COMBINING TARGETED SAMPLING AND BACTERIAL SOURCE TRACKING (BST) DURING CALM AND STORMY CONDITIONS

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Abstract. In April 2004, high numbers of fecal enterococci triggered a beach advisory on Sea Island, GA. Targeted sampling, which finds fecal contamination much like the children's game of "hot" and "cold," was combined with three bacterial source tracking (BST) methods: *Enterococcus* speciation, the presence or absence of a human virulence factor in *Enterococcus faecium*, and fluorometry. During calm (i.e non-runoff) conditions, the likely contamination sources were wildlife feces and leaking sewer lines located on a creek of St. Simons Island, GA. Fluorometry quickly identified malfunctioning sewer lines. A test for human virulence factor was positive. During stormflow (i.e. runoff) conditions, the likely contamination sources were wildlife feces and effluent from two pipes. A test for human virulence factor was negative. Because the percentage of *Ent. faecalis* from the pipes was high (>30%), fecal contamination from wild birds was likely. This is the first report of targeted sampling during stormy conditions, and the first time fluorometry has been combined with targeted sampling.

INTRODUCTION

High numbers of fecal enterococci were observed on the south beach of Sea Island on the coast of Georgia and these numbers triggered a beach advisory in April 2004. The University of Georgia was asked to identify the source(s) of fecal contamination reaching the island. The technique chosen was targeted sampling combined with three BST methods: *Enterococcus* speciation, the presence or absence of a human virulence factor in *Ent. faecium* isolates, and fluorometry. The major problem with BST is its cost, and to reduce the cost we developed targeted sampling as a prelude to BST. Targeted sampling, which works much like the children's game of "hot" and "cold," has four steps. The first step is to divide the sampling protocol into two events, one for baseflow (calm) and one for stormflow (stormy) conditions. One important component of stormy conditions is runoff, which typically increases fecal bacteria levels 10- to 100-fold (Solo–Gabriele et al., 2000). The second step is to conduct intensive sampling(s) of the contaminated waterway during calm conditions, collecting as many samples as possible in one day to reduce temporal variability. The third step is to combine the fecal bacterial numbers with GPS data, ensuring that the sample locations are accurately plotted on a map. The fourth step is to conduct BST in "hotspot" areas (i.e., sites containing high fecal bacteria). Targeted sampling is then repeated for stormy conditions. Targeted sampling has been successfully conducted on the Sapelo River in Georgia during calm conditions (Kuntz et al., 2003), but no previous studies have been conducted during stormy conditions.

The best BST approach is to select one (or more) phenotypic (i.e., expressed characteristics), genotypic (i.e., DNA-based), or chemical methods to identify the host origin of the fecal contamination. This "toolbox" approach allows one to select the BST method(s) best-suited for each location based on (i.e., cost, time, reproducibility, discriminatory power, ease of interpretation, and ease of performance). Given the absence of agricultural animals on Sea Island, the simplest, quickest, and least expensive BST methods were a phenotypic method, *Enterococcus* speciation; a genotypic method, the presence or absence of a human virulence factor in *Ent. faecium* isolates; and a chemical method, fluorometry. In the case of the phenotypic method, the percentage of *Ent. faecalis* is determined. High percentages of *Ent. faecalis* are associated only with humans and some wild birds (Wheeler et al., 2002). In the case of the genotypic method, the presence or absence of a human virulence factor in *Ent. faecium* isolates is determined by a polymerase chain reaction (PCR; Scott et al., 2005). Finally, in the case of the chemical method, water is analyzed for the presence of optical brighteners, the colorless dyes in laundry detergents that fluoresce

under ultraviolet light. Hagedorn et al. (2003) evaluated the ability of a fluorometer to detect these fluorescent compounds in detergent residues associated with human wastes in estuarine and coastal zone environments, and concluded that when fluorometry was supported by fecal indicator bacterial counts, it was an inexpensive BST method to detect human wastes.

In addition, a recent BST study suggests that sediments may serve as fecal enterococci reservoirs (Feng et al., 2004). In our own research, water and sediment was recently tested for numbers of fecal enterococci in Academy Creek near Brunswick, Georgia. The sediment sample contained > 3,100 fecal enterococci g^{-1} dry weight while the number of fecal enterococci in the water was below detectable levels (< 10 per 100mL; P. G. Hartel, unpublished). Therefore, sediment sampling was included in the Sea Island study.

The objective of this study was to conduct targeted sampling, combined with one or more of three BST methods, *Enterococcus* speciation, the presence or absence of a human virulence factor in *Ent. faecium* isolates, and fluorometry to identify the sources of fecal contamination reaching Sea Island. Samplings were conducted during calm and stormy conditions.

METHODS

Targeted sampling during calm and stormy conditions was conducted from sea kayaks and a University of Georgia Marine Extension Service (MAREX) research vessel. Targeted sampling during stormy conditions was conducted on 3 May 2004; targeted sampling during calm weather conditions was conducted between 12 and 14 May 2004. Targeted sampling and fluorometry were only conducted on Village Creek and its tributaries.

Location coordinates were taken with a GPS device (Model GPSMAP 175, Garmin International Inc., Olathe, KS). Turbidity was recorded with a turbidity meter (HF Scientific Inc., Fort Myers, FL), and dissolved oxygen (DO), salinity, temperature, and pH were recorded with a Hydrolab Quanta (Austin, TX). Locations of each sampling site were converted to an ArcView 3.2 shapefile and incorporated into a GIS database.

Water samples were collected in 500-mL Whirl-Pak bags (Nasco, Modesto, CA). Sediment samples were collected with an ethanol-disinfected spoon and placed into sterile 500-mL polypropylene bottles. Water and sediment samples were placed on ice and processed within 6 hours with the Enterolert™ System (IDEXX Laboratories, Westbrook, ME) as described by Hartel et al. (2004). Because the Enterolert™ system yields an unacceptable rate of false positive wells (McDonald et al., 2003), each Most-Probable-Number (MPN) was "confirmed" by further testing the contents of each positive (fluorescing) well (Hartel et al., 2004). For fecal enterococci, the federal limit for a single grab sample is 104 fecal enterococci per 100 mL (USEPA, 2002) and this limit was the one chosen for the study.

All confirmed fecal enterococci from the Quanti-tray wells were speciated as described by Wheeler et al. (2002).

Water or sediments were collected from four locations (during calm conditions) and were analyzed for the presence or absence of a human virulence factor in *Ent. faecium* isolates. Approximately 100 enterococcal isolates were obtained from positive Quanti-tray wells at each location and each set of isolates was spotted on a 0.45-µm membrane filter resting on a 5-cm petri plate with mEI agar (Becton-Dickinson). The four plates were incubated at 41±0.5 °C for 24 hours and then sent overnight to the Biological Consulting Service of North Florida (Gainesville, FL).

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. Water was continually pumped through the detector when readings were taken. For fluorometry, any site with an optical density >100 was considered positive (Hagedorn et al., 2003).

For DO, a reading of 4.0 mg L^{-1} at all times is considered necessary to support warm water species of fish (Georgia Department of Natural Resources, 1999). Because Georgia's streams are naturally characterized by low topographic relief, extensive floodplain swamps, and rich organic bottom sediments, DO levels often fall below this established criterion (Davie, 2001). Hypoxia occurs when DO levels are <2.0 mg L^{-1} (Breitburg, 2002). Therefore a DO limit of <3.0 mg L^{-1} was arbitrarily chosen as the study cutoff.

CONCLUSIONS

Calm Conditions

All sites on the oceanside of Sea Island and Hampton River had high DO, low fluorescence and low fecal enterococci levels (Fig. 1A). Numbers of fecal enterococci from the oceanside sediment were <1 g^{-1} dry weight.

Village Creek and its tributaries (Sites 37 to 73) had two sites with high fecal enterococci only, two sites with both low DO and high fluorescence, and five sites with low DO, high fluorescence, and high fecal enterococci. The five sites (Sites 68, 69, 70, 71, and 72) were on Blackbanks Creek located off St. Simons Island. Fecal enterococci isolated from Blackbanks Creek tested positive for presence of the human virulence factor. The Village Creek sediment sample had 228 fecal enterococci g^{-1} dry weight. Gould's Inlet and Postell Creek had three

sites exceeding the limit for fecal enterococci. Fecal enterococci from Postell Creek were negative for the presence of the human virulence factor.

Stormy Conditions

Sites on the beachside of Sea Island and Hampton River had high DO, low fluorescence and low fecal enterococci with the exception of Sites 26 and 37 (Fig. 1B).

Seven of 10 sites on Village Creek (Sites 38 to 47) exceeded the limit for fecal enterococci, with numbers decreasing with increasing distance from Site 47 (two county-maintained pipes emanating from Sea Island).

DISCUSSION

There were three likely sources of contamination to the south beach of Sea Island. The first source was runoff from two county-maintained pipes emanating from Sea Island adjacent to the causeway during stormy conditions. Sediment collected near the pipes contained high numbers of fecal enterococci. Because the percentage of *Ent. faecalis* in Village Creek reached 30%, and tested negative for the human virulence factor, wild birds were the likely source.

The second likely source was human fecal contamination from Blackbanks Creek during calm conditions. This creek contained the only sites with low DO, high fluorescence, high numbers of fecal enterococci, and tested positive for the human virulence factor. Therefore, the source of this contamination is likely humans.

The third likely source of fecal contamination was wildlife in the marsh. This source was common to both calm and stormy conditions.

This study is the first targeted sampling conducted during stormflow conditions, and to combine DO and fluorometry with targeted sampling during either calm or stormy conditions. Combining targeted sampling with BST made BST less costly. The Sea Island study cost $10,000.

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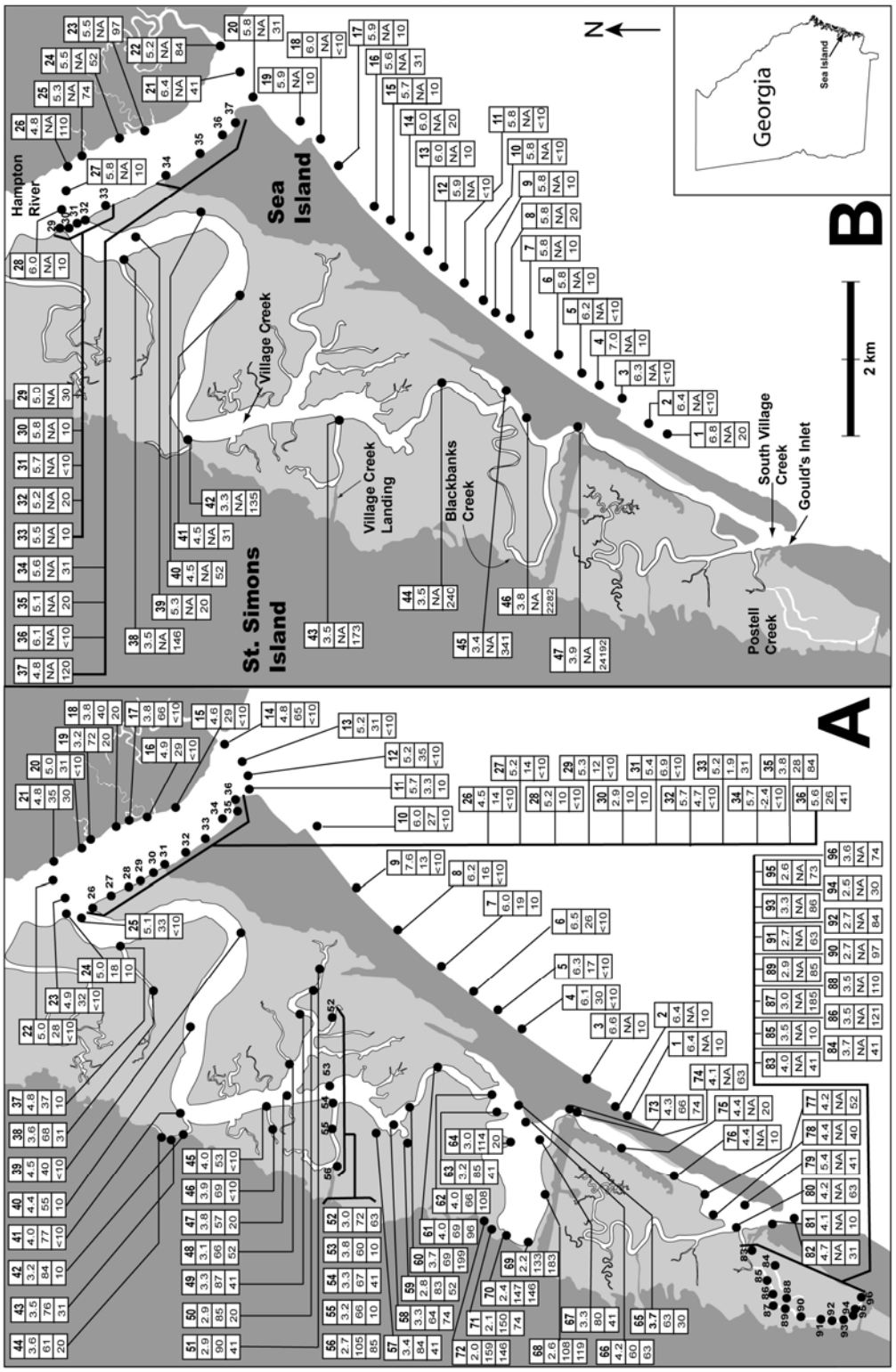

Figure 1. Location of sampling sites around Sea Island during A) calm (12 to 14 May 2004) and B) stormy (3 May 2004) conditions. Each location shows the site number (top number), dissolved oxygen (top middle number; mg L^{-1}), fluorometry (bottom middle number; optical density), and fecal enterococci (bottom number; number per 100 mL). Fluorometry was not conducted in Postell Creek or Blackbanks River during calm conditions, or any site during stormy conditions. Outlined white areas define creeks and the Atlantic Ocean, light gray areas define marsh, and dark gray areas define dry land. The scale is 1:11,000.