IMPROVING FLUOROMETRY AS A SOURCE TRACKING METHOD TO DETECT HUMAN FECAL CONTAMINATION IN GEORGIA WATERS

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Abstract. In a continuing effort to develop inexpensive source tracking methods to detect human fecal contamination in environmental waters, we combined targeted sampling with fluorometry. Targeted sampling works by identifying hotspots of fecal contamination through multiple samplings over ever-decreasing distances. Fluorometry identifies human fecal contamination by detecting optical brighteners, primarily from laundry detergents. On St. Simons Island, targeted sampling and fluorometry identified two hotspots of fecal contamination. One hotspot was confirmed as fecal contamination from humans, but the other was not, most likely because of background organic matter fluorescence. Adding a 436-nm emission filter to the fluorometer reduced this background fluorescence by >50%, and with this filter in place, the second hotspot was identified as fecal contamination from birds. As long as a fluorometer is equipped with a 436-nm emission filter, targeted sampling combined with fluorometry may be a relatively inexpensive method to identify human fecal contamination in water.

INTRODUCTION

Microbial source tracking identifies the host origin of fecal contamination in water. Our effort has concentrated primarily on developing inexpensive methods because most of the communities in which we work cannot afford expensive methods. One way we reduced the cost of source tracking was to develop targeted sampling (Kuntz et al., 2003), where hotspots of fecal contamination are identified by multiple samplings over ever-decreasing distances. Targeted sampling has been successfully combined with an expensive genotypic source tracking method, ribotyping, in estuarine waters during calm (baseflow) conditions (Kuntz et al., 2003), and combined with several inexpensive source tracking methods in marine and estuarine waters during both calm and stormy (stormflow) conditions (McDonald et al., 2006).

One problem with targeted sampling is that it cannot identify specific sources of fecal contamination. One relatively inexpensive source tracking method to identify human fecal contamination is fluorometry. Fluorometry works by detecting optical brighteners, which are compounds added to laundry detergents to compensate for undesirable yellowing in clothes (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 and wastewater treatment plants.

Conducting fluorometry in Georgia waters is difficult because background organic matter fluorescence. 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 (Hagedorn et al., 2005), one way to solve this problem of background fluorescence may be to change the filter in the fluorometer from a broad spectrum 410 to 600-nm emission filter to a more restrictive 436-nm filter.

We conducted a study combining targeted sampling and fluorometry in Georgia waters. We confirmed the fluorometric data by determining the percentage of *Enterococcus faecalis* and detecting the *esp* (enterococcal surface protein) gene. High percentages (≥30%) of *Ent. faecalis* are usually associated only with humans and some wild birds (Wheeler et al., 2002) and the *esp* gene in *Ent. faecium* isolates is detected in 97% of human sewage and septic samples and not in any nonhuman animal feces (Scott et al., 2005).

METHODS

St. Simons is one of Georgia's barrier islands. Since April 2004, the beach on the southern end of the island has had numerous beach advisories due to high numbers of the fecal indicator bacteria, fecal enterococci.

Targeted sampling requires multiple samplings over ever-decreasing distances. To satisfy this requirement, three samplings were conducted. The first sampling was conducted at the southern end of the island near St. Simons Village on 3 July 2005, and 66 water samples were obtained from the surf zone and the storm drains

emptying into Postell Creek, in the interior of the island, and into the Atlantic Ocean. Fluorometric values were obtained with each water sample.

A second sampling was conducted only on upper Postell Creek on 27 Apr 2006, and 23 water samples were obtained, 17 from the creek and six from storm drains. A third sampling of a storm drain system was conducted on 12 Jun 2006 and 19 water samples were obtained.

The Most Probable Number (MPN) of fecal enterococci, *Enterococcus* speciation, and detecting the *esp* gene were determined according to McDonald et al. (2006). Total organic carbon (TOC) was determined by a high temperature combustion method. The limit of fecal enterococci for a single grab sample is 104 per 100 mL of water, and this limit was the one chosen to identify sites of concern.

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 to 600 nm) as described by the manufacturer. For the second and third samplings, the 410 to 600-nm emission filter was replaced with a 436-nm emission filter. Water samples were processed according to McDonald et al. (2006). Hagedorn et al. (2005) suggested that any site with a fluorometric value >100 was positive for optical brighteners, and this limit was the one selected to identify sites of concern.

RESULTS

All surf zone samples from St. Simons beach (Sites #6-14) contained ≤52 fecal enterococci per 100 mL (Fig. 1A and 1B) and had fluorometric values <100. Sites #1-5, #15-17, and #64A-66A were either ditches, storm drains, or a pond. With the exception of Site #1, all were sites of concern for fluorometry, and, with the exception of the storm drains at Sites #2 and #64A, all were sites of concern for fecal enterococci as well. The storm drain at Site #2 had low numbers of fecal enterococci (41 per 100 mL), but had the highest fluorometric value (662 units) of all the sites sampled. Site #3 is a ditch that drains into Site #4, a pond frequented by ducks. The percentage of *Ent. faecalis* from this ditch was 33%, and the *esp* gene was not detected. Site #5 is a storm drain located at Beachview and Mallory Streets, and contained the highest number of fecal enterococci (19,863 per 100 mL) among all the sites in the first sampling. The percentage of *Ent. faecalis* from this ditch was only 20%, but the *esp* gene was detected.

High fecal enterococcal numbers and fluorometric values were also observed in Postell Creek. In lower Postell Creek (Sites #18-32), fecal enterococcal numbers decreased with distance from Site #32 (1,259 fecal enterococci per 100 mL), which was furthest upstream, to Site #18, the mouth of Postell Creek (31 fecal enterococci per 100 mL) with few exceptions (Sites #19-21 and #24). The major exception, Site #21, drained the section of Postell Creek where high counts of fecal enterococci from storm drains at Sites #15-17 were located. Like fecal enterococci, fluorometric values also decreased with distance from Site #32 (320 units) to Site #18 (65 units).

In upper Postell Creek (Fig. 1B), all sites were of concern for fecal enterococci, and ranged from 246 (Site #56A) to 5,794 (Site #60A) fecal enterococci per 100 mL. Excluding storm drains, fluorometric values were consistently high, averaging 323 units. When fecal enterococci were obtained from upper Postell Creek, the percentage of *Ent. faecalis* was 56% and the *esp* gene was not detected.

The second sampling of upper Postell Creek was repeated with the fluorometer equipped with a 436-nm emission filter (Fig. 1C). All sites were of concern for fecal enterococci. However, with the 436-nm emission filter in place, only the six storm drain samples exceeded 100 fluorometric units. Excluding these storm drains, the average fluorometric value for samples collected from upper Postell Creek dropped from an average of 323 units to an average of 49 units. Two storm drain samples, Site #61B and #62B (near Arnold Road), had an average of 4,674 fecal enterococci per 100 mL and an average fluorometric value of 191 units. The next storm drain, Site #63B (The Grands), had >24,192 fecal enterococci per 100 mL, but a slightly lower fluorometric reading (130 units). Finally, three storm drain samples, Sites #64B, #65B, and #66B (near East Beach Causeway) had between 5,475 and >24,192 fecal enterococci per 100 mL and an average fluorometric value of 113 units. Percentages of *Ent. faecalis* exceeded 30% at all three locations, but the *esp* gene was not detected. Total organic carbon concentrations ranged from 6 to 16 mg L^{-1}.

The third sampling was of the storm drain system identified as having human fecal contamination in the first targeted sampling (Fig. 1D). In between first and third samplings, work was conducted on the storm drain by the county Department of Engineering Services. When resampled, counts at the highest part of the storm drain around the work site (Sites A, B, C, and D) ranged from 20,630 to >241,920 fecal enterococci per 100 mL. Total organic carbon concentrations at Sites A, B, and D were also extremely high, and ranged from 165 to 911 mg L^{-1}. From the work site, fecal enterococcal counts generally decreased with increasing distance until counts were 51 fecal enterococci per 100 mL in the retention pond (Site O; same as Site #4 in Fig. 1A). Similarly, TOC concentrations also generally decreased with increasing distance from the work site to 16 mg L^{-1} in the retention pond. Beyond the retention pond, where the water flowed out a storm drain to the sea, counts of fecal bacteria were

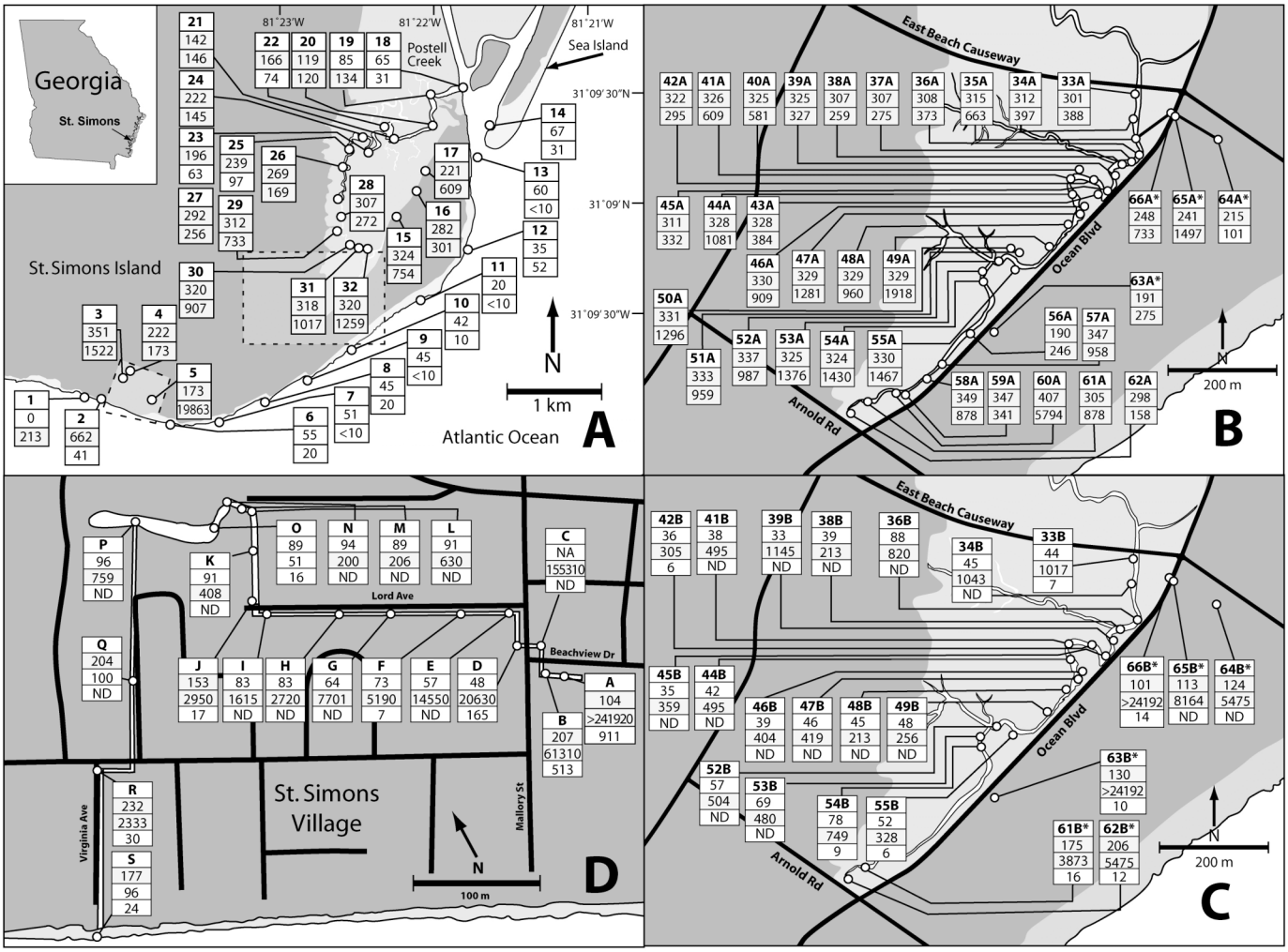

Fig. 1. Location of sampling sites in and around St. Simons, Georgia, during calm (baseflow) conditions. In A, the dashed box at the bottom is shown in D and the dashed box to the right is shown in B and C. In A and B (first sampling), each location shows the site number (top number), fluorometry (middle number; no dimension), and fecal enterococci (bottom number; number per 100 mL). If the site number has an asterisk, then it is a storm drain. If the fluorometric value is >100, or the number of fecal enterococci exceeds the maximum allowable for a grab sample (>104 fecal enterococci per 100 mL), then the site is of concern and the number is shaded. In C (second sampling) and D (third sampling), each location shows the site number (top number), fluorometry (top middle number; no dimension), fecal enterococci (bottom middle number; number per 100 mL), and total organic carbon (bottom number, mg L^{-1}). ND, not done.

variable, ranging from 96 to 2,333 fecal enterococci per 100 mL. Fluorometric values were of concern at five sites (Sites A, B, J, R, and S). Percentages of *Ent. faecalis* at three locations, Sites C, J, and R, were <30% and the *esp* gene was not detected at any of the three locations.

DISCUSSION

Previous studies have already shown that targeted sampling is able to identify hotspots of fecal contamination quickly and easily in marine and estuarine waters during calm and stormy conditions (Kuntz et al., 2003, McDonald et al., 2006). Here, targeted sampling was combined with fluorometry to identify human fecal contamination specifically.

During the first sampling, there was a consensus on the absence of fecal contamination for Sites #1, #2, and #6-14 because both enterococcal counts and fluorometric values were low. However, for other sites, the data were contradictory. For Site #5, the storm drain at Beachview and Mallory Streets, the source of fecal contamination was likely to be humans. Fluorometric values were high (173 units) and the *esp* gene was detected, but this detection was not supported by high percentages of *Ent. faecalis* (20%). If humans were a source, then the

percentage of *Ent. faecalis* should have exceeded 30%. However, low percentages of *Ent. faecalis* (15 and 23%) have been observed in the primary effluent of two of 32 Delaware wastewater treatment plants (P. G. Hartel, unpublished data), so this result was possible but unusual. For Site #3, a storm drain emptying a duck pond (Site #4), the percentage of *Ent. faecalis* was high (>30%) and the *esp* gene was not detected, which suggested a bird source, but a high fecal enterococcal MPN and a high fluorometric value (222 units) suggested a human source. The remaining sites, all in Postell Creek, could not be resolved for the same reason: a high percentage of *Ent. faecalis* (56%) and no detection of the *esp* gene suggested a bird source, while consistently high fecal enterococcal MPNs and high fluorometric values (average 323 units) in the upper portion of the creek suggested a human source.

Some of these contradictions were resolved during the second sampling. When the fluorometer was equipped with a 436-nm filter, the average fluorometric value of upper Postell Creek decreased from 323 to 49 units. This reduction meant that data from this location no longer conflicted because the combination of low fluorometric value (<100 units), high percentage of *Ent. faecalis* (56%), and no detection of the *esp* gene all suggested that the source of the high numbers of fecal enterococci was likely to be birds, not humans.

However, not all contradictions (high fecal enterococcal MPNs and high fluorescence on the one hand, and high percentage of *Ent. faecalis* and the *esp* gene not detected on the other hand) were resolved. These sites included two storm drains on Postell Creek during the second targeted sampling and most of the storm drain in St. Simons Village during the third targeted sampling. Two reasonable explanations for these contradictory results are: a) regrowth of fecal bacteria in the storm drain sediment, and b) the presence of other organic compounds (e.g., motor oil) that are known to fluoresce at the same wavelengths as optical brighteners. One possible solution to the second problem may be to expose the water samples to ultraviolet light. Optical brighteners photodegrade quickly in sunlight (Kramer et al., 1996) and it may be possible to distinguish between optical brighteners and organic matter based on their photodecay rates (C. Hagedorn, personal communication).

The reason for combining targeted sampling and fluorometry was to identify human fecal contamination inexpensively. For fluorometry, the major cost was the purchase of an expensive field fluorometer. However, once the fluorometer was purchased, the operating costs were low. A less expensive handheld fluorometer is currently being tested. In any case, combining targeted sampling and fluorometry still appear to offer a promising method for identifying human fecal contamination inexpensively.

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