Exposing water samples to ultraviolet light improves fluorometry for detecting human fecal contamination

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ABSTRACT

Fluorometry identifies human fecal contamination by detecting optical brighteners in environmetal waters. Because optical brighteners are sensitive to sunlight, we determined if we could improve fluorometry by exposing water samples to ultraviolet (UV) light to differentiate between optical brighteners and other fluorescing organic compounds. Optical brighteners were likely present when the relative percentage difference in fluorometric value of the water before and after UV light exposure was >30% (glass cuvettes, 30 minute exposure) or >15% (polymethacrylate cuvettes, 5 minute exposure). In a blind study, we correctly identified the presence or absence of optical brighteners in 178 of 180 (99%) of the samples tested with a more expensive field fluorometer and in 175 of 180 (97%) of the samples tested with a less expensive handheld fluorometer. In the field, the method correctly identified two negative and three positive locations for human fecal contamination. When combined with counts of fecal bacteria, the new fluorometric method may be a simple, quick, and easy way to identify human fecal contamination in environmental waters.

INTRODUCTION

Microbial source tracking (MST) identifies sources of fecal contamination in environmental waters using a variety of chemical, genotypic, and phenotypic methods. Our efforts have focused on developing inexpensive MST methods because most communities we advise cannot afford expensive methods. As a result, we developed targeted sampling (Kuntz *et al.* 2003), a MST method that, in conjunction with local knowledge, uses multiple samplings over ever-decreasing distances to identify hotspots of fecal contamination. This sampling minimizes bacterial genotypic changes with flow (Hartel *et al.* 2004), time (Jenkins *et al.* 2003), geography (Hartel *et al.* 2002), and animal diet (Hartel *et al.* 2003). Targeted sampling can identify most sources of fecal contamination quickly and easily based on simple observation.

However, targeted sampling does not work when a) a single source is not visually obvious or b) multiple sources are observed. Therefore, we were interested in combining targeted sampling with other inexpensive MST methods to address these two conditions. Because leaking sewer lines and malfunctioning septic drainfields are easier to remedy than most other sources of fecal contamination (e.g., wildlife), we focused first on inexpensive methods to identify human fecal contamination.

One potentially inexpensive MST method for identifying human fecal contamination is detecting optical brighteners (also called fluorescent whitening agents) in environmental waters. These optical brighteners come primarily from laundry detergents, where the brighteners in the detergent emit light in the blue range (415 to 445 nm), thereby compensating for undesirable yellowing in clothes (Kaschig 2003). In the United States, 97% of laundry detergents contain the optical brightener DSBP (4,4'-bis (2-sulfostyry) biphenyl) and DAS1 (4,4'-diamino-

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2,2'-stilbene-disulfonic acid; Hagedorn *et al.* 2005). Because household plumbing systems mix effluent from washing machines and toilets together, optical brighteners are associated with human sewage in septic systems (Boving *et al.* 2004) and wastewater treatment plants (Poiger *et al.* 1998). The idea, then, was to combine targeted sampling with detecting optical brighteners to identify human fecal contamination. With this combination, there are four possible contamination scenarios: 1) high concentrations of optical brighteners and high counts of fecal bacteria, which suggests a malfunctioning septic drainfield or leaking sewer pipe, 2) high concentrations of optical brighteners and low counts of fecal bacteria, which suggests gray water in the storm water system, 3) low concentrations of optical brighteners and high counts of fecal bacteria, which suggests other warm-blooded animals or a human source from something like an outhouse, and 4) low concentrations of optical brighteners and low counts of fecal bacteria, which suggests no source of fecal contamination.

There are three possible approaches for detecting optical brighteners in water, each with its own advantages and disadvantages. The first approach is to leave cotton pads in environmental waters for a period of time (thereby taking advantage of optical brighteners' affinity to bind to cellulose) and then to expose the pads to UV light (Sargent and Castonguay 1998). If optical brighteners are present, then the pads will fluoresce. This approach is inexpensive and easy to use, but has low sensitivity. The second approach is to use high performance liquid chromatography (HPLC; Shu and Ding 2005), a method that has excellent sensitivity, but is expensive and uses an instrument that requires a high degree of technical skill to operate. The third approach is to use a fluorometer, an instrument that is relatively inexpensive, easy to use, and has excellent sensitivity (Hagedorn *et al.* 2005). Given its apparent lack of disadvantages, this instrument was the one chosen for our studies.

Unfortunately, when fluorometry is combined with counts of fecal bacteria, the results can be contradictory. On the one hand, there are several instances when combining fluorometry and bacterial counts were successful (e.g., Kerfoot and Skinner 1981, Hagedorn *et al.* 2005, McDonald *et al.* 2006); on the other hand, there were other instances when combining fluorometry and bacterial counts were unsuccessful (e.g., Close *et al.* 1989, Wolfe 1995).

Although there are undoubtedly cases when combining fluorometry and bacterial counts were unsuccessful because the sampling was simply too far from the suspected source, the most likely reason for these failures is background fluorescence from other organic compounds, especially organic matter (Wolfe 1995).

For organic compounds other than organic matter, several anthropogenic compounds are known to fluoresce. For example, radiator flush water fluoresces (Pitt 2001), as do a variety of compounds from pulp and paper production, and food processing plants (Gregor *et al.* 2002). For organic matter, it has long been known that organic matter in water fluoresces when exposed to UV light (Kalle 1949), and in fact, fluorescence has been used to measure total organic carbon in water (Smart *et al.* 1976). One partial solution for organic matter-rich waters is to change the emission filter in the fluorometer from a broad spectrum (410 to 600 nm) to a narrow spectrum filter (e.g., 436 nm). Because organic matter has broadband, featureless emission spectra (Chen and Bada 1992) and the emission spectra of optical brighteners are in the 415- to 445-nm range, changing the filter to this narrow wavelength reduces background organic matter fluorescence by >50% (Hartel *et al.* In press-a).

Nevertheless, even with a fluorometer equipped with a narrow wavelength emission filter, background fluorescence from organic compounds is still a problem. One simple solution may be to expose the water samples to UV light. Because optical brighteners photodecay in a matter of hours when exposed to sunlight (Kramer *et al.* 1996), it may be possible to exploit the differences in photodecay rates between optical brighteners and these other organic compounds. Therefore, in hopes of improving fluorometry for MST, we conducted a study to determine the effect of UV light exposure on water samples with different concentrations of optical brighteners and organic matter, as well as a number of organic compounds likely to be present in environmental waters. In the case of field studies where the concentrations of optical brighteners were unknown, we tried to confirm the fluorometric data by measuring the optical brightener concentrations with HPLC. Finally, in keeping with the idea of making fluorometry as inexpensive as possible, we also tested a relatively inexpensive handheld fluorometer as a possible substitute for a more-expensive field fluorometer.

METHODS

Sampling Locations

There were five sampling locations in the United States, two in Georgia and three in Virginia. Locations identified as having high fecal enterococcal counts were those that exceeded the USEPA maximum of 104 fecal enterococci per 100 ml for a grab sample (USEPA 2002a). In Virginia, sites identified as having high fluorescence were those water samples that exceeded a limit of 100 fluorometric units (Dickerson *et al.* In press). For the sake of consistency, the same limit was adopted in Georgia.

The two Georgia locations were the Barbour Island River, next to Barbour Island (31°35'00"N, 81°14'05"W), and a storm drain located in St. Simons Village (31°08'06"N, 81°23'25"W) on St. Simons Island. Barbour Island River is located to the north of Sapelo Sound and to the west of St. Catherines Island on the Georgia Coast. This tidal river is the site of commercial clam beds, and its waters have historically had low numbers of fecal indicator bacteria (<14 fecal coliforms per 100 ml; Brooks Good, Georgia Department of Natural Resources, personal communication). Fluorometry has never been conducted at this site. This site was chosen as the first negative control. St. Simons Island, one of Georgia's barrier islands, is approximately 17 km long and 4 km wide at its widest point. At the southern end of the island is St. Simons Village, which had a storm drain previously identified as having fecal contamination with humans (Hartel *et al.* In press-a). The human fecal contamination was confirmed with the esp gene (Scott *et al.* 2005).

The three locations in Virginia were all beaches located on the same peninsula containing the cities of Newport News, Hampton Roads, and Hampton. The first beach, Hilton Beach on the northern shore of the James River, is on the west side of the peninsula; the second beach, Anderson Beach near the confluence of the James River with the Chesapeake Bay, is on the tip of the peninsula; and the third beach, Buckroe Beach on the western shore of the Chesapeake Bay, is on the east side of the peninsula. All three beaches are within 10 km of each other.

Hilton Beach (37°01'67"N 76°27'88"W) is located in an older residential neighborhood of Newport News, and includes approximately 75 m of waterfront. Effluent containing high enterococcal counts and high fluorescence from a storm drain near

the eastern end of the beach had been previously identified as having fecal contamination from humans in 2004 and was remediated in 2005 (Dickerson *et al.* In press). The human fecal contamination was confirmed with antibiotic resistance analysis. Anderson Beach (37°14'20"N, 76°30'39"W) is located in Hampton Roads and includes approximately 800 m of waterfront. Unlike Hilton Beach, effluent containing high enterococcal counts but low fluorescence from several sampling sites had been previously identified as having human fecal contamination in 2004, but had been remediated in 2005 (Dickerson *et al.* In press). Finally, Buckroe Beach (37°02'81"N 76°17'25"W) is located in Hampton and includes some 1500 m of shoreline. Its waters had low enterococcal counts and low fluorescence, and human fecal contamination has not been observed at this location (Dickerson *et al.* In press). Buckroe Beach was chosen as the second negative control.

Sample Collection

Georgia locations were sampled in 2006; Virginia locations were sampled in 2004 (before remediation) and 2005 (after remediation). At each sampling location, four chemical and physical characteristics, pH, salinity, temperature, and dissolved oxygen, were recorded with a Hydrolab Quanta (Austin, TX). A fifth characteristic, total organic carbon (TOC), was determined with a high-temperature combustion method (Method 5310B; Clesceri *et al.* 1998). Single grab samples for TOC (>10 ml) were collected in sterile glass containers, and the containers were placed on ice in a cooler for transport to the laboratory. At the laboratory, the samples were frozen at -20°C until they were thawed for analysis. Because the packing material in the HPLC column was 0.5 µm, all thawed water samples were passed through a 0.45-µm filter. By definition (Clesceri *et al.* 1998), this filtration removed particulate organic carbon (POC), and therefore, dissolved organic carbon (DOC), not TOC, was actually measured.

For bacterial counts and fluorometry, duplicate water samples were collected aseptically in stand-up, 120-ml (4 oz.) Whirl-Pak bags (Nasco, Modesto, CA) and were placed on ice in a cooler for transport to the laboratory. Samples for bacteria and fluorometry were kept in the dark at 4°C and were processed within 6 and 24 hours, respectively.

The fecal indicator bacteria for estuarine and marine waters are the fecal enterococci (USEPA

2002a). In Georgia, numbers of fecal enterococci were estimated with a Most-Probable-Number (MPN; Enterolert system, IDEXX Laboratories, Westbrook, ME); in Virginia, numbers of fecal enterococci were counted with membrane filtration. For MPN analyses, water samples were diluted with sterile distilled water to 10^{-1} in sterile manufacturer-supplied polystyrene bottles and were treated as per the manufacturer's instructions. The number of fluorescing (positive) wells was converted to a MPN value based on the dilution factor and manufacturer-supplied MPN tables.

For membrane filtration analyses, duplicate water samples were serially diluted, and each dilution was passed separately through a 0.45-µm filter. Each filter was transferred to a 50-mm Petri dish containing mEI agar (Becton-Dickinson, Sparks, MD), and was incubated for 24 hours at 41°C (Method 1600; USEPA 2002b). After incubation, each presumptive enterococcal colony (those with blue halos) was picked with a sterile toothpick, and a portion of the colony was transferred to a separate well in a 96-microwell plate, each well containing 0.2 ml Enterococcosel (Becton-Dickinson) broth. After incubating the plate for 24 hours at 37°C, all wells exhibiting a black color (esculin hydrolysis positive) were recorded as positive for enterococci.

Fluorometry

Fluorometry was conducted with two fluorometers, one a more expensive field fluorometer (Model 10-AU; Turner Designs, Sunnyvale, CA) and the other, a less expensive handheld fluorometer (Aquafluor; Turner Designs). Both fluorometers were originally set to detect long wavelength optical brighteners (excitation, 360 nm; emission, 410 to 600 nm) as described by the manufacturer. The emission filter was replaced with a 436-nm emission filter in the field fluorometer and a 445-nm emission filter in the handheld fluorometer. This narrow wavelength reduces background organic matter fluorescence in locations where organic matter concentrations are high (Hartel *et al.* In press-a).

To calibrate the field fluorometer, the sensitivity was set to the medium range. In Georgia, the negative control was distilled water and the positive control was distilled water amended with 100 µl of commercial liquid laundry detergent (Tide, Proctor and Gamble, Cincinnati, OH) L^{-1}. At a sensitivity setting of 50%, 100 µl of detergent L^{-1} was equal to a fluorometric value of 100 units. In Virginia, the calibration of the field fluorometer was done with an actual optical brightener as described by Dickerson *et al.* (In press). Sampling was conducted in both the flow-through (field fluorometer only) and discrete sampling modes. In the case of discrete samples for the field fluorometer, individual water samples were analyzed in 25 inside diameter (ID) by 150-mm glass cuvettes (Turner Designs). These cuvettes require a minimum water sample of 30 ml. The handheld fluorometer was calibrated in the same way, except that the instrument had no sensitivity setting and the discrete samples were analyzed in square (10 by 10 mm) by 48 mm (height) polymethacrylate cuvettes. These cuvettes required a minimum water sample of 3.5 ml. Water samples were brought to room temperature (20 to 25°C). For the field fluorometer, water samples were read within 30 seconds to avoid heating effects by the internal UV lamp; for the handheld fluorometer, the instrument recorded the fluorometric value automatically within a few seconds.

Fluorometer Laboratory Experiments

Five laboratory experiments were conducted, the first four with organic matter, and the fifth with other organic compounds. In the first experiment, the optimal photodecay time of optical brighteners was determined using the glass cuvettes of the field fluorometer and exposing the sample to varying times of UV light. There were four concentrations of organic matter (0, 10, 20, and 40 mg L^{-1}) and three concentrations of commercial detergent (0, 50, and 200 µl L^{-1}). The organic matter was Suwannee River Natural Organic Matter (52.5% C, International Humic Substances Society, St. Paul, MN). Samples were mixed in distilled water and were prepared in two sets, one exposed to UV and one not. Two UV lights (Model ULV-225D, Ultra Violet Products, Upland, CA) were used, each light containing two 25-watt bulbs, which irradiated the samples at 365 nm. Cuvettes were sandwiched between the two lights in an upright, handmade test tube rack, which held the cuvettes at their top and bottom edges to minimize any shading. Because organic matter fluorescence is temperature dependent (Smart *et al.* 1976), all studies were conducted in a 20°C incubator with fans under the UV lights to reduce any heat buildup. Non-UV samples were treated in the same manner as the UV treatment except the UV lights were turned off. After an initial fluorometric reading, fluorometric values were recorded every 15 minutes for 2 hours, and once at 3 and 4 hours. Each combination of laun-

dry detergent and organic matter was done in triplicate.

In the second experiment, the first experiment was repeated with the handheld fluorometer. There were two other modifications. First, this fluorometer used polymethacrylate cuvettes (Sigma–Aldrich, St. Louis, MO), and because they are transparent to UV light, the UV exposure times were reduced to 0, 5, 10, 15, and 30 minutes. Second, given the small drop in fluorometric values for the non-UV treatment over the 4-hour limit in the first experiment and the short exposure time in this experiment, the non-UV treatment was not done.

After determining the optimal UV exposure times in the first two experiments, the third experiment used these times to determine the relative percentage difference in fluorometric values before and after UV light exposure. Separate portions of the experiment were conducted for laundry detergent and for two natural organic matters. In the first portion, 11 different concentrations of commercial detergent, from 0 to 100 µl L^{-1} in 10 µl increments, were either exposed to UV light or left unexposed. This portion was conducted with the field fluorometer only. In the second portion, six concentrations (0, 5, 10, 15, 20, and 25 mg L^{-1}) of Nordic Reservoir (53.2% C, International Humic Substances Society) and Suwannee River Natural Organic Matters were either exposed to UV or left unexposed. This portion of the experiment was conducted with both the handheld and field fluorometers. Fresh preparations of laundry detergent and organic matters were kept in the dark to minimize inadvertent UV light exposure before the start of the experiment. Four replicates were done for both portions of the experiment.

In the fourth experiment, a single blind experiment was conducted to determine the presence or absence of optical brighteners in water samples containing known amounts of organic matter and laundry detergent. Both the field and handheld fluorometers were tested. Distilled water was amended 0, 10, or 20 mg Suwannee River Natural Organic Matter L^{-1} and then spiked either 50 or 100 µl laundry detergent L^{-1} or left unspiked. For each organic matter concentration, 15 cuvettes were spiked with one of the two laundry detergent concentrations and 15 cuvettes were left unspiked. Each cuvette was numbered randomly between 1 and 30 before giving the cuvettes to another investigator with no prior knowledge of the sample. The second investigator determined if the sample contained optical brighteners or not based on the appropriate exposure to UV

light for the handheld or field fluorometer. In this manner, any false positive and false negatives could also be identified.

In the fifth experiment, various automotive and industrial compounds likely to be in urban environmental waters were tested for their fluorescence before and after UV light exposure. Eight car care products, fourteen car fluids, and two laundry detergents were each purchased locally. One septic tank effluent was obtained from a Virginia Tech onsite research facility in Kentland, VA, and two sewage influents and effluents were each obtained from wastewater treatment plants located in Christiansburg and Radford, VA. Optical brighteners, DSBP-like Tinopal CBS-X (4,4' distyryl biphenyl) and DAS1, were obtained from Sigma–Aldrich (St. Louis, MO) as positive controls. The products were added to water to determine if the compound fluoresced or not. Fluorescence was measured with the field fluorometer only, which was outfitted with the 436-nm emission filter. To expose each cuvette to UV light, each cuvette was placed in a tray that held the cuvettes at their top and bottom edges to minimize any shading. The tray was placed on a shelf at an angle and irradiated at 365 nm for 4 hours with a commercial UV system (Black-Ray XX15BLB UV, Ultra Violet Products). All studies were conducted in an air-conditioned room (20°C) to reduce any heat buildup, and all samples were tested in duplicate.

Fluorometer Field Experiment

In environmental waters, the concentrations of DOC and the optical brighteners are unknown. Therefore, environmental water samples were tested for DOC according to standard methods (Clesceri *et al.* 1998), and the concentrations of optical brighteners were determined with HPLC.

For HPLC, a stock solution of DAS1 (1000 µg ml^{-1}), as well as mixtures of all working standards, were prepared in HPLC-grade acetonitrile. Stock solutions were stored at -20°C in the dark. To extract optical brighteners from environmental waters, a glass fiber filter (0.45-µm pore size, 25-mm diameter, Whatman, Florham, NJ) was prerinsed with 10 ml of HPLC-grade methanol, followed by 10 ml of HPLC-grade water. Each environmental water sample was brought to room temperature and was passed through a prerinsed filter. The water was adjusted to pH 8.0 with 0.1 *N* NaOH. Solid phase extraction C18 cartridges (Extra-Clean, 1.0 g, surface area 493 m g^{-1}, Alltech, Deerfield, IL)

were pre-rinsed once with 10 ml of HPLC-grade methanol and twice with 10 ml of HPLC-grade water. A 60-ml syringe and syringe adapter were attached to the cartridge, and the entire assembly mounted on a vacuum manifold. A total of 100-ml water sample or standard was added to the syringe assembly, which was pulled through the cartridge under vacuum at a flow rate of 1 to 2 drops per second. After the sample had passed through the cartridge, a 10-ml sample of HPLC-grade water was added to the syringe assembly. The extracted water was discarded and a 10-ml disposable glass test tube placed under each syringe assembly. Optical brighteners were eluted with 5 ml of acetonitrile (flow rate, <1 drop per second) into the disposable glass test tube. The test tubes were placed in a 40°C water bath, and the eluate in each tube was evaporated to dryness under a gentle stream of nitrogen gas. The test tube was placed on ice (to minimize evaporation) and 200 µl of acetonitrile was added to the test tube. After vortexing, each resuspension was transferred with a glass Pasteur pipet to a 250-µl polypropylene insert contained in a 2-ml autosampler vial. The vial was sealed with a cap containing a Teflon-lined insert.

Samples were analyzed with a SpectraSystem HPLC (Thermo Finnigan, San Jose, CA) consisting an autosampler (Model AS3000) integrated with a gradient pump (Model P4000) and Hypersil column (10 cm x 0.46 cm ID, 5 µm packing, Agilent, Bellefonte, PA). The mobile Phase A consisted of 100% HPLC-grade acetonitrile and mobile Phase B consisted of 100% HPLC grade water. The flow rate was 0.4 ml minute^{-1} and the injection volume was 20 µl. Elution was performed at room temperature (18 to 22°C) with a 1:1 acetonitrile:water solution switching to 1:2 acetonitrile:water in 10 minutes. A fluorescence detector (Model FL3000), operating at an excitation wavelength of 350 nm and an emission wavelength of 430 nm, was used to detect any fluorescence. The chromatographic data were analyzed with a proprietary software program (ChromQuest 4.0 System).

Solid phase extraction did not did not eliminate the fluorescent signal from organic matter. To determine how much fluorescent signal from organic matter was still remaining after extraction, HPLC-grade water was amended with 1, 2, 5, 10, 15, and 20 mg of Suwannee River Natural Organic Matter or Nordic Reservoir Natural Organic Matter L^{-1}, and the water samples were extracted normally.

Because the 0.45-µm filter removed particulate organic carbon, all amended water samples were analyzed for DOC. Three replicates for each organic matter concentration were used. Depending on the DOC of the environmental water samples, the appropriate amount of fluorescence was subtracted from each sample's total fluorescence.

Statistics

For the first and second laboratory experiments, repeated measures analyses were applied to both sets of data with SAS (Version 9.1, Cary, NC). Both the first experiment (11 measurements during a 4-hour time period) and the second experiment (five measurements during a 30-minute time period) were analyzed for two main effects (within and between subjects) for three concentrations of laundry detergent (0, 50 and 200 µl L^{-1}) and four levels of organic matter (0, 10, 20, 30, and 40 mg L^{-1}) in order to evaluate the change in fluorometric value over time. However, before the analyses were performed, the data were analyzed for normality and homogeneity of variance in order to select the appropriate transformations and statistics.

For the third laboratory experiment, the relationships among the two fluorometers (Aquafluor and Model 10-AU), six concentrations of organic matter (0, 5, 10, 15, 20 and 25 mg L^{-1}), and two different natural organic matters (Nordic Reservoir and Suwannee River) were analyzed with various general linear models using PROC GLM (SAS, Version 9.1). Because each sample was measured before and after UV exposure (i.e., 5 minutes for the Aquafluor and 30 minutes for Model 10-AU), the data were expressed in terms of relative percent difference in fluorometric readings before and after UV exposure (i.e., [fluorometric reading before UV exposure minus fluorometric reading after UV exposure] divided by the fluorometric reading before UV exposure X 100).

RESULTS

Physical and Chemical Characteristics

All values for pH, salinity, temperature, and dissolved oxygen from the five sampling locations were within a normal range (Table 1). The salinity for the storm drain in St. Simons Village was low (0.2 ppt) because the drain collected fresh water.

Table 1. The pH, salinity, temperature, and dissolved oxygen of marine and fresh waters at or around US locations in Georgia and Virginia during calm (base-flow) conditions. Dissolved organic carbon values are given in Table 5.

Location	pH	Salinity ppt	Temp °C	Dissolved Oxygen mg L^{-1}
Georgia				
Barbour Island River	7.3	32.4	29.5	4.0
St. Simons Village Storm Drain	6.7	0.2	26.3	4.8
Virginia				
Hilton Beach	7.4	10.5	20.3	7.8
Anderson Beach	8.2	15.6	19.5	8.2
Buckroe Beach	8.4	18.3	24.0	7.0

Fluorometry Laboratory Experiments

In the first laboratory experiment, the optimal duration of UV light exposure was determined for the field fluorometer. When no detergent was added and the treatments were not exposed to UV light, significant differences in fluorometric values were observed among the three organic matter concentrations (10, 20, and 40 mg L^{-1} of distilled water; Figure 1A). Therefore, as expected, organic matter fluoresced. Little to no decrease in fluorometric values was observed over the four-hour period. However, when these same organic matter concentrations were exposed to UV light, the fluorometric values decreased significantly over the 4-hour period, with the greatest decrease occurring during the first 30 minutes (Figure 1B). No fluorescence was observed in distilled water not amended with organic matter whether exposed to UV light or not (negative control).
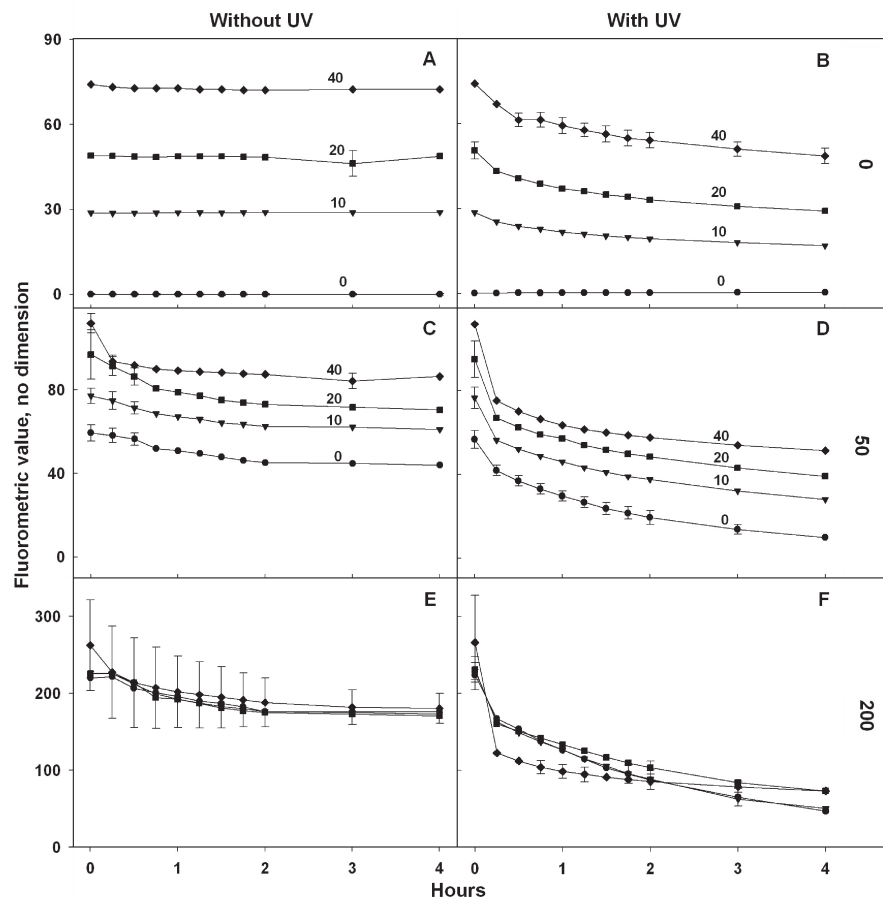

Figure 1. Decline in fluorometric value of distilled water amended with 0 (top), 50 (middle), and 200 (bottom) µl of commercial laundry detergent per liter, and 0 (circles), 10 (triangles), 20 (squares), and 40 (diamonds) mg of Suwannee River natural organic matter per liter without (A, C, and E) and with (B, D, and F) increasing UV light exposure. The 30-ml water samples were each contained in glass cuvettes designed for the Turner Designs Model 10-AU fluorometer. The air temperature was 20°C. Each point is the average of three replicates. Error bars, 1 standard deviation. Where no error bars are shown, the symbol was larger than the error bars.

When detergent was added to the water (final concentration, 50 µl L^{-1}), fluorescence values increased significantly (Figure 1C) compared to treatments with no detergent. Therefore, as expected, the detergent contained optical brighteners and they fluoresced. When not exposed to UV, this fluorescence decreased slowly to an average of -24% over a four-hour period. Once exposed to UV light, the fluorometric values decreased rapidly compared to the non-UV treatment, with the most rapid decrease occurring in the first 15 minutes and a total decrease of -65% in 4 hours (Figure 1D). However, differences among the treatments were not significant until after 30 minutes.

When even more detergent was added to the water (final concentration, 200 µl L^{-1}) and the treatments not exposed to UV, little differences were observed among the treatments during the four-hour exposure, except that the fluorometric values were higher than for the same treatments amended with 50 µl of detergent L^{-1}, and there was a noticeable decrease in fluorescence during the first 15 minutes for the treatment containing 40 mg of organic matter L^{-1} (Figure 1E). Similar results were observed with UV light exposure except the UV light produced a greater decrease (average -75% over four hours; Figure 1F). When all the treatments were summed, the minimal optimum time to observe significant differences between the UV and non-UV treatments was 30 minutes, and this time was adopted as the standard UV exposure time for field fluorometer samples.

In the second laboratory experiment, the optimal duration of UV light exposure was determined for the handheld fluorometer. When no detergent was added, small decreases in fluorometric values were observed among the three organic matter concentrations (10, 20, and 40 mg L^{-1} distilled water) during the 30-minute exposure (Figure 2). When detergent was added to the water (final concentration, 50 µl L^{-1}), fluorescence values increased significantly compared to treatments with no detergent. Once exposed to UV light, the fluorometric values of the samples decreased rapidly, with the most rapid decrease occurring in the first five minutes. When even more detergent was added to the water (final concentration, 200 µl L^{-1}), results were similar to the 50 µl of optical brightener L^{-1}, except initial fluorometric values were higher and little differences were observed among the treatments. When all the treatments were summed, the minimal optimum time to observe sig-
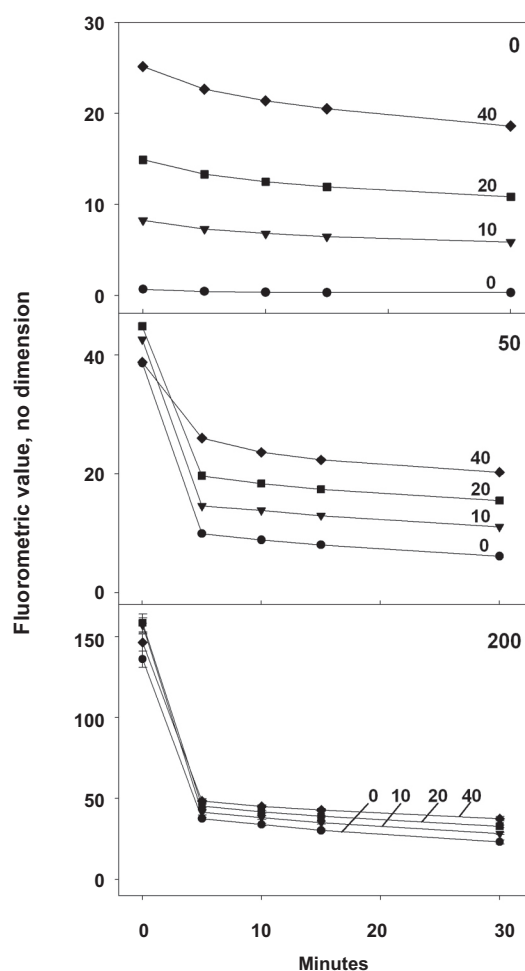

Figure 2. Decline in fluorometric value of distilled water amended with 0 (top), 50 (middle), and 200 (bottom) µl of laundry detergent per liter, and 0 (circles), 10 (triangles), 20 (squares), and 40 (diamonds) mg of Suwannee River natural organic matter per liter with increasing UV light exposure. Each point is the average of three replicates. Error bars, 1 standard deviation. Where no error bars are shown, the symbol was larger than the error bars.

nificant differences was five minutes and this time was adopted as the standard UV exposure time for handheld fluorometer samples.

In the third laboratory experiment, the relative percentage decrease in fluorometric value before and after UV light exposure was determined for a commercial laundry detergent and two natural organic matters. No fluorometric value was observed when no detergent or no organic matter was added to water (negative controls), and fluorometric values were observed when either detergent or organic matter was added to water, regardless of the fluorometer or the origin of the organic matter. When measured

Table 2. Relative percentage difference in fluorometric values of distilled water containing various concentrations of two types of natural organic matter from the Nordic Reservoir and the Suwannee River before and after 30 minutes of UV light exposure in glass cuvettes [10-AU field fluorometer, Turner Designs, Sunnyvale, CA], and before and after 5 minutes of UV light exposure in polymethacrylate cuvettes [handheld fluorometer, Aquafluor, Turner Designs]. Each value represents the average of four replicates. NA = not applicable; SD = standard deviation.

Measurement Method and Organic Matter Source	Total Organic Matter (mg L^{-1})						Average ±1SD
	0	5	10	15	20	25	
Fluorometric Values (no dimension)							
10-AU Field Fluorometer–Nordic Reservoir Natural Organic Matter							
Before 30 min of UV light	0	11.3	20.3	28.7	36.3	42.7	NA
After 30 min of UV light	0	7.7	14.3	19.5	25.5	30.8	NA
Percent Difference	NA	31.5	29.2	32.2	29.8	27.8	30.1 ±1.8
10-AU Field Fluorometer–Suwannee River Natural Organic Matter (from Figure 4)							
Before 30 min of UV light	0	13.4	25.0	34.0	42.0	48.7	NA
After 30 min of UV light	0	8.8	16.3	23.0	30.7	35.9	NA
Percent Difference	NA	34.5	34.9	32.3	27.0	26.3	31.0 ±4.1
Handheld Fluorometer–Nordic Reservoir Natural Organic Matter							
Before 5 min of UV light	0	5.8	8.6	11.2	15.4	17.6	NA
After 5 min of UV light	0	5.3	7.8	10.0	14.1	16.0	NA
Percent Difference	NA	8.7	9.4	11.0	8.3	9.1	9.3 ±1.0
Handheld Fluorometer–Suwannee River Natural Organic Matter							
Before 5 min of UV light	0	5.4	10.3	16.8	19.3	23.2	NA
After 5 min of UV light	0	4.6	8.9	14.9	16.9	20.4	NA
Percent Difference	NA	13.6	13.6	11.3	12.3	12.0	12.5 ±1.0

with the field fluorometer, the relative percentage decreases before and after 30 minutes of UV exposure for 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µl of commercial laundry detergent L^{-1} of water were 65, 59, 60, 61, 58, 54, 54, 60, 50, and 58%, respectively. The average relative percentage decrease was 58 ±4%. In contrast, the average relative percentage decreases for both Suwannee River and Nordic Reservoir natural organic matter analyzed with the field fluorometer (glass cuvettes) were significantly lower (30 and 31%, respectively; Table 2). A value of 30% was adopted as a working guideline (i.e., if the relative percentage decrease was >30%, then the sample was positive for optical brighteners; if the relative percentage decrease was ≤30%, then the sample was negative for optical brightener). For the organic matters analyzed with the handheld fluorometer (polymethacrylate cuvettes), the average decrease was approximately 9% for Nordic Reservoir natural organic matter, and 13% for Suwannee River natural organic matter, and >15% was arbitrarily adopted as a standard cutoff.

Because the sensitivity of the field fluorometer could be adjusted, fluorometric values obtained with the field fluorometer were significantly higher than handheld fluorometer for different concentrations of organic matter. However, the relative percentage differences decreased significantly with higher organic matter concentrations for the field fluorometer ($p < 0.0001$) and not for the handheld fluorometer ($p = 0.21$). Therefore, the relative percentage differences of the fluorometric values across the organic matter concentrations (5 to 25 mg L^{-1}) were more stable for the handheld fluorometer than for the field fluorometer. There were also significant differences between Nordic Reservoir and Suwannee River natural organic matters for both the field fluorometer ($p < 0.0004$) and the handheld fluorometer ($p < 0.0003$). Even though the differences between the organic matters were significant, overall the relative percentage differences were still small (<6%). The interactions between the two natural organic matters and organic matter concentration were significant for the field fluorometer ($p < 0.0007$) and not for the handheld fluorometer ($p = 0.22$).

Table 3. Single blind experiment to determine the presence or absence of optical brighteners in randomly numbered, distilled water samples amended with 0, 10, or 20 mg Suwannee River natural organic matter per liter and 15 samples spiked with either 50; or 100 µL laundry detergent per liter; or 15 samples left unspiked. Both a field fluorometer [Model 10-AU, Turner Designs, Sunnyvale, CA] and a handheld fluorometer [Model Aquafluor, Turner Designs, Sunnyvale, CA] were tested. A second investigator recorded a water sample as positive or negative for optical brighteners if the fluorometric value dropped >30% (Model 10-AU) or >15% (Aquafluor) after UV light exposure.

Fluorometer	Possible detergent (µl L^{-1})	Organic matter (mg L^{-1})	Detergent Added		No Detergent Added	
			correctly identified	incorrectly identified	correctly identified	incorrectly identified
Model 10-AU	100	0	15	0	15	0
	100	10	15	0	13	2
	100	20	15	0	15	0
	50	0	15	0	15	0
	50	10	15	0	15	0
	50	20	15	0	15	0
Aquafluor	100	0	13	2	15	0
	100	10	15	0	13	2
	100	20	15	0	15	0
	50	0	15	0	15	0
	50	10	15	0	14	1
	50	20	15	0	15	0

In the fourth experiment, a single blind experiment was conducted with both the field and handheld fluorometers to determine the presence or absence of optical brighteners in water samples with known amounts of organic matter and laundry detergent using the working guidelines established in the third experiment. For the field fluorometer, only 2 of 180 water samples (1%) were misidentified. In this instance, two samples containing 10 mg of organic matter L^{-1} and no detergent were falsely identified as having optical brighteners (Table 3). For the handheld fluorometer, only 5 of 180 water samples (3%) were misidentified. In this instance, two samples containing 100 µl of detergent and no organic matter were incorrectly identified as having no optical brighteners, and three samples containing 10 mg of organic L^{-1} and no detergent were falsely identified as having optical brighteners. The experiment also determined that both field and handheld fluorometers were effective to a minimum threshold of 20 mg of organic matter L^{-1} (≈10 mg of organic C L^{-1}) and 50 µl of detergent.

In the fifth and final experiment, the effect of emission filter and UV light was tested on various organic compounds likely to be found in urban environmental waters. The fluorometric values of the two optical brighteners (positive control), DSBP and DAS1, were unaffected by the emission filter, and decreased significantly after 4 hours of UV light exposure (-48 and -44%, respectively; Table 4). Because they contain optical brighteners, similar results were observed for the septic tank effluent, the two sewage influents and effluents, and the two commercial laundry detergents. Of the 8 car care products, 13 car fluids, and one natural product (crude oil), only diesel fuel, 1 wash soap (Mr. Clean), and crude oil fluoresced ≥50 fluorometric units with 436-nm emission filter. When these compounds were exposed to 4 hours of UV light, only the wash soap was affected (124 to 75 fluorometric units, a 40% decrease); neither diesel fuel nor crude oil was affected by UV light. Therefore, of the car care products and car fluids, with the exception of one wash soap, the compounds either did not fluoresce, or, if they did fluoresce, their fluorescence was unaffected by exposure to UV light.

Table 4. Fluorescence of various car care products, car fluids, laundry detergents, septic tank effluent, and sewage influents and effluents observed with a 436-nm emission filters before and after 4 hours of ultraviolet (UV) light exposure. Optical brighteners, DSBP and DAS1, were included as positive controls.

Category	Brand or Virginia Location	Amount per Liter of Water	436-nm Emission Filter Fluorometric Units (no dimension)		Decline (%)
			Before UV	After UV	
Optical Brighteners					
DSBP	Sigma–Aldrich	10 µg	183	96	48
DAS1	Sigma–Aldrich	10 µg	201	112	44
Laundry Detergents					
Detergent	All™	100 µl	181	75	59
Detergent	Food Lion™	500 µl	421	215	49
Septic Tank Effluent, Sewage Influents, and Sewage Effluents					
Septic Tank Effluent	Blacksburg	undiluted	127	45	65
Sewage Influent	Eastern	undiluted	431	212	51
Sewage Effluent	Eastern	undiluted	271	148	45
Sewage Influent	Southwestern	undiluted	336	177	47
Sewage effluent	Southwestern	undiluted	198	102	49
Car Care Products					
Cleaner	Next Dimension	10 ml	10	10	0
Engine Cleaner	CRC Chemicals™	10 ml	0	1	0
Engine Cleaner	Gunk™	10 ml	20	21	0
Glass Treatment	Rain X™	10 ml	13	11	15
Glass Treatment	Prestone™	10 ml	8	9	0
Wash Soap	Mr. Clean™	500 µl	124	75	40
Wash Soap	Purple Magic™	500 µl	40	41	0
Wash Soap	Turtle™	500 µl	23	20	5
Car Fluids					
Antifreeze	Prestone™	10 ml	7	6	14
Antifreeze	Advance Auto™	10 ml	9	10	0
Brake Fluid	Valvoline™	10 ml	7	8	0
Diesel Fuel		10 ml	114	115	0
Gasoline	Shell	10 ml	11	10	9
Oil (crude)		10 ml	298	294	1
Oil (motor)	Pennzoil	10 ml	10	10	0
Oil (racing)	Valvoline	10 ml	2	3	0
Oil (synthetic)	Mobil 1	10 ml	6	6	0
Oil Additive	CD2 Chemicals	10 ml	17	18	0
Radiator Flush	Zerex	10 ml	10	11	0
Radiator Additive	Red Line	10 ml	15	13	13
Steering	Advance Auto	10 ml	3	4	0
Transmission	Valvoline	10 ml	6	7	0

Fluorometry Field Experiment

In the field experiment, two locations known to be negative for human fecal contamination and three locations known to be positive for human fecal contamination were tested for their numbers of fecal indicator bacteria, as well as the presence or absence of optical brighteners by fluorometry and HPLC. Unfortunately, the HPLC data could not be interpreted without ambiguity because of organic matter interference. Distilled water amended with Suwannee River natural organic matter produced HPLC peaks relative to their TOC concentration at the same retention time as DAS1 (two minutes), and peak heights with the other natural organic matter, Nordic Reservoir were similar (data not shown). Attempts to change the HPLC protocol to reduce this interference were unsuccessful.

For the first of the two negative control locations, Barbour Island River, 1 of the 2 samples exceeded 104 fecal enterococci per 100 ml, but neither of the two samples showed a spike in fluorescence (>100 units; Table 5). Similarly, for the second of the two negative control locations, Buckroe Beach, neither of the two samples exceeded 104 fecal enterococci per 100 ml nor showed a spike in fluorescence. Although neither Barbour Island River nor Buckroe Beach samples would normally be exposed to UV light because they showed no fluorescent hotspots, exposing the samples to UV light resulted in maximum drop of ≤15% at each location.

In contrast to the negative control locations, all three locations identified as positive for human fecal contamination had samples with both high numbers of fecal enterococci (>104 fecal enterococci per 100 ml) and high fluorometric values (>100 units).

In contrast to the negative control sites, all samples from the three positive locations showed a large percentage decrease in optical brightener units after UV exposure (>30% for field fluorometer, Virginia; >15% for handheld fluorometer, Georgia). However, after the two Virginia sites positive for human fecal contamination were remediated, both sites had low numbers of fecal bacteria (≤20 fecal enterococci per 100 ml) and low fluorescence (≤64 units). When water samples from the two remediated sites were exposed to UV light, the relative percentage decreases were all ≤15%.

DISCUSSION

Exposing an environmental water sample to UV light may represent a simple, quick, and easy way to identify the presence or absence of optical brighteners in that sample. Previously, results on combining

Table 5. Number of fecal enterococci, dissolved organic carbon (DOC), fluorometric value of water sample before and after ultraviolet (UV) light exposure, and the relative percentage decline in UV units from five locations: two negative (Barbour Island River and Buckroe Beach) and three positive (St. Simons Village, Hilton Beach, and Anderson Beach) for human fecal contamination. Values are also given for two positive locations (Hilton Beach and Anderson Beach) that were subsequently remediated. For a location to be considered positive for human fecal contamination, the number of fecal enterococci must exceed the limit for a grab sample (>104 per 100 ml) and be confirmed positive for human fecal contamination with another MST test (e.g., *esp* gene). In addition, the type of fluorometer is identified for each location and whether the percentage decline exceeds the limit (>15% for the handheld and >30% for the field fluorometer) for a sample to be considered positive for optical brighteners.

Location/Sample	Fecal Enterococci (per 100 ml)	Dissolved Organic Carbon (mg L^{-1})	Fluorometric Units (no dimension)		Relative Decrease (%)	Fluorometer Type	Exceeds Limit
			Before UV	After UV			
Barbour Island River, Georgia							
BR1	74	7	13	11	15	Handheld	No
BR2	121	7	13	11	15	Handheld	No
Buckroe Beach, Virginia							
BB1	44	26	28	24	14	Field	No
BB2	35	ND	31	28	8	Field	No
St. Simons Village, Georgia							
SSV1	61,310	512	198	130	34	Handheld	Yes
SSV2	>241,920	764	199	138	31	Handheld	Yes
Hilton Beach, Virginia (before remediation)							
HB1	41,800	30	126	53	58	Field	Yes
HB2	136,000	ND	237	116	51	Field	Yes
Anderson Beach, Virginia (before remediation)							
AB1	5,040	21	149	86	42	Field	Yes
Hilton Beach, Virginia (after remediation)							
HB1	<10	ND	28	24	14	Field	No
HB2	<10	ND	64	57	11	Field	No
Anderson Beach, Virginia (after remediation)							
AB1	20	ND	27	23	15	Field	No

fluorometry and counts of fecal bacteria were contradictory because the combined method appeared to work for some investigators (e.g., Kerfoot and Skinner 1981, Hagedorn *et al.* 2005, McDonald *et al.* 2006) but not for others (e.g., Close *et al.* 1989, Wolfe 1995). These contradictions led some investigators to conclude that fluorometry should not be recommended as a MST method (Boving *et al.* 2004). These contradictions were likely caused by background fluorescence from organic compounds in the water, and our study focused on resolving these contradictions by being able to differentiate optical brighteners from other organic compounds.

This differentiation involved two steps. The first step was to add a narrow wavelength emission filter to the fluorometer, which reduced background fluorescence from organic matter (Hartel *et al.* In press-a). Nevertheless, adding narrow wavelength filter did not eliminate background fluorescence entirely, and a second step, exposing the environmental water sample to UV light, was required. This step was based on the idea that optical brighteners photodecay rapidly when exposed to sunlight (Kramer *et al.* 1996). Therefore, a series of experiments was conducted to test UV light exposure as a possible way to differentiate between fluorescence from optical brighteners and other organic compounds.

In the case of organic matter, the optimal time for exposing environmental water samples to UV light was 30 minutes for the field fluorometer and 5 minutes for the handheld fluorometer. The differences in the exposure times were expected because the field fluorometer used glass cuvettes, which were mostly opaque to the UV wavelength used here, and the handheld fluorometer used polymethacrylate cuvettes, which were transparent to the UV wavelength used here. Once the appropriate UV exposure times were determined, the relative percentage decreases between optical brighteners in commercial laundry detergent and Suwannee River and Nordic Reservoir natural organic matter before and after UV exposure were compared. The relative percentage photodecay was significantly greater for optical brighteners in the laundry detergent compared with either Suwannee River or Nordic Reservoir natural organic matter. Therefore, assuming a water sample shows a significant increase in fluorometric units from surrounding waters (and is thereby suspicious under the auspices of targeted sampling), a practical working guideline was developed: if the percentage drop in fluorometric value after UV light exposure

was >30% (glass cuvettes) or >15% (polymethacrylate cuvettes), then optical brighteners were likely present in the water sample. This working guideline was tested in a blind study with water samples containing varying amounts of organic matter and commercial laundry detergent. Under these conditions, the ability of an investigator to correctly identify the presence or absence of optical brightener in unknown water samples was 99% for the field fluorometer and 97% for the handheld fluorometer.

In the case of other organic compounds like car care products and car fluids, the results showed that most of these compounds did not fluoresce at the same wavelengths of optical brighteners and can be ignored. With the exception of one wash soap (Mr. Clean), all remaining fluorescing compounds could be eliminated by exposing the compound to UV light (i.e., crude oil and diesel fuel). In contrast to optical brighteners and detergents, neither crude oil nor diesel fuel was affected by UV light exposure. The one fluorescing wash soap likely contained optical brighteners, but in Virginia and most other United States, commercial car washing facilities are prohibited from discharging their effluent into storm drains. In contrast to the car care products and car fluids, the septic tank effluent and the sewage influents and effluents not only showed fluorescence, but also large percentage decrease in fluorometric values after UV exposure. This large percentage drop was expected because these influents and effluents contain optical brighteners. For example, for a typical optical brightener like DAS1, concentrations are typically 10.5 µg L^{-1} in raw sewage, 6.9 µg L^{-1} after primary treatment, 2.4 µg L^{-1} after secondary treatment, and 0.5µg L^{-1} upon discharge to surface waters (Poiger *et al.* 1998). Again, the drop in fluorometric values demonstrated the potential usefulness of optical brighteners as a surrogate for potential human-derived fecal contamination in water.

When the UV exposure method was tested in five locations, three with environmental waters likely positive for human fecal contamination and two not, the results showed high numbers of fecal indicator bacteria (>104 fecal enterococci per 100 ml) and high fluorometric values (>100 units) in the three positive locations (St. Simons Village, Hilton Beach, and Anderson Beach), and low numbers of fecal enterococci and low fluorometric values in the two negative control locations (Barbour Island River and Buckroe Beach). Although HPLC could not confirm the presence or absence of optical brighteners as a

source of these fluorometric values (because of unexpected organic matter interference with the method), the results of the UV exposure were consistent with the presence of optical brighteners. Thus, waters at the three positive locations had the fluorescence of their waters decrease >30% (glass cuvettes) or >15% (polymethacrylate cuvettes) when they were exposed to UV light. When the two positive Virginia locations were remediated, the fluorescence in the water samples not only dropped below the 100-unit cutoff, but also only decreased ≤15% after UV exposure. Similarly, the negative controls all decreased ≤15% after UV exposure. However, it is important to note that the water samples from these negative sites, remediated or not, would never be exposed to UV because they did not exceed the fluorometric cutoff.

Besides equipping the fluorometer with a narrow wavelength emission filter, the combined method of targeted sampling and fluorometry required that three criteria be met. First, common sense dictated that both fecal indicator bacteria and fluorometric signals were likely to be diluted with increasing distance from the source, therefore, multiple samplings over ever-decreasing distances (i.e., targeted sampling) were necessary to identify most fecal bacterial and fluorometric hotspots. In the case of fecal bacterial hotspots, Hartel *et al.* (In press-b) observed that sampling from bridges and roads helped only in identifying reaches of a freshwater creek with fecal bacterial problems, and that targeted sampling was required to identify the fecal bacterial hotspots, which were typically found in small tributaries. In the case of fluorometry, Hagedorn *et al.* (2005) observed that most fluorescent plumes, particularly those from malfunctioning septic drainfields, were only a few square meters in size. In fact, in tidal areas, fluorescent plumes were undetectable unless sampling was conducted during an ebb tide.

Second, a suitable TOC cutoff was needed to determine fluorometric hotspots. A practical working guideline was <40 mg of TOC L^{-1}, because above this limit, organic matter sequesters most of the fluorescence from optical brighteners (Hartel *et al.* In press-a). Because POC could potentially interfere with the HPLC packing material, the environmental waters from the five locations tested here were filtered through a 0.45-μm filter and only DOC values were determined. However, considering that POC is typically 10% of DOC values (Alberts *et al.* 1990),

the TOC of four of the five environmental waters tested would be within the guideline. The two storm drain samples in St. Simons Village would exceed the guideline and would require confirmation with a different MST test. In this case, the water from Site SSV2 was examined for the presence *esp* gene (Scott *et al.* 2005) and tested positive for human fecal contamination (Hartel *et al.* In press-a).

Third, a suitable cutoff value for fluorometry was needed to determine fluorometric hotspots. Although >100 fluorometric units was a satisfactory cutoff to identify fluorometric hotspots here, this cutoff requires some flexibility. Some waters extremely low in TOC (<5 mg L^{-1}) may require a lower fluorometric cutoff value. For example, in studies of the Mayagüez Bay and the Yagüez River in Puerto Rico, where the TOC was <2 mg L^{-1}, the cutoff value was lowered to >30 fluorometric units because the background fluorescence was consistently <10 units. Two sites on the Yagüez River, one with 15,800 *Escherichia coli* per 100 ml and a fluorometric spike of 60 units, and another with 14,830 *E. coli* per 100 ml and a fluorometric spike of 90 units, were both subsequently confirmed positive for human fecal contamination (Hartel *et al.* In press-a) with the *esp* gene (Scott *et al.* 2005).

Fluorometry had four disadvantages. First, the list of organic and inorganic compounds tested here was not exhaustive and there may be other compounds that interfere with fluorometry that were not tested. For example, magnesium in seawater contributes to fluorescence (Willey 1984), but this inorganic ion was not tested. Hagedorn *et al.* (2005) tested a variety of marine and freshwater algae for their fluorescence at the same wavelengths optimal for optical brighteners, and although none fluoresced, more species need to be tested.

Second, although optical brighteners degrade quickly when exposed to sunlight (Kramer *et al.* 1996), there is evidence that optical brighteners persist in sediment (Poiger *et al.* 1998). It is not clear how well fluorometry will work in turbid waters or when sediments are resuspended, especially when high winds or stormflow conditions disturb the sediment. Here, the sampling was done in relatively clear waters during baseflow conditions when the sediments were not disturbed. Although most leaking sanitary sewers and malfunctioning septic drainfields flow regardless of other environmental conditions and detection of their optical brighteners would be optimal during

baseflow conditions, there are conditions where sanitary sewers and septic drainfields fail only during stormflow conditions.

Third, not enough is known about storm drains to interpret the fluorometric data easily. For example, fecal indicator bacteria may regrow in storm drains (Ferguson and Getrich 2006). In addition, commercial concerns and private individuals may wash their cars or sidewalks with detergents containing optical brighteners that also drain into the storm drain system, legally or illegally. Finally, because there is little to no sunlight in the storm drains, there is likely little to no photodegradation of optical brighteners in these drains. This scenario opens up the possibility that the presence of optical brighteners could represent long-past fecal contamination. These complicating factors suggest that collecting water samples from storm drains over a period of time would be desirable.

Fourth, fluorometry required that the TOC of the fluorometric hotspot be known, an analysis that is not always easily available or inexpensive. Even so, if the TOC limit for fluorometry is 40 mg L^{-1}, then most environmental waters are below this limit. For example, in the southeastern United States, where waters are characterized by low topographic relief, extensive floodplain swamps, and rich organic bottom sediments, DOC concentrations average from 5 to 28 mg of C L^{-1} in estuaries (Alberts and Takács 1999), with POC concentrations averaging about 10% of DOC values (Alberts *et al.* 1990). In Georgia, only storm drains and some blackwater creeks and rivers are likely to exceed this level of C.

The use of HPLC to identify optical brighteners did not work because of organic matter interference. This method requires further refinement to ensure optical brighteners and not organic matter is being observed. Fortunately, other methods (e.g., detection of the *esp* gene; Scott *et al.* 2005) exist as alternatives to confirm fluorometric results.

Both the field and handheld fluorometers were equally satisfactory in determining the presence of optical brighteners. Each fluorometer had its advantages and disadvantages. The handheld model was considerably lighter (<0.5 kg with batteries) than the field model (approximately 25 kg when mounted on backpack with lawnmower battery as an electrical source and excluding the sampling pole), which made the handheld model easier to handle and to carry. However, the handheld model had no flow-

through cell, and the field fluorometer may be better suited than the handheld model when continuous readings are desirable. Although the handheld fluorometer had more stable fluorometric readings over a range of organic matter concentrations, the field fluorometer also has a sensitivity adjustment that may be helpful in some waters. The most important consideration for our studies was cost. Because the handheld fluorometer was approximately one-seventh of the field fluorometer's cost, it should be recommended when cost considerations are paramount. In fact, given the cost of a handheld fluorometer, UV light, and two pipetters (one 100-µl pipetter for the calibration standard and one 5- or 10-ml pipetter for loading cuvettes), and considering the cost of disposable items like gloves, pipet tips, and polymethacrylate cuvettes, the results suggest that it may be possible to conduct fluorometry (with its UV light modifications) at a reasonable cost. When these costs are combined with those costs necessary to enumerate fecal indicator bacteria under the auspices of targeted sampling and the cost of TOC analyses, it may be possible to identify sources of human fecal contamination relatively inexpensively on a permanent basis.

Provided certain criteria were met for combining targeted sampling and fluorometry, UV light exposure was a simple, quick, and easy method to confirm the presence or absence of optical brighteners in waters and, when combined with counts of fecal indicator bacteria, the presence or absence of human fecal contamination in a water sample. The criteria for targeted sampling and fluorometry included: a) installing a narrow wavelength emission filter in the fluorometer, b) following the guidelines of targeted sampling, c) determining the water's TOC, and d) establishing a suitable fluorometric cutoff value depending on the water's TOC value. Once these criteria were met, any water sample exceeding the fluorometric cutoff could be exposed to UV light (30 minutes for glass cuvettes and 5 minutes for polymethacrylate cuvettes), and if the percentage decrease in fluorometric value was >30% in the glass cuvettes or >15% in the polymethacrylate cuvettes, then the water likely contained optical brighteners. If the environmental water contained high numbers of fecal indicator bacteria as well, then the water was likely positive for human fecal contamination, probably from a leaking sewer or malfunctioning septic drainfield.

As currently envisioned, targeted sampling requires bacteriological enumeration before fluoro-

metric results are considered. However, in cases where an investigator was only interested in detecting human fecal contamination, it may be possible to reverse this order and conduct fluorometry first to identify plumes of optical brighteners and then confirm these plumes with counts of fecal indicator bacteria. Such sampling would reduce bacteriological costs even more by requiring fecal bacterial enumeration only when absolutely necessary.

Regardless whether fluorometry is used first or not, the combination of targeted sampling and fluorometry appears to be an elegant method for identifying human fecal contamination quickly and easily when UV light exposure is added to the protocol. In addition to testing more organic matters and other organic compounds, and determining the effect of sediment (especially during stormflow conditions), future studies need to be conducted across a broader geographic area to discover the limitations of the working guidelines and the minimum thresholds for the fluorometers to identify a fluorometric hotspot. Such studies are in progress.

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ACKNOWLEDGMENTS