Multi-tiered approach using quantitative polymerase chain reaction for tracking sources of fecal pollution to Santa Monica Bay, California

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Abstract

The ubiquity of fecal indicator bacteria such as *Escherichia coli* (*E. coli*; EC) and *Enterococcus* sp. (ENT) in urban environments makes tracking fecal contamination extremely challenging. A multi-tiered approach was used to assess sources of fecal pollution in Ballona Creek, an urban watershed that drains to Santa Monica Bay (SMB) near Los Angeles, CA. A mass-based design at six mainstem sites and four major tributaries over a six hour time period was used to assess: Tier 1) the flux of ENT and EC using culture-based methods; Tier 2) ENT using quantitative PCR (QPCR), and detection and/or quantification of additional markers of human fecal contamination including *Bacteroides* sp. human specific marker and enterovirus, using quantitative reverse transcriptase PCR (QRTPCR); and Tier 3) the specific types of enteroviral genomes found via sequence analysis. Sources and concentrations of fecal indicator bacteria were ubiquitously high throughout Ballona Creek, with no single tributary dominating fecal inputs. The flux of ENT and EC averaged 10^9 to 10^{10} cells h^{-1}, and was as high at the head of watershed as at the mouth prior to discharge into SMB. In addition, there was a consistent detectable signal of the *Bacteroides* human specific marker, with 86% of the samples taken over the extent over the study period testing positive. Enteroviruses were quantifiable in 14 of 36 samples (39%), with the highest concentrations at the site furthest upstream, Cochran Avenue. These results indicated the power of using multiple approaches to assess and quantify fecal contamination in freshwater conduits to high use, high priority recreational swimming areas.

Introduction

Santa Monica Bay (SMB), California, is home to some of the most popular beaches in the world. It is located adjacent to metropolitan Los Angeles where more than 50 million beachgoers visit SMB shorelines every year, which is more than all other beaches in California combined (SMBRC 2005). However, there are serious concerns about beach water quality because of continued exceedences of water quality thresholds based on fecal indicator bacteria such as total coliforms, fecal coliforms or *Escherichia coli* (*E. coli*; EC) and *Enterococcus* (ENT), particularly in areas impacted by urban runoff. Thirteen percent of the shoreline mile-days in SMB exceeded water quality thresholds between 1995 and 2000 with over 50% of these exceedances located near storm drains (Pina *et al.* 1998). The public health risk associated with urban runoff has been directly demonstrated through epidemiology studies. Haile *et al.* (2003) demonstrated that swimmers near storm drain discharges in SMB had a higher likelihood of respiratory and/or gastrointestinal symptoms compared to swimmers more than 400 m from a storm drain.

Despite the impairments to water quality and risks to human health, identifying and eliminating the sources of bacteria responsible for the beach warnings remains elusive. The difficulty in identifying and eliminating the sources of bacteria results from two important factors. First, the traditional indicators of fecal pollution upon which the water quality thresholds were developed are not specific to humans. These fecal indicator bacteria can be shed from any warm-blooded organism, including wild and domesticated animals (Geldreich 1978).

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Therefore, source tracking turns into a challenging scenario when these diffuse and frequently intermittent or episodic fecal releases occur. The second difficulty when identifying and eliminating sources of fecal indicator bacteria is their ubiquity in urban environments. Finally, unlike many of the human pathogens of concern, fecal indicator bacteria may survive and even grow in the environment (e.g., Kinzelman *et al.* 2004, Solo-Gabrielle ett al. 2000, Weiskel *et al.* 1996).

Viruses are one tool that could prove useful in source tracking studies because they include many pathogens of concern, and they are generally species-specific. Viruses are known to cause a significant portion of waterborne disease from water contact, mostly from ingestion of sewage contaminated water and seafood (Fogarty *et al.* 1995). Until recently, however, methods for virus detection and quantification have relied on cell culture based approaches that are much too slow to be effective source tracking tools. Recently developed molecular techniques, such as Quantitative Reverse Transcriptase PCR (QRTPCR) can detect and quantify viral genetic material directly from water samples. Results of tests conducted previously in southern California (Fuhrman *et al.* 2005, Noble and Fuhrman 2001a, Tsai *et al.* 1993, 1994), in Florida (Griffin *et al.* 1999, Rose *et al.* 1997), and Europe (Pina *et al.* 1998) using conventional RTPCR or PCR have detected a host of genetic material from human specific viruses including enterovirus, hepatitis A virus, rotavirus, and adenovirus in urban runoff discharges or seawater samples.

A different approach would be to use alternative bacterial indicators for source tracking that might be much more abundant in urban discharges. For example, *Bacteroides* sp., makes up approximately one-third of the human fecal microflora, considerably outnumbers the fecal coliforms, EC, and ENT. *Bacteroides* sp. are obligate anaerobes, so there is little concern over persistence or regrowth in the environment. More importantly, human specific *Bacteroides* sp. markers have been developed, increasing the value of this potential indicator (Bernhard and Field 2000a, b; Dick and Field 2004).

Both virus and alternative bacterial indicators such as *Bacteroides* sp. have been shown to be potentially useful source tracking tools. Griffith *et al.* (2003) and Noble *et al.* (2003) concluded that genetic based methods, such as PCR consistently provided the best information when attempting to conduct source tracking on mixed source samples. To date, however, no single method has all of the traits to be the consummate source-tracking tool. Therefore, a multi-tiered multi-indicator approach has been recommended by some investigators (Boehm *et al.* 2003, Stewart *et al.* 2003). By using multiple tools, investigators can utilize the strengths of each to ascertain inputs and track fates that will ultimately lead to successful management solutions.

The objective of this study was to identify the contributions and quantify the loading of fecal contamination to the SMB using a multi-tiered approach. The first tier included traditional fecal indicator bacteria measurements. The second tier included molecular assays developed and conducted for ENT, *Bacteroides* sp .human specific marker, and enterovirus. These methods rely on conventional PCR, QPCR, or QRTPCR, which have not been previously applied in conjunction with one another for source tracking studies in urban watersheds. The third tier was to sequence the enterovirus from the field samples with the greatest concentrations to determine the likely type of enterovirus amplified in the assay. The multi-tiered approach was applied using a mass-based design to quantify inputs and flux through an urban watershed to the beach. The multi-tiered approach was applied using a mass-based design to quantify inputs and flux through an urban watershed to the beach.

Methods

This study quantified inputs of flow, bacteria concentrations and virus genomic equivalents, then tracked them through an urban watershed over time. This mass-based design was applied in the Ballona Creek watershed, the largest tributary to SMB. Ballona Creek is over 85% developed and currently has the largest inputs of fecal indicator bacteria to SMB (Figure 1).

Samples were collected at six mainstem, and four major tributaries, to Ballona Creek. The six mainstem sites extended from Cochran Avenue (where the system daylights from the underground storm drainage system) to Inglewood Avenue (located at the head of tide just prior to discharge into SMB; Table 1). The four tributaries represented the four largest hydrodynamic inputs to the system and were located in reaches between each of the mainstem sampling sites. Flow was calculated as the product of flow rate and wetted cross-sectional area

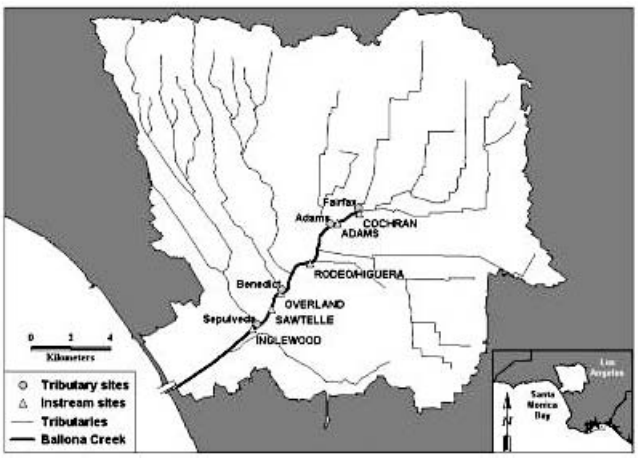

Figure 1. Map of the Ballona Creek watershed in Los Angeles, Calif. Tributary and main-stem sampling sites for the water quality study are indicated. (Inset) Santa Monica Bay, in Southern California.

(Viessman *et al.* 1989). Doppler area-velocity sensors (Teledyne ISCO, Los Angeles, CA) were used to measure flow rate . Pressure transducers that measure stage, along with verified as-built cross sections, were used to estimate wetted cross-sectional area. One minute instantaneous flow was logged electronically during the entire six hour sampling period. Both the area-velocity sensors and pressure transducers were calibrated prior to sampling.

One hour composite water samples were collected immediate downstream of flow measurement devices at each site (see GPS coordinates Table 1) between 8:00 a.m. and 2:00 p.m. on August 26, 2004. The six hour sampling period corresponds to the approximate hydrodynamic travel time from Cochran Avenue to Inglewood Avenue (Ackerman *et al.* 2003). The hourly 4-L composite samples at each site were created after combining ten individual 400-ml grab samples collected every 6 minutes into a single container. In total, 60 composite samples were collected at Ballona Creek as a result of sampling for 6 hours at 10 different sites.

After collection, samples were placed on ice and transported immediately to the University of Southern California for processing. For each composite sample, 100 ml of water was devoted to indicator bacteria analysis, and 200-600 ml of sample volume was vacuum filtered through replicate 47-mm 0.4-ìm polycarbonate (PC) filters (Poretics, Inc., Livermore, CA) using a filter funnel and receiver (Millipore, Inc., Bedford, MA) for ENT analyses by QPCR or *Bacteroides* by conventional PCR, as suggested by Haugland *et al.* (2005). In addition, replicate filtrations were also conducted using 47-mm Type HA (Millipore, Inc., Bedford, MA) mixed cellulose ester, 0.45-µm pore size filters for enterovirus analysis as suggested by Fuhrman *et al.* (2005). For each filter, the volume filtered was the maximum filterable within ca. 10 minutes of starting the filtration. The total volume filtered was dependent on the location and turbidity of each individual sample, and the filter volumes were carefully recorded to the nearest 1 ml.

Table 1. Sampling sites along the mainstem and major tributaries of Ballona Creek.

Site	Description	GPS Coordinates (NAD 83 datum)
Cochran Ave.	mainstem	34° 02.662" N 118° 21.237" W
Fairfax Drain	tributary	34° 02.298" N 118° 22.136" W
Adams Ave.	mainstem	34° 02.009" N 118° 22.494" W
Adams Drain	tributary	34° 02.009" N 118° 22.494" W
Rodeo/Higuera	mainstem	34° 01.305" N 118° 22.693" W
Benedict Box Channel	tributary	34° 00.925" N 118° 23.432" W
Overland Ave.	mainstem	33° 00.429" N 118° 23.771" W
Sawtelle Ave.	mainstem	33° 59.816" N 118° 24.164" W
Sepulveda Channel	tributary	33° 59.512" N 118° 24.693" W
Inglewood Ave.	mainstem	33° 59.394" N 118° 24.696" W