2009 Investigation of Spatial and Temporal Distribution of Human-specific *Bacteroidales* marker in Malibu Creek, Lagoon and Surfrider Beach

University of California, Los Angeles Study
Principal Investigators: Jennifer Jay and Richard F. Ambrose
Graduate Student Researchers: Vanessa Thulsiraj and Steven Estes

Introduction

WQ impairment near urbanization & Malibu as an important site

Fecal pollution, human and non-human, is a major cause of water impairment in coastal areas. However, our understanding of fecal pollution in coastal ecosystems, as well as our ability to identify and mitigate its sources, is greatly limited by the uncertainties surrounding its behavior in two major reservoirs: wetlands and beach sediments. Fecal indicator bacteria (FIB) and pathogens can enter coastal creeks and rivers from upland sources, but near-shore beach sources are also significant reservoirs (Desmarais et al., 2002; Davies et al., 1995; Craig et al., 2004; Gerba and McLeod 1976), and coastal wetlands have been shown to both increase (Ferguson et al., 2005; Grant et al., 2001; Gersberg et al., 1995; Sanders et al., 2005) and decrease (Evanson and Ambrose 2006) the levels of FIB in water.

Understanding whether there is a relationship between populations of FIB and human-specific *Bacteroidales (*HSB) in wetlands is important for determining impact that these environments pose to human health in coastal bathing waters. Recent work in Southern California has shown that coastal wetlands can be a source of FIB due to wildlife congregation (Lu et al., 2008), regrowth of FIB in sediment (Lee et al., 2006; Davies et al., 1995), and scouring of sediment. Although contrasting conclusions exist it is important to obtain a better understanding of how coastal wetlands influence FIB. Malibu is an important study site and serves as a template to investigate how wetlands specifically influence human-specific factors. This information can assist managers and policymakers to better understand how wetland-related decisions impact beach closure days and consequently local economies and human health.

Our understanding of the fate of fecal pollution in coastal ecosystems, as well as our ability to identify and mitigate its sources, is greatly hindered by limitations in the detection of microbial contamination (in terms of analysis times and host-specificity) and by uncertainties surrounding its behavior in watershed and beach sediments. Both FIB and pathogens appear to have greater persistence in sediments than they do in water, and have been shown to grow in this environment (Lee et al., 2006; Desmarais et al., 2002). Present efforts to identify fecal contamination sources employ a tiered approach in which traditional fecal indicator bacteria (FIB) levels are used to inform advanced host-specific investigation. However, a persistent lack of correlation between FIB and human-specific *Bacteroidales* markers (HSM), high temporal variability in water quality parameters, and long analysis times obscure these endeavors. Rapid detection methods and alternative, host-specific fecal indicators are at the forefront of current coastal water quality protection efforts.

Use of Bacteroidales for fecal source identification

Rapid detection methods and alternative indicators are clearly needed for the development of effective recreational water initiatives (Gregory et al., 2006). Current techniques are primarily culture-based, requiring a minimum of 18 hours for analysis. This delay could result in swimmers being exposed to poor water quality or an unnecessary beach closure, thereby impacting their personal health or the local economy. Among these explored technologies are quantitative polymerase chain reaction (qPCR) (Khan et al., 2007; McDaniels et al., 2005; Shanks et al., 2008; Siefring et al., 2008), fluorescent in situ hybridization (Lee and Deininger 2004; Field and Samadpour 2007), enzymatic methods (Scott et al., 2002), flow cytometry (Griffith et al., 2003; Paster et al., 1994) and immumomagnetic separation/ATP quantification (Gerba 2000). Quantitative PCR is advantageous because it is one of the mentioned rapid processes (3-5 hour processing time), and host-specific.

Microbial source tracking (MST) is an actively growing and important area of research, as information on host-specific sources of fecal contamination can be the key to successful remediation efforts (Bernhard and Field 2000b; Dick et al., 2005a). Griffith et al (2003) compared twelve MST techniques and found detection of *Bacteroidales* to be the most effective method to detect human fecal pollution in various mixtures of fecal sources. Members of the order *Bacteroidales* are found exclusively in endothermic organisms, and reside within feces, the digestive tract, and other body cavities (Dick et al., 2005b). *Bacteroidales* levels in human sewage are orders of magnitude higher than levels of fecal coliform bacteria (Dick and Field 2004). These organisms are obligate anaerobes, and thus do not have the potential for regrowth in the environment that confounds the use of *E. coli* and enterococci as indicators (Seurinck et al., 2005). Most importantly, *Bacteroidales* organisms from different fecal sources exhibit distinct genetic sequences, thus allowing the development of host-specific nucleic acid-based assays. Significant research has been directed toward the development of conventional and quantitative PCR methods for *Bacteroidales* markers specific to human, bovine, pig, horse and dog as well as to universal *Bacteroidales* (Kildare et al., 2007; Shanks et al., 2008; Carson et al., 2005; Nobel et al., 2006; Santoro and Boehm 2006). These assays have been applied to fecal source tracking in many environments: urban watersheds coastal beaches (Boehm 2007; Boehm et al., 2002), freshwater lakes and rivers (Lund 1996), groundwater (Reischer et al., 2008), and agriculturally impacted estuaries and bays (Shanks et al., 2006; Gourmelon et al., 2007).

This study examines the spatial and temporal distribution of human-specific *Bacteroidales* marker (HBM) in lower Malibu Creek, Lagoon and Surfrider Beach under specific hydrologic conditions. Specifically, this work identifies the distribution of fecal indicator bacteria and HBM during wet and dry weather, when the lagoon is open and closed. We investigate whether detectable concentrations of HBM are present in the Lagoon, and if concentrations of HBM correlate with fecal indicator bacteria.

Materials and Methods

Site description of Surfrider Beach and Malibu Creek

The Malibu Creek watershed (109 mi^2) is partially developed (mixed use 17%, 83% open), with 90,000 residents in five cities and unincorporated Los Angeles County. Reaches of the creek are

impaired for bacterial contamination, and Surfrider Beach, with over 10,000 visitors on a typical summer weekend day, has frequent postings for impaired water quality. Fecal sources include non point sources and wildlife. Possible human sources of fecal contamination include septic systems and disinfected discharge from Tapia Wastewater Reclamation Facility into Malibu Creek (discharging only in the winter). Malibu Creek ends in a 13-acre lagoon, so birds are likely an important fecal source at Surfrider Beach as well. The beach is currently sampled daily for FIB. Additionally, Heal the Bay's Stream Team has gathered long-term nutrient and FIB data at 17 locations throughout this watershed, showing increasing nutrient and FIB levels with distance downstream in each of four subwatersheds.

Sample design and collection

For this study, a snapshot of bacteria concentrations was measured from the lower Malibu Creek watershed. Surface water samples were collected from a total of 20 sample locations throughout Malibu Creek, Lagoon and Surfrider Beach (Fig 1). Water samples were collected with sterile 500mL Nalgene bottles attached to a sampling pole, or by submerging a sterile 2L Nalgene bottle. Samples taken from flowing storm drains collected end-of-pipe discharges. Samples were stored on ice and transported to the laboratory within 6 hours for immediate analysis. Samples were taken during wet and dry weather, while Malibu Lagoon was open and discharging to Surfrider Beach. Additional sampling occurred during a transitional phase where the Lagoon had been previously closed, but opened overnight and was open during time of sampling. The third hydrologic condition investigated was during dry weather while the Lagoon was closed due to the formation of a sand berm, which prevented flow from the Lagoon to the beach.

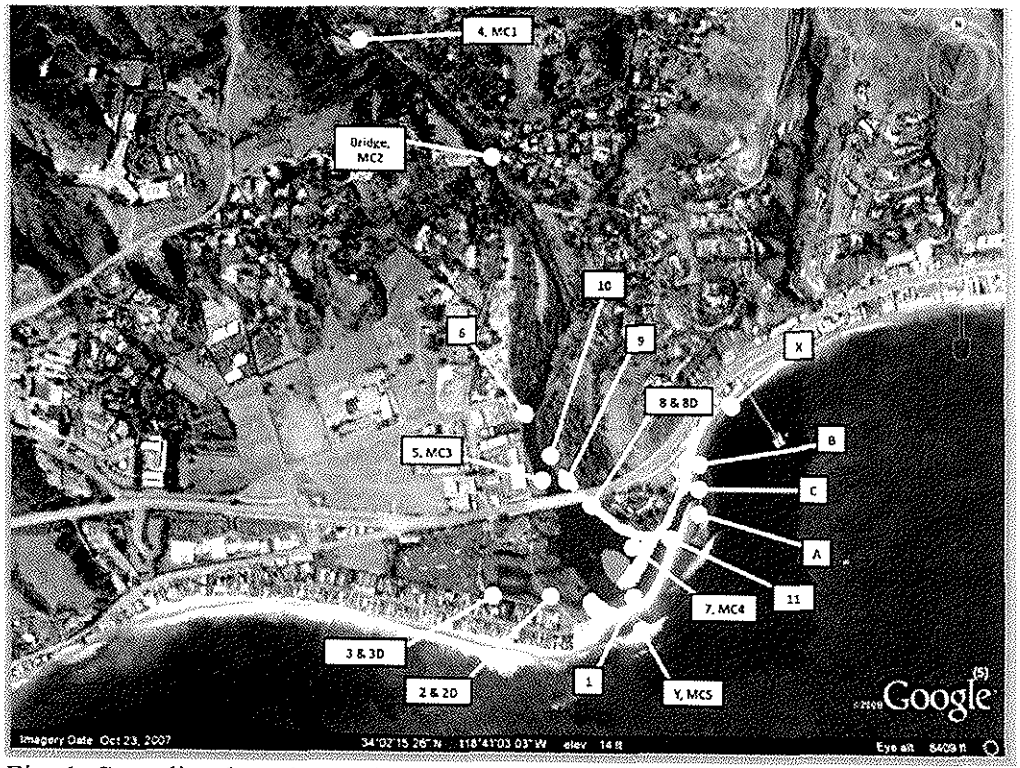


Fig. 1. Sampling locations within the Malibu Watershed. Samples were taken from Malibu Creek (Site 4, Bridge), Lagoon (Site 1-4, 5-11)Surfrider Beach (Site A, B, C, X, Y). Sample sites 2D, 3D, and 8D represent storm drain discharges.

FIB analysis by traditional methods

Water samples were measured for three types of fecal indicator bacteria including total coliform (TC), *Escherichia coli* (EC), and enterococci (ENT) using IDEXX Laboratories, Inc. (Westbrook, ME) defined substrate tests commercially known as Colilert-18 and Enterolert in a 97 well quantitray format. Ten fold and 100-fold dilutions of water samples were used as recommended by the manufacturer.

Geochemical analysis of water samples

Water samples were analyzed for nitrate and ammonia using the Hach Spectrophotometer (model DR 280). Combo by Hanna (model H 18130), a multi-parameter probe, was used to measure total dissolved solids (TDS), water temperature, electrical conductivity and pH. Dissolved oxygen concentrations were measured by the YSI 55 DO probe (model 55/12 FT). Water samples were also analyzed for total suspended solids (TSS) in the lab using Environmental Sciences Section Method 340.2.

Extraction of DNA

DNA was extracted from filters using the Mobio UltraClean Fecal DNA Kit. DNA was extracted according to manufacturer's protocol with the addition of 90 seconds of bead-beating in lieu of 10 minutes vortexing, as listed in the protocol. DNA extracts were stored at -20°C until they could be processed for human-specific *Bacteroidales*. The concentration of extracted DNA from each sample was measured fluorometrically (Stratagene, La Jolla, CA (Santoro and Boehm 2007)) using the Quant-It PicoGreen double-stranded DNA reagent kit (Invitrogen, Carlsbad, CA). Each sample was measured and total DNA(ng) was found by reference to Lambda DNA standards (n=8 ranging from 0.1 to 25 ng µl-1). Prior work in Jay lab has shown that superior DNA amount and quality are obtained with no further purification steps.

Quantitative PCR analysis of Bacteroidales

Detection of 16S rRNA gene markers for human-specific *Bacteroidales* were performed using qPCR (Stratagene, Inc., Mx3000P), by the SYBR Green-based method, with DNA primers HF183F and HF183R (Dick and Field 2005; Seurinck et al., 2005). Each qPCR mixture (total volume 25 µl) contained approximately1-2 ng DNA (diluted in known volume of RNase-free water) and ~13 µl master mix (2× SYBR Green, Stratagene, Inc.; 140 µM each primer, Operon Biotechnologies, Huntsville, AL). Sample were run in duplicate and converted to concentration by reference to *Bacteroidales* standards (a *Bacteroidales* plasmid DNA kindly provided from the Furman laboratory (n=8 ranging from 2×100 to 2×10^6 copies µl-1). Samples "spiked" (i.e., positive controls) with 1-µl (2×10^5 copies) *Bacteroidales* standard are used to estimate low rates of recovery and possible inhibition by contaminants in DNA extracts. In the case of interference, samples were diluted two-fold and reprocessed (Nobel et al., 2006). Negative controls were run with every reaction, and consist of all elements except target DNA.

Results

Fecal Indicator Bacteria Results

Over the entire study period (February to July 2009), a total of 70 water samples were taken from up to 20 different sample locations within lower Malibu Creek, Lagoon and Surfrider Beach. During this time, fecal indicator bacteria were above the water quality single sample standard in 50%, 54% and 39% for TC, EC and ENT. The greatest number of exceedances occurred at site 3 and 3D for total coliforms, however the single highest level (241957 MPN/100ml) for TC was measured from site 6 (lagoon) during wet weather on February 16, 2009. For *E. coli*, sites 2 and 3 showed the greatest number of exceedances, with 4/4 samples above the threshold for both sample locations. Several sites had maximum EC concentrations above the detection limit (>24196 MPN/100ml), including sites 7, 8 and 2D. The greatest number of exceedances for enterococci was measured at site 3D, however highest concentrations of ENT (19863MPN/100ml) was measured during wet weather from sample site 7, during dry weather when the lagoon was closed.

Fecal indicator bacteria concentrations were found to be high throughout the Malibu Creek, Lagoon and Surfrider Beach during a storm event on February16, 2009. This was the only time that elevated fecal indicator bacteria concentrations were measured upstream in Creek waters or in ocean water samples in this study. The mean concentration during wet weather was measured as 69280 MPN/100ml for TC, 1328 MPN/100ml for EC and 3755 MPN/100ml for ENT. Highest percent exceedance was measured during wet weather. Samples exceeded water quality standards 87% of the time for both TC and EC and 100% for ENT. All samples collected exceeded standards for all three indicators except for two samples. Water samples exceeded water quality standards 87% (13/15) and 100% (15/15) of the time for EC and ENT. Samples collected from site 8, taken within the lagoon, and site 2D, stormwater runoff did not exceed standards for total coliforms and *E. coli.*

In March 2009, field sampling occurred during dry weather while the lagoon was breached. FIB concentrations were typically below the health standard of 400MPN/100mL and 104MPN/100mL for *E. coli* and enterococci. However, specific hot spots were found within Malibu Lagoon under this hydrologic condition. Exceedances for FIB occurred at four sample locations (2, 3, 7, 8) within the lagoon and adjacent to two storm drains (3D and 8D) in March. FIB levels were above health limits in 22% (TC and ENT) and 28% (EC) of samples collected. Mean concentrations for this sampling field campaign were 19924 MPN/100ml TC, 699 MPN/100ml EC, and 371MPN/100ml ENT. However, median concentrations were all below the recreational water quality threshold (TC 2006, EC 168 and ENT 10 MPN/100ml).

During dry weather, transitional open lagoon conditions, elevated levels of FIB occurred in 10 sample locations resulting in 22%, 44% and 6% exceedance for TC, EC and ENT respectively. Slightly larger number of exceedances were observed for E. coli, however exceedances were much lower for enterococci in the May sampling event. Samples were above the health standard for E. coli at sites 2, 3, 6, 8-11, and 3D. Highest FIB concentrations were measured from site 3 (EC 1552 MPN/100ml) and site 3D (EC 2307MPN/100ml, ENT 1004 MPN/100ml). The largest number of exceedances occurred within Malibu Lagoon, although one ocean water sample (site X) and one upstream sample (site Bridge) were above the water quality limit for total coliforms. Both of these samples did not exceed the single sample standard for any other indicator organism. Mean sample concentrations were 12861 MPN/100ml TC, 531 MPN/100ml EC and 6

MPN/100ml ENT. Median concentrations were again lower than mean values, with values of 3189 MPN/100ml TC, 206 MPN/100ml ENT, and 20 MPN/100ml ENT.

After closure of the lagoon, fecal indicator bacteria concentrations increased, and higher level of exceedences were observed for all three indicators (74% for TC, 63% for EC, and 37% for ENT). Despite exceedances in the lagoon throughout the study period, FIB levels did not exceed health standards for samples collected in Malibu Creek and at Surfrider Beach. The lowest mean concentrations for Surfrider Beach ocean water samples were observed during the closed lagoon, dry weather condition in July 2009 (60 MPN/100ml TC, 20 MPN/100ml EC, and 2 MPN/100ml ENT). And although concentrations were intermediate to high (10^3 to $10^{4.7}$ MPN/100ml) for total coliforms in upstream locations, site 4 and Bridge, these sites never exceeded standards for either *E. coli* or enterococci throughout the sampling period. Mean concentrations for the closed lagoon, dry weather condition were 17096 MPN/100ml TC, 10297 MPN/100ml EC, and 2096 MPN/100ml ENT. Median concentrations were still high, with both TC and EC above the single standard sample threshold (24196 MPN/100ml TC, 5172 MPN/100ml EC and 74 MPN.100ml ENT).

Human-specific HF183 Bacteroidales Results

The human-specific HF183 *Bacteroidales* marker (HBM) concentrations were measured from water samples taken within the Malibu Creek, Lagoon and Surfrider Beach. The human-specific HF183 *Bacteroidales* marker was analyzed during open and closed lagoon, dry and wet weather conditions. Duplicate concentrations were averaged and reported as the value for positive samples. A total of 80 water samples were analyzed for the HBM. Forty-four samples were taken during dry weather while the lagoon was breached (open and transitional). Human-specific *Bacteroidales* marker was not detected in any of the samples taken during this condition. During wet weather open lagoon conditions in February 14.3% (2/14) of samples were positive for HBM. Concentrations of the HBM measured at sites 3 and 5 were 452 copies/100ml and 880 copies/100ml. In July, during dry weather closed lagoon conditions 3 of 22 (13.6%) samples were positive for the HBM. Sample locations 3, 6 and 7 all located within the Malibu lagoon had HBM concentrations of 121 copies/100ml, 55c opies/100ml and 210 copies/100ml.

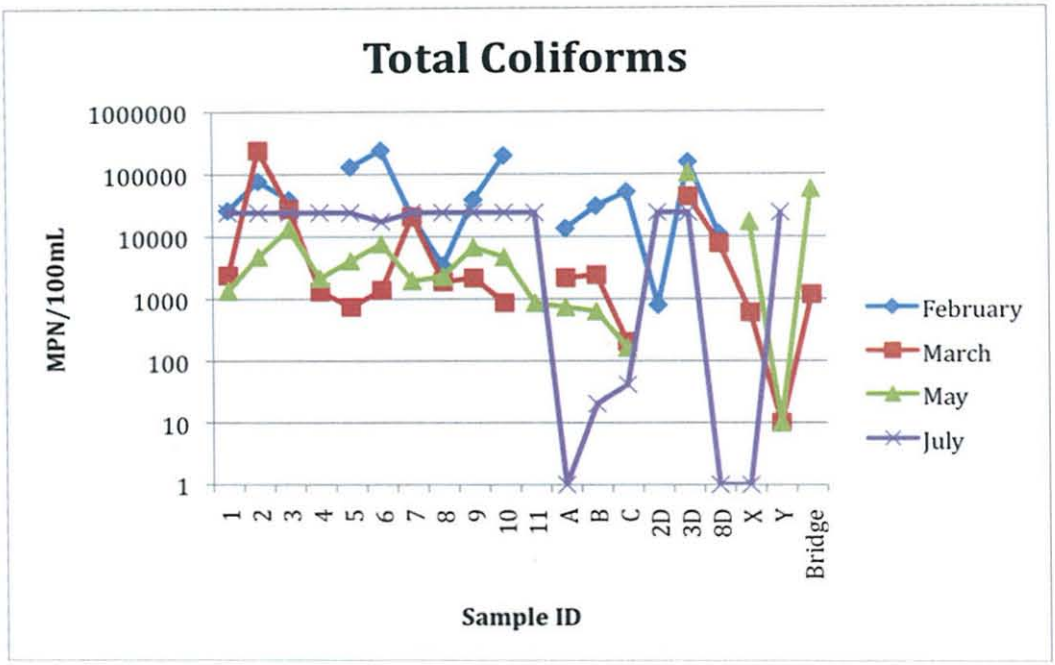
In addition to the four field sampling days at the 20 locations throughout the watershed, 5 sample sites were measured during a two-week sampling campaign in the months of April and May. Samples were taken at sites 4, 5, 7, Y and Bridge on April 29 2009. Samples taken from sites 4, 5, 7, and the Bridge did not have detectable concentrations of the human-specific *Bacteroidales* marker. Sample site Y, an ocean water sample taken from Surfrider Beach at the mouth of the lagoon, was sampled on April 29 and 30th as well as May 5th and 7th. Site Y did not have detectable concentrations of HBM in the additional four samples taken during the two-week period.

Conclusion

Of the 80 water samples analyzed within the Malibu watershed, five samples were positive for the human-specific HF183 *Bacteroidales* marker. The five positive samples were measured from 4 sample locations, all located within the Malibu lagoon. Site 3 was positive for the HBM during wet and dry weather. Concentrations at this site ranged between 121 – 452 copies/100ml. Other sites that were positive for Malibu Lagoon included sites 5, 6, and 7. Concentrations at these

sites ranged between 55 – 880 copies/100ml, which is equivalent to 0.00005 - .0009% sewage. The highest percent exceedance of FIB and HBM concentrations were measured during wet weather. During the study, 93.8% of the samples did not have detectable concentrations of HBM. These data do not rule out any particular potential sources of human fecal contamination. The human-specific *Bacteroidales* marker was not measured in any of the lower Malibu Creek samples and ocean water samples taken from Surfrider Beach.

Figures


Total Coliforms
MPN/100mL
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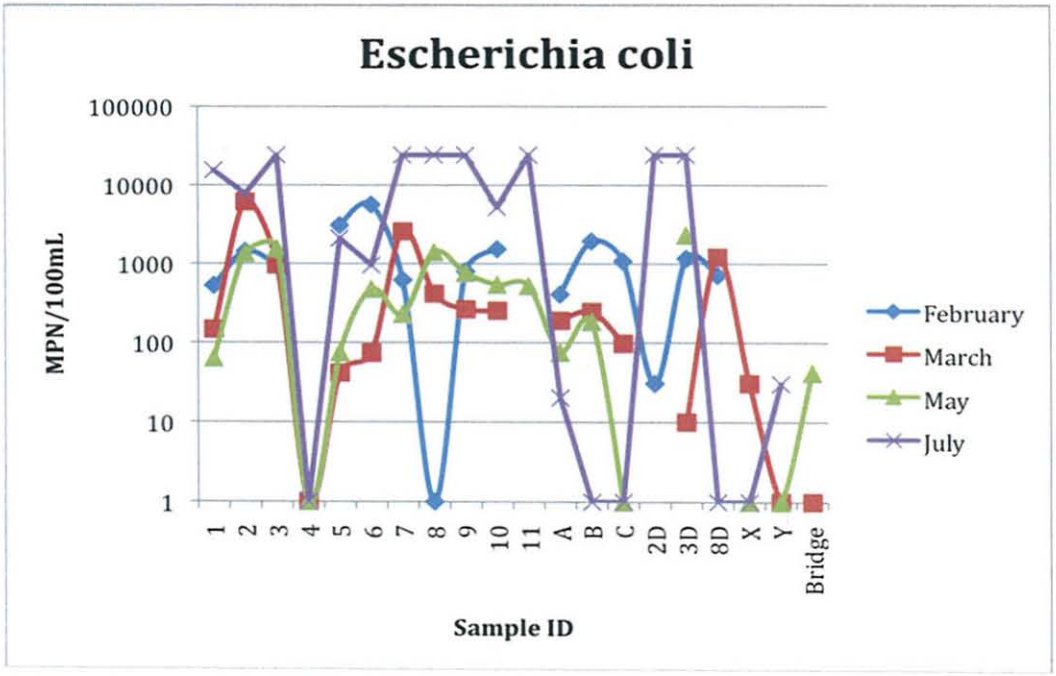
Escherichia coli
MPN/100mL
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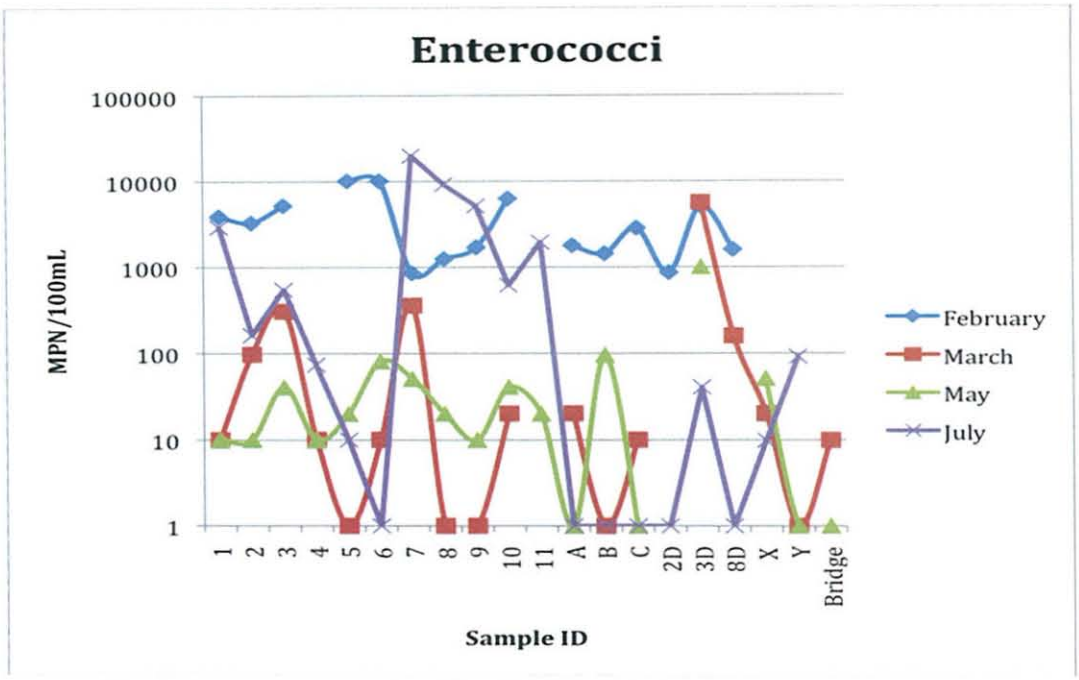
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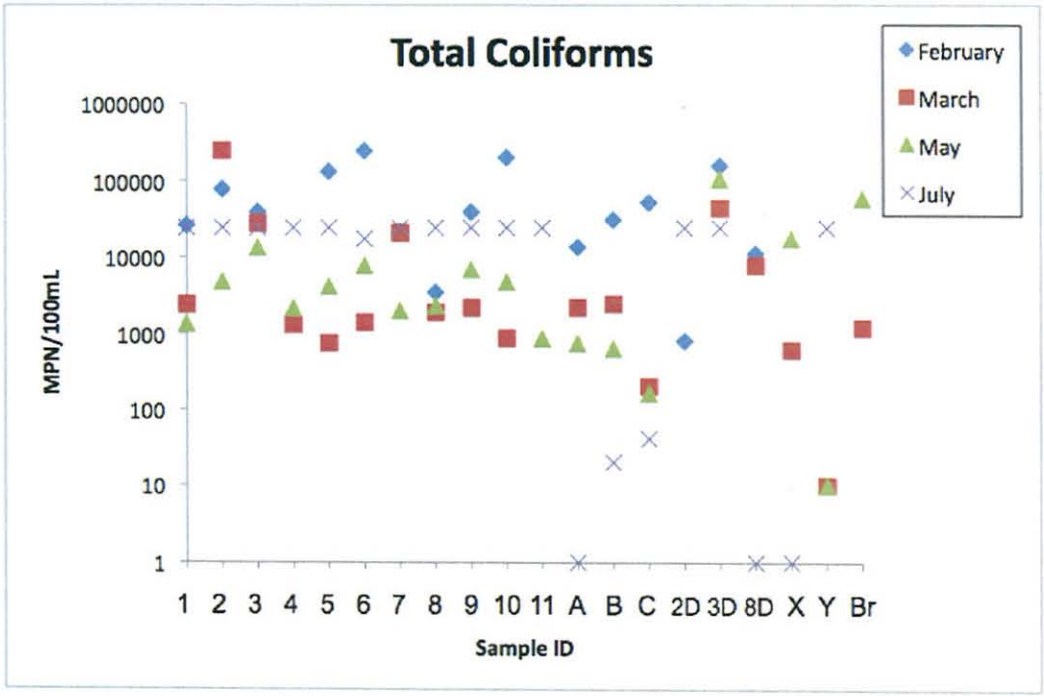
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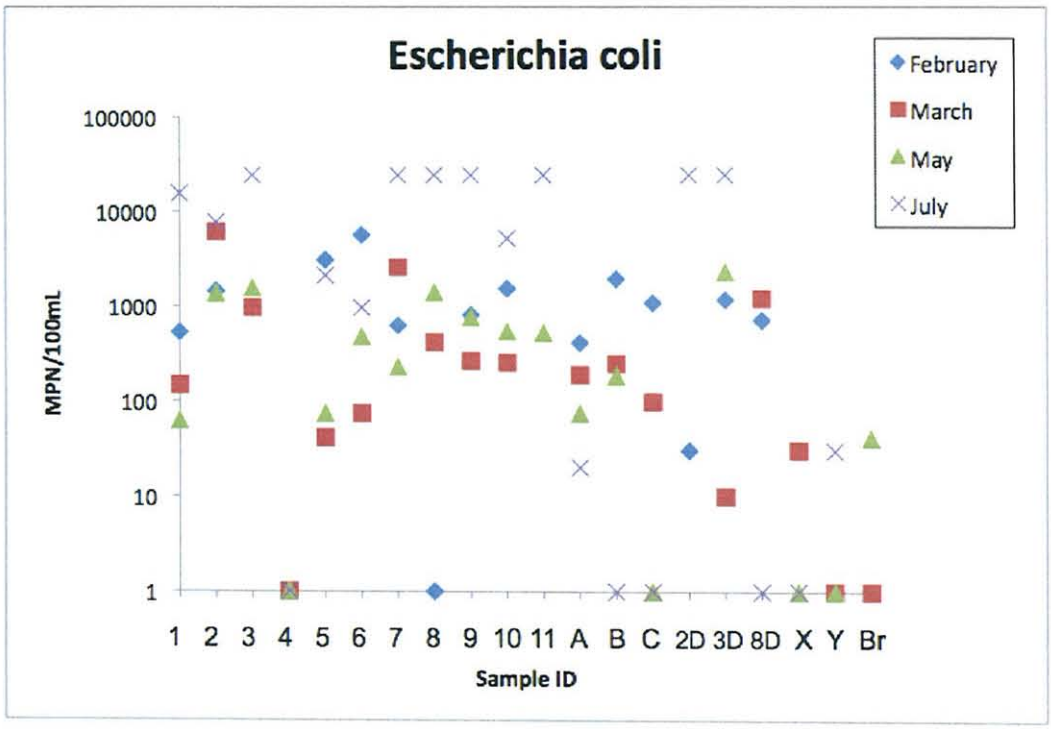
Escherichia coli
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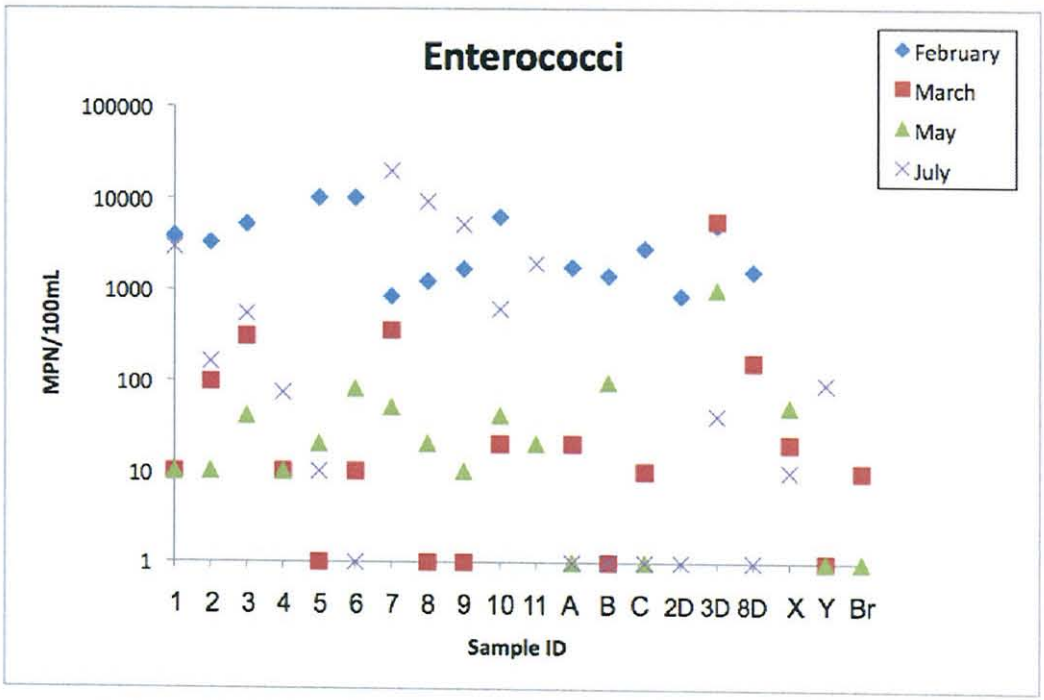
Enterococci
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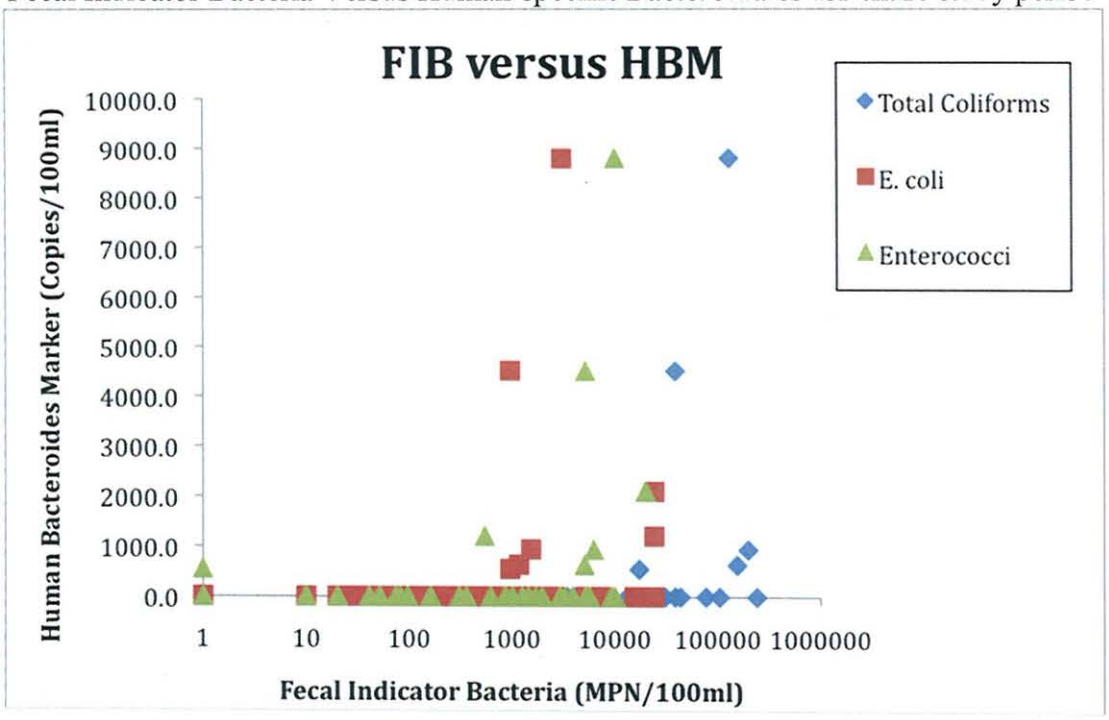
Sample ID

Fecal Indicator Bacteria Versus Human-specific *Bacteroidales* for entire study period



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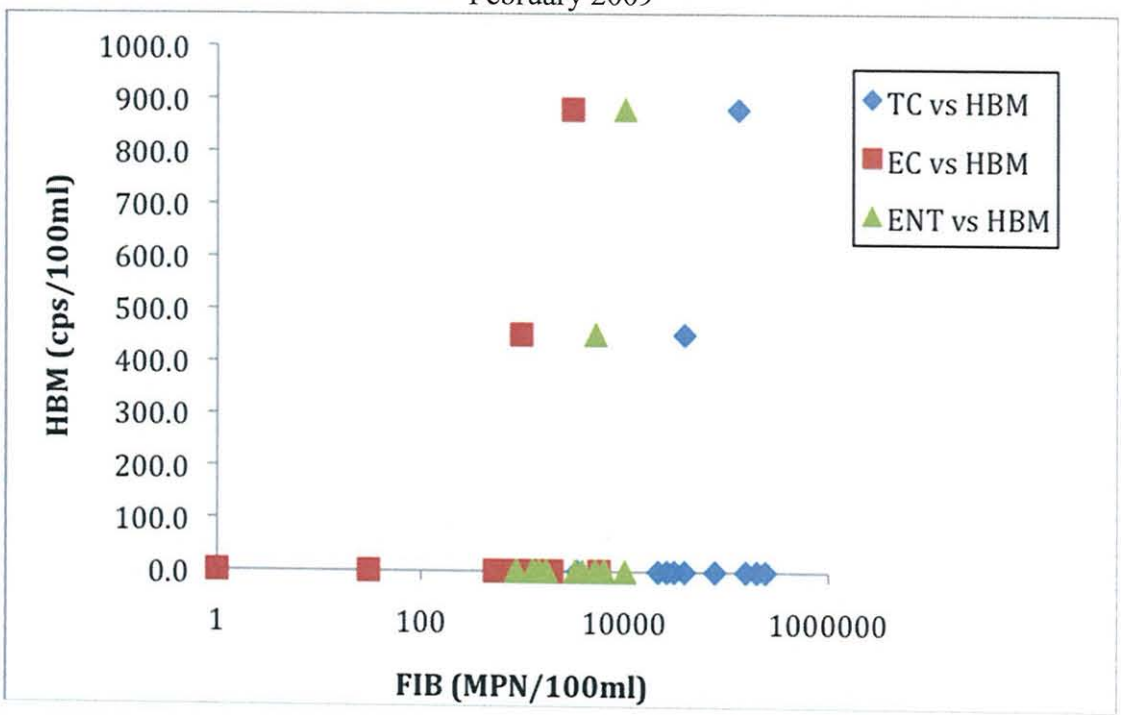

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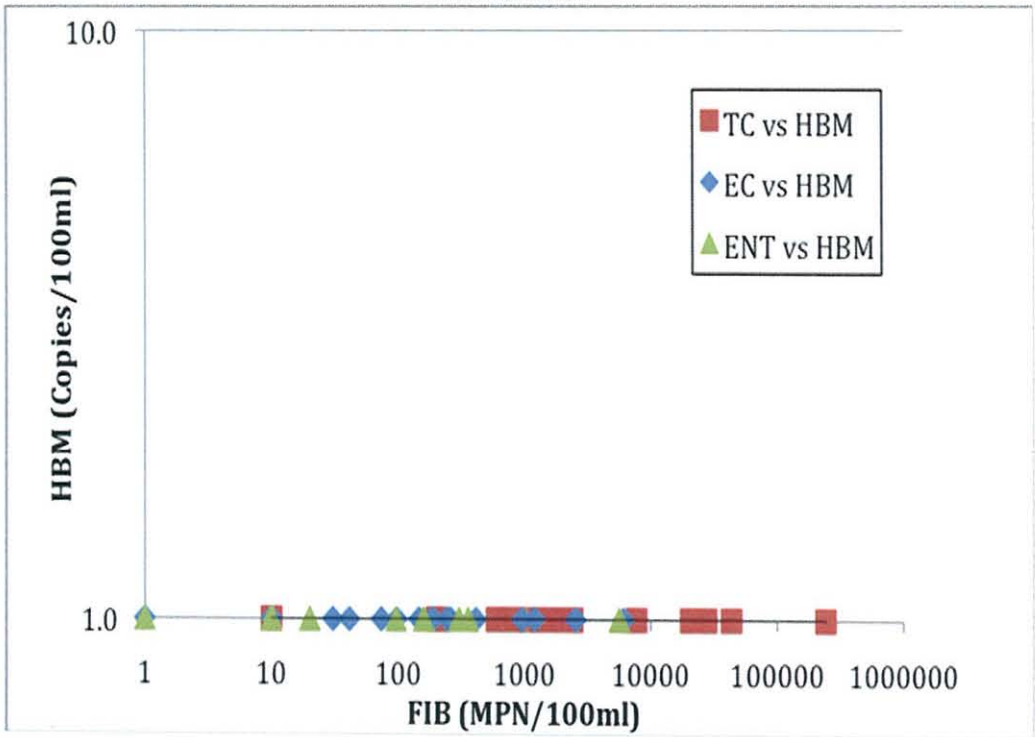
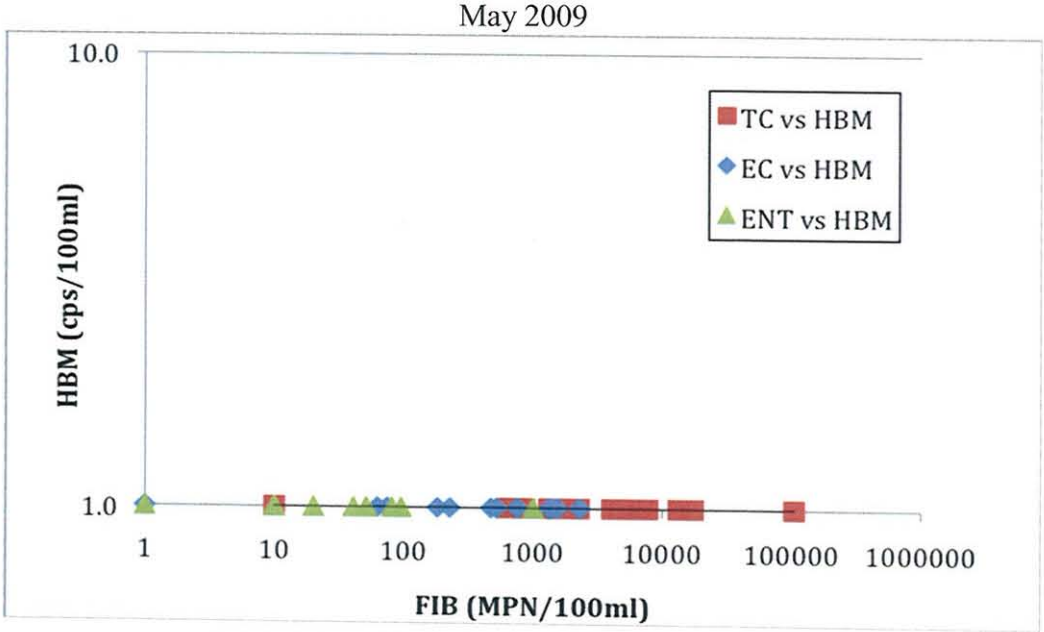
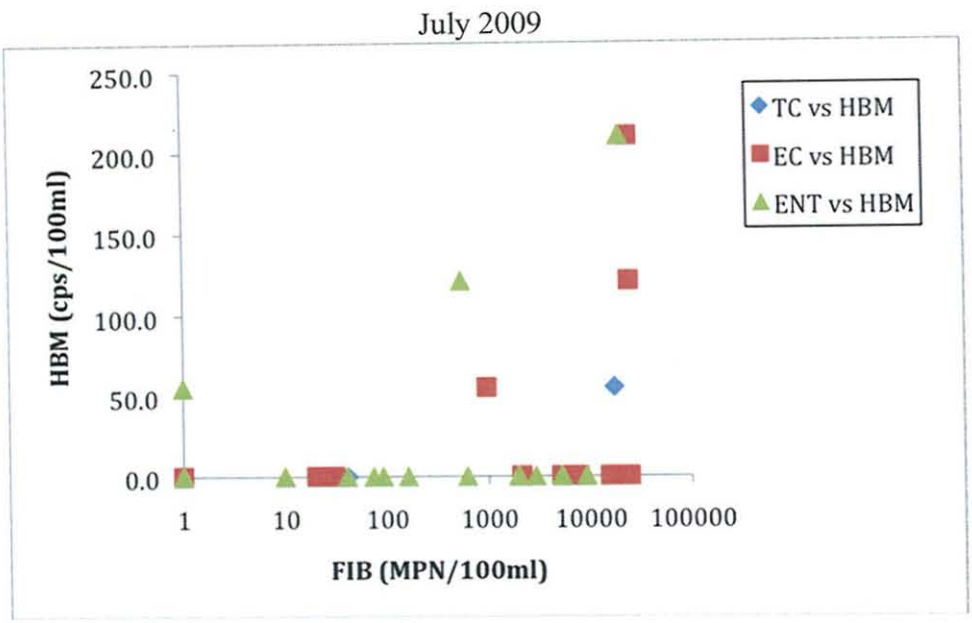
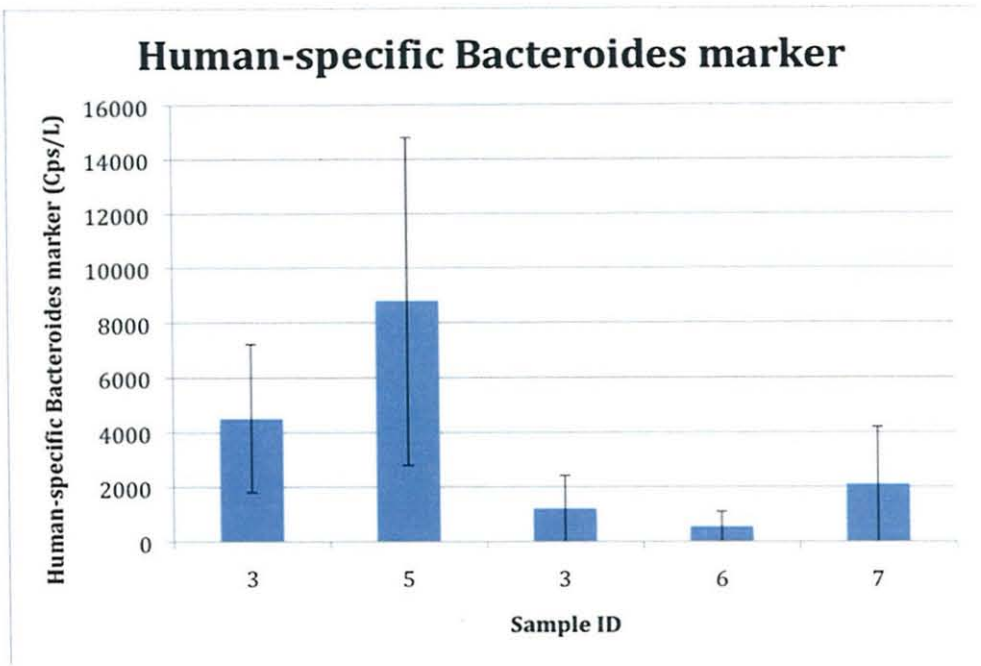


May 2009







Average HBM concentrations from duplicates are graphed above. The error bars represent the minimum and maximum concentrations measured from each sample processed. (Sample 3 and 5 on the left were taken in February, and samples 3, 6 and 7 on the right were taken in July).

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