yes	No	No	Yes
	Feb-06	No	No	No	No	No
	Jun-06	Yes	Yes	No	Yes	No
	Jul-06	Yes	Yes	No	No	No
	Aug-06	Yes	No	No	No	No

The PCR results indicated that ruminant fecal contamination was found during December 2005 at BC-NBU, FC-17, HW-DT and NB-GLR. In addition, ruminant fecal contamination was found at every station sampled during the month of June 2006. Four of the eight samples that were positive in June 2006 corresponded with stormwater runoff from rain events, namely the Bradley Creek (BC-NBU and BC-SB) and Howe Creek (HW-DT and HW-GP) stations. A horse farm is located in the area that drains to BC-SB, so runoff of horse fecal matter into Bradley Creek is likely the cause of that positive sample. The upstream stations of FC-17, FOY, HW-DT, and HW-GP all had at least one sample that was positive for ruminant fecal contamination, which is to be expected with the fairly large deer population in New Hanover County. Five out of 12 of the samples positive for ruminant fecal contamination were directly associated with rain events. PC-BDDS was the only station where no positives were found.

Human fecal contamination was found at NB-GLR during the month of December 2005. WC-MB was positive in January 2006. BC-SB, FC-17 and HW-DT were all positive for human fecal contamination in June 2006. FOY was the only human positive during the month of July 2006. BC-NBU and HW-DT were both positive in August 2006, and PC-BDDS was the only human positive in January 2007. Both NB-GLR and PC-BDDS have sewer-line pump stations located close to those stations, and WC-MB is a marina where several people live on houseboats. Septic systems are utilized in the Futch Creek area draining to FC-17 and FOY.

T-RFLP Results

Upon completion of DNA fingerprinting, the MiCA T-RFLP PAT+ was used to determine the *Bacteroides* groups present in each sample. The PAT+ uses the resultant peak areas and fragment sizes, along with the forward and reverse primer and restriction enzyme information, to perform an analysis on the structure of the microbial community present in each sample. The resultant information makes it possible to link some of the fragment sizes to known bacterial groups and sources.

The only identified peaks that were host-specific were human-borne, however, some peaks were found that are shared between human and avian species, and some that are shared between human and ruminant sources. These peaks could have been the result of fecal bacteria pollution from either host in those cases. While it was possible to determine that human or mixed human/animal sources did account for 13 of the 40 *Bacteroides* groups present in all of the samples, the majority of the fragment sizes were not present (i.e. unidentifiable) in the MiCA T-RFLP PAT+ system. Based on the peaks that were identified, host-specific group percentages were calculated for each station and month that T-RFLP was conducted (Table 5).

According to the percentages, 6 of the 25 samples had profiles where 50% or more of the peaks were human-borne, with the two peaks present in the HW-GP July 2006 sample being 100% human-borne. If the parameters are expanded to include the number of samples where 25% or more of the peaks were human-borne, it jumps to 14 of the 25 samples, or 56%.

Many of the high percentages in the “other” category coincide with rain events, signaling that stormwater runoff of animal-borne fecal bacteria is the most likely cause. These were seen at BC-SB (February, June and July 2006), FC-17 (January and August 2006), HW-DT (June and July 2006), HW-GP (June 2006), NB-GLR (July 2006), PC-BDDS (January 2007), and WC-MB (January 2006). Seven of these eleven instances corresponded with high fecal coliform bacteria levels as well, which have been linked to rainfall events (Young and Thackston 1999; Mallin et al. 2002). Several of the high percentage totals found for human fragments also coincided with high levels of fecal coliform bacteria in the water. HW-DT (December 2005, June and July 2006), HW-GP (December 2005, July 2006), and NB-GLR (December 2005 and February 2006) all had fecal coliform levels that exceeded the state standard during the indicated months and all had a high reported percentage of human-borne fragments present in the T-RFLP profiles.

Table 5. Host-specific *Bacteroides* group percentages based on T-RFLP results. Bolded entries are samples that were taken during rain events.

Station	Month	Human	Human/Avian	Human/Ruminant	Other
BC-NBU	Dec-05	15.28			84.72
	Jan-06				
	Feb-06				
	Jun-06				
	Jul-06	42.16			57.84
	Aug-06	27.61			72.39
BC-SB	Dec-05				
	Jan-06				
	Feb-06	11.90		5.09	83.01
	Jun-06	20.80			79.20
	Jul-06	52.90			47.10
	Aug-06				
FC-17	Dec-05	6.33			93.67
	Jan-06	16.61	4.89		78.50
	Feb-06				
	Jun-06	15.03			84.97
	Jul-06				
	Aug-06				100.00
FOY	Dec-05				
	Jan-06				
	Feb-06				
	Jun-06	27.94			72.06
	Jul-06	9.98		12.12	77.90
	Aug-06				
HW-DT	Dec-05	38.43		5.27	56.30
	Jan-06				
	Feb-06				
	Jun-06	26.45			73.55
	Jul-06	58.49			41.51
	Aug-06	25.84		15.39	58.77
HW-GP	Dec-05	25.58		5.33	69.09
	Jan-06				
	Feb-06				
	Jun-06	16.71			83.29
	Jul-06	100.00			
	Aug-06				
NB-GLR	Dec-05	57.48			42.52
	Jan-06				
	Feb-06	50.60		10.24	39.16
	Jun-06	17.07			82.93
	Jul-06	17.18		11.29	71.53

	Aug-06				
PC-BDDS	**Jan-07** Feb-07 Mar-07 **Apr-07** May-07 Jun-07	8.38		4.68	86.94
WC-MB	Dec-05 **Jan-06** Feb-06 Jun-06 **Jul-06** Aug-06	55.91 24.75			44.09 75.25

T-RFLP also resulted in a profile corresponding to each sample, with peaks of varying fragment sizes and heights representing the different bacterial groups present in the sample and their relative abundance. Of those peaks, the peaks that were identified using the MiCA T-RFLP PAT+ are labeled with their fragment size and host-specificity. Figure 2 shows the profiles from the NB-GLR fingerprinting results as an example.

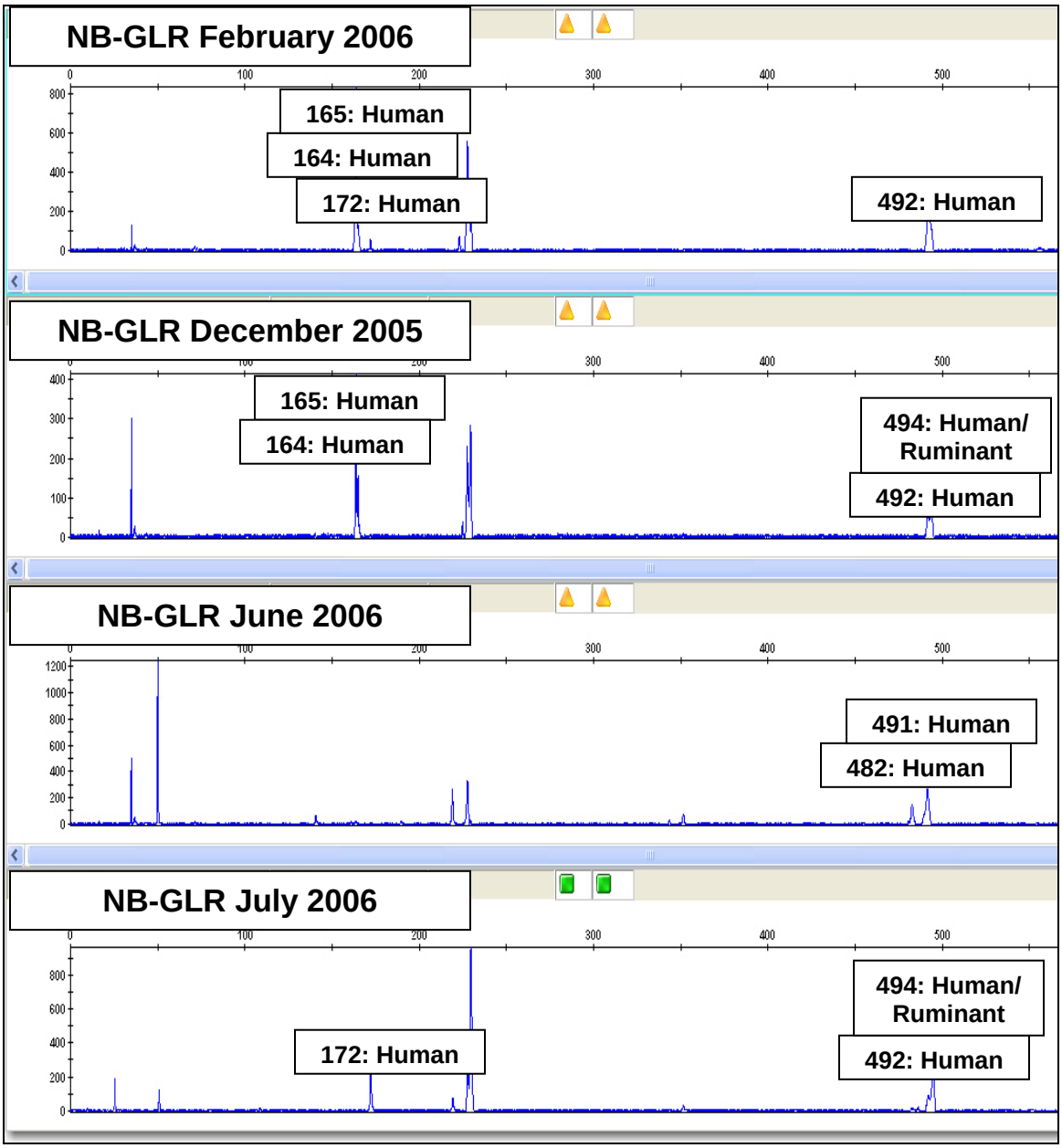

Figure 2: T-RFLP profiles from NB-GLR for the months of February (top) and June (third) and July (bottom) 2006, and December (second) 2005.

In an effort to obtain a positive control sample to use for comparison, one sample was taken from each of four different sampling locations on Hewletts Creek immediately following a major sewer-line failure in November 2006. DNA was extracted from each of these samples and they were processed for fingerprinting as well. The resultant profiles are provided in Figure 3.

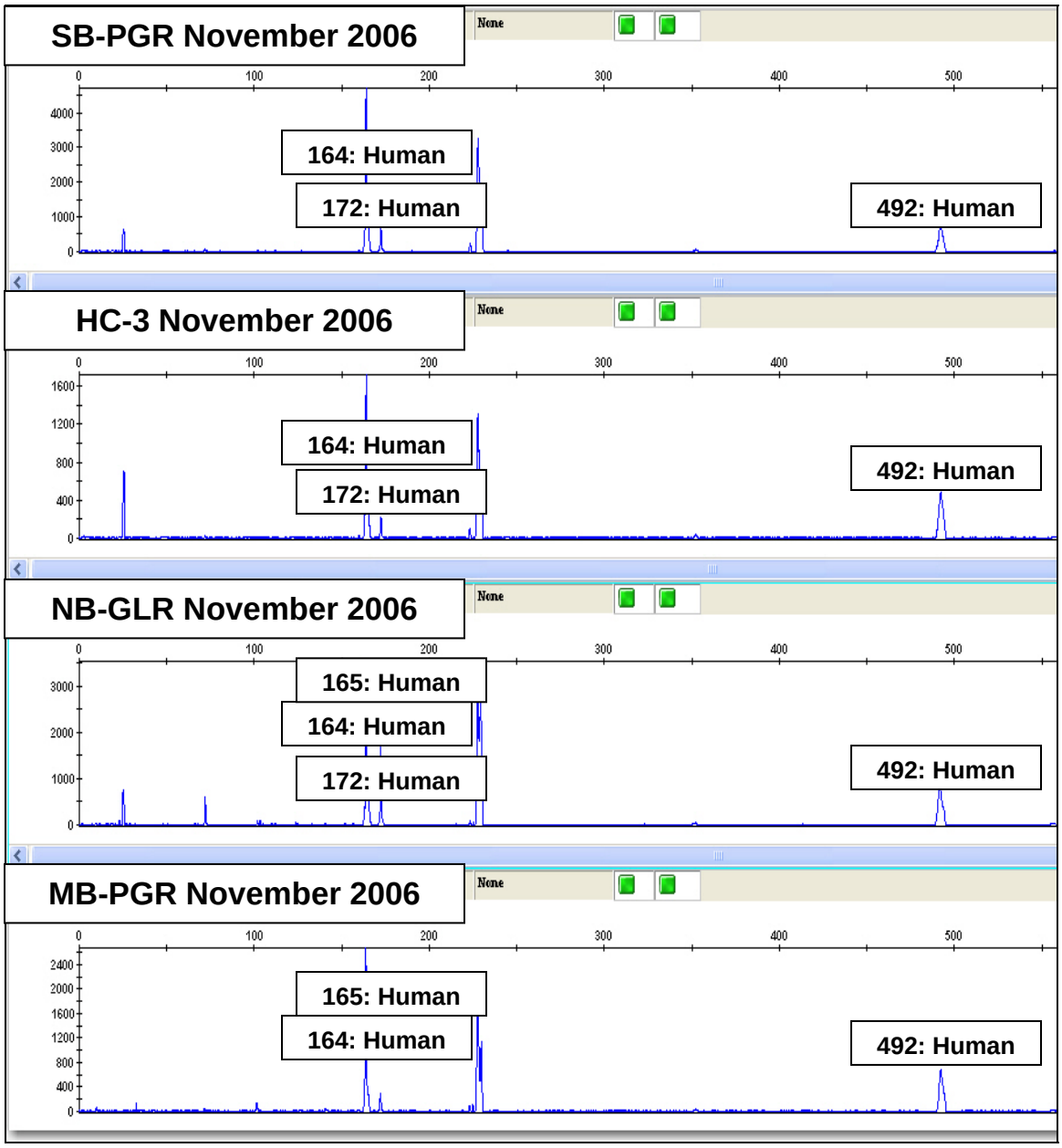

Figure 3: T-RFLP profiles from Hewletts Creek stations SB-PGR (top), HC-3 (second), NB-GLR (third) and MB-PGR (bottom) for the November 2006 sewage spill.

In these positive control profiles, it is possible to see the consistency in the peaks that have been identified. Human peaks 164 and 492 were found in all four of the sewage spill samples, 172 was found in three of the samples, and 165 was found in two of them. Some or all of these fragment sizes were found to be present in the experimental T-RFLP profiles. This positive control offers a good basis for comparison for the other tidal creeks.

Correlation analysis was used to compare host-specific *Bacteroides* group percentages with physical and chemical parameters collected on-station in conjunction with the bacterial samples. The results (Table 6) present the strength and direction of the relationship between two variables (the correlation coefficient r), and the associated

probability *P*). Of these correlations, only a few are statistically significant, meaning they have an associated *P*-value of 0.05 or less. Human-specific peaks were found to have a significant negative correlation with salinity and conductivity, and a significant positive correlation with nitrate/nitrite and chlorophyll *a*. Human/ruminant fragments were also found to be significantly negatively correlated with salinity. The unidentifiable other fragments were found also to have a significant negative correlation with salinity, as well as with pH and dissolved oxygen.

Table 6. Correlation analysis results comparing physical parameters, nutrients, and chlorophyll *a* concentrations to host-specific group percentages. Presented as Pearson correlation coefficient (r) and probability (*P*); bolding represents significant at $P < 0.05$.

Host Specificity	Statistical Value	Temperature	Conductivity	Salinity	Dissolved Oxygen	pH
Human	r	0.160	**-0.348**	**-0.350**	-0.109	-0.23
	P	0.248	**0.010**	**0.003**	0.437	0.091
Human/Avian	r	-0.121	0.085	0.085	0.079	0.093
	P	0.382	0.540	0.542	0.568	0.503
Human/Ruminant	r	0.154	-0.130	**-0.114**	-0.010	-0.083
	P	0.266	0.349	**0.041**	0.941	0.550
Other	r	0.249	-0.245	**-0.310**	**-0.280**	**-0.354**
	P	0.069	0.074	**0.023**	**0.041**	**0.009**

Table 6 cont.

Host Specificity	Statistical Value	Turbidity	Nitrate	Orthophosphate	chlorophyll *a*
Human	r	0.110	**0.368**	0.012	**0.292**
	P	0.428	**0.006**	0.932	**0.032**
Human/Avian	r	-0.064	0.053	-0.012	-0.069
	P	0.646	0.703	0.934	0.620
Human/Ruminant	r	-0.090	-0.015	0.131	0.138
	P	0.519	0.916	0.344	0.318
Other	r	0.140	0.110	0.017	0.014
	P	0.314	0.428	0.905	0.921

CONCLUSIONS

More than 85% of the samples were positive for the presence of general *Bacteroides* groups. However, not all stations were positive for *Bacteroides* during every month of sampling, despite being positive for fecal coliform bacteria. It is possible that some human error may have occurred during the extraction or PCR processes to affect the viability of the DNA, or PCR inhibitors may have been present in the solution.

Canine fecal contamination was found at seven of the nine stations, but was not nearly as prevalent as expected. Only 12 of the 54 samples were positive for canine fecal contamination, which is less than 23%. The observed lack of a larger canine fecal contamination is a positive sign that the City of Wilmington and the Town of Wrightsville Beach, both in New Hanover County, have well-working pet waste management regulations and systems in place to encourage pet owners to clean-up after their pets. Developing such a program on a county level as well would be beneficial.

More than 18% of the samples were positive for human fecal contamination. 20% of these positive samples were from Futch Creek (FC-17 and FOY), an area that still has residents utilizing individual septic systems. Further sample processing through T-RFLP also identified human-specific fragment sizes and their relative abundance in these positive samples. Two of the creeks studied, Bradley and Hewletts, experienced sewage spills in 2005, 2006 and 2007 (Mallin et al. 2007; Tavares et al. 2008). Principal component analysis revealed that human fecal contamination was positively associated with a group of factors including temperature, turbidity, nitrate/nitrite, and chlorophyll *a* concentrations, and negatively related to conductivity, salinity, and pH. Fecal bacteria live in the guts of warm-blooded animals and grow well in comparable conditions, which explains a positive relationship with temperature. Fecal coliforms are also very salinity sensitive, which explains why there was a negative correlation with salinity and conductivity; i.e. they die faster in saltier water than fresh water. Upper creek stations are where the highest fecal coliform counts in general are typically found in this urbanizing creek system; these stations are also characterized by elevated turbidity, nitrate and chlorophyll *a* concentrations (Mallin et al. 2000). In some instances human-sourced fecal coliforms can originate from the same source(s) as nitrate; i.e. septic system leachate and sewer line or pump station leaks. In other situations the nitrate (which stimulates algal growth as chlorophyll *a*) may arrive from stormwater runoff into an area containing human sourced fecal bacteria.

Ruminant fecal contamination was found mainly in the four upstream areas, as well as five of the six creeks sampled. Like the canine fecal contamination, 12 of the 54 samples were positive for ruminant sources of fecal bacteria pollution. Of the 12 positives, four of them were found following a June 2006 rain event. Principal component analyses showed that the human/ruminant specific fecal bacteria was related to a similar group of variable as the human alone, with a positive association with chlorophyll *a*, nitrate, temperature, and turbidity, and a negative association with the other physical parameters, particularly conductivity and salinity. Of these, correlation analysis only found the negative correlation with salinity as being statistically significant.

The one fragment size identified as human/avian had no positive association with the factors associated with the other host-specific fragments, such as turbidity, nutrients and chlorophyll *a*, and none of the correlation coefficients were found to be statistically significant. Fecal coliform bacteria from waterfowl are likely to be deposited either directly on water or in associated marshes, and are thus not necessarily associated with stormwater runoff. There should be no positive relationship with turbidity, a symptom of land runoff.

The presence of human fecal bacteria pollution is of tremendous concern. New Hanover County has a well-known history of sewer line and pump station problems. Old lines are incapable of accommodating all of the new connections and lines have failed on a number of occasions. Evidence of human fecal bacteria pollution is indicative of further leaks in the sewer system, septic system failures (where they are present), or general persistence of human-specific *Bacteroides* bacteria in the creeks themselves, as has been seen in past research (Conboy and Goss 2001); human-sourced fecal bacteria have persisted in elevated numbers in the sediments of some of these creeks following spills (Mallin et al. 2007; Tavares et al. 2008). Background research is needed to help identify the unknown fragment sizes and make further conclusions on a course of action for watershed management.

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16.0 Appendix A. North Carolina Water Quality standards for selected parameters (NCDEHNR 1996). We note that these standards are general, and differ with designated water body use. Details can be found at within the N.C. Division of Water quality website at: http://h2o.enr.state.nc.us/csu/documents/ncactable290807.pdf

Parameter	Standard
Dissolved oxygen	5.0 ppm (mg/L)
Turbidity	25 NTU (tidal saltwater) 50 NTU (freshwater)
Fecal coliform counts	14 CFU/100 mL (shellfishing waters), and more than 10% of the samples cannot exceed 43 CFU/100 mL. 200 CFU/100 mL (human contact waters)
Chlorophyll *a*	40 ppb (μg/L)

CFU = colony-forming units
mg/L = milligrams per liter = parts per million
μg/L = micrograms per liter = parts per billion

17.0 Appendix B. UNCW ratings of sampling stations in Wilmington watersheds based on 2008, where available, for chlorophyll *a*, dissolved oxygen, turbidity, and fecal coliform bacteria (human contact standard) based on North Carolina state chemical standards for freshwater or tidal saltwater, *fecal coliform based on contact standard.

G (good quality) – state standard exceeded in ≤ 10% of the measurements
F (fair quality) – state standard exceeded in 11-25% of the measurements
P (poor quality) – state standard exceeded in >25% of the measurements

Watershed	Station	Chlor *a*	DO	Turbidity	Fecal coliforms*
Barnard's Creek	BNC-RR	G	G	G	P
Bradley Creek	BC-CA	G	G	G	P
	BC-SB	F	F	G	P
	BC-NB	G	F	G	P
	BC-76	-	P	G	F
Burnt Mill Creek	BMC-KA1	G	P	G	P
	BMC-KA3	G	F	G	P
	BMC-AP1	F	F	G	P
	BMC-AP3	P	G	G	P
	BMC-WP	P	G	G	P
	BMC-PP	P	P	G	P
Greenfield Lake	GL-LC	-	P	G	-
	GL-JRB	-	P	G	-
	GL-LB	-	P	G	-
	GL-2340	P	P	G	P
	GL-YD	P	F	G	P
	GL-P	P	G	G	P
Hewletts Creek	HC-3	G	P	G	G
	NB-GLR	G	P	F	P
	MB-PGR	G	G	G	P
	SB-PGR	G	P	G	P
	PVGC-9	G	P	G	P
Howe Creek	HW-FP	G	G	G	G
	HW-GP	G	F	G	F
	HW-DT	F	F	G	P
Motts Creek	MOT-RR	G	F	G	P
Smith Creek	SC-CH	-	P	P	G
Whiskey Creek	WC-MLR	G	P	G	G

18.0 Appendix C. GPS coordinates for the Wilmington Watersheds Project sampling stations used during various years.

Watershed	Station	GPS coordinates	
Barnard's Creek	BNC-RR	N 34.15873	W 77.93795
Bradley Creek	BC-CA	N 34.23257	W 77.86658
	BC-CR	N 34.23077	W 77.85235
	BC-SB	N 34.21977	W 77.84578
	BC-SBU	N 34.21725	W 77.85410
	BC-NB	N 34.22150	W 77.84405
	BC-NBU	N 34.23265	W 77.92362
	BC-76	N 34.21473	W 77.83357
Burnt Mill Creek	BMC-KA1	N 34.22207	W 77.88506
	BMC-KA3	N 34.22280	W 77.88601
	BMC-AP1	N 34.22927	W 77.86658
	BMC-AP2	N 34.22927	W 77.89792
	BMC-AP3	N 34.22927	W 77.90143
	BMC-WP	N 34.24083	W 77.92419
	BMC-PP	N 34.24252	W 77.92510
Futch Creek	FC-4	N 34.30127	W 77.74635
	FC-6	N 34.30298	W 77.75070
	FC-8	N 34.30423	W 77.75415
	FC-13	N 34.30352	W 77.75790
	FC-17	N 34.30378	W 77.76422
	FOY	N 34.30705	W 77.75707
Greenfield Lake	GL-SS1	N 34.19963	W 77.92447
	GL-SS2	N 34.20038	W 77.92952
	GL-LC	N 34.20752	W 77.92980
	GL-JRB	N 34.21260	W 77.93140
	GL-LB	N 34.21445	W 77.93553
	GL-2340	N 34.19857	W 77.93560
	GL-YD	N 34.20702	W 77.93120
	GL-P	N 34.21370	W 77.94362
Hewletts Creek	HC-M	N 34.18230	W 77.83888
	HC-2	N 34.18723	W 77.84307
	HC-3	N 34.19023	W 77.85083
	HC-NWB	N 34.19512	W 77.86155
	NB-GLR	N 34.19783	W 77.86317
	MB-PGR	N 34.19807	W 77.87088
	SB-PGR	N 34.19025	W 77.86472
	PVGC-9	N 34.19165	W 77.89175

Howe Creek	HW-M	N 34.24765	W 77.78718
	HW-FP	N 34.25443	W 77.79488
	HW-GC	N 34.25448	W 77.80512
	HW-GP	N 34.25545	W 77.81530
	HW-DT	N 34.25562	W 77.81952
Motts Creek	MOT-RR	N 34.15867	W 77.91605
Pages Creek	PC-M	N 34.27008	W 77.77133
	PC-OL	N 34.27450	W 77.77567
	PC-CON	N 34.27743	W 77.77763
	PC-OP	N 34.28292	W 77.78032
	PC-LD	N 34.28067	W 77.78495
	PC-BDDS	N 34.28143	W 77.79417
	PC-WB	N 34.27635	W 77.79582
	PC-BDUS	N 34.27732	W 77.80153
	PC-H	N 34.27508	W 77.79813
Smith Creek	SC-23	N 34.25795	W 77.91967
	SC-CH	N 34.25897	W 77.93872
Whiskey Creek	WC-NB	N 34.16803	W 77.87648
	WC-SB	N 34.15935	W 77.87470
	WC-MLR	N 34.16013	W 77.86633
	WC-AB	N 34.15967	W 77.86177
	WC-MB	N 34.15748	W 77.85640

19.0 Appendix D. University of North Carolina at Wilmington reports and papers concerning water quality in New Hanover County's tidal creeks.

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