Identifying Sources of Fecal Contamination Inexpensively with Targeted Sampling and Bacterial Source Tracking


ABSTRACT

Most bacterial source tracking (BST) methods are too expensive for most communities to afford. We developed targeted sampling as a prelude to BST to reduce these costs. We combined targeted sampling with three inexpensive BST methods, Enterococcus speciation, detection of the esp gene, and fluorometry, to confirm the sources of fecal contamination to beaches on Georgia's Jekyll and Sea Islands during calm and stormy weather conditions. For Jekyll Island, the most likely source of contamination was bird feces because the percentage of Ent. faecalis was high (30%) and the esp gene was not detected. For the Sea Island beach during calm conditions, the most likely sources of fecal contamination were leaking sewer lines and wildlife feces. The leaking sewer lines were confirmed with fluorometry and detection of the esp gene. For the Sea Island beach during stormflow conditions, the most likely sources of fecal contamination were wildlife feces and runoff discharging from two county-maintained pipes. For the pipes, the most likely source of contamination was bird feces because the percentage of Ent. faecalis was high (30%) and the esp gene was not detected. Sediments were also a reservoir of fecal enterococci for both Jekyll and Sea Islands. Combining targeted sampling with two or more BST methods identified sources of fecal contamination quickly, easily, and inexpensively. This combination was the first time targeted sampling was conducted during stormy conditions, and the first time targeted sampling was combined with enterococcal speciation, detection of the esp gene, and fluorometry.

Bacterial source tracking (BST) identifies sources of bacterial fecal contamination by various phenotypic (e.g., antibiotic resistance analysis; Wiggins et al., 1999), genotypic (e.g., REP and BOX polymerase chain reaction [PCR]; Dombek et al., 2000), and chemical (e.g., fecal steroids; Leeming et al., 1996) methods. The major problem with most of these methods is their cost, which precludes many communities from being able to afford BST methodology. We developed targeted sampling as a prelude to BST to reduce this cost (Kuntz et al., 2003).

Targeted sampling works much like the children’s game of “hot” and “cold,” and has five steps. The first step is to separate the sampling for two different flow conditions, one for baseflow and another for stormflow. Because the terms baseflow and stormflow do not de-

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scribe tidal waters well, the terms calm and stormy are used here. This separation is necessary because fecal bacteria typically increase 10- to 100-fold during stormy conditions (Solo-Gabriele et al., 2000; Gregory and Frick, 2001; Hartel et al., 2004). In this manner, targeted sampling accommodates changes in bacterial numbers with different flow conditions. The second step is to talk with the persons knowledgeable about the to-be-sampled areas (e.g., riverkeepers) to identify potential sources of fecal contamination. The third step is to combine this knowledge with targeted sampling of the contaminated waterway, collecting as many samples from the water body and tributaries (as appropriate) in 1 d. Collecting all samples in 1 d is helpful because nearly 70% of water quality exceedences are single-day events (Leecaster and Weisberg, 2001) and the sampling reduces bacterial changes with time (Jenkins et al., 2003). Each sampling location is identified through a global positioning system (GPS). The fourth step is to place the data in a geographic information system (GIS) database to identify hotspots of fecal contamination on a map. The targeted sampling is repeated at the hotspots as necessary to limit the potential source(s) of fecal contamination to as small a geographic area as possible. Limiting the samples to a small geographic area reduces bacterial changes with geography (Hartel et al., 2002) and animal diet (Hartel et al., 2003). The fifth step may be to conduct BST. In most cases, persistent sources of fecal contamination are obvious, and BST is unnecessary; however, in some cases, BST may be necessary to discern between or among a small number of not-so-obvious sources or to confirm an obvious source. If BST is conducted, then the method is selected based on a “toolbox” approach (USEPA, 2005), where the best BST method is selected after considering each method's cost, reproducibility, discriminatory power, ease of interpretation, and ease of performance. Targeted sampling, combined with the expensive genotypic BST method, ribotyping, has been successfully conducted on the tidal Sapelo River in Georgia during calm conditions (Kuntz et al., 2003). Targeted sampling in estuarine and n marine waters during stormy conditions has never been conducted.

In April 2004, high numbers of fecal enterococci triggered beach advisories for Saint Andrews Park on Jekyll Island and the south beach of Sea Island, GA. Both St. Andrews Park and the south beach of Sea Island have only a limited number of potential sources

Abbreviations: BST, bacterial source tracking; DO, dissolved oxygen; GIS, geographic information system; GPS, global positioning system; MAREX, Marine Extension Service; MPN, most probable number; PCR, polymerase chain reaction.
of fecal contamination, most likely humans and birds. Considering these two potential sources and the cost, the most appropriate and least expensive BST methods were Enterococcus speciation, a phenotypic method; detection of the esp (enterococcal surface protein) gene in Ent. faecium, a genotypic method; and fluorometry, a chemical method. In the case of the Enterococcus speciation, fecal enterococci are speciated phenotypically and the percentage of Ent. faecalis determined. High percentages (>30%) of Ent. faecalis are generally associated only with humans and some wild birds (Wheeler et al., 2002; Kuntz et al., 2004). In the case of the esp gene, the gene is detected in Ent. faecium isolates with a PCR assay. Because the gene is detected in 97% of sewage and septic samples and not in any livestock waste lagoons or in nonhuman animal feces (Scott et al., 2005), the detection of this gene in environmental waters is a good index of human fecal contamination. Finally, in the case of fluorometry, water is analyzed for the presence or absence of optical brighteners, which are added to most dishwashing and laundry detergents and fluoresce under ultraviolet light. Hagedorn et al. (2003) evaluated the ability of a fluorometer to detect human wastes in estuarine and coastal zone environments, and concluded that when fluorometry was supported by high counts of fecal indicator bacteria, it may be an inexpensive BST method to detect human wastes.

Our objective was to identify the sources of fecal contamination inexpensively at St. Andrews Park and Sea Island during calm and stormy weather conditions using targeted sampling and two or more BST methods: Enterococcus speciation, the detection of the esp gene, and fluorometry. Dissolved oxygen (DO) was also used to identify hotspots of excessive nutrients that might be associated with leaking sewer lines or malfunctioning septic drainfields. Finally, because recent BST studies suggest that beach sands may serve as reservoirs of fecal indicator bacteria (Clean Beaches Council, 2005), sediment samples were also collected. Sampling was always conducted on an ebbing high tide because these sampling conditions yield the highest enterococcal numbers (Boehm and Weisberg, 2005).

MATERIALS AND METHODS

Sampling Locations and Dates

Jekyll Island is one of Georgia’s barrier islands. St. Andrews Park is located on the southern tip of the island facing St. Andrews Sound. The park beach is approximately 1.3 km long and is bounded by Beach Creek at the northern end and the tip of Jekyll Island at the southern end. On 21 Apr. 2004, targeted sampling was conducted during calm conditions in the waters around St. Andrews Park. A total of 39 water samples was taken along the beach and farther out into St. Andrews Sound. The samples collected farther out in the sound formed a “box” around the park to identify sources of fecal contamination from the sound. On the same day, samples of water and low-tide sediment were also taken from two locations. On the following day, 22 water samples were obtained from Beach Creek during calm conditions and an ebbing high tide.

On 24 Feb. 2005, targeted sampling was conducted during stormy conditions. A total of 35 water samples was obtained along the beach, out into St. Andrews Sound, and from Beach Creek. Sampling began on an ebbing spring high tide, following a 5.1-mm rainfall in a 2-h period just before the sampling. Winds from the southwest ranged from 26 to 32 km h⁻¹.

Sea Island, also one of Georgia’s barrier islands, is relatively small, approximately 8 km long and 2.4 km wide at its widest point. The island is linked to another barrier island, St. Simons Island, by a causeway to the east. The south beach of Sea Island is located on the southern tip facing the Atlantic Ocean.

Targeted sampling during stormy conditions was conducted first because stormy conditions occurred on the first sampling day (3 May 2004). The total rainfall was 35.6 mm. A total of 47 samples was obtained before the storm event ended; 20 water samples were taken from the ocean side of Sea Island, 17 from Hampton River, and 10 from Village Creek. On 12 to 14 May 2004, targeted sampling was conducted around Sea Island during calm conditions. A total of 96 water samples was obtained: 24 samples from Postell Creek and Gould’s Inlet on 12 May 2004, 36 samples from Village Creek and its tributaries (including Blackbanks Creek) on 13 May 2004, and 36 samples from the Hampton River and the ocean side of Sea Island on 14 May 2004. Samples of water and low-tide sediment were also taken from two locations on 13 May 2004.

Chemical and Physical Characteristics

At each sampling point, the location coordinates were taken with a GPS device (Model GPSMAP 175, Garmin International Inc., Olathe, KS). Sampling site coordinates were converted to ArcView 3.2 shapefiles and incorporated into a GIS database. Turbidity at each location was recorded with a turbidity meter (HF Scientific Inc., Fort Myers, FL), and DO, salinity, temperature, and pH were recorded with a Hydrofab Quanta (Austin, TX).

For locations where DO measurements were used to identify sites of concern, a limit of <3.0 mg L⁻¹ was arbitrarily chosen as the cutoff. A reading of 4.0 mg L⁻¹ at all times is considered necessary to support warm-water fish species (Georgia Department of Natural Resources, 1998). Hypoxia occurs when DO levels are <2.0 mg L⁻¹ (Breitburg, 2002).

Fecal Enterococcal Sampling

With one exception, the samples were collected from a University of Georgia Marine Extension Service (MAREX) research vessel. The exception was Postell Creek near Sea Island, where the water depth was too shallow for the MAREX vessel and sea kayaks were used. Water samples were collected in 500-mL (18-oz) Whirl-Pak bags (Nasco, Modesto, CA). Sediment samples 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 h using the Enterolert System (IDEXX Laboratories, Westbrook, ME). Water samples were diluted with sterile distilled water to 10⁻¹ in sterile manufacturer-supplied polystyrene bottles. Sediment samples were serially diluted with sterile distilled water to 10⁻¹, 10⁻², and 10⁻³ in the same type of bottle. A package of powdered Enterolert 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 Quanti-tray was incubated for 24 h at 41 ± 0.5°C. 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 most probable number (MPN) value based on the dilution factor and manufacturer-supplied MPN tables. For fecal enterococci, the federal limit for a single grab sample of water is 104 fecal enterococci per 100 mL (USEPA, 2002). This limit was chosen to identify sites of concern.

Fecal enterococcal counts in sediments were expressed on a per-gram dry weight basis. Each sediment sample was thoroughly shaken in the collection bottle, and three 10-mL subsamples were poured into preweighed aluminum weighing boats. The sediments were dried at 95°C for at least 24 h before the boats were reweighed. The weights of the three sediments were averaged and were divided into the MPN value as appropriate. There is no federal limit for the number of fecal enterococci in sediment.

**Enterococcus Speciation and Detecting the esp Gene**

The BST methods of Enterococcus speciation and detection of the esp gene require Ent. faecalis and Ent. faecium isolates, respectively. For Enterococcus speciation, isolates were obtained from two locations at St. Andrews Park and three locations at Sea Island. The two St. Andrews Park locations were Beach Creek and just offshore of the park. Samples were only collected during calm conditions and consisted of separate water and sediment samples. The three Sea Island locations were Hampton River, Postell Creek, and Village Creek. Samples were collected during both calm and stormy conditions, except for Postell Creek, which was collected during calm conditions only, and consisted of water samples, except for Village Creek, which consisted of a sediment sample only. For detection of the esp gene, isolates were obtained from two locations at St. Andrews Park and five locations at Sea Island. The two locations at St. Andrews Park were Beach Creek and just offshore of the park, and the five Sea Island locations were Blackbanks Creek, Postell Creek, Village Creek, two countymaintained pipes adjacent to the Sea Island causeway, and just offshore of the ocean side of Sea Island. All samples consisted of water samples, except the sample from offshore of the ocean side of Sea Island, which consisted of a combined water and sediment sample.

To obtain enterococcal isolates, positive (fluorescing) 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 aseptically from each well with a pipette, and the portion was spotted into one well of a 96-well microtiter plate containing Enterococcus agar (Becton Dickinson, Sparks, MD). The plate was incubated for 24 h at 35°C. Wells positive for esculin hydrolysis (black color) were streaked onto 5-cm plates containing brain heart infusion agar with 6.5% NaCl. Plates were incubated at 35°C in Ziploc bags (DowBrands, Indianapolis, IN) to maintain high humidity levels. After 48 h, colonies on the plates (positive growth) were subjected to a catalase test with 8.82 M H₂O₂ to ensure that each isolate was catalase negative. Quantitray wells containing bacteria that conformed to the 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. Two wells of the 96-well plate were reserved for an American Type Culture Collection (ATCC) control, Ent. faecalis ATCC no. 19433, two wells were reserved for Ent. faecium ATCC no. 19434, and two 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 a medium specific for the identification of Ent. faecalis and Ent. faecium according to the flowchart described by Manero and Blanch (1999). Plates were incubated at 35°C and reactions were recorded after 72 h. Isolates exhibiting reactions inconsistent with the identification of either Ent. faecalis or Ent. faecium were recorded as "other enterococci.”

In the case of esp gene detection, at least 100 isolates of Ent. faecium were spotted onto a 0.45-μm membrane contained on 5-cm petri plates with mEl agar (Becton-Dickinson) for each of the two locations at St. Andrews Park and each of the five locations at Sea Island. The plates were incubated at 41 ± 0.5°C for 24 h and were sent by overnight mail to Biological Consulting Service of North Florida (Gainesville, FL) for testing. 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**

Fluorometric measurements were only conducted on water samples from Sea Island during calm conditions. Fluorometry was conducted with a field fluorometer (Model 10-AU-005, Turner Designs, Sunnyvale, CA) set to detect long wavelength optical brighteners as described by the manufacturer. Seawater was continually pumped through the detector with a pump mounted on the MAREX research vessel. Because sea kayaks did not have pump capabilities, fluorometry was not conducted on Postell Creek. To ensure stable optical density readings, the fluorometer was kept within 20° of level. The fluorometer was zeroed with Atlantic Ocean water such that the background optical density read between −10 and +10. Samples were collected on an ebbing tide to facilitate capturing land-based runoff. The instrument was checked with water samples containing laundry detergent of known concentrations to ensure that the low limit of detection of the fluorometer was stable. For fluorometry, any site with an optical density >100 was considered positive (Hagedorn et al., 2003), and this limit was the one chosen for identifying sites of concern.

**Statistical Analysis**

Because the data were not normally distributed, nonparametric tests were conducted. For St. Andrews Park, Spearman's correlation coefficient was determined between fecal enterococcal counts and NTU (nephelometric unit) values; for Sea Island, Spearman's correlation coefficients were determined between fecal enterococcal counts and DO samples, and, separately, between fecal enterococcal counts and fluorometric readings. Enterococcal counts below the limit of detection (<10 fecal enterococci per 100 mL) were treated as "ties" values (i.e., having the same numeric value) and were assigned average weights in the same manner as equivalent quantitative values.

**RESULTS**

**St. Andrews Park**

Little difference was observed in water pH either among the sampling locations or between calm and stormy conditions in St. Andrews Park (Tables 1A and 1B). Because the sampling for stormy conditions occurred during the rainier and colder month of February, both salinity and temperature were lower than the sampling that occurred
Table 1. Physical and chemical characteristics of seawater at or around (A) St. Andrews Park (Jekyll Island) during calm conditions on 21 and 22 April 2004, (B) St. Andrews Park (Jekyll Island) during stormy conditions on 24 Feb. 2005, (C) Sea Island during calm conditions on 12–14 May 2004, and (D) Sea Island during stormy conditions on 3 May 2004.

<table>
<thead>
<tr>
<th>Location and condition</th>
<th>Salinity</th>
<th>Temperature</th>
<th>pH</th>
<th>Avg. NTU†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. St. Andrews Park, calm conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beach Creek</td>
<td>30.4</td>
<td>21.4</td>
<td>7.8</td>
<td>20</td>
</tr>
<tr>
<td>St. Andrews Park</td>
<td>30.4</td>
<td>21.4</td>
<td>7.8</td>
<td>46</td>
</tr>
<tr>
<td>Jekyll River</td>
<td>30.4</td>
<td>21.4</td>
<td>7.8</td>
<td>22</td>
</tr>
<tr>
<td>B. St. Andrews Park, stormy conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beach Creek</td>
<td>26.8</td>
<td>15.4</td>
<td>7.7</td>
<td>36</td>
</tr>
<tr>
<td>St. Andrews Park</td>
<td>27.4</td>
<td>15.6</td>
<td>7.7</td>
<td>56</td>
</tr>
<tr>
<td>Jekyll River</td>
<td>26.2</td>
<td>15.4</td>
<td>7.6</td>
<td>54</td>
</tr>
<tr>
<td>C. Sea Island, calm conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oceanside</td>
<td>33.9</td>
<td>24.8</td>
<td>8.0</td>
<td>51</td>
</tr>
<tr>
<td>Hampton River</td>
<td>32.9</td>
<td>25.0</td>
<td>7.7</td>
<td>30</td>
</tr>
<tr>
<td>Postell Creek</td>
<td>32.9</td>
<td>24.8</td>
<td>7.4</td>
<td>33</td>
</tr>
<tr>
<td>Village Creek</td>
<td>31.8</td>
<td>25.3</td>
<td>7.3</td>
<td>14</td>
</tr>
<tr>
<td>D. Sea Island, stormy conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oceanside</td>
<td>32.8</td>
<td>23.5</td>
<td>7.9</td>
<td>31</td>
</tr>
<tr>
<td>Hampton River</td>
<td>32.0</td>
<td>23.4</td>
<td>7.8</td>
<td>56</td>
</tr>
<tr>
<td>Postell Creek</td>
<td>ND²</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Village Creek</td>
<td>29.1</td>
<td>23.5</td>
<td>7.4</td>
<td>30</td>
</tr>
</tbody>
</table>

† NTU, nephelometric units. ‡ ND, not done.

During calm conditions in the drier and warmer month of April. Furthermore, stormy conditions disturbed the sediment, and the average NTU was at least doubled for Beach Creek and the Jekyll River, except for the surf zone of St. Andrews Park, which was only slightly higher during stormy conditions (57 compared with 46 NTU). When targeted sampling was conducted during calm weather conditions, with the exception of Site 33 (20 fecal enterococci per 100 mL), all St. Andrews Park beach sites (Sites 22–45) contained 10 or <10 (below the limit of detection) fecal enterococci per 100 mL (Fig. 1A). With the exception of one site (Site 57, 20 fecal enterococci per 100 mL), all sites defining the “box” around St. Andrews Park (Sites 46–60) contained 10 or <10 fecal enterococci per 100 mL. Two-thirds of the sites in the lower reach of Beach Creek (Sites 7–21) had 20 or fewer fecal enterococci per 100 mL; the remaining one-third of the sites in the upper reach (Sites 1–6) had counts between 52 and 228 fecal enterococci per 100 mL, generally decreasing from the upper to the lower reach of the creek. Three sites (Sites 1, 2, and 3) exceeded the USEPA allowable number of 104 fecal enterococci per 100 mL of seawater for a grab sample, and were the only sites of concern. Numbers of fecal enterococci were 4110 g⁻¹ dry weight for Beach Creek sediment and 1110 g⁻¹ dry weight for St. Andrews Park sediment.

When targeted sampling was conducted during stormy conditions and an ebbing spring tide on 24 Feb 2005, a total of 35 water samples was collected. Of 15 water samples from St. Andrews Park (Sites 10–24, Fig. 1B), 11 (73%) exceeded the USEPA allowable maximum number of 104 fecal enterococci per 100 mL, with fecal enterococcal numbers generally decreasing from north to south, away from Beach Creek. The numbers of fecal enterococci in the nine Beach Creek sites (Sites 1–9) also exceeded the USEPA maximum, ranging from 171 to 428 fecal enterococci per 100 mL, and generally decreased from the upper to the lower reach of the creek. With the exception of Sites 34 and 35 just north of Beach Creek, all the remaining nine sites defining the “box” around the park (Sites 25–35) had 30 or fewer fecal enterococci per 100 mL. When turbidity was compared with MPN values for fecal enterococci from all sampling points regardless of weather conditions, the correlation was not significant ($r^2 = 0.03, P = 0.001$).

A total of 266 enterococcal isolates was speciated during calm conditions (Table 2A). The percentage of *Ent. faecalis* was high in the water of Beach Creek (37%) and diminished to 24% in the water of St. Andrews Park. In contrast, the percentages of *Ent. faecalis* were lower in sediment and more similar between Beach Creek and St. Andrews Park (12 vs. 15%). Therefore, the percentages of *Ent. faecalis* observed in the water were not the same as those observed in the sediments. When *Ent. faecium* isolates from Beach Creek and offshore St. Andrews Park were analyzed for the *esp* gene, the gene was not detected at either location.

**Sea Island**

Little differences were observed among salinity, water temperature, and water pH either among the sampling locations or between calm and stormy conditions because the sampling dates were close to each other (3 May 2004 vs. 12–14 May 2004; Tables 1C and 1D). Stormy conditions approximately doubled the turbidity on the Hampton River and Village Creek compared with calm conditions, while in the surf zone on the ocean side of Sea Island, turbidity measurements were actually higher during calm conditions than during stormy conditions.

For targeted sampling during calm conditions, with the exception of Site 30 (2.9 mg DO L⁻¹), all sites on the ocean side of Sea Island (Sites 1–10) and the Hampton River sites (Sites 11–36) had high DO (≥3.0 mg L⁻¹), low fluorescence (<100 optical density), and low fecal enterococcal counts (<104 fecal enterococcal counts per 100 mL; Fig. 2A). Numbers of fecal enterococci obtained from the ocean side of Sea Island were <1 g⁻¹ dry weight. Therefore, with the exception of Site 30, neither the ocean side of Sea Island nor the Hampton River had any sites of concern. Fecal enterococci isolated from ocean side Sea Island water and sediment were negative for the *esp* gene.

In contrast to the single site of concern on the Hampton River and the ocean side of Sea Island, the 37 sites in Village Creek and its tributaries (Sites 37–73) had 10 sites of concern: two sites with a low DO only (Sites 50 and 51), two sites with high fecal enterococci only (Sites 60 and 62), two sites with both low DO and high fluorescence (Site 56 and 71), and four sites with low DO, high fluorescence, and high fecal enterococci (Sites 68, 69, 70, and 72). Of these 10 sites, one was near Village Creek Landing (Site 56), four (Sites 50, 51, 60, and 62) were on Village Creek, and the remaining five (Sites 68, 69, 70, 71, and 72) were on Blackbanks Creek. *Enterococcus faecium* isolates obtained from Blackbanks Creek tested positive for the presence of the *esp* gene.
A Creek sediment sample (at Site 73) had 228 fecal enterococci g$^{-1}$ dry weight, but these enterococci tested negative for the presence of the esp gene.

Blackbanks River (Sites 74–82) had no sites of concern, while Postell Creek (Sites 83–96) had six sites with low DO (Sites 89, 90, 91, 92, 94, and 95) and three sites with high fecal enterococci (Sites 86, 87, and 88). Fluorometry was not conducted on Blackbanks River or Postell Creek. Enterococcus faecium isolates from Postell Creek were negative for the esp gene.

A total of 290 enterococcal isolates was speciated from water around Sea Island during calm conditions (Table 2B). Only water from Hampton River had ≥30% Ent. faecalis.

For targeted sampling during stormy conditions, with the exception of Site 26 (110 fecal enterococci per 100 mL) and Site 37 (120 fecal enterococci per 100 mL), all the oceanside sites of Sea Island (Sites 1–20) and Hampton River (Sites 21–37) had high DO and low numbers of fecal enterococci (Fig. 2B). Both Sites 26 and 37 drained small tidal creeks. The average number of fecal enterococci for the north side of the Hampton River (83 fecal enterococci per 100 mL) was higher during stormy conditions than calm conditions (16 fecal enterococci per 100 mL).

In contrast to the ocean side of Sea Island and the Hampton River, seven of the 10 sampling sites on Village Creek (Sites 38–47) exceeded the limit for fecal enterococci during stormy conditions. This result was similar to those observed during calm conditions; however, Village Creek sampling sites during stormy conditions were dissimilar from Village Creek sampling...
Table 2. Number and percentage of *Enterococcus faecalis* and other enterococci from water and sediment samples at or around (A) St. Andrews Park (Jekyll Island) during calm conditions, (B) Sea Island during calm conditions, and (C) Sea Island during stormy conditions. Percentages may not add up to 100 because of rounding.

<table>
<thead>
<tr>
<th>Location</th>
<th>Isolates</th>
<th><em>Enterococcus faecalis</em></th>
<th>Other enterococci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no.</td>
<td>no. of organisms (%)</td>
<td></td>
</tr>
<tr>
<td>A. St. Andrews Park, calm conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beach Creek water</td>
<td>84</td>
<td>31 (37)</td>
<td>53 (63)</td>
</tr>
<tr>
<td>St. Andrews Park water</td>
<td>17</td>
<td>4 (24)</td>
<td>13 (76)</td>
</tr>
<tr>
<td>Beach Creek sediment</td>
<td>99</td>
<td>12 (12)</td>
<td>87 (87)</td>
</tr>
<tr>
<td>St. Andrews Park sediment</td>
<td>66</td>
<td>10 (15)</td>
<td>56 (85)</td>
</tr>
<tr>
<td>B. Sea Island, calm conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hampton River water</td>
<td>24</td>
<td>8 (33)</td>
<td>16 (67)</td>
</tr>
<tr>
<td>Postell Creek water</td>
<td>122</td>
<td>32 (26)</td>
<td>90 (74)</td>
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<tr>
<td>Village Creek water</td>
<td>144</td>
<td>28 (18)</td>
<td>118 (82)</td>
</tr>
<tr>
<td>C. Sea Island, stormy conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hampton River water</td>
<td>64</td>
<td>13 (20)</td>
<td>51 (80)</td>
</tr>
<tr>
<td>Postell Creek water (ND)</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>Village Creek water (pipe discharge)</td>
<td>179</td>
<td>54 (30)</td>
<td>125 (70)</td>
</tr>
</tbody>
</table>

† ND, not done.

during calm conditions because the sites showed a decrease in fecal enterococcal counts with increasing distance from Site 47 (number of fecal enterococci per 100 mL): Site 47 (24192), Site 46 (2282), Site 45 (341), Site 44 (240), Site 43 (173), Site 42 (135), and Site 41 (31). Site 47 was runoff discharging from two pipes adjacent to the Sea Island causeway. *Enterococcus faecium* isolates from these two pipes were negative for the esp gene. Because the storm event ended, it was not possible to obtain fecal enterococcal counts for Blackbanks River or Blackbanks Creek.

A total of 243 enterococcal isolates was speciated from water around Sea Island during stormy conditions (Table 2C). During stormy conditions, water from Village Creek had 30% *Ent. faecalis* (54 of 179 isolates), in contrast to Hampton River, which had 20% *Ent. faecalis* (13 of 64 isolates). A significant negative correlation ($r^2 = -0.62, P < 0.0001, n = 148$) was observed between DO readings and enterococcal counts during calm and stormy conditions. Similarly, a significant positive correlation ($r^2 = 0.66, P < 0.0001, n = 71$) was observed between fluorometric readings and enterococcal counts during calm conditions.

DISCUSSION

When targeted sampling was combined with two or more of three BST methods—enterococcal speciation, detection of the *esp* gene, and fluorometry (Table 3)—
the combination was able to identify sources of fecal contamination quickly, easily, and inexpensively. Targeted sampling, by playing a microbiological version of the children’s game of “hot” and “cold,” reduced fecal bacterial changes with flow, time, and geography to a point where the environmental complexity was reduced, and BST methods were able to quickly and easily identify sources. Previously, targeted sampling of an estuarine area had been combined with an expensive BST method, ribotyping, during calm conditions (Kuntz et al., 2003). Here, targeted sampling of estuarine areas was combined with two or more inexpensive BST methods during both calm and stormy conditions. Including technician and boat time, the total cost to conduct the targeted sampling and the BST analyses of St. Andrews Park and Sea Island was US$5000 and US$10,000, respectively.

In the case of one estuarine area, St. Andrews Park, the most likely major source of fecal contamination during stormy conditions was bird feces from Beach Creek. No fecal contamination was observed in waters outside the park during calm or stormy conditions, therefore sources of fecal contamination must be near or inside the park. During calm conditions, no sources of fecal contamination were observed in the water either near or inside the park except for the extreme upper reach of Beach Creek; however, during stormy conditions with an ebbing spring tide, large numbers of fecal enterococci were observed in the water coming from Beach Creek into St. Andrews Park. Fecal enterococcal numbers decreased north and south of the creek, and most of St. Andrews Park was in violation of the state standard (>104 fecal enterococci per 100 mL). Runoff and tidal forcing probably caused this pulse of fecal contamination from Beach Creek. Fecal bacteria typically increase 10- to 100-fold after stormy conditions in estuarine areas (Solo-Gabriele et al., 2000) and after tidal forcing, where feces deposited above the normal but below the maximal high-tide mark are brought into the water by ebbing spring tides (Boehm and Weisberg, 2005).

The major source of fecal enterococci to Beach Creek is likely to be bird feces because the percentage of Ent. faecalis exceeded 30% and such a high percentage is associated with the highest percentage from humans and wild birds (Wheeler et al., 2002). Because Beach Creek is surrounded entirely by marsh with no human habitation, the esp gene was not detected in the Ent. faecium isolates, birds are the most likely fecal source; however, the percentages of Ent. faecalis in all wildlife species has not been tested, so it is possible that Ent. faecalis isolates came from other wildlife sources. It is also possible that pet and wildlife feces on the beach above the normal high-tide mark contributed to fecal enterococcal numbers in the water column during storm runoff and tidal forcing conditions.

In the case of a second estuarine area, Sea Island, there were three likely sources of fecal contamination to the south beach of Sea Island. The first and most likely source was runoff water discharging from two pipes adjacent to the Sea Island causeway during stormy conditions. This runoff contained a high number of fecal enterococci (24,192 per 100 mL), which dominated Village Creek northward during stormy conditions. Because Sea Island is an island, it is likely that this fecal contamination also flowed southward to the south beach where the beach advisory was issued: however, no hydrologic data exist to confirm this flow. Similar to St. Andrews Park, the percentage of Ent. faecalis in Village Creek was high (30%) and the esp gene was not detected in Ent. faecium isolates, therefore birds were the likely source.

The second likely source was human fecal contamination from Blackbanks Creek during calm conditions. This creek was unusual because it contained the only sites with low DO (≤3.0 mg L⁻¹), high fluorescence (≥100), and high numbers of fecal enterococci (>104 per 100 mL). Low levels of DO are often associated with high nutrient concentrations and eutrophication (Breitburg, 2002). A significant negative correlation ($r^2 = -0.62$) existed between DO and fecal enterococcal counts. Furthermore, high fluorescence is associated with human wastes in estuarine and coastal zone environments (Hagedorn et al., 2003), and we observed a significant positive correlation ($r^2 = 0.66$) between fluorescence and fecal enterococcal counts. When Ent. faecium isolates were tested, the esp gene was detected. Therefore, fecal contamination from humans is likely. Blackbanks Creek has homes on county sewer lines or septic systems nearby and they are the likely source.

The third likely major source of fecal contamination was wildlife in the marsh. This source was common to both calm and stormy conditions. During calm conditions, there were six sites of concern on Village Creek and nine sites of concern on Postell Creek. The likely source was wildlife because many of these sites are located deep in the marsh far from human habitation, the percentage of Ent. faecalis was <30%, and the esp gene was not detected.

<table>
<thead>
<tr>
<th>Location (condition)</th>
<th>Dissolved O₂</th>
<th>Ent. faecalis</th>
<th>Fluorometry</th>
<th>esp gene</th>
<th>Suspected source</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Andrews Park</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beach Creek (calm)</td>
<td>ND†</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>wildlife</td>
</tr>
<tr>
<td>Sea Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postell Creek (calm)</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>wildlife</td>
</tr>
<tr>
<td>Blackbanks Creek (calm)</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>human</td>
</tr>
<tr>
<td>Village Creek (stormy)</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>wildlife</td>
</tr>
</tbody>
</table>

† ND, not done.
Although targeted sampling in an estuarine environment during stormy conditions identified sources of fecal contamination not evident during calm conditions, targeted sampling during stormy conditions also had two additional obstacles. Besides the common obstacles of targeted sampling (to know the hydrology of the area, to sample during daylight hours, and to sample during an ebbing tide), stormy conditions are ephemeral and require immediate boat availability. These obstacles made sampling during stormy conditions more difficult than during calm conditions. Indeed, a complete targeted sampling of Sea Island during stormy conditions was not accomplished because of these obstacles. Conditions would be even less favorable if tidal forcing caused by spring tides was important. Nevertheless, the results of the targeted sampling during stormy conditions were instructive because high numbers of fecal enterococci occurred in Beach Creek and near the Sea Island causeway only under these conditions.

Neither the St. Andrews Park nor the Sea Island targeted samplings considered sediment as a reservoir for fecal enterococci. Sediments have long been known as reservoirs of fecal bacteria (Stephenson and Rychert, 1982), but are not usually considered in BST studies. Although numbers of fecal enterococci were low in the Sea Island oceanside sediment (<1 per 100 mL), they were much higher in the sediments of Village Creek (228 g⁻¹ dry weight), Beach Creek (4110 g⁻¹ dry weight), and St. Andrews Park (1110 g⁻¹ dry weight). The large numbers of fecal enterococci in the sediment may potentially create beach advisories when the sediments are disturbed (Clean Beaches Council, 2005), even if only wind disturbs the sediment (Hartel et al., 2005). Because the turbidity values typically doubled during stormy conditions for both St. Andrews Park and Sea Island nonsurf zones, it is likely that sediment contributed to fecal enterococcal numbers during stormy conditions. Furthermore, given the differences in fecal enterococcal numbers and the percentages of *Enterococcus* species between St. Andrews Park and Beach Creek sediments, sediments may also affect any BST method using enterococcal speciation (e.g., Wheeler et al., 2002); however, it is important to note that some research suggests that no significant correlation exists between turbidity measurements and fecal enterococci numbers (McDonald et al., 2003), and no significant correlation was observed here ($r^2 = 0.03$, $P = 0.001$). Nevertheless, the results suggest that sediments need to be considered for targeted sampling and BST studies whenever they are relevant.

Another important issue with sediments is the source of their fecal enterococci. Because preliminary data suggest that fecal enterococci do not survive in moist sediments (Feng et al., 2004), these sediments must be continually resupplied with fecal enterococci from outside sources. For St. Andrews Park, it is likely that the sediment was resupplied with fecal enterococci from bird feces coming from Beach Creek during stormy conditions. This scenario would explain why the numbers of fecal enterococci were fourfold higher in Beach Creek sediment than in St. Andrews Park sediment. It is also possible that the sediment is resupplied with fecal enterococci from marine invertebrates (Signoretto et al., 2004) and plants. *Escherichia coli* and enterococci grow in freshwater green algae (Whitman et al., 2003), and we have observed large numbers of fecal enterococci associated with the marine alga, sea lettuce (*Ulva* sp.; Hartel, unpublished data, 2004).

This study was the first to combine targeted sampling with DO measurements, enterococcal speciation, detection of the *esp* gene, and fluorometry. The “toolbox” approach used a variety of inexpensive chemical and BST genotypic tests to identify sources of fecal contamination. The genotypic method for detecting the *esp* gene and the phenotypic method of determining the percentage of *Ent. faecalis* were complementary, and may be particularly appropriate in distinguishing fecal contamination between humans and wild birds. The use of fluorometry to identify sources of human fecal contamination was also particularly noteworthy because, after the initial significant expense of purchasing the fluorometer, the samples were quick and inexpensive to process. Although high concentrations of organic matter in Georgia waters produced a high background signal, the method was still able to identify human fecal contamination in Blackbanks Creek, which was later confirmed with the detection of the *esp* gene. More studies of this type are needed to ensure that the method works for stormy conditions in marine waters as well as for calm and stormy conditions in fresh water. Such studies are currently in progress.

### CONCLUSIONS

Targeted sampling, when combined with two or more of three BST methods—enterococcal speciation, detection of the *esp* gene, and fluorometry—was able to identify sources of fecal contamination quickly, easily, and inexpensively. The targeted sampling was the first to be conducted during both calm and stormy conditions in estuarine areas. As a prelude to BST, targeted sampling quickly and easily identified hotspots of fecal contamination around St. Andrews Park (Jekyll Island) and Sea Island under both conditions. Because bacterial changes with flow, time, and geography were minimized, the costs associated with BST methods to identify the sources were also minimized. These lowered costs should make BST methodology more affordable to most communities. Although targeted sampling during stormy conditions identified sources of fecal contamination not seen during calm conditions, targeted sampling during stormy conditions was more difficult than during calm conditions because of two additional obstacles, the ephemeral nature of storms and the need for immediate boat availability.

Sediments were a large potential reservoir of fecal enterococci and need to be considered in both targeted sampling and BST studies. With respect to targeted sampling, state regulatory agencies may wish to avoid sampling where sediments are disturbed, or to note these conditions when considering whether or not to issue beach advisories. With respect to BST methods, sediments may
affect enterococcal speciation (i.e., isolation of *Ent. faecalis* and *Ent. faecium*).

This study was the first to combine targeted sampling with enterococcal speciation, detection of the *esp* gene, and fluorometry. Using a variety of inexpensive chemical and BST genotypic tests in a "toolbox" approach to identify sources of fecal contamination was useful, particularly in identifying sources of human fecal contamination, which, in contrast to difficult-to-reduce fecal contamination from wildlife or birds, can be more easily mitigated or eliminated. In the case of fecal contamination from wildlife or birds, states may wish to treat marshes more like a wildlife refuge where the beauty of the marsh is emphasized, high numbers of fecal enterococci are accepted, and human activities like swimming are discouraged, particularly during runoff or when tidal forcing occurs.

**ACKNOWLEDGMENTS**

We thank Elizabeth Cheney, Paul Christian, James Gilbert, and Robert Overman for their assistance. This research was partially funded by grants from the Coastal Resources Division of the Georgia Department of Natural Resources, The Sea Island Company, and the Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET) of the National Oceanic and Atmospheric Administration (NOAA).

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