Combining targeted sampling and fluorometry to identify human fecal contamination in a freshwater creek

Peter G. Hartel, Karen Rodgers, Gwyneth L. Moody, Sarah N. J. Hemmings, Jared A. Fisher and Jennifer L. McDonald

ABSTRACT

Many bacterial source tracking (BST) methods are too expensive for most communities to afford. In an effort to develop an inexpensive method of detecting human sources of fecal contamination in a freshwater creek during baseflow and stormflow conditions, we combined targeted sampling with fluorometry. Targeted sampling is a prelude to BST and finds sources of fecal contamination by continued sampling and resampling over ever-decreasing distances. Fluorometry identifies human fecal contamination in water by detecting fluorescing compounds, optical brighteners, from laundry detergents. Potato Creek, a freshwater creek in Georgia (U.S.A.), had three reaches identified as containing high numbers of fecal bacteria, and these reaches were sampled by targeted sampling and fluorometry. Targeted sampling quickly and inexpensively identified humans, cattle, and dogs as the major sources of fecal contamination in the first, second, and third reaches, respectively. Fluorometric values were consistent with these identifications, but high fluorometric values were sometimes observed in areas with no fecal contamination. One likely cause of these false-positive signals was fluorescence from organic matter. For targeted sampling, the cost of each sample was $6, with a one-time equipment cost of $3,650; for fluorometry, the cost of each sample was negligible, with a one-time equipment cost of $14,250. This was the first study of this relatively inexpensive combination in freshwater during both baseflow and stormflow conditions.

Key words | bacterial source tracking, *Escherichia coli*, fluorescent whitening agents, microbial source tracking, optical brighteners, organic matter

Peter G. Hartel (corresponding author)
Karen Rodgers
Gwyneth L. Moody
Sarah N. J. Hemmings
Jared A. Fisher
Department of Crop & Soil Sciences,
University of Georgia,
3111 Plant Sciences, Athens, GA 30602-7272,
USA
Tel.: +1 706 542 0898
Fax: +1 706 542 0914
E-mail: *pghartel@uga.edu*

Jennifer L. McDonald
Marine Extension Service,
University of Georgia,
715 Bay Street,
Brunswick, GA 31520-4601,
USA

NOMENCLATURE

ATCC   American Type Culture Collection
BST   bacterial source tracking
GIS   geographic information system
GPS   global positioning system
MPN   Most Probable Number

INTRODUCTION

Bacterial source tracking (BST) identifies sources of fecal contamination by various phenotypic, genotypic, and chemical methods. Many of these methods are too expensive

for most communities to afford. One way to reduce the cost is to use targeted sampling as a prelude to BST (Kuntz *et al.* 2003). Targeted sampling works much like the children's game of "hot" and "cold." Briefly, the method separates sampling for two different flow conditions, one for baseflow and another for stormflow, to accommodate potential changes in fecal sources during the different flow conditions (e.g., Hartel *et al.* 2004). Next, the method combines local knowledge with 1-day samplings to reduce bacterial changes with time (Jenkins *et al.* 2003). Once each sampling location is identified through the global positioning system (GPS), the data is placed in a geographic information system (GIS) database, and hotspots of fecal contamination are identified

and resampled. Limiting the samples to a small geographic area reduces bacterial changes with geography (Hartel *et al.* 2002) and animal diet (Hartel *et al.* 2003). In most cases, persistent sources of fecal contamination are visually obvious and BST is unnecessary. In the few cases where persistent sources of fecal contamination are not obvious or it is necessary to confirm persistent sources, then a BST method can be selected on the basis of a "toolbox" approach (USEPA 2005), an approach where each method's cost, reproducibility, discriminatory power, ease of interpretation, and ease of performance is considered (Simpson *et al.* 2002).

Targeted sampling has been successfully combined with an expensive genotypic BST method, ribotyping, in estuarine waters during calm (baseflow) conditions (Kuntz *et al.* 2003), and successfully combined with three inexpensive BST methods in marine waters during both calm (baseflow) and stormy (stormflow) conditions (McDonald *et al.* 2006). Targeted sampling has never been combined with a BST method in freshwater during both baseflow and stormflow conditions.

One relatively inexpensive BST method to identify or confirm human sources of fecal contamination is fluorometry. Fluorometry is a chemical BST method which identifies human fecal contamination by detecting optical brighteners (also called fluorescent whitening agents) in water. Optical brighteners are compounds added to laundry detergents and typically represent 0.15% of the total detergent weight (Hagedorn *et al.* 2005). As highly substituted ring (aromatic) structures, they are excited (activated) by light in the near-UV range (360 to 365 nm) and emit light in the blue range (415 to 445 nm; Kaschig 2003). In this emission range, they compensate for undesirable yellowing of clothing. In the United States, 97% of laundry detergents contain either the optical brightener DSBP (4,4'-bis(2-sulfostyry)biphenyl) or DAS1 (4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulfonate) (Hagedorn *et al.* 2005). Although these water-soluble compounds have a high affinity for cellulosic material, a small but significant percentage is lost to the wastewater. Because household plumbing systems mix effluent from washing machines and toilets together, optical brighteners are associated with human sewage in septic systems and wastewater treatment plants. Once in the wastewater, these compounds persist until they are exposed

to sunlight, whereupon they photodegrade in a matter of hours (Kramer *et al.* 1996). Below wastewater treatment plants, concentrations of optical brighteners average $0.5\,\mu g\ L^{-1}$ in fresh waters (Poiger *et al.* 1998) and a few $\mu g\ L^{-1}$ in marine waters (Hayashi *et al.* 2002).

Detecting optical brighteners alone is limited as a BST tool because this detection may or may not be associated with fecal contamination. For example, the effluent from a wastewater treatment plant may contain optical brighteners, but unless the wastewater treatment plant fails to disinfect its effluent sufficiently, it will not contain viable fecal bacteria, and the plant does not represent a source of human fecal contamination. Therefore, detecting optical brighteners needs to be combined with numbers of fecal bacteria (Table 1).

The data for combining fluorometry and counts of fecal bacteria are contradictory because the combination appears to work for some investigators (e.g., Kerfoot & Skinner 1981; Hagedorn *et al.* 2005) but not for others (e.g., Close *et al.* 1989; Wolfe 1995). Therefore, combining fluorometric measurements with counts of fecal bacteria may or may not have potential as a BST method for detecting human fecal contamination. To investigate this possibility further, we conducted a study to evaluate combining targeted sampling and fluorometry in a freshwater creek during baseflow and stormflow conditions. To confirm fluorometry, we used detection of the *esp* (enterococcal surface protein) gene. The *esp* gene in *Ent. faecium* isolates is detected in 97% of human sewage and septic samples but not in any non-human animal feces (Scott *et al.* 2005).

Table 1 │ Likely cause(s) of fecal contamination when certain numbers of fecal bacteria and optical brightener (fluorometric units) are observed

Fecal bacteria numbers	Optical brightener	Likely cause(s)
High	High	Failing onsite waste disposal system or leaking sewer pipe
High	Low	Human (e.g., outhouse) or other warm-blooded animals
Low	High	Gray water in storm water system
Low	Low	No evidence of fecal contamination

MATERIALS AND METHODS

Water and sediment sampling locations and times

The Potato Creek watershed covers approximately $380\,km^2$ within the upper Flint River watershed of Georgia (Absher 2003) and because of its size, targeted sampling was conducted in a series of three nested samplings, each sampling encompassing an ever-decreasing distance. The first targeted sampling divided Potato Creek into reaches marked by easily accessible points, usually bridges. This sampling was the same as the traditional sampling methodology employed by the U. S. Geological Survey or U. S. Environmental Protection Agency to identify specific reaches with persistently high numbers of fecal bacteria. The second targeted sampling involved either walking or kayaking reaches with high numbers of fecal bacteria to identify specific hotspots of fecal contamination. If the source of fecal contamination was unclear, then a third targeted sampling was conducted only around these hotspots.

Each targeted sampling requires determining fecal bacterial counts during both baseflow and stormflow conditions. Because weather conditions are often variable, multiple samplings may need to be conducted until a coherent picture of the sources of fecal contamination emerges. However, in some instances, if the sources of fecal contamination are clear (e.g., cattle standing in the stream), then only a single sampling is needed.

The first targeted sampling of Potato Creek was conducted by an engineering firm who identified three reaches with persistently high numbers of the fecal indicator bacterium, *Escherichia coli*: (a) an unnamed tributary near Griffin, Georgia (henceforth, Griffin reach), (b) Potato Creek near Meansville, Georgia (henceforth, Meansville reach), and (c) Potato Creek near Thomaston, GA (henceforth, Thomaston reach; Figure 1). Since conducting multiple samplings over a period of several months was expensive, only a baseflow sampling was conducted during this first overall targeted sampling.

The Griffin reach is a narrow (between 1 and 2 m wide), shallow (typically, <20 cm deep), tributary in the headwaters of Potato Creek within the city of Griffin, GA. The reach is located in a residential neighborhood, and the homes are on a sewer system. Many drainage pipes and
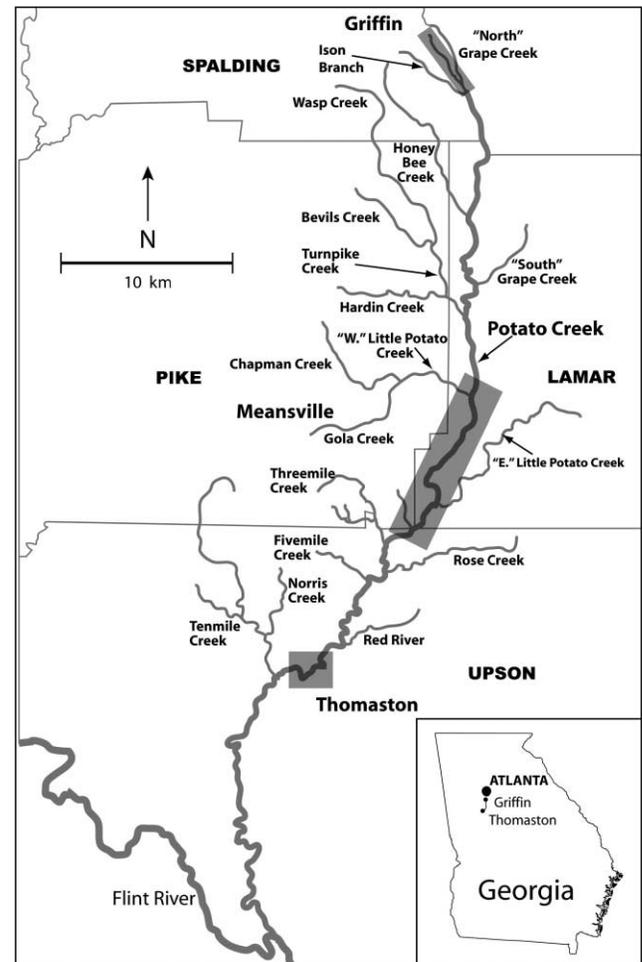

Figure 1 | Location of three reaches of Potato Creek identified by an engineering firm as having elevated numbers of the fecal indicator bacterium, *Escherichia coli* (light gray areas; from north to south: Griffin, Meansville, and Thomaston). Some tributaries have names in quotes to distinguish them from other tributaries with the same name. The creek flows from north to south. The inset shows the location of Potato Creek in Georgia (U.S.A.).

a few small tributaries (<0.2 m wide) also drain into the creek. The reach was sampled three times during baseflow (6 Jul 2004, 25 Jan 2005 and 16 Dec 2005) and twice during stormflow conditions (8 Sep 2004 and 1 June 2005). No fluorometer was available before October 2004, therefore, fluorometry was conducted for all the samplings except the initial baseflow and stormflow samplings.

The Meansville reach of Potato Creek is east of the town of Meansville, GA, between the bridges of two roads, Turner Bridge Road and C.W. Allen Road. Farms, especially cattle and dairy farms, are present in this largely rural area. Although the reach is fenced to prevent cattle from getting

into Potato Creek proper, cattle have unrestricted access to some of the small tributaries of Potato Creek. The reach was sampled once during baseflow (20 April 2005) and once during stormflow conditions (22 June 2005). Tributaries near C. W. Allen Road were resampled on 22 Jun 2005 and 18 Jul 2005.

The Thomaston reach of Potato Creek is in the town of Thomaston, GA, between County Road and Hannahs Mill Road. The reach is located in a mixed neighborhood consisting of both residential and commercial properties, and it is notable because it contains the intake for the Thomaston Water Treatment Plant. The creek is deeper and wider here compared to the Griffin reach. A small number of pipes of undetermined origin were observed, although few of them were discharging during the two samplings. Samples were collected both during baseflow (11 May 2005) and stormflow conditions (6 Jul 2005).

Chemical and physical characteristics of Potato Creek

At each sampling point, the location coordinates were taken with a GPS device (Model 76CS, Garmin International Inc., Olathe, KS). Locations of each sampling site were converted to ArcView 3.2 shapefiles and incorporated into a GIS database. Turbidity was recorded with a turbidity meter (HF Scientific Inc., Fort Myers, FL), and dissolved oxygen (DO), temperature, and pH were recorded with a Hydrolab Quanta (Austin, TX). For DO, a limit of $4.0\,\mathrm{mg\,L^{-1}}$ was used to identify sites of concern because this limit is a minimum considered necessary to support warm water fish species (Georgia Department of Natural Resources 2002). Total organic carbon was determined with a Carlo Erba C/N analyzer, and NO_3^--N was determined by standard methods (*Standard Methods for the Examination of Water and Wastewater* 1998).

Sampling for *Escherichia coli*

All samples were collected aseptically on foot or from inflatable kayaks. Water samples were collected in 500-ml (18 oz.) Whirl-Pak bags (Nasco, Modesto, CA). Sediment samples (uppermost few millimeters only) were collected with an ethanol-disinfected spoon and were placed into sterile 500-ml polypropylene bottles. Water and sediment samples were placed on ice and processed within 6 hours using the Colilert System™ (IDEXX Laboratories, Westbrook, ME), which uses the Most-Probable-Number (MPN) method. The Colilert system estimates the number of total coliforms (not recorded) and *E. coli* per 100 ml. Water samples were diluted with sterile distilled water to 10^{-1} in sterile manufacturer-supplied polystyrene bottles. A package of powdered Colilert medium was added to each bottle. After the medium was dissolved, the contents of each bottle were added to a Quanti-tray, a sterile disposable panel containing 97 wells. Each Quanti-tray was mechanically sealed, which distributed the sample uniformly into the wells. Each Colilert Quanti-tray was incubated at $37 \pm 0.5°C$ and after 18 to 22 hours, fluorescing (positive) wells were counted under a 365-nm UV light (Model EA-160, Spectronics Corp., Westbury, NY). The number of positive wells was converted to a MPN value based on the dilution factor and manufacturer-supplied MPN tables.

Sediment samples were allowed to resettle for 1 hour, after which the overlying water was removed aseptically. The sediment was thoroughly shaken in the collection bottle, and 10-g samples were serially diluted with sterile distilled water to 10^{-1} and 10^{-3} in sterile manufacturer-supplied polystyrene bottles for enumeration of *E. coli* as described previously. To express the number of *E. coli* on a per g dry weight basis, three additional 10-ml sediment samples were each poured into preweighed aluminum weigh boats and were dried at 95°C for at least 24 hours before the boats were reweighed. The weights of the three sediments were averaged and were divided into the MPN value as appropriate.

The fecal indicator bacterium was *Escherichia coli*. Although it is recommended by the USEPA (1986) as an effective predictor of gastrointestinal illness in freshwater, the State of Georgia has yet to identify the number of *E. coli* permitted in various environmental waters. Therefore, an arbitrary limit of 235 *E. coli* per 100 ml was chosen for a single grab sample because it is the recommended limit of *E. coli* in fresh water with primary contact (USEPA 1986).

Enterococcus speciation and detection of the *esp* gene

Detection of the *esp* gene requires obtaining *Ent. faecium* isolates. These isolates were obtained from another group

of fecal indicator bacteria, the fecal enterococci, by the same protocol used for *E. coli*, except that the Enterolert System™ (IDEXX Laboratories) was used and the incubation temperature for the Quanti-trays was 41 ± 0.5°C. After 24 hours, positive (fluorescing) Enterolert Quanti-tray wells were labeled with an acetate marker. The back of the Quanti-tray was surface-disinfected with 70% ethanol, and each well was punctured with a separate sterile pipette tip. A 10-μl portion was removed from each well with a pipette, and the portion was spotted into one well of a 96-well microtiter plate containing Enterococcosel agar (Becton Dickinson, Sparks, MD). The plate was incubated for 24 hours at 35°C. Wells positive for esculin hydrolysis (black color) were streaked onto 5-cm plates containing Brain Heart Infusion agar (Becton Dickinson) with 6.5% NaCl. Plates were incubated at 35°C in Ziploc bags (DowBrands, Indianapolis, IN) to maintain high humidity levels. After 48 hours, colonies on the plates (positive growth) were subjected to a catalase test with 8.82 M H_2O_2 to ensure that each isolate was catalase negative. Quanti-tray wells containing bacteria that conformed to this USEPA definition of fecal enterococci (hydrolyzed esculin, grew on brain heart infusion agar with 6.5% NaCl, and were catalase negative; USEPA 2002) were speciated.

To speciate the enterococci, an isolate was randomly picked with a sterile plastic stab from the plate containing brain heart infusion agar with 6.5% NaCl. Each isolate was suspended in 125 μl of saline–phosphate buffer contained in a well of a 96-well microtiter plate. Three wells of the 96-well plate were reserved for an American Type Culture Collection (ATCC) control, *Ent. faecium* ATCC #19434, and three wells were reserved for randomly placed uninoculated controls. The isolates were inoculated with a sterile polypropylene replicator (Sigma Chemical Co., St. Louis, MO) into separate microtiter plates, each containing medium specific for the identification of *Ent. faecium* according to the flowchart described by Manero & Blanch (1999). Plates were incubated at 35°C and reactions were recorded after 72 hours. Isolates exhibiting reactions consistent with the identification of *Ent. faecium* were spotted onto a 0.45-mm membrane contained on 5-cm Petri plates with mEI agar (Becton-Dickinson). The plates were incubated at 41 ± 0.5°C for 24 hours and were sent by overnight mail to Biological Consulting Service of North Florida (Gainesville, FL). The *Ent. faecium* isolates were analyzed for the presence of the *esp* gene with the appropriate positive and negative controls as described in Scott *et al.* (2005).

Fluorometry

Fluorometry was conducted with a field fluorometer (Model 10-AU-005, Turner Designs, Sunnyvale, CA) set to detect long wavelength optical brighteners (excitation, 360 nm; emission, 410–600 nm) as described by the manufacturer. Water samples were refrigerated in the dark at 4 to 10°C and processed within 1 day. Each water sample was analyzed at room temperature (20 to 25°C) and read within 30 seconds to avoid heating the sample by the UV lamp. The fluorometer was zeroed with distilled water (negative control) and 100 mg of commercial laundry detergent (Tide, Procter & Gamble, Cincinnati, OH) L^{-1} was arbitrarily set to equal 100 fluorometric units (positive control). Under normal circumstances, environmental water containing > 100 fluorometric units is considered positive (Hagedorn *et al.* 2005), but Georgia waters contain high amounts of organic matter, and a higher value, > 150 fluorometric units, was chosen for identifying sites of concern.

RESULTS

General chemical and physical characteristics

None of the general physical and chemical characteristics of water samples taken from Potato Creek during Summer 2005 baseflow conditions was unusual. The pH of the water was relatively neutral (pH 6.3 to 7.1), and DO levels were above the 4.0 mg L^{-1} cutoff for impairment (Table 2).

Griffin reach

Three baseflow samplings were conducted off this reach, but only the second and third baseflow samplings were combined with fluorometry because the fluorometer was not available until March 2005. The first baseflow sampling was conducted during the summer (Jul 2004), and the second and third baseflow samplings were conducted

Table 2 | Average physical and chemical characteristics of some water samples taken from Potato Creek during Summer 2005 baseflow conditions at four different locations: (1) Grandview Drive in Griffin, (2) below the Griffin Wastewater Treatment Plant (WWTP) outfall, (3) Little Potato Creek near Meansville, and (4) intake for the Thomaston Water Treatment Plant

Location	pH	Temp.	Turbidity	DO[a]	Organic C	NO$_3^-$-N
		°C	NTU		mg L^{-1}	
Grandview Drive	7.1	18.9	11	4.8	1.7	0.23
Griffin WWTP	6.1	18.0	38	7.5	1.4	0.57
Little Potato Creek	6.3	20.4	31	7.9	ND[b]	ND
Thomaston intake	6.3	21.0	22	7.9	4.0	0.15

[a]DO, dissolved oxygen; [b]ND, not done.

during the winter (Jan and Dec 2005). Only the first two baseflow samplings are shown (Figure 2(A)).

During the first baseflow sampling, 23 samples were obtained (Sites 1A to 23A), five of which were from tributaries or pipes. All 23 samples exceeded 235 *E. coli* 100 per ml, and almost half (11 of 23 sites; 48%) exceeded 1,000 *E. coli* per 100 ml. Dogs in kennels near the creek were observed below Sites 1A and 4A, and a broken household sewer pipe over the creek was observed at Site 8A. However, no effluent was draining from the broken sewer pipe. When *Ent. faecium* isolates were tested for the presence of the *esp* gene at Site 23A, the gene was not detected. When the baseflow sampling was repeated for the first time in the winter (Jan 2005), 20 samples were obtained (Samples 1B to 20B), seven of which were obtained from tributaries or pipes. Only six samples, including three from tributaries or pipes, contained >235 *E. coli* per 100 ml and only one sample (Site 20B) contained >1,000 *E. coli* per 100 ml. None of the sites exceeded 150 fluorometric units. When the baseflow sampling was repeated for the second time in winter (Dec 2005), 20 samples were obtained, and the results were similar to the previous winter sampling: only one sample exceeded 235 *E. coli* per 100 ml and no sample exceeded 150 fluorometric units (data not shown).

Two stormflow samplings were conducted, but only the second sampling was combined with fluorometry (Figure 2(B)). During the first stormflow sampling (Sep
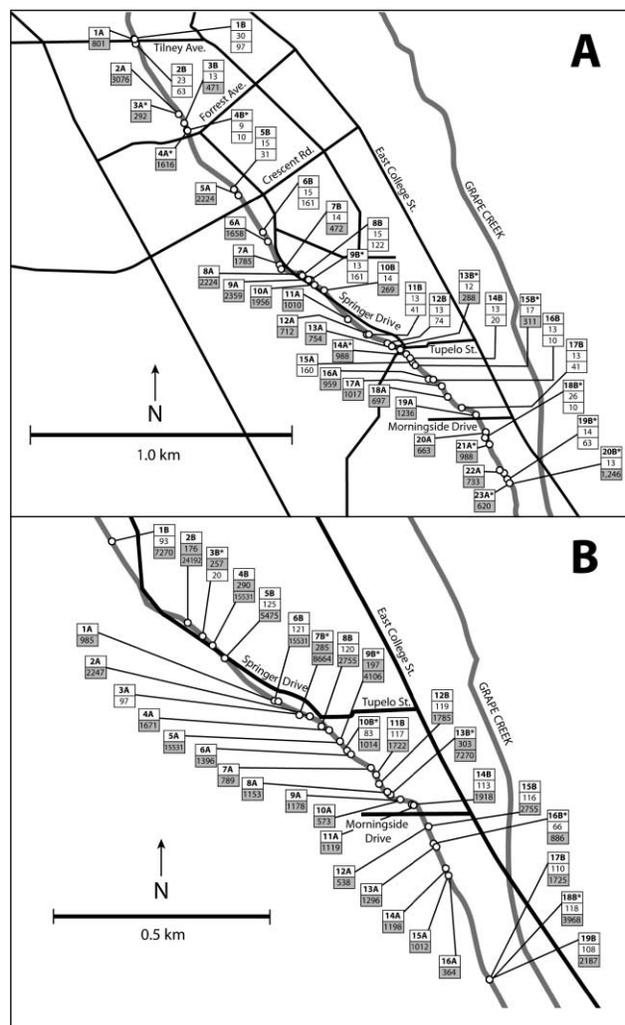

Figure 2 | Location of the sampling sites in the Griffin reach during baseflow (A) and stormflow (B) conditions. The reach runs from north to south. Two samplings were conducted for each flow condition. The first sampling shows the site number (top number with the letter (A) and number of *E. coli* per 100 ml (bottom number). The second sampling shows the site number (top number with the letter (B), fluorometric value (middle number), and number of *E. coli* per 100 ml (bottom number). If a tributary or pipe was sampled, then the site number is identified with an asterisk. If the fluorometric value exceeds 150 units or the number of *E. coli* exceeds 235 per 100 ml, then each number is shaded in light gray.

2004), 16 samples were obtained (Sites 1A to 16A). Except for Site 3A, all samples exceeded the limit for *E. coli*, and, in fact, 10 of the remaining 15 samples were >1,000 *E. coli* per 100 ml. During the second stormflow sampling (Jun 2005), 19 samples were obtained (Sites 1B to 19B), of which seven were from tributaries or pipes. With the exception of one tributary (Site 3B), all samples exceeded the limit for *E. coli*, and 18 of the remaining 19 samples were >1,000 *E. coli* per

100 ml. Six of the 19 samples exceeded 150 fluorometric units, of which four were from tributaries or pipes. However, one of these six samples (Site 3B), a tributary, had only 20 *E. coli* per 100 ml. The broken household sewer pipe (Site 2B) was underwater and was associated with a fluorometric reading of 176 and 24,192 *E. coli* per 100 ml. Immediately downstream of this site (Site 4B), the water sample had a fluorometric reading of 290 units and 15,531 *E. coli* per 100 ml.

Meansville reach

Fifteen samples (Sites 1 to 15) were obtained between Route 18 and Turner Bridge Road during a baseflow sampling (Mar 2005) to determine the potential sources of fecal contamination from upstream of the actual Meansville reach (Figure 3). None of the 15 samples exceeded 235 of *E. coli* per 100 ml, but one location (Site 15) exceeded 150 fluorometric units. One baseflow (April 2005) and one stormflow (Jun 2005) sampling were then conducted on the Meansville reach. During the baseflow sampling, 20 sites were sampled (Sites 1A to 20A), of which 14 were tributaries. Only two samples, one from Potato Creek (Site 3A) and one from a tributary (Site 10A) were of concern. Only one sample (Site 20B) exceeded 150 fluorometric units.

During the stormflow sampling, 21 sites were sampled (Sites 1B to 21B), of which 17 were tributaries. In contrast to baseflow conditions where only two sites were of concern, ten sites, composed of one Potato Creek sample and nine tributary samples, were of concern during stormflow conditions. There was a sharp increase in the numbers of *E. coli* below Site 13B, where numbers of *E. coli* increased from 201 to $\geq$ 1,500 *E. coli* per 100 ml. Despite the increase in the number of sites of concern, none of the 21 samples had fluorometric values >150 units.

Under the guidelines of targeted sampling, the three main tributaries in this section of the reach were resampled (18 Jul 2005) and 12 samples were obtained (Figure 3, inset). All three tributaries drained pastures, but cows were observed in only one pasture. In the two pastures where no cows were observed, all four samples (Sites 1 to 4) had <235 *E. coli* per 100 ml, whereas in the one pasture with cows, 7 of 8 samples (Sites 5 to 12) had >235 *E. coli* per 100 ml. Cows were observed standing in the tributary. Sediment from this tributary contained 1,182 *E. coli* per g

dry weight. One of the samples from the pasture with cows, Site 12, and two of three samples from one of the other two pastures, Sites 2 and 3, had fluorometric values >150 units. However, none of these three samples contained high enough *E. coli* numbers to be of concern.

Thomaston reach

One baseflow (May 2005) and one stormflow (Jul 2005) sampling were conducted in the reach (Figure 4). During the baseflow sampling, 21 samples were collected (Sites 1A-21A), six of which were from tributaries. Only two samples exceeded 235 *E. coli* per 100 ml, neither of which was from a tributary. The highest count in Potato Creek was 299 *E. coli* per 100 ml (Site 13A). No samples had fluorometric values >150 units. In addition, five tributaries (Letters A through E) were sampled, one of which (Letter A) contained 1,086 *E. coli* per 100 ml. None of the five tributary sites had fluorometric values >150 units. Because this unnamed tributary alongside Hillside Drive (Letter A) was also the only tributary sample associated with high numbers in Thomaston reach, under the guidelines of targeted sampling, it was scheduled for resampling.

Numbers of *E. coli* increased in the reach during stormflow conditions. Of the 18 samples collected (Site 1B to 18B) during these conditions, eight of 12 Potato Creek samples and four of six tributary samples exceeded the *E. coli* limit. One (Site 12B) had 3,255 *E. coli* per 100 ml. With one exception, none of the 18 samples had fluorometric values >150 units. The exception was Site 15B, which contained runoff from the parking lot of a shopping center, and had a fluorometric value of 385 units; however, the sample contained only 41 *E. coli* per 100 ml.

The unnamed tributary alongside Hillside Drive was resampled during both baseflow (17 May 2005) and stormflow conditions (1 Jun 2005). Of eight samples obtained during baseflow conditions, only the sample from Site 1 contained >235 *E. coli* per 100 ml (Figure 4, inset). The fluorometric value was 72 units. According to neighbors, Site 1 is a kennel containing approximately 20 to 30 dogs. When the unnamed tributary was resampled during stormflow conditions, the numbers of *E. coli* were 2,481 per 100 ml below the kennel and 377 per 100 ml
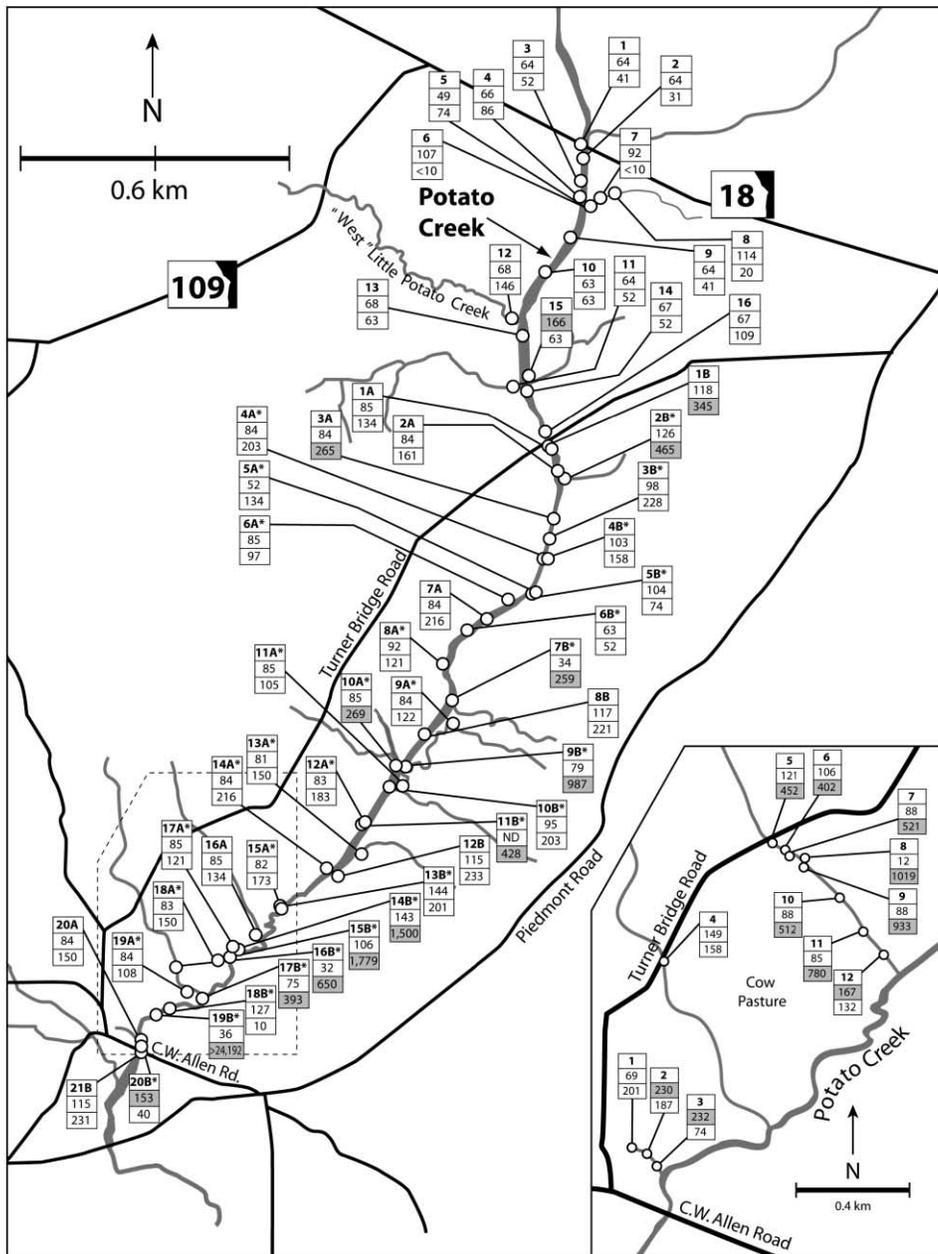
Figure 3 | Location of sampling sites in the Meansville reach during baseflow and stormflow conditions. The reach runs from north to south. Each site shows the site number (top number), fluorometric value (middle number), and number of *E. coli* per 100 ml (bottom number). The site number is identified with the letter A for sampling during baseflow conditions, B for sampling during stormflow conditions, and an asterisk if a Potato Creek tributary was sampled. An additional baseflow sampling above Turner Bridge Road has site numbers with no letters. If the fluorometric value exceeds 150, or the number of *E. coli* exceeds 235 per 100 ml, then the number is shaded in light gray. The inset shows resampling of selected tributaries with persistent fecal contamination.

above the kennel (data not shown). Sediment obtained directly below the kennel contained 160,582 *E. coli* per g dry weight of sediment. When *Ent. faecium* isolates in the sediment were tested for the presence of the *esp* gene, it was not detected.

DISCUSSION

Targeted sampling

This study was the first targeted sampling in fresh water during baseflow and stormflow conditions. The results were
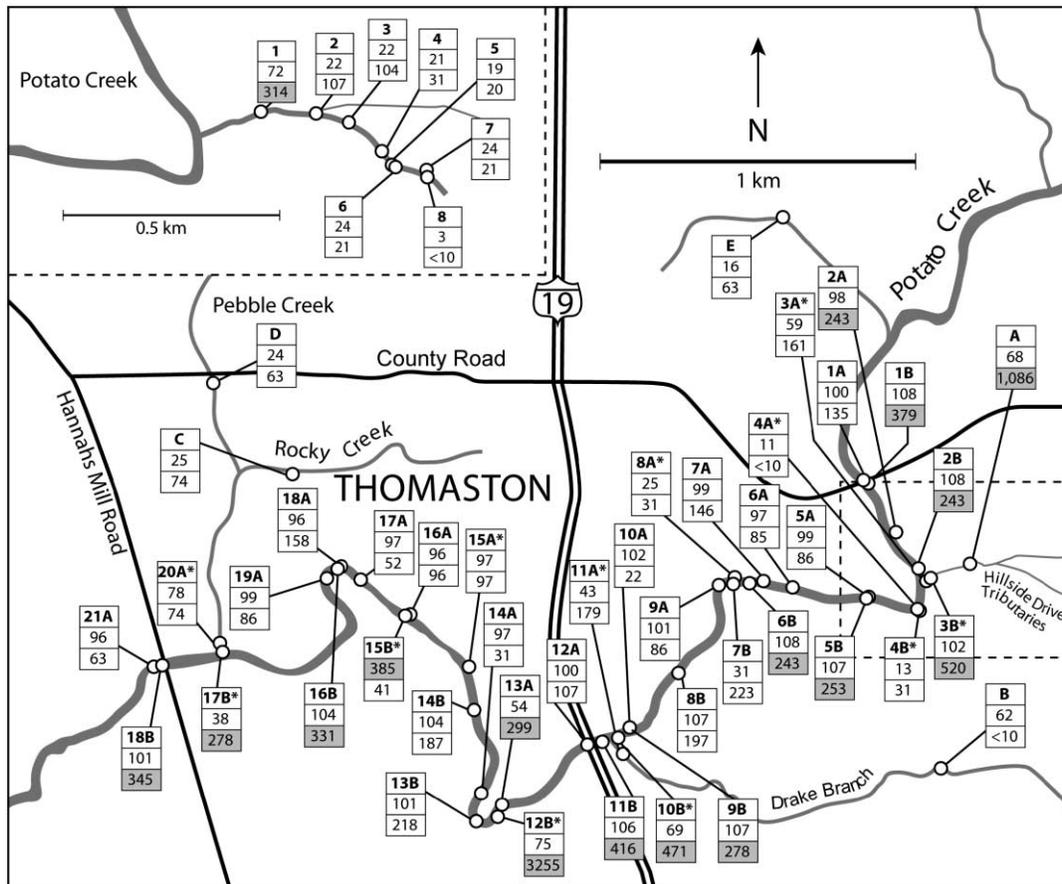
Figure 4 | Location of sampling sites during baseflow and stormflow conditions in the Thomaston reach of Potato Creek. The reach runs from west to east. Each site shows the boldface site number (top number), fluorometric value (middle number), and number of *E. coli* per 100 ml (bottom number). The site number is identified with the letter A for sampling during baseflow conditions, B for sampling during stormflow conditions, and an additional asterisk if a tributary was sampled. Sites sampled separately from a vehicle are identified with a boldface top letter and no number. The dashed line shows the location of the inset. If the fluorometric value exceeds 150 units or the number of *E. coli* exceeds 235 per 100 ml, then the number is shaded in light gray. The inset shows resampling of a selected tributary along Hillside Drive with persistent fecal contamination.

similar to that for marine and estuarine conditions (McDonald *et al.* 2006) except *E. coli*, not fecal enterococci, was the fecal indicator bacterium, and tidal influences were not a consideration. Like the marine and estuarine conditions, the method quickly and inexpensively identified some sources of fecal contamination. In the first targeted sampling, three reaches of Potato Creek, arbitrarily identified as Griffin, Meansville, and Thomaston, were identified as having persistent high numbers of fecal bacteria (baseflow only). In accordance with the targeted sampling protocol, only these three reaches were resampled during baseflow and stormflow conditions.

In the case of resampling the Griffin reach, the reach was indeed persistently contaminated with high numbers of

E. coli during both base and stormflow conditions. As expected, fecal contamination was much worse during stormflow than during baseflow conditions; such increases have been observed in Georgia fresh waters previously as runoff brings new sources of fecal material into the reach (Gregory & Frick 2001; Hartel *et al.* 2004). Targeted sampling identified a broken home sewer line as a potential source of fecal contamination during both base- and storm-flow conditions, as well as potential fecal contamination from dog kennels during stormflow conditions. It is possible that urban wildlife contributed to these numbers as well. During baseflow conditions, higher numbers of *E. coli* were observed in the summer than in winter. This result may be because conditions for bacterial survival are better in summer than in

winter. Even though *E. coli* has long been known to survive best in cool, moist conditions (Van Donsel *et al.* 1967), any freezing would have reduced *E. coli* numbers significantly.

In the case of the Meansville reach, the initial sampling showed that fecal contamination to the Meansville reach was not coming from Potato Creek upstream. Therefore, the source must be downstream from Turner Bridge Road. Targeted sampling showed that cows were the major source of contamination to this reach. When tributaries near C. W. Allen Bridge were sampled, high *E. coli* numbers were observed and cows were observed standing in the tributary. These results suggest that best management practices such as creating riparian filter strips, building non-riparian shade, and providing cattle with alternative water sources away from the tributaries (Byers *et al.* 2005) would be helpful in reducing fecal contamination. Such practices should reduce sediment loads in the creek as well (not measured here).

In the case of the Thomaston reach, the major source of identifiable fecal contamination was a dog kennel located on Hillside drive. Targeted sampling showed that high numbers of *E. coli* in the unnamed tributary were not obtained in the water above this kennel, but were obtained in the water and sediment below the kennel. In addition to this identifiable source, other unidentifiable sources (e.g., wildlife) were likely to contribute fecal contamination to this Potato Creek reach during baseflow and stormflow conditions. For example, high numbers of *E. coli* were observed in Potato Creek above the point where the unnamed tributary from Hillside Drive enters Potato Creek, as well as in several tributaries.

Fluorometry

Fluorometry worked, but appeared to have some limitations. On the positive side, fluorometry confirmed a broken household sewer line crossing over the creek in the Griffin reach. During baseflow conditions, no effluent was visible, bacterial counts were low, and fluorometric values were low. The *esp* gene was not detected, confirming the fluorometric result. During stormflow conditions, the pipe was underwater and the high water was likely flooding the sewer system. High counts of *E. coli* (24,192 per 100 ml) and a high fluorometric value (176 units) were detected in the water below the pipe as

well as locations further downstream. In the Meansville reach, targeted sampling identified cows as the major source of fecal contamination, and there were no instances of high bacterial counts and high fluorometric values consistent with human fecal contamination. Finally, in the Thomaston reach, targeted sampling identified a dog kennel as the major source of fecal contamination, and, like Meansville, there were no instances of high bacterial counts and high fluorometric values consistent with human fecal contamination. In addition, the *esp* gene was not detected in the Thomaston sample. Therefore, whenever high bacterial counts were observed, positive fluorometric values were observed during conditions where they were expected, and, were not observed when they were not expected.

On the negative side, there were five instances (Table 3) where high fluorometric values were observed and bacterial counts were low. Using the fluorometry key (Table 1), these sites would be identified as having gray water, where effluent would contain laundry detergents, but not human feces. In the case of Griffin and Thomaston, both urban environments, gray water was a possible source, but more likely sources were diesel fuel and motor oil from nearby asphalt roads. Both diesel fuel and motor oil sources contain compounds that fluoresce at approximately the same wavelength as optical brighteners (C. Hagedorn, personal communication, 2005). Indeed, the site in the Thomaston reach was associated with runoff from a local shopping center parking lot.

Although diesel fuel and motor oil are likely sources of fluorescence in the Griffin and Thomaston water samples, they

Table 3 │ Reach, conditions, and site number of five locations where high fluorometric values (>150 units) and low numbers of *Escherichia coli* (<235 per 100 ml) occurred

Reach (flow condition)	Site No.	Fluorometric units	*E. coli* per 100 ml
Griffin (stormflow)	3B	257	20
Meansville (baseflow)	2 (inset)	230	187
Meansville (baseflow)	3 (inset)	232	74
Meansville (baseflow)	12 (inset)	167	132
Thomaston (stormflow)	15B	385	41

were unlikely sources in the three Meansville water samples, which came from tributaries draining cow pastures. In these instances, the most likely reason for the high fluorometric readings was background fluorescence from organic matter. It has long been known that organic matter in water fluoresces when exposed to UV light (Kalle 1949), and in fact, fluorescence can measure total organic carbon (Smart *et al.* 1976). This fluorescence may be a particular problem in the southeastern United States waters, which are characterized by low topographic relief, extensive floodplain swamps, and rich organic bottom sediments. In addition to organic matter, another possibility is ammonium and nitrate from cow manure. Fluorescence is positively correlated with ammonium and nitrate (Chen & Bada 1992). One possible solution which would reduce this background fluorescence may be to restrict the emission wavelength of the fluorometer. Since organic matter fluorescence in environmental waters has broadband, featureless emission spectra (Chen & Bada 1992) and the emission spectra of optical brighteners are in the 415- to 445-nm range, restricting the emission filter in the fluorometer within this wavelength range may improve the resolution of fluorometry to detect optical brighteners. Studies to restrict the wavelength of the emission filter are in progress.

CONCLUSIONS

The major problem with BST is its cost, and many communities cannot afford most BST methods. Therefore, concentrating BST research on inexpensive methods may be meritorious. Here, an inexpensive prelude to BST, targeted sampling, was combined with a relatively inexpensive BST method, fluorometry, to identify human sources of fecal contamination. Both methods identified human fecal contamination in the one reach where it was observed. For targeted sampling, each IDEXX sample costs $6, in addition to one-time costs for a sealer ($3,500), a UV light ($150), and an incubator (normally available for free at wastewater treatment facilities). For fluorometry, the cost of each sample is negligible, with one-time costs for a field fluorometer ($14,250). If the sources of fecal contamination are unknown, then conducting targeted sampling and confirming human fecal contamination with fluorometry may be a relatively inexpensive method of source tracking. Conversely, if human

fecal contamination is suspected, then using fluorometry to identify sources of optical brighteners and confirming hotspots with fecal counts may be yet another inexpensive alternative. Importantly, both methodologies place a premium on common sense (e.g., identifying obvious sources like cows and broken sewer pipes) and local knowledge (e.g., runoff from parking lots) to minimize costs. In the case of fluorometry, changing the emission filter to reduce background organic matter fluorescence may be helpful for waters with high concentrations of organic matter.

REFERENCES

Absher, C. 2003 Fecal coliform TMDL implementation: A case study. *Stormwater* **4**(6), 62–76.

Byers, H. L., Cabrera, M. L., Matthews, M. K., Franklin, D. H., Andrae, J. G., Radcliffe, D. E., McCann, M. A., Kuykendall, H. A., Hoveland, C. S. & Calvert, V. H. II 2005 Phosphorus, sediment, and *Escherichia coli* loads in unfenced streams of the Georgia Piedmont, USA. *J. Environ. Qual.* **34**, 2293–2300.

Chen, R. F. & Bada, J. L. 1992 The fluorescence of dissolved organic matter in seawater. *Mar. Chem.* **37**, 191–221.

Close, M. E., Hodgson, L. R. & Todd, G. 1989 Field evaluation of fluorescent whitening agents and sodium tripolyphosphate as indicators of septic tank contamination in domestic wells. *N.Z.J. Mar. Freshwater Res.* **23**, 563–568.

Georgia Department of Natural Resources 2002 *Rules and Regulations for Water Quality Control*. Environmental Protection Division, Chapter 391-3-6, available at: http://rules.sos.state.ga.us/docs/391/3/6/03.pdf (verified 22 March 2007).

Gregory, M. B. & Frick, E. A. 2001 Indicator-bacteria concentrations in streams of the Chattahoochee River National Recreation Area, March 1999–April 2000. In *Proceedings of the 2001 Georgia*

Water Resources Conference, 26–27 Mar 2001 (ed. K. J. Hatcher), Univ. of Georgia, Athens. pp. 510–513.

Hagedorn, C., Saluta, M., Hassall, A. & Dickerson, J. 2005 Fluorometric detection of optical brighteners as an indicator of human sources of water pollution: Development as a source tracking methodology. *Environ. Detect. News* **2**, 1–13.

Hartel, P. G., Summer, J. D., Hill, J. L., Collins, J. V., Entry, J. A. & Segars, W. I. 2002 Geographic variability of *Escherichia coli* ribotypes from animals in Idaho and Georgia. *J. Environ. Qual.* **31**, 1273–1278.

Hartel, P. G., Summer, J. D. & Segars, W. I. 2003 Deer diet affects ribotype diversity of *Escherichia coli* for bacterial source tracking. *Water Res.* **37**, 3263–3268.

Hartel, P. G., Frick, E. A., Funk, A. L., Hill, J. L., Summer, J. D. & Gregory, M. B. 2004 Sharing of ribotype patterns of *Escherichia coli* isolates during baseflow and stormflow conditions. *U.S. Geological Survey, Scientific Investigations Report 2004–5004*. 10 p, available at: http://pubs.usgs.gov/sir/2004/5004/pdf/SIR_2004-5004.pdf (verified 8 July 2006).

Hayashi, Y., Managaki, S. & Takada, H. 2002 Fluorescent whitening agents in Tokyo Bay and adjacent rivers: Their application as anthropogenic molecular markers in coastal environments. *Environ. Sci. Technol.* **36**, 3556–3563.

Jenkins, M. B., Hartel, P. G., Olexa, T. J. & Stuedemann, J. A. 2003 Putative temporal variability of *Escherichia coli* ribotypes from yearling steers. *J. Environ. Qual.* **32**, 305–309.

Kalle, K. 1949 Fluoreszenz und Gelbstoff im Bottnischem and Finnishchen Meerbusen. *Deutsche Hydrographische Zeitschrift*, **2**, 9–124 (in German).

Kaschig, J. 2003 Fluorescent whitening agents (FWAs) in laundry detergents. In: *Proceedings of the Second Symposium on Detergents, Damascus, Syria*, available at: http://www.cibasc.com/image.asp?id = 9568 (verified 19 May 2006).

Kerfoot, W. B. & Skinner, S. M. Jr. 1981 Septic leachate surveys for lakeside sewer need evaluation. *J. Water Pollut. Control Fed.* **53**, 1717–1725.

Kramer, J. B., Canonica, S., Hoigné, J. & Kaschig, J. 1996 Degradation of fluorescent whitening agents in sunlit natural waters. *Environ. Sci. Technol.* **30**, 2227–2234.

Kuntz, R. L., Hartel, P. G., Godfrey, D. G., McDonald, J. L., Gates, K. W. & Segars, W. I. 2003 Targeted sampling protocol with *Enterococcus faecalis* for bacterial source tracking. *J. Environ. Qual.* **32**, 2311–2318.

Manero, A. & Blanch, A. R. 1999 Identification of *Enterococcus* spp. with a biochemical key. *Appl. Environ. Microbiol.* **65**, 4425–4430.

McDonald, J. L, Hartel, P. G., Gentit, L. C., Belcher, C. N., Gates, K. W., Rodgers, K., Fisher, J. A., Smith, K. A. & Payne, K. A. 2006 Identifying sources of fecal contamination inexpensively with targeted sampling and bacterial source tracking. *J. Environ. Qual.* **35**, 889–897.

Poiger, T., Field, J. A., Field, T. M., Siegrist, H. & Giger, W. 1998 Behavior of fluorescent whitening agents during sewage treatment. *Water Res.* **32**, 1939–1947.

Scott, T. M., Jenkins, T. M., Lukasik, J. & Rose, J. B. 2005 Potential use of a host associated molecular marker in *Enterococcus faecium* as an index of human fecal pollution. *Environ. Sci. Technol.* **39**, 283–287.

Simpson, J. M., Santo Domingo, J. W. & Reasoner, D. J. 2002 Microbial source tracking: State of the science. *Environ. Sci. Technol.* **36**, 5279–5288.

Smart, P. L., Finlayson, B. L., Rylands, W. D. & Ball, C. M. 1976 The relation of fluorescence to dissolved organic carbon in surface water. *Water Res.* **10**, 805–811.

Standard Methods for the Examination of Water and Wastewater 1998 20th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

U.S. Environmental Protection Agency (USEPA) 1986 *Ambient Water Quality Criteria for Bacteria–1986*. USEPA Rep. 440/5-84-002. USEPA, Cincinnati, OH.

U.S. Environmental Protection Agency (USEPA) 2002 *Implementation Guidance for Ambient Water Quality Criteria for Bacteria*, available at: www.epa.gov/ost/standards/bacteria/bacteria.pdf (accessed 17 Mar 2003; verified 15 Dec 2005). USEPA, Office of Water, Washington, DC.

U.S. Environmental Protection Agency (USEPA) 2005 *Microbial Source Tracking Guide Document*. EPA/600-R-05-064. Office of Research and Development, Cincinnati, OH.

Van Donsel, D. J., Geldreich, E. E. & Clarke, N. A. 1967 Seasonal variations in survival of indicator bacteria in soil and their contribution to storm water pollution. *Appl. Microbiol.* **15**, 1362–1370.

Wolfe, T. M. 1995 *A Comparison of Fecal Coliform Densities and Fluorescent Intensities in Murrells Inlet, a Highly Urbanized Estuary and in North Inlet, a Pristine Forested Estuary*. M.S. Thesis. University of South Carolina, Columbia.