TESTING FOR OPTICAL BRIGHTENERS AND FECAL BACTERIA TO DETECT SEWAGE LEAKS IN TIDAL CREEKS

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Abstract: Three New Hanover County, North Carolina, Tidal Creeks were sampled for the presence of optical brighteners and fecal coliform bacteria. Optical brighteners, compounds added to nearly all modern laundry detergents, may indicate pollution from septic and sewer line leaks. When coupled with fecal bacteria sampling, testing for optical brighteners may strengthen identification of human fecal contamination. Samples were collected from Futch, Hewletts and Bradley Creeks during the months of September, October, and November of 2007. Optical brighteners were analyzed using a fluorometer. Fecal bacteria were analyzed using a membrane filtration (mFC) method. Over the entire data set there was a significant relationship between optical brightener values and fecal coliform bacteria counts. The relationship was also significant for Hewletts and Bradley Creek when analyzed individually, but not for Futch Creek. Hewletts Creek suffered from several sewage spills in 2005 and 2006, and Bradley Creek in 2007. Futch Creek is influenced by septic systems and previous testing found local septic systems functioning properly. Thus, simultaneous testing for fecal bacteria and optical brighteners is a viable procedure for use in detecting human-soured fecal bacteria in water bodies.

Key words: Optical brightener; fecal coliform bacteria, tidal creeks, Southeastern North Carolina, water quality.

INTRODUCTION

Many tidal creeks in the southeastern United States are currently and in some cases rapidly becoming urbanized by housing, marinas, shopping malls, and golf courses (Van Dolah et al. 2008). This urbanization causes an increase in population and coastal tourism (USEPA 1992). Unfortunately, development along tidal creeks often leads to degraded water quality along with increased human health risk (Mallin et al. 2000a; Holland et al. 2004; Van Dolah et al. 2008). Pathogenic enteric bacteria may be sourced from the excrement of humans or animals (Dadswell 1993) and contaminated water can cause human illness or death by means of direct contact or consumption of shellfish (USFDA 1995).

One indicator for estimating fecal pathogenic bacteria presence is the abundance of fecal coliform bacteria (Dadswell 1993; Ford and Colwell 1996; Rees et al. 1998). The North Carolina Water Quality standard for fecal coliform counts is 200 CFU/100mL (human contact waters) and 14 CFU/100 mL (shell fishing waters), with no more than 10% of the samples exceeding 43 CFU/100mL (NCDEHNR 1996). One important source by which fecal coliform bacteria can enter tidal creeks and other coastal waters is stormwater runoff, which has been documented as a significant fecal pollution source in North and South Carolina (Mallin et al. 2000a; Holland et al. 2004).

Spills and Leaks from sewer lines and septic systems represent other pollution source(s) that can negatively affect microbiological water quality in tidal creeks. Spills and major leaks from sanitary sewer systems can be major human health issues and cause significant ecological damage (Mallin et al. 2007). Poorly-placed and/or poorly-functioning septic systems are also a major pollution problem in many areas, including southeastern North Carolina (Cahoon et al. 2006), the Outer Banks (Mallin et al. 2006) and the west coast of Florida (Lipp et al. 2001). Failing septic systems in New Hanover County, North Carolina may also be a pollution source to local water bodies as some 1215 New Hanover county residents still use septic systems (personal correspondence, Catherine Timpy, New Hanover County Health Department). As development continues, additional measures for detecting fecal bacteria and sources in coastal waterways should be included to protect public health. Testing for optical brighteners may prove to be an inexpensive and effective way to locate and address sites of sewer line and septic systems leaks.

Optical brighteners, compounds added to laundry detergents, adsorb to clothing and form a light reflective layer creating the appearance of whiter whites and brighter colors. These compounds are excited by light in the near UV range (360-365nm) and emit light in the blue range (400-440nm). After light absorption, fluorescence is given off during the second excited state and can be measured by a fluorometer. Ninety seven percent of all laundry detergents in the United States contain one or both of two types of fluorescent whitening agents; FWA-1 also called DAS1 or FB-28, or FWA-2 referred to as DSBP or Tinopal CBS-X. Both compounds have been determined as safe for primary consumers. Aside from laundry detergents, optical brighteners are also added to textiles, plastics, synthetic fibers and many kinds of paper. Other uses include medical, chemical and petroleum applications (Hagedorn et al. 2005a). Presence of optical brighteners and fecal coliform bacteria in a waterway may indicate an input of human origin since household plumbing systems combine wastewater from toilets and washing machines. Detection of both fecal coliform bacteria and optical brightener contamination suggests one of four scenarios (Table 1).

Outflow from wastewater treatment plants should neither contain optical brighteners nor fecal coliform bacteria; both are destroyed at the plant by disinfection procedures such as chlorination and UV light. Although it is generally known that optical brighteners biodegrade at a slow rate, studies conflict on their photo-decay rates. (URS Corp. 2004) noted that optical brighteners photo-decay rates. One study noted that optical brighteners photo-decay and biodegrade at relatively slow rates allowing them to be present in a waterway long enough to be detected (URS Corp. 2004). Alternatively, optical brighteners have displayed photo-decay in a matter of hours when exposed to UV light (Kramer et al. 1996). Although it was not the case in our sampling sites, detection of optical brighteners is possible even in areas without laundry facilities since certain soaps and almost all brands of toilet paper contain optical brighteners.

Currently there are several methods for detecting optical brighteners in waterways. The first involves placing cotton pads in waterways (typically smaller streams or creeks), and allowing them to adsorb optical brighteners for a period of time. After collection, pads are dried and exposed to UV light. They will fluoresce if optical brighteners are present. This method is both inexpensive and simple, yet yields low sensitivity (Sargent and Castonguay, 1998). Another approach is high performance liquid chromatography; a method which offers an instrument of high sensitivity yet is expensive and requires a trained technician (Shu and Ding, 2005). The final approach is to use a fluorometer, an instrument that offers ease of use, moderate expense and high sensitivity (Hagedorn et al. 2005b).

SITE DESCRIPTIONS

Bradley Creek, Hewletts Creek, and Futch Creek are three tidal creeks which have been monitored since 1993 by researchers at UNC Wilmington's Aquatic Ecology Laboratory. Each of the three creeks has a salinity gradient between 35 ppt, where they meet the ICW, to fresh water 3-5 km upstream (Fig.1). Primary marsh vegetation is black needlerush *Juncus roemerianus* in the oligohaline areas, and marsh cordgrass *Spartina alterniflora* in the mesohaline to polyhaline areas. The three creeks were chosen due to their watershed population and land use factors. Out of five tidal creeks in New Hanover County, the Bradley and Hewletts Creek watersheds are ranked first and second for population density, developed land and percentage of impervious cover, while Futch Creek is ranked fifth. Futch Creek drains into the ICW and is located on the New Hanover-Pender County line (Fig. 1). Six stations were sampled by boat during 2007 (Fig. 1; Table 2). The Bradley Creek watershed is the largest in the area and drains into the Atlantic Intracoastal Waterway (ICW). Six stations, including both fresh and brackish sites were sampled from shore for fecal coliform bacteria and optical brighteners during fall 2007 (Fig. 1; Table 3). Hewletts Creek drains into the ICW and consists of both tidal and freshwater sites. This watershed has the second highest human population, percent watershed developed area, and percent impervious surface coverage in New Hanover County (Mallin et al. 2000a); it also has frequent elevated fecal bacteria counts and algal blooms (Mallin et al. 2000a; Mallin et al. 2004). Generally speaking, Futch Creek maintains good water quality when compared to other tidal creeks in the county (Mallin et al. 2000a; Mallin et al. 2004).

METHODS

Methodology for detecting optical brighteners in New Hanover County tidal creeks was based on Hartel et al. (2007a), and Hartel et al. (2007b). Optical brightener samples were collected monthly at or near high tide for three months. Bradley and Hewletts Creek were collected from shore, while Futch samples were taken by boat. Optical brightener samples were collected by filling Nalgene 125mL opaque collection bottles 10 cm below the surface midstream facing upstream. Bottles were acid washed and triple rinsed before sampling. Samples were refrigerated in the dark at 8º C and read within eight days. Water was tested for temperature, salinity, dissolved oxygen, conductivity, pH, and turbidity at each site using a YSI 6920 (YSI, Incorporated, Cincinnati, OH).

Fluorometry was performed with a laboratory fluorometer (Model 10-AU-000, Turner Designs, Sunnyvale, California). A kit was added to the fluorometer that included a lamp (10-049) emitting near UV light at 310-390 nm, a filter (10-069R) for the 300-400nm light range, and finally a 436 nm filter was added to greater decrease background fluorescence (Hartel 2007a). A standard curve was created using 100 mg of Tide (Procter and Gamble, Cincinnati, Ohio) in one liter of deionized water. Another standard curve was created using 100mg of Tide in one liter of ambient water from Bradley Creek. Tide is a commonly used laundry detergent known to contain optical brighteners. The fluorometric value of 100 was equal to 100mg of Tide in both 1 L of deionized water and ambient water, when the fluorometer was adjusted to an 80% sensitivity scale. Finding the standard curve with ambient water indicated that fluorescence was from optical brighteners and not organic matter. Each sample was read in triplicate at room temperature after 10 seconds to minimize degradation of optical brighteners by UV light (Hartel 2007b).

Fecal coliform samples were collected by filling pre-autoclaved containers ca. 10 cm below the surface, facing upstream. Samples were stored on ice and processed within six hours. Fecal coliform concentrations were determined using a membrane filtration (mFC) method (APHA 1995); samples were assayed and read in triplicate. Fecal coliform and optical brightener sampling at Futch Creek during the month of November was performed by Coastal Planning and Engineering of North Carolina, Inc. All other sampling was performed by the Aquatic Ecology Lab at UNCW Center for Marine Science.

After all data were compiled, exploratory data analyses, including regression and correlation, were used and scatter plots were constructed to determine if significant relationships existed between optical brightener readings and fecal coliform bacteria counts. Data were normalized by log-transformation and statistically analyzed using JMP to determine if significant correlations existed between optical brighteners and fecal coliform bacteria. Data was analyzed as a whole and also by individual creek.

RESULTS

During the study fecal coliform counts ranged between 1 and 7000 CFU/ 100ml, with the highest value recorded at uppermost station (FC-17). Fluorometric values ranged from 2.0-6.4 (table 2). One possible explanation for the high fecal numbers in the month of November may be that the first recordable rain event (0.89 cm) in eighteen days occurred within 24 hours of sampling. Site FC-17 was no longer sampled under the current tidal creeks program and therefore no data are available for November. No significant correlation between fecal counts and optical brighteners was found in Futch Creek, ($R^2 = -0.073$ at a p-value of 0.8425. This number was not significant at a critical value of <0.05.

Fecal coliform counts from Bradley Creek ranged from 1-1100 CFU/ 100ml; flourometric values ranged from 2.8-27 (table 3). Station BC-SBU was not sampled during the month of October because of lack of water. The October fecal coliform count of 1100 CFU/100ml was considered an outlier and removed from the data set when statistical analyses were performed. The Pearson's Product Moment analysis yielded a correlation coefficient $R^2 =$ of 0.6962 (p-value of 0.003), indicating a significant relationship between optical brightener numbers and fecal coliform bacteria at Bradley Creek (Fig. 3).

Hewlett's Creek fecal coliform counts ranged from 2-1115 CFU/ 100ml and flourometric values ranged 1.8-25.7 (table 4). The Pearson's Product Moment correlation coefficient (R^2) found a value of 0.8837 (p-value of 0.0001), indicating a highly significant relationship between optical brightener values and fecal coliform bacteria at Hewletts Creek during time of our study (Fig. 4).

A correlation analysis of the entire data set yielded a Pearson Product Moment correlation coefficient of $r^2 = 0.324348$ (p value of 0.0248) indicating that there was a significant correlation between overall optical brighteners and fecal coliform bacteria within the three combined creeks. A scatter plot and regression analysis indicated a significant but weak relationship existed between the two parameters for the combined data sets (Fig. 5).

DISCUSSION

The strong correlations between fecal coliform abundance and optical brightener concentrations found at Hewletts Creek and Bradley Creek indicated that some of the fecal bacterial contamination in these creeks likely came from human wastewater in the form of sewage leaks or spills or septic system leakage. Hewletts Creek has suffered several sewage spills over the last three years. We note that the last publicized major spill was in November 2006, nine months before our investigation, but construction of the new line was ongoing during our sampling. (Burkholder et al. 1997) and (Mallin et al. 2007) indicated that fecal and enterococcus bacteria counts will persist in the sediments longer than formerly believed, while optical brighteners degrade slowly if not exposed to UV light (URS Co. 2004). It is possible that the optical brighteners detected at Hewletts Creek and Bradley Creek could be sourced from either past spills or current sewer line leaks.

Stormwater runoff is likely the principal source of fecal bacteria to Bradley Creek because of the high percentage of developed land and impervious cover in the watershed (Mallin et al. 2000a). In terms of fecal bacteria, Bradley Creek is considered one of the most polluted creeks in the county (Mallin et al. 2000a). It was first closed to shellfishing in 1947, and among the county's five tidal creeks, it contains the highest population, percentage of developed land, and percentage of impervious cover (Mallin et al. 2000a). A sewage spill occurred in the north branch of this creek near Wrightsville Avenue in July 2007. A recent PCR-based study performed at the UNCW's Aquatic Ecology Lab indicated human sourced fecal bacteria in water samples taken at station BC-NBU in both July and August 2006, and at station BC-SB in June 2006 (Spivey et al. unpublished).

Fluorometric values found at Futch Creek were consistently low each month, with high fecal counts occurring only after a rain event. The data implied a low likelihood of human sourced fecal bacteria with past data supporting this theory. In an effort to reduce fecal coliform bacterial concentrations by increasing circulation from the ICW, two channels were dredged at the mouth of Futch Creek during 1995 and 1996. The results of this project included a significant increase in salinity in the creek and significantly lower fecal counts which allowed the lower creek to be reopened for shellfishing (Mallin et al. 2000b). The septic systems used by Futch Creek residents were examined concurrent with a study on the effects of dredging (Mallin et al. 2000b); no infrastructure problems were found. Fecal bacteria found in Futch Creek are most likely coming from the high population of wildlife which reside there.

Optical brighteners are not the only chemicals, synthetic or man-made that fluoresce. Other substances including organic matter, radiator flush, and a variety of substances from pulp and paper production also fluoresce (summarized in Hartel et al. 2007b). Measures taken to cut out background fluorescence from organic matter in the fluorometric process as described in the methods. It would be advisable for further experiments to elaborate on this process, searching for additional ways to decrease background fluorescence. Further improvement to the source investigation process should include a thorough investigation of the watershed in question, utilizing, if available, maps containing detailed and accurate information on septic system and sewer line locations. A further refinement could include collecting samples at low tide instead of high tide. Although it may make sampling more difficult (in the New Hanover tidal creeks boat passage is impossible at low tide), it will decrease dilution caused by tidal waters, making sources easier to locate. A further step, although currently expensive and time consuming, would be to test the fecal bacteria samples using one or more chemical, genotypic, or phenotypic methods and determine what kind of animal (human, bird, mammal, etc.) the bacteria was sourced from.

A significant relationship was not found between optical brightener values and fecal coliform bacteria counts at Futch Creek. However, significant positive correlations between these two parameters were identified at sewage-impacted Hewletts Creek and Bradley Creek. These experiments further elaborate the value of using optical brighteners (Hagedorn et al. 2005a; Hartel et al. 2007a) as a tool in detecting human sources of fecal microbial pollution in waterways.

Past data has indicated a strong inverse relationship between fecal coliform counts and salinity in area tidal creeks. This relationship is thought to be due to decreased coliform survival in higher salinity waters, increased flushing near the Intracoastal Waterway (ICW) and headwaters being closer to pollution sources (Mallin et al. 2000a).

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FIGURE CAPTIONS

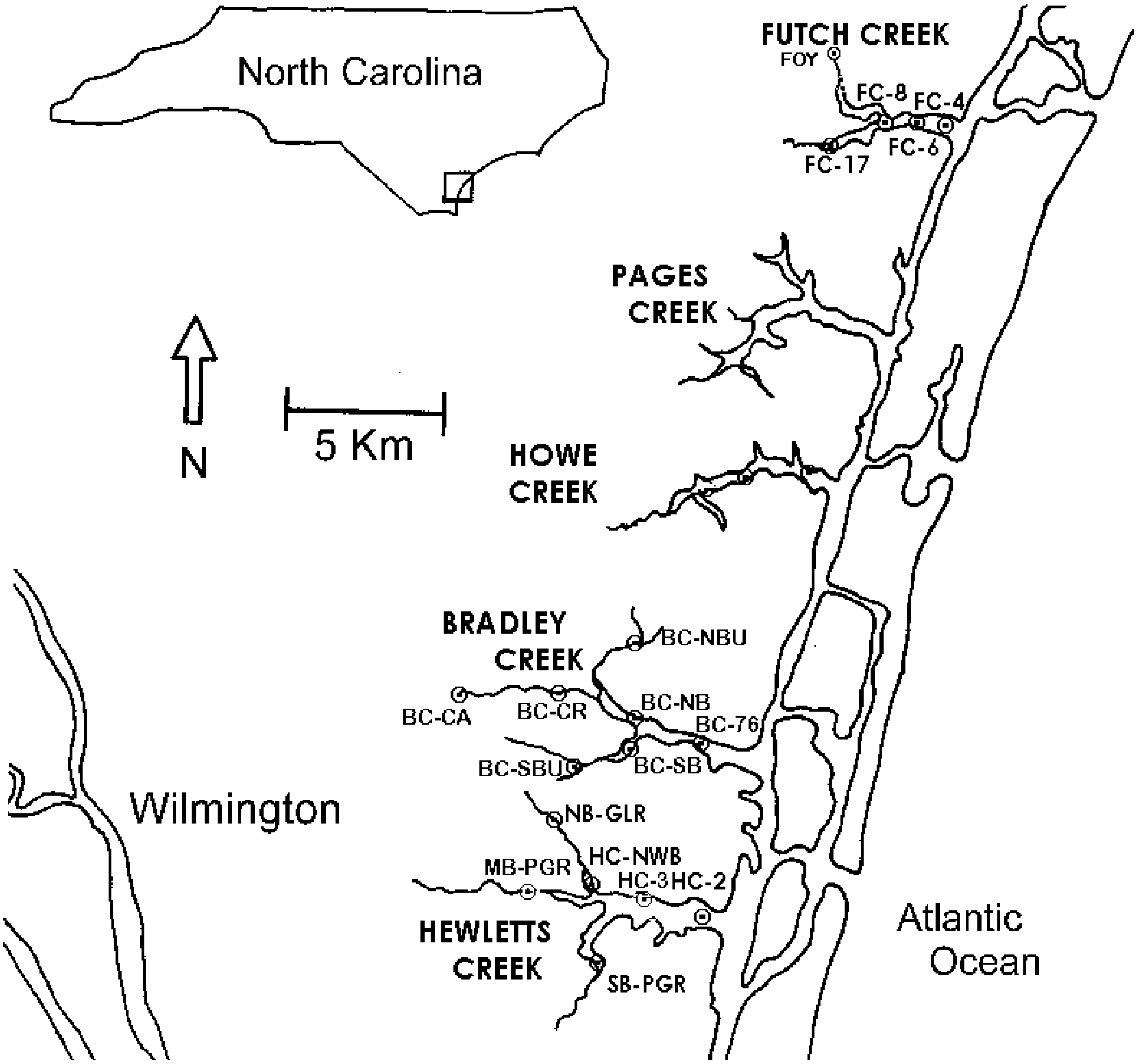
FIG. 1. Tidal creek system in New Hanover County, North Carolina, USA. Abbreviations are: CFR, Cape Fear River, FUT, Futch Creek; PAG, Pages Creek; HOW, Howe Creek; BRD, Bradley Creek; HEW, Hewletts Creek; WIS, Whiskey Creek; WB, Wrightsville Beach.

FIG.2. Scatter plot displaying the non-significant relationship between optical brightener values and fecal coliform bacteria at Futch Creek.

FIG. 3. Scatter plot displaying the positive relationship between optical brighteners and fecal coliform bacteria at Bradley Creek.

FIG. 4. Scatter plot displaying the positive relationship between optical brighteners and fecal coliform bacteria at Hewletts Creek.

FIG. 5. Scatter plot of optical brightener values vs. fecal coliform bacteria when data from all three creeks were combined


North Carolina
FUTCH CREEK
FOY
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FC-4
FC-6
FC-17
PAGES
CREEK
N
5 Km
HOWE
CREEK
BRADLEY
CREEK
BC-NBU
BC-CA
BC-CR
BC-NB
BC-76
BC-SBU
BC-SB
Wilmington
NB-GLR
HC-NWB
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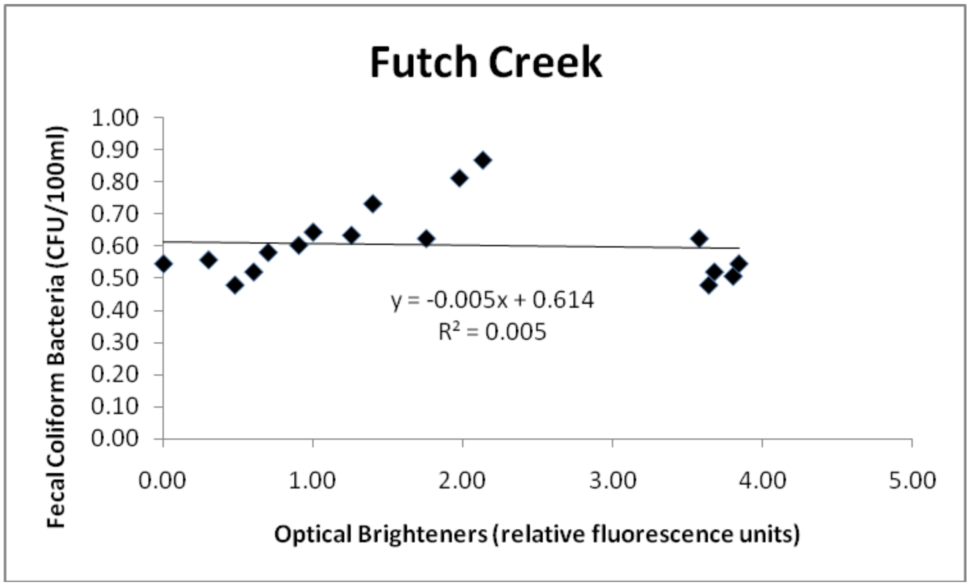
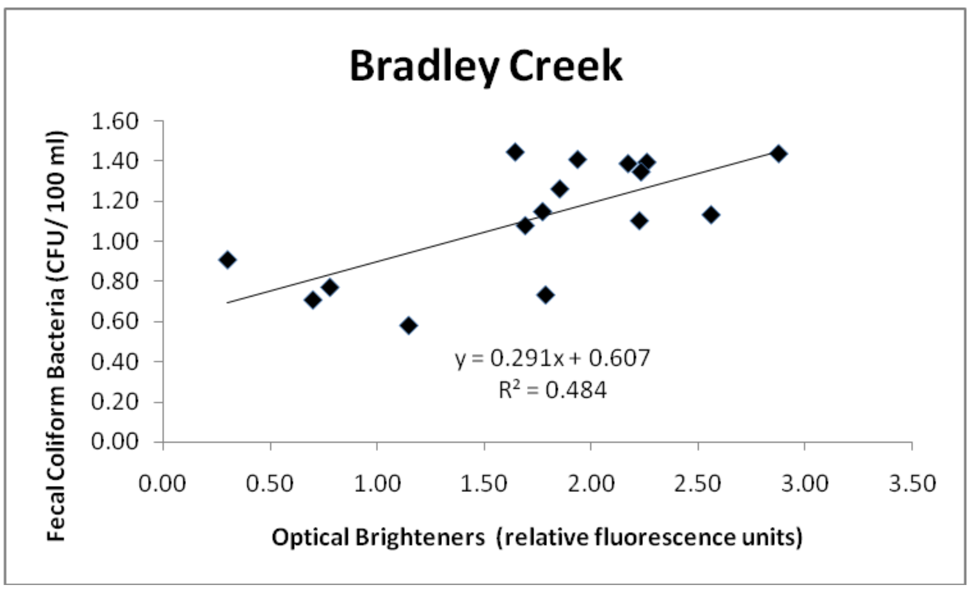
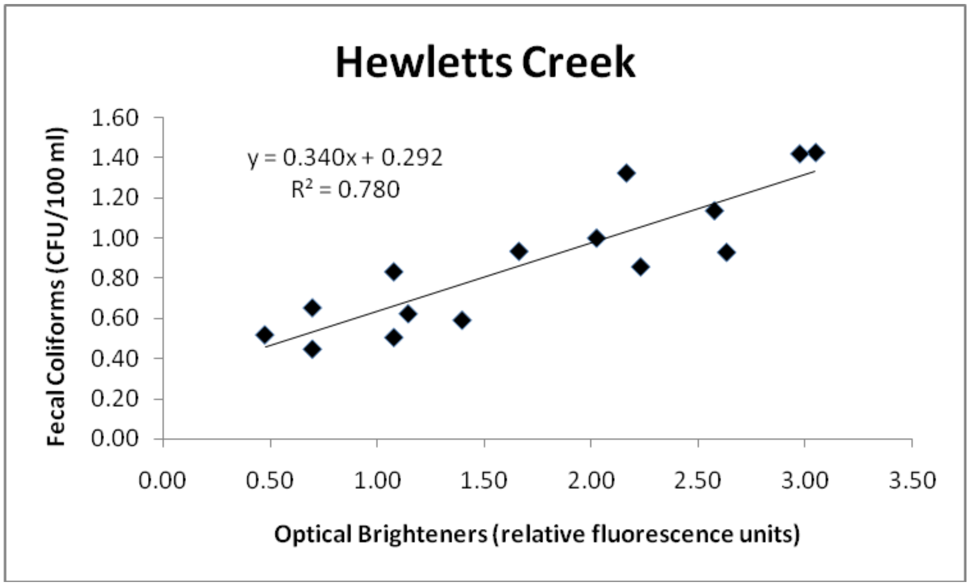
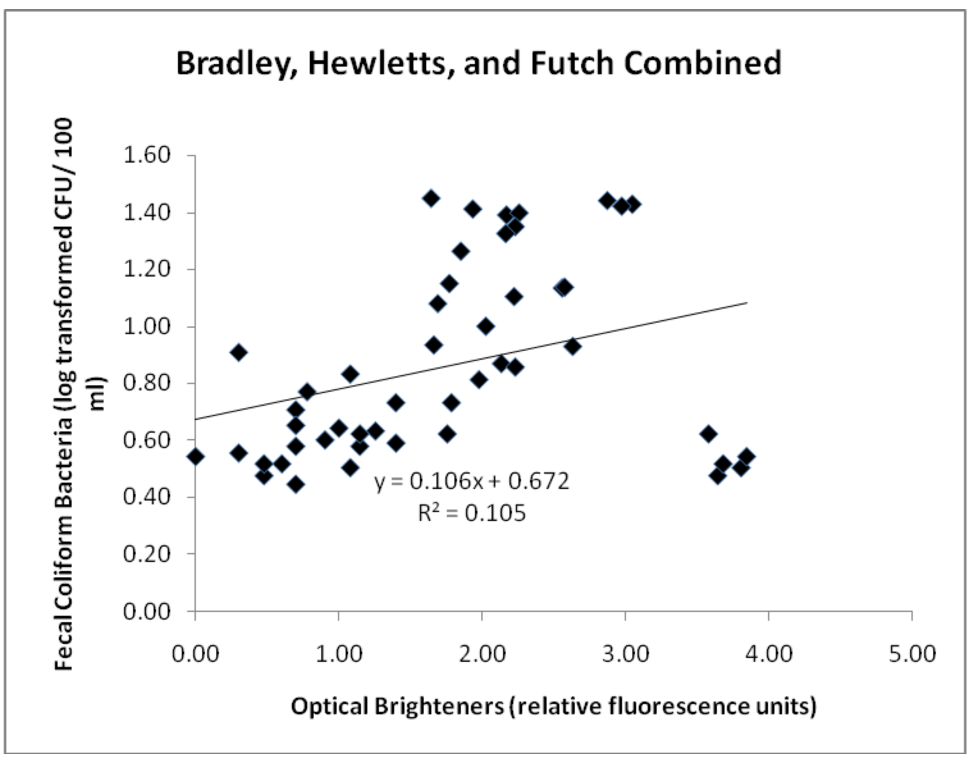

Futch Creek
Fecal Coliform Bacteria (CFU/100ml)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
1.00
2.00
3.00
4.00
5.00
Optical Brighteners (relative fluorescence units)
y = -0.005x + 0.614
R² = 0.005


Bradley Creek
Fecal Coliform Bacteria (CFU/ 100 ml)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
Optical Brighteners (relative fluorescence units)
y = 0.291x + 0.607
R² = 0.484



Hewletts Creek
y = 0.340x + 0.292
R² = 0.780
Fecal Coliforms (CFU/100 ml)
Optical Brighteners (relative fluorescence units)
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
0.50
1.50
2.00
2.50
3.00
3.50



Bradley, Hewletts, and Futch Combined
y = 0.106x + 0.672
R² = 0.105
Fecal Coliform Bacteria (log transformed CFU/ 100 ml)
Optical Brighteners (relative fluorescence units)
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
2.00
3.00
4.00
5.00

Table 1
Probable cause for combined fecal bacteria and optical brightener contamination.
(modified from Hartel et al. 2007a).

Fecal bacteria	Optical brightener	Probable cause
High	High	Sewer pipe leak or failing septic system
High	Low	Other warm-blooded mammal source or human waste from another source or outhouse
Low	High	Gray water in storm water system
Low	Low	Background fluorescence of insignificant contamination

Table 2. Futch Creek fecal coliform (FC) and optical brightener (OB) data. FC as CFU/100 mL, OB as mean of three readings.

2007	Station	FC	OB as mean ± SD of three readings
9/13	Fc-4	7	3.0± 0.1
10/31	Fc-4	2	2.0± 0.0
11/16	Fc-4	3800	3.2± 0.1
9/13	Fc-6	56	3.2± 0.1
10/31	Fc-6	3	2.3± 0.0
11/16	Fc-6	6400	2.2± 0.1
9/13	Fc-8	17	3.3± 0.1
10/31	Fc-8	0	2.5± 0.1
11/16	Fc-8	4800	2.3± 0.0
9/13	Fc-13	94	5.5± 0.0
10/31	Fc-13	1	2.6± 0.1
11/16	Fc-13	4400	2.0± 0.0
9/13	Fc-17	135	6.4± 0.1
10/31	Fc-17	9	3.4± 0.0
9/13	FOY	24	4.4± 0.0
10/31	FOY	4	2.8± 0.0
11/16	FOY	7000	2.5± 0.1

Table 3. Bradley Creek fecal coliform (FC) and optical brightener (OB) data. FC as CFU/100 mL, OB as a mean of three readings.

Date	Station	FC	OB as mean ± SK of three readings
9/21	BC-76	1	7.1± 0.1
10/16	BC-76	4	4.1± 0.1
11/27	BC-76	13	2.8± 0.1
9/21	BC-SB	43	27.0± 0.1
10/16	BC-SB	166	11.7± 0.1
11/27	BC-SB	58	13.1± 0.1
9/21	BC-NB	147	23.5± 0.1
10/16	BC-NB	5	4.9± 0.1
11/27	BC-NB	60	4.4± 0.1
9/21	BC-SBU	745	26.5± 0.5
11/27	BC-SBU	85	24.7± 0.6
9/21	BC-CR	360	12.6± 0.2
10/16	BC-CR	1100	8.2± 0.2
11/27	BC-CR	48	11.0± 0.2
9/21	BC-NBU	180	23.9± 0.2
10/16	BC-NBU	70	17.3± 0.3
11/27	BC-NBU	169	21.3± 0.2

Table 4. Hewletts Creek fecal coliform (FC) and optical brightener (OB) data. FC as CFU/100 mL, OB as mean of three readings.

Date	Station	FC	OB	Standard deviation
9/13	HC-2	2	2.3	0.1
10/15	HC-2	24	2.9	0.1
11/25	HC-2	4	1.8	0.1
9/13	HC-3	13	3.2	0.1
10/15	HC-3	4	3.5	0.1
11/25	HC-3	11	2.2	0.1
9/13	MB-PGR	1115	25.7	0.5
10/15	MB-PGR	940	25.3	0.2
11/25	MB-PGR	145	20.1	0.2
9/13	NB-GLR	375	12.7	0.1
10/15	NB-GLR	45	7.6	0.1
11/25	NB-GLR	427	7.5	0.1
9/13	SB-PGR	105	9.0	0.1
10/15	SB-PGR	11	5.8	0.0
11/25	SB-PGR	169	6.2	0.1