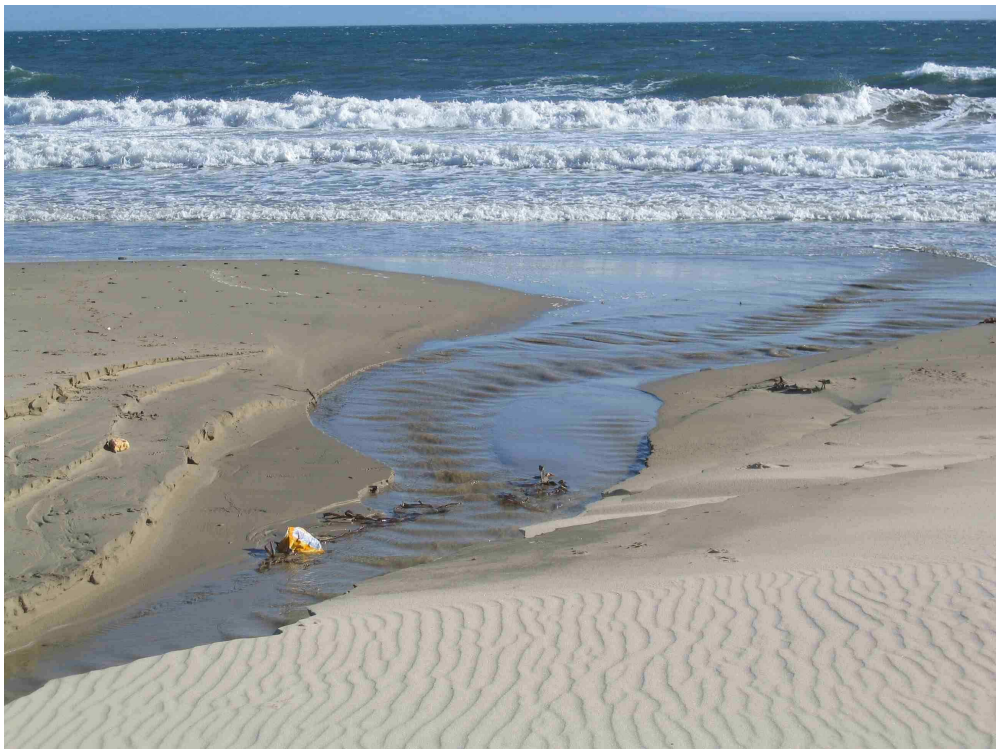
Microbiological Water Quality Investigation for Las Palmas Creek, Hope Ranch, Santa Barbara, CA

Final Report



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June 16, 2006

UNIVERSITY OF CALIFORNIA
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A Group Project submitted in partial satisfaction of the requirements for the degree of Master's in Environmental Science and Management for the Donald Bren School of Environmental Science and Management by

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As authors of this Group Project report, we are proud to archive it on the Bren School's web site such that the results of our research are available for all to read. Our signatures on the document signify our joint responsibility to fulfill the archiving standards set by the Donald Bren School of Environmental Science and Management.

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The Group Project is required of all students in the Master's of Environmental Science and Management (MESM) Program. It is a four-quarter activity in which small groups of students conduct focused, interdisciplinary research on the scientific, management, and policy dimensions of a specific environmental issue. This Final Group Project Report is authored by MESM students and has been reviewed and approved by:

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June 16th, 2006

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ABSTRACT

Hope Ranch Beach in Santa Barbara resembles other southern California beaches in that it occasionally exhibits high levels of fecal indicator bacteria (FIB), especially during storm events, which may result in beach closures. Some of this pollution may be attributable to Las Palmas Creek, which drains into Hope Ranch Beach. The high baseline concentrations of FIB in Las Palmas Creek can increase by three orders of magnitude during rain events. Using a combination of industry standard assays for FIB, as well as polymerase chain reaction (PCR)-based DNA analysis, this investigation establishes baseline microbiological water quality data at Las Palmas Creek and Hope Ranch Beach, and suggests the potential sources of its contamination. Water samples were collected weekly at six sites during the summer of 2005, followed by sporadic "flush" samples taken during rain events in winter 2005-2006. PCR analysis focused on identifying indicators for human, herbivore, and horse waste, as well as identifying the potential pathogen *Cryptosporidium parvum*. In order to locate probable inputs and potential reservoirs for fecal waste contamination, statistical analyses were performed to identify significant exceedances of FIB levels in environmental samples, using the state standard for recreational marine waters as a benchmark, as well as significant relationships between soil texture and FIB concentration. The results suggest that both herbivores and soil reservoirs could be contributing to the contamination of Las Palmas Creek. This project will assist the Hope Ranch Beach Committee in evaluating and addressing water quality along the creek, and in turn allow the Homeowners Association to tailor any future water quality investigations and/or management plans to the acquired data.

INTRODUCTION

Global Problem

Pollution in the human environment is a worldwide problem, especially in rapidly urbanizing coastal regions where contamination is diffuse, and its sources often unidentified. The primary concern in these areas is human health, where contaminants of concern include waterborne pathogens directly responsible for human illness. Secondary effects of water pollution on humans include, but are not limited to, impacts on fisheries, which suffer from reduced yield under conditions ranging from mercury contamination to nutrient loading.

Historically, the regulations governing inputs of bacterial pollution into the watershed have emphasized the control of publicly-owned treatment works (POTWs), and other industrial sources of storm water discharges, as required by the 1987 amendments to the federal Clean Water Act (CWA) (US EPA Ag Center 2006). However, the application of these National Pollution Discharge Elimination System (NPDES) permits does not address non-point sources of contamination, such as sewer-line extensions, land wastewater applications, and waste lagoons, as well as agricultural discharge (US EPA Ag Center 2006). Modern water quality problems are often attributed to managers neglecting, or at least failing to adequately control, other non-point sources such as fertilizer runoff, animal wastes, and septage from residential runoff. Total Maximum Daily Loads (TMDLs), standards required by the CWA and developed and enforced by local resource management agencies, are designed to target non-point sources, such as those from agricultural practices, and ensure that national waters are "fishable" and "swimable." However, regional TMDL water quality targets are less heavily enforced than the national permitting system used to curb point source inputs into a watershed, and are rarely applied to smaller catchments lacking in federal and state jurisdiction because they are not designated "waters of the U.S." (US EPA Ag Center 2006).

Fecal pollution, in particular, often arises from diffuse sources and has significant implications for water quality. In 2002, 87% of beach closings reported nationwide were attributable to FIB levels, but only 38% of these closings were based on a known source of contamination (NOAA 2006). In Santa Barbara, a beach warning or closure occurs when FIB levels in a weekly water sample exceed the standards set by state law AB 411, following the procedures established by the Santa Barbara County Department of Environmental Health Services (SBCEHS 2006). However, while ocean water quality receives ample attention in the region, some freshwaters remain relatively unstudied, especially in privately owned areas. Even in low-density developments that exist outside of the central Santa Barbara downtown, perennial dry-weather creek water quality can pose a threat to human health, regardless of the availability of, and the conclusions drawn from, FIB data (personal communication, Rob Almy, Santa Barbara County Water Agency). The presence of this threat is supported by epidemiological studies, while anecdotal information provided by local beachgoers has suggested a correlation between ocean water recreation and subsequent illness.

Available Techniques

In the last twenty years, water quality experts have sought and developed rapid methods for detecting impaired waters. FIB counts traditionally serve as a cue for scientists, policymakers, and the public to recognize the magnitude of contamination in their watersheds. These decision-makers have also relied upon previously established epidemiological relationships between FIB concentrations and frequencies of illness to decide what levels of pollution require careful monitoring and corrective action. FIB assays are used as an alternative to direct detection of pathogens that may be present in the water and provide a low-cost and generally robust method for evaluating water quality. However, they are not specific to the sources of pollutants of concern and introduce a 24-hour delay between sampling and results. Furthermore, no single assay, or combination of assays, can reflect the presence of all of the potential pathogens in a coastal environment (NEMI 2002).

To get a better understanding of the causes of pollution in a watershed, environmental managers are turning to more specific forms of tracking contamination than FIB quantifications. Source-tracking is a field in which water quality scientists study water samples for a specific contaminant. The main objective of microbial source-tracking is to trace the origin of the waste back to some defined, often upstream, physical source. Potential contaminants include those from urban/suburban runoff, rural sources (livestock), pets, and wildlife, in addition to indigenous signals present in the environment and in reservoirs such as the sediments in the streambed (US EPA 1997).

Discerning between sources of fecal contamination becomes the ultimate goal in source tracking, and yet, there is still no single "silver bullet" with which to identify, locate, and eliminate a particular pollutant (US EPA 2005). The simplest source- tracking methods may include chemical analyses of water samples to locate compounds such as caffeine or detergents to indicate the present of human waste in water (US EPA 2005). Another source-tracking technique is the use of the fecal coliforms to fecal streptococci ratio, which was originally understood to allow scientists to differentiate between human and animal sources of fecal pollution (Loaiciga and Renehan 1999).

Once novel in the water quality sciences, DNA-based techniques for tracking sources of pollution are presently becoming an integral, if still unofficial, part of the United States Environmental Protection Agency's TMDL program (US EPA 2005). Today's advanced microbial source-tracking techniques rely on the isolation of a targeted source identifier – usually a gene sequence – that may be amplified through the use of polymerase chain reaction (PCR). This process can potentially identify a pathogen or indicator as specific as a particular taxonomic assemblage within a classification at the species level, and if necessary, can be cloned and sequenced in further study to confirm the precise presence of an organism of concern in an environmental water sample (US EPA 2005).

Such PCR-dependent studies may address two central assessment and management issues for the human use of, and recreation in, coastal waters: (1) the origin/source of fecal indicator bacteria (i.e. human or animal waste), and (2) the occurrence of pathogens that may or may not correlate with fecal indicator bacteria levels. To procure such information on water

quality, a complete microbial source-tracking study generally involves the use of various conventional (FIB-counting) as well as emerging (DNA-based) techniques to determine if indicator bacteria originate from human waste or other sources. Typically a suite, or tier, of analyses is used to explain the potential sources and to suggest the health risks posed by the contamination (Boehm *et al.* 2003; US EPA 2005).

Regional Applications

Hope Ranch Beach in unincorporated Santa Barbara, California, has experienced its share of warnings and closures since weekly water quality testing began in 1996, but the origin of FIB counts, and especially the degree of fecal contamination of creek and coastal waters, remains largely unknown up until the initiation of this project (personal communication, Rob Almy, Santa Barbara County Water Agency). The desire of the Hope Ranch community to obtain a baseline understanding of Las Palmas Creek's microbiological water quality and of the magnitude of potential human health effects of recreating at the beach has driven the need for this project, including a final set of creek management recommendations that will aim to address any local water quality problems.

In Hope Ranch, the proximity of horse trails to Las Palmas Creek suggests the potential for contamination of the water by equine waste. The recreational activities known to take place in or along the creek, or especially at the surf zone where the creek drains to the ocean, introduce some degree of human health concern attributable to zoonotic diseases. Such diseases are characteristically transmitted between vertebrate animal and human through contact with an animal's feces, urine, saliva, blood, and milk (Center for Disease Control 2006). Zoonotic diseases that are known to infect humans through horse feces (or water contaminated with it) are of special relevance in horse-friendly communities such as Hope Ranch, and include campylobacteriosis, cryptosporidiosis, giardiasis, and leptospirosis (Center for Disease Control 2006).

The second major potential source of microbial contamination that Hope Ranch residents have expressed concern over is the possible presence of human fecal waste in recreational waters, as waste water from all the homes in the community are treated by septic systems and not by a municipal sewage facility. An illness of potential concern that has been associated with both human as well as animal feces is salmonellosis. The food poisoning attributed to the bacterium *Salmonella* spp. normally results from direct contact with sewage or indirect contact with contaminated water or food (Center for Disease Control 2006). While these pathways are more likely in urbanized areas served by municipal sewage lines than in low-density residential site such as the environs of Las Palmas Creek, they represent the possibility for other such waterborne contaminants to make their way from septic leach fields to the recreational water supply.

The tools available in the microbial source-tracking field are diverse in specificity as well as sensitivity, and they can supplement a baseline water quality investigation with insight into causes of pollution and potential remedies. However, the intricacies of any particular watershed, such as the Hope Ranch area and Las Palmas Creek, ensure that source-tracking alone cannot positively identify every source of pollution, nor coerce action on behalf of the local residents. Cooperative management efforts remain a significant part of maintaining the

quality of water resources in and around the creek, for Hope Ranch residents and for other watershed groups across the region, state, and world. This report begins the process by presenting and analyzing the results of a water quality investigation for June 2005-March 2006.

PROBLEM STATEMENT

Project Questions

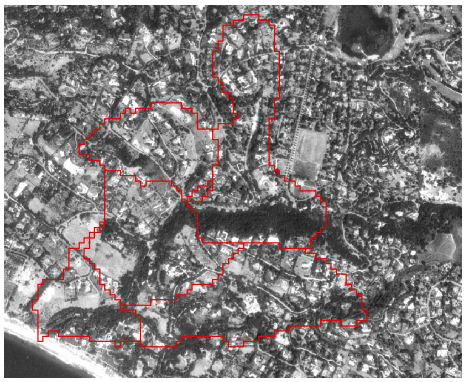
This study was designed to collect data in response to the following questions:

- First, what are the risks to human health, especially that of recreational users of Hope Ranch Beach, which result from the water quality conditions (as indicated by the level of FIB and presence/absence of *Cryptosporidium parvum*) along Las Palmas Creek?
- Second, how might the Beach Committee best manage the creek to mitigate this risk, based on the probable sources of contamination as identified through this project?

Site Description

The Hope Ranch neighborhood is situated on unincorporated coastal land between the southern California cities of Goleta and Santa Barbara (Figure 1). Unlike typical suburban developments, the parcels in Hope Ranch usually consist of multiple acres of landscaped or partially forested land, and many also stable horses and occasionally other farm animals. The neighborhood is affluent, and has a network of horse trails that run throughout the neighborhood. One of these trails runs the entire length of Las Palmas Creek, a perennial stream that parallels Las Palmas Drive and discharges most runoff for Hope Ranch into the surf zone (Figure 2). All homes are served by septic tanks or dry wells for on-site wastewater treatment (personal communication, Willie Brummett, Santa Barbara County Environmental Health Services).

(above)
Figure 1: Regional Map
Goleta, Hope Ranch, and Santa Barbara, CA.



(left)
Figure 2: Vicinity Map
Red line outlines main catchments draining into Las Palmas.

Water from the Hope Ranch neighborhood does not drain into a Federally- or State-recognized blue-line stream, as is the case with larger watersheds in the vicinity, such as Arroyo Burro (USDA 1981). Partially for this reason, less is known about Las Palmas Creek and its relationship to Hope Ranch Beach than other creeks in the Santa Barbara area and the beaches they drain into. In particular, during dry weather, the sources of baseflow that generate Las Palmas Creek are unknown. The creek does not appear to be fed from any obvious input, such as the manmade Laguna Blanca Lake, which is situated within the La Cumbre Country Club about a half mile north of the creek's headwaters. Summer flow may be due to recharge from landscape irrigation, or may be the remnants of core flow from the rainy season (personal communication, Rob Almy). During winter rain events, the roads channel most surface runoff into Las Palmas Creek, which remains an unmodified channel within in a vegetated tree corridor. The creek banks are often steep and composed of clay, which produces rapid runoff and a high erosion hazard (USDA 1981).

Concerns and Stakeholders

Over the past ten years, the Hope Ranch Beach Committee has expressed concern that Las Palmas is transporting animal or human waste into the surf zone. Surfers and swimmers have attributed ear, eye, or gastrointestinal infections to recreation at the beach (personal communication, Ken Young, Hope Ranch beach Committee). Furthermore, the County Department of Environmental Health Services has noted 10, 6, and 8 percent exceedances of fecal indicator bacteria (FIB) levels above state standards in 2003, 2004, and 2005, respectively. These numbers do not set Hope Ranch Beach far apart from the respective county-wide averages of 9, 6, and 14 percent for the same three consecutive years (SBCEHS, "Beach Data Comparison: Percent Exceedances for 1998-2005").

Because there are a variety of known or suspected inputs into the creek, attributing high FIB counts to either horseback riders or faulty septic system(s) has not been easily justified without sound scientific data. For this reason, in 1999, Professor Hugo Loaiciga, a licensed engineer, was contracted to conduct a study on the creek that continued into the year 2000. Using culture-dependent methods, he determined that horses were the likely source of FIB in the water (Loaiciga and Renehan 2000). Since then, many of the residents involved in the community have made environmental health a priority for Hope Ranch. Under this motivation, in spring 2005, four graduate students were recruited in an attempt to further characterize the baseline water quality at, and leading up to, the surf zone at Hope Ranch Beach from June 2005 to February 2006.

The focus of the project was refined to include quantifying FIB, as well as identifying potential sources, along the creek, and proposing a preliminary calculation of the risk associated with the observed FIB concentrations. Through interim meetings with the Hope Ranch Beach Committee and a final report, the project has also identified a need for community education on local water quality issues and conditions. The results presented below present a current understanding of known indicator and illness-causing contaminants, suggest their potential sources, and finally, provide a valuable set of information on which future water quality studies can be based. The results also provide a framework for making management recommendations for all who live by and use Las Palmas Creek and Hope Ranch Beach.

METHODS

Sampling Phases

For the purposes of this project, field collection began in June 2005 with the first weekly dry season sample and ended in February 2006 with the final set of storm samples. It was therein divided up into two distinct phases: summer and winter.

Weekly summer sampling began on June 22, 2005 and ended on September 6, 2005. Samples were taken on Mondays to coincide with data collected by Santa Barbara County's Ocean Water Monitoring Program. Additionally, one set of soil and sediment samples were collected per month during summer sampling.

Winter sampling occurred during and shortly after three distinct winter storms. Storms were selected for sampling and analysis if the following criteria were met: (1) the National Oceanic and Atmosphere Administration (NOAA) National Weather Service predicted more than 1 inch of precipitation throughout the duration of the storm, and (2) at least 0.25 inches of rain fell within the first 4 hours. During each storm event, three samples were taken at every site: one within 4 hours after rain began (referred to as the "rising limb" of the hydrograph, as illustrated in Figure 3), one 4 hours after the rain ended (or the next morning if rain ceases during the night, referred to as the "falling limb" of the hydrograph), and one 6 days after the end of the storm event. Specifically, water samples were taken on October 17, 18, and 24 of 2005; December 31, January 3, and January 9 of 2005 and 2006; and February 27 and 28 and March 5 of 2006. One additional set of dry-weather soil and sediment samples was collected during the winter period on January 30, 2006.

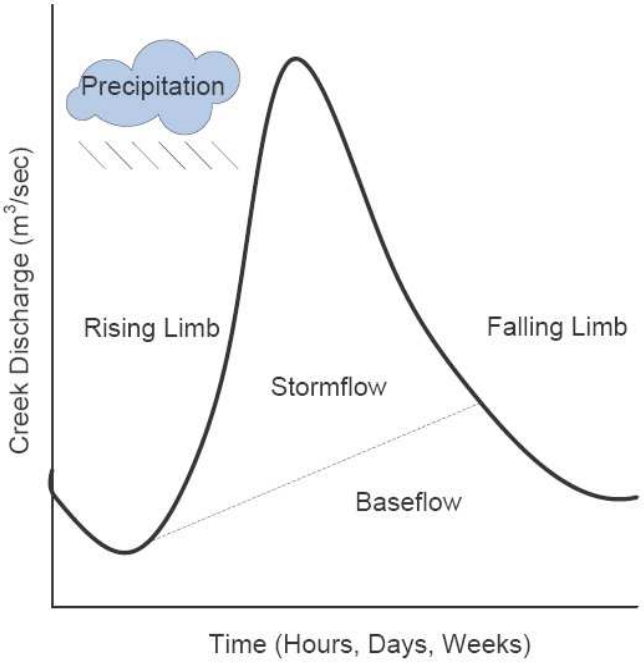


Figure 3: Hydrograph. The curving line is a graphical representation of how creek discharge changes as a storm progresses and ends. Creek discharge increases after the start of a storm, during a brief period of time known as the "rising limb." The rapid runoff that characterizes this period is called a "flush," which also is the colloquial name given to large storm events. As a storm weakens and ends, creek discharge decreases, known as the "falling limb." After the storm ends, surface flow, or "stormflow" runs off, leaving the creek to be recharged with groundwater, or "baseflow."

Sampling Locations

Six sites were sampled from the headwaters of Las Palmas to the ocean at Hope Ranch Beach: 5 along the creek and 1 at the ocean (Figure 4). Site 1 is located at the creek-surf mixing zone where the water is ankle-deep. The end of the concrete channel that discharges the creek water into the ocean was designated Site 2. Site 3 is located under the bridge on the Beach Trail, a horse trail that starts at the entrance to the Beach parking lot. Site 4 is located at the intersection of Via Bendita and Las Palmas Road on the Via Bendita side, just beyond where the creek flows through a pipe under the road. Site 5 is located near the intersection of Via Cayente and Las Palmas Road, just above where the runoff channel from Via Cayente enters the creek. Finally, Site 6 is near the intersection of Via Tranquila and Las Palmas Road, under the first bridge on the creek trail, at the end of the pipe that guides the creek under the bridge. Pictures of the sites are available in Appendix 1.

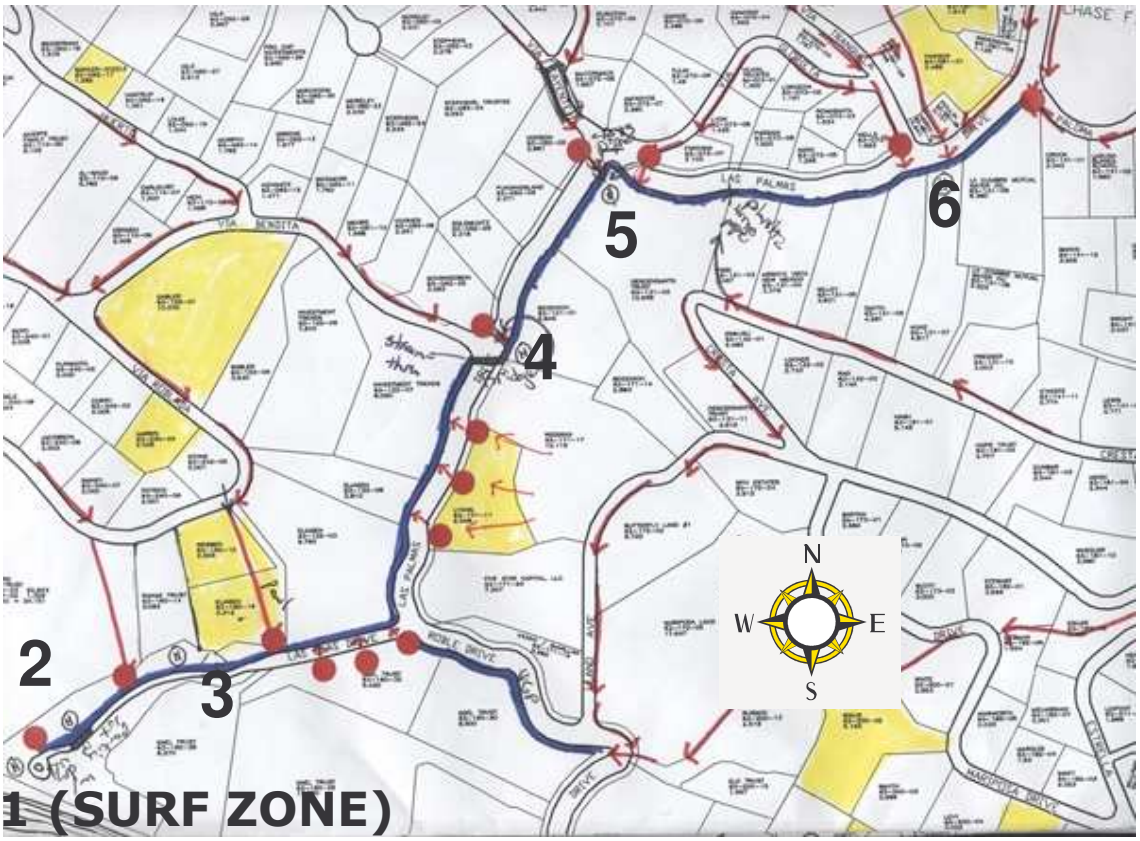


Figure 4: Sampling Map
The blue line represents Las Palmas Creek (approximately 1.5 miles in length), and the numbers correspond to the sampling sites used during the present study. The red arrows indicate the direction of surface runoff from the neighborhood during storm events, and the red dots represent the location of the pipes that drain into Las Palmas Creek during wet weather. Finally, the polygons represent housing parcels, and yellow parcels indicate properties that stable horses.

Background Conditions

At each of the sampling locations, the research team monitored the creek for temperature, pH, dissolved oxygen (DO), salinity, and discharge (Q). These background parameters were measured during every sampling event, unless otherwise noted.

Temperature and pH were measured with an Oakton pH/mV/°C Meter (pH 10 Series). A YSI Inc. Oxygen, Conductivity, Salinity, and Temperature Meter (Model 85/100 FT) meter was used to measure DO and salinity. The probes were placed 2 inches below the water surface 1-2 feet away from the sample collection site and read after allowed to stabilize for 2-5 minutes.

Flow was measured with a Flo-Mate Portable Flowmeter (Model 2000). When the flow of the creek at Site 2 was solely comprised of pipe discharge during the summer months, flow was recorded using the average fill time for a 5-gallon bucket for 10 fills. A section of the creek at Sites 3-5 was selected to measure flow during each sampling event. The ideal section was characterized by laminar, unidirectional flow with few obstructions upstream and downstream and a fairly smooth bottom to minimize turbulence. A vertical cross-section of depth and flow was measured at the center of 11 equal laminar increments perpendicular to the direction of flow. Flow was calculated as the summation of the flow in each increment ([increment width] x [depth] x [velocity]) for the entire cross-section.

Sample Collection

At each site, three liters of water were poured through Miracloth (Calbiochem) to remove large debris, and stored in sterile polypropylene containers at 4 degrees C until arrival at the laboratory. Samples were analyzed with IDEXX reagents within 6 hours of acquisition, and 250mL of each filtered water sample was archived in a sterile polypropylene bottle at -20 degrees C for possible future analyses. Figure 5 provides an outline of how each three-liter sample was utilized for laboratory analysis, the steps of which are explained in the rest of this section.

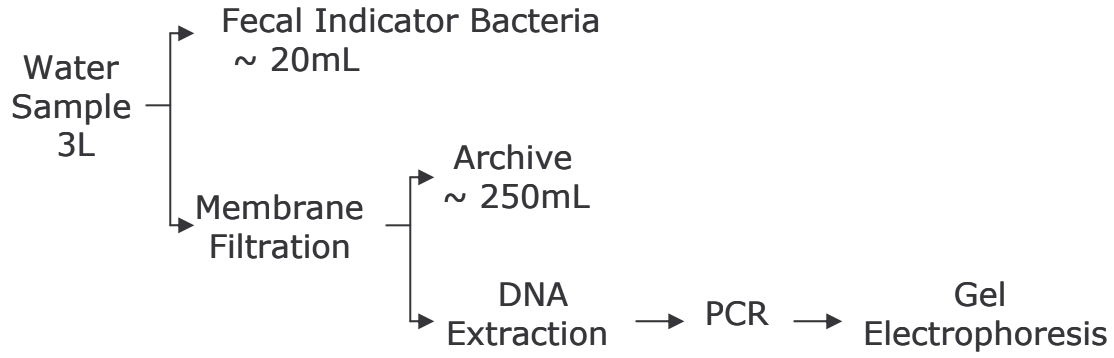


Figure 5: Water Sample Allocation and Analysis Scheme

In addition to water samples, four-ounce volumes of soil were collected at each site with sterile polypropylene scoops from multiple areas on the creek bank and homogenized to create a representative sample of the creek walls. Sediment was collected from the saturated stream bed in a similar manner, except that excess water was poured off before storing the sample. These samples were stored in sterile polycarbonate jars or sterile 4-ounce Whirl-Pak bags (Nasco) at 4 degrees C until arrival at the laboratory.

Fecal Indicator Bacteria Quantification

Within six hours of sample acquisition, fecal indicator bacteria (FIB) in water, soil, and sediment were quantified using Colilert and Enterolert reagents in the Quanti-Tray 2000 format (IDEXX Laboratories). During the summer season and during the post-storm periods in the winter, samples were diluted 1:10 in 100 mL, while during the winter rains samples were diluted 1:100 in 100mL prior to FIB analysis.

FIB were quantified in soil and sediment by first suspending 10 grams (wet weight) of the media in 40mL of water, and then shaking or vortexing vigorously for 2 minutes. This solution was then diluted 1:10 in 100mL and used as per IDEXX instructions for water with Enterolert and Colilert assays. No effort was made to remove suspended soil particles from the water. Water content was measured in soil and sediments by drying a known weight of the sample at 105ºC overnight, and recording the difference between the original and dried samples (modified from personal communication, Dr. Patricia Holden, UCSB). Most probable number (MPN) of organisms per gram was calculated by dividing the resulting FIB concentration by the dry soil weight of the soil sample per 10 mL.

Sources of Positive Controls: Human specific Bacteroides prevotella, Rhodococcus coprophilus, Horse specific Bacteroides, Giardia lamblia, and Cryptosporidium parvum

As human specific *Bacteroides prevotella* is expected to reside in human waste, a positive control was obtained from fresh confluent on August 8, 2005 from a public restroom at the Santa Barbara Botanical Gardens, which is representative of the heterogeneous waste one would expect to find in the septic systems located in the Hope Ranch setting. Material for a second, sewage-based positive control was taken on August 12, 2005 from the El Estero Wastewater Treatment Plant in Santa Barbara, CA. Through PCR analysis, described in detail below, it was confirmed that *Bacteroides prevotella* was present in both samples.

Rhodococcus coprophilus and horse specific *Bacteroides* samples were obtained from fresh horse manure, collected on August 17, 2005 from both a private Hope Ranch stable and from the trail running alongside the creek. Samples were obtained from multiple piles and then manually homogenized to create a representative manure sample. As with human specific *Bacteroides*, *R. coprophilus* was confirmed to be present in the sample through PCR analysis.

Infectious *Giardia lamblia* and *Cryptosporidium parvum* cyst isolates were purchased from Waterborne Inc. (New Orleans, LA), and provided as 10^6 cysts in 4ml of solution (PBS, antibiotics, and 0.01% Tween 20).

DNA Extraction and Quantification

Water samples were filtered through a 0.22um, 47mm-diameter nylon membrane to capture suspended microbes. DNA was extracted from these membranes using the UltraClean Water DNA Isolation Kit (Mo Bio Laboratories, Inc.), which combines bead beating and chemical lysing to burst cell membranes. Three freeze-thaw cycles (5 minutes at -80 degrees C, 5 minutes at 37 degrees C) were employed to encourage any remaining cyst walls to burst (personal communication, Dr. Robert Atwill, UC Davis). The DNA extraction followed the Mo Bio instructions without modifications.

An ethanol precipitation step followed the extraction process to concentrate DNA. The 3mL DNA elution product was mixed with 6ml chilled 100% ethanol, 200ul NaCl, and 4ul poly-acryl carrier. This solution was chilled at -20 degrees C for 20 minutes, followed by a 20-minute spin-down at 2500xG. The solution was decanted slowly to avoid disturbing the pellet, and then allowed to dry overnight in a desiccator. DNA was resuspended in 50ml of 0.1x elution buffer.

Soil, sediment, fecal material, and cyst isolate DNA were extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc.). This method employed bead-beating with chemical lysis to burst cells. The following amounts of each substance were added to the bead tubes: 0.25g soil, 0.5g sediment, 0.25 fecal material, and 200ul cyst stock solution. Samples were freeze-thawed 3 times (5 minutes at -80 degrees C, 5 minutes at 37 degrees C) to aid cyst wall lysis, if present (personal communication, Dr. Robert Atwill, UC Davis). The DNA extraction followed the Mo Bio instructions without modifications, except that 50mL of elution buffer was used to elute the DNA rather than 100mL to avoid a DNA concentration step.

DNA was quantified using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). The samples were assayed according to the company's protocol, using a 0-1000 ng/ul scale. A 0-25 pg/ul scale was employed for pure cyst extractions due to the small amounts of DNA expected from this extraction, as per Invitrogen's instructions.

PCR Analysis of Microbial DNA

PCR is a process that replicates small amounts of DNA exponentially. DNA extracted from the microorganisms in the environmental sample, known as "template," is mixed with a "master mix" containing reagents necessary for PCR: Taq polymerase, forward and reverse primers, dNTPs, magnesium chloride, and buffer. Taq polymerase is an enzyme that assembles DNA from polymers, or dNTPs, which are the basic building blocks of DNA: adenine (A), guanine (G), cytosine (C), and thymine (T). Primers are used in the reaction to locate sections of genes or segments of other nucleic acids for PCR amplification, and in microbial source-tracking, to specifically target fragments of DNA called "DNA markers," each of which is unique to a microbial species or group of organisms that must be present in order to amplify DNA through this process (Qiagen 2002).

Taq polymerase also relies on a temperature cycling process in order to amplify DNA; this process is accomplished using a device called a thermocycler. At about 95 degrees C, double-stranded DNA will separate into single strands, which is called the "denaturing" step. The

sample is then cooled to about 50-60 degrees C to allow the primers to attach to the single-stranded DNA, known as "annealing." The temperature is then typically raised to 72 degrees C to allow the Taq to reconstruct the double-stranded DNA fragment from the dNTPs, called "elongation." This process is then repeated 30-50 times, and can create hundreds of thousands of copies of the original DNA marker. The optimal magnesium chloride concentration and annealing temperature are the two most critical elements of a successful PCR protocol, and may require optimization when adapting a published protocol (Qiagen 2002).

Primers for polymerase chain reaction (PCR) were chosen based on the rationale presented in Appendix 2. Forward and reverse primers for human specific *B. prevotella*, *R. coprophilus*, horse specific *Bacteroides*, *Cryptosporidium* spp., and *G. lamblia* are listed in Table 1 below.

The human specific *B. prevotella* PCR mixture consisted of 10ng template, 10pmol of each primer, 200uM of each dNTP, 1.5mM $MgCl_2$, 640ng/uL BSA, 5ul 1x Qiagen PCR Buffer, and 1.25 units Qiagen Taq polymerase in a 50uL volume. Reactions were carried out in a PCR Sprint Thermocycler (ThermoHybaid, SPRT001 Issue 3) under the following conditions: 35 cycles of 94°C for 30 seconds, 53°C for 1 minute, and 72°C for 2 minutes, with a final 6 minute extension at 72°C (Bernhard and Field 2000a). PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide and compared to a 1kb ladder (Promega).

The *R. coprophilus* PCR mixture consisted of 7ng template, 5pmol of each primer, 150uM of each dNTP, 2.5mM $MgCl_2$, 100ng/uL BSA, 5uL 1x Qiagen PCR Buffer, and 2.5 units Qiagen Taq polymerase in a 50uL volume. Reactions were carried out in a PCR Sprint Thermocycler with the following amplification conditions: 40 cycles of 1 minute at 94°C, 1 minute at 65°C, 1 minute at 72°C followed by a final 8 minute extension at 72°C (Savill *et al.* 2001). PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide and compared to a 1kb ladder (Promega).

The horse specific *Bacteroides* PCR mixture consisted of 10ng template, 10pmol of each primer, 200uM of each dNTP, 1.5mM $MgCl_2$, 600ng/uL BSA, 5uL 1x Qiagen PCR Buffer, and 1.25 units Qiagen Taq polymerase in a 50uL volume. Reactions were carried out in a PCR Sprint Thermocycler under the following conditions: 30 cycles of 95°C for 1 minute, 53°C for 45 seconds, and 72°C for 1 minute (Dick *et al.* 2005). PCR products were electrophoresed on a 2% agarose gel stained with ethidium bromide and compared to a 50bp mini ladder (Fisher BioReagents).

The *Cryptosporidium* spp. PCR mixture consisted of 10ng template, 5pmol of each primer, 200uM of each dNTP, 2mM $MgCl_2$, 100ng/uL BSA, 5uL 1x Qiagen PCR Buffer, and 1 unit Qiagen Taq polymerase in a 50uL volume. Reactions were carried out in a PCR Sprint Thermocycler under the following conditions: a 10 minutes initial denaturing step at 95°C, 45 cycles of 94°C for 1 minute, 56°C for 90 seconds, and 72°C for 90 seconds, followed by a final 7 minute extension at 72°C (Champliaud *et al.* 1998). PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide and compared to a 1kb ladder (Promega).

The primer protocol for identifying *G. lamblia* was not consistently functional, and therefore was not used in the analyses.

Table 1: PCR Primers Used

Species	Forward (5'-3')	Reverse (5'-3')	Source
B. prevotella (Human)	ATCATGAGTTCACATGTCCG	CAATCGGAGTTCTTCGTG	Bernhard 2000
R. coprophilus	GGGTCTAATACCGGATATGACCAT	GCAGTTGAGCTGCGGGATTTCACA	Savill 2001
Bacteroides. (Horse)	CCAGCCGTAAAATAGTCGG	CAATCGGAGTTCTTCGTG	Dick 2005
Cryptosporidium spp.	CCGAGTTTGATCCAAAAAGTTACGAA	TAGCTCCTCATATGCCTTATTGAGTA	Laxer 1991
G. lamblia	AAGCCCGACGACCTCACCCGCAGTGC	GAGGCCGCCCTGGATCTTCGAGACGAC	Caccio 2002

For the purposes of understanding the temporal viability of these DNA markers, a literature review was conducted to determine the approximate longevity of the organisms in the aquatic environment, which represents the length of time a DNA marker will remain viable after submersion in water (see "Published Longevity" in Table 2). The viability of each DNA marker in the Hope Ranch environment was not analytically determined in this study.

In addition to documenting the published sensitivity of each PCR protocol, the group performed a basic sensitivity analysis for each primer to roughly determine the number of DNA markers required to return a positive signal, as reported under "Determined Sensitivity" in Table 2. Each analysis was duplicated with and without a freeze-thaw step during the extraction process to ensure that this step did not decrease the strength of the signal. For human specific *B. prevotella*, two concentrations of Total Coliforms in confluent from the Holden laboratory were averaged to estimate approximately how much confluent would be necessary to reach the AB 411 single sample standard in 2 liters of water from Las Palmas Creek. The water was then filtered and DNA extracted as per the methods for creek water documented above. The general sensitivities of *R. coprophilus* and horse specific *Bacteroides* were determined by suspending 0.5 and 0.15 grams of wet horse manure in 2 liters of Las Palmas creek water separately, and then filtering and extracting DNA from each. The sensitivity for the *Cryptosporidium* spp. protocol was determined by changing the amount of DNA used in the PCR reactions: 96pg/ul, 48pg/ul, and 19pg/ul of DNA extracted from pure *C. parvum* cysts.

Table 2: Longevity of DNA markers in aquatic environment and sensitivity of PCR assays. Sensitivity reported by published literature, as well as the sensitivity that was roughly determined by the current study.

	Published Longevity	Published Sensitivity	Determined Sensitivity	Citation
Human specific *B. prevotella*	8 days	$1.4*10^{-6}$ g dry feces/L	200 ul confluent	Seurinck *et al.* 2005 Bernhard *et al.* 2000
R. coprophilus	2 weeks	40 cells	0.15 g wet manure	Long *et al.* 2003 Savill *et al.* 2001
Horse specific *Bacteroides*	8 days	100 template copies	0.15 g wet manure	Seurinck *et al.* 2005 Dick *et al.* 2005
Cryptosproridium spp.	12 weeks @ 25°C	1 cyst	19 pg/ul	Carey *et al.* 2004 Rochelle *et al.* 1997

Gel Electrophoresis

All PCR products were visualized with gel electrophoresis to determine the presence or absence of a particular DNA marker. This marker is the segment of DNA that is amplified,

or replicated, hundreds of times during one PCR assay until it gains enough mass to retain an ethidium bromide stain and fluoresce under ultraviolet (UV) light. Each marker is unique to an organism of interest and signifies the presence of the organism in the original sample. During electrophoresis, the negatively charged DNA is exposed to a current from the negative anode, which allows the DNA to migrate towards the positive cathode. The DNA will migrate at different rates depending on the size of the DNA marker, where larger markers will travel shorter distances than markers of smaller mass within the same timeframe. The successful amplification and electrophoresis of a DNA marker is commonly referred to as a "hit." If the DNA marker was successfully PCR amplified, this hit is visualized when the gel is exposed to UV light by appearing as a bright band situated at the same lateral distance from the anode as the positive control (a sample known to contain the DNA marker of interest in the reaction). A negative control, also referred to as "a blank," is a sample containing no DNA that is used in PCR and electrophoresis to ensure that none of the reagents were contaminated with DNA markers and to also decrease the chance of misinterpreting false positives as hits.

Each gel electrophoresis run typically contained the PCR products from two sampling events on separate migration rows. The first well in each row was loaded with a DNA "ladder" for identifying the correct band size and a positive control in the second well for verifying the successful PCR amplification of the DNA marker of interest. A negative control is loaded into the final well as a quality control measure. A simplified diagram of gel electrophoresis is shown in Figure 6.

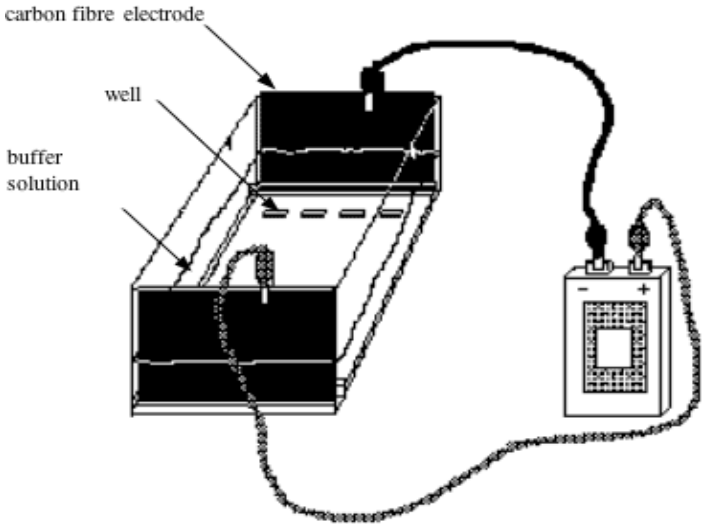


Figure 6: Typical Laboratory Set-Up for Gel Electrophoresis
Courtesy of http://www-saps.plantsci.cam.ac.uk/worksheets/scotland/dna.htm.

Soil Texture Analyses

On January 30, 2006, soil samples were collected for texture analyses. In total, twelve samples were collected from the six various sites to represent the soil horizons in the following manner: site 1 (1 sample), site 2 (1), site 3 (3), site 4 (3), site 5 (2), and site 6 (2). For soil sample collection sites, see Appendix 1.

A texture analysis was performed using a procedure adapted from the methods of the lab of Dr. Oliver Chadwick, UCSB Geography Department. After drying the soil for approximately 24 hours at 105°C, the soil was ground using a mortar and pestle, and passed through a #10 sieve (2mm). The material remaining on the sieve was discarded as the particles are too large to be considered part of soil. Next, approximately 40g of oven dried soil were then added to 1L bottles; the exact mass from each sample was recorded. To each sample bottle 100mL of the dispersant sodium hexametaphosphate (50g/L) was added and the bottles were shaken at 300rpm for ~20 minutes.

Following agitation, the individual soil/dispersant mixtures were poured through a #270 sieve (0.53um) into a 1L graduated cylinder with a cross-sectional area of 27.8cm^2. To ensure all particles <0.53um would pass through, a wet-sieve technique was employed (this process separates the sand from the silt and clay fraction). The soil remaining on the sieve (the sand fraction) was then transferred into a 105°C oven and allowed to dry overnight for sand analyses.

Silt/Clay Analysis

The graduated cylinders were then filled with deionized water until the volume of the liquid reached 1L. The cylinders were then capped using Parafilm and carefully shaken end over end for one minute. Next, after noting the time, the Parafilm was removed and an ASTM 152H hydrometer was suspended in each cylinder. Hydrometer measurements were then taken after 40sec, 3min, 10min, 30min, 60min, and 7hr, and at 10am the following morning.

To determine sedimentation rate based on particle size the following equations were employed:

$$D = K\sqrt{\frac{L}{t}}$$

where D = diameter of soil particles
K = 0.01286 [constant based on particle density (2.65) and temperature (25°C)]
L = distance (cm)
t = time (min)

where L is defined as

$$L = 16.29 - 0.164R$$

and R is the hydrometer reading at time t. Percent finer is then defined as: R/initial sample mass.

Sand Analysis

First, the mass of the oven-dried soil of the sand fraction remaining from the wet sieve was recorded. The samples were again ground using a mortar and pestle. Then, samples were passed through, in order, #35 (500um), #60 (250um), #80 (180um), and #120 (125um) sieves. Measurements of soil remaining of each sieve were carefully recorded, as well as the amount that passed through the #120 sieve. (The latter was used to ensure no mass was lost during the process.)

Percent finer is defined as:

#35 = 1 – (mass remaining on sieve / initial sample weight)
#60 = #35 – (mass remaining on sieve / initial sample weight)
#80 = #60 – (mass remaining on sieve / initial sample weight)
#120 = #80 – (mass remaining on sieve / initial sample weight)

Organic Matter

Samples from the twelve locations were dried at 105°C for ~24 hours in weighing tins to provide the oven-dried weight. The oven-dried weights were recorded and samples were replaced in the oven at 450°C for 24 hours. Weights were again recorded.

Percent organic matter is defined as:

(oven-dried weight – final weight) / oven-dried weight.

RESULTS

Water

Background Conditions

The summer field conditions – pH, temperature, and dissolved oxygen (DO) – assessed throughout Las Palmas Creek show a general increase in pH as well as DO as the sites progress downstream (Figures 7-9). Flow measurements also consistently increased with proximity to the ocean (Figure 10). This is most likely due to baseflow recharging the stream at lower elevations, since there were no visible signs of surface runoff during the summer sampling period. Flow did not demonstrate much variability during the first phase of the study. However, increases in running water under higher flow conditions may partially contribute to higher DO levels observed downstream when compared to sites located farther from the surf zone (US EPA 1997). Furthermore, the maximum DO at a given site varies with temperature (US EPA 1997).

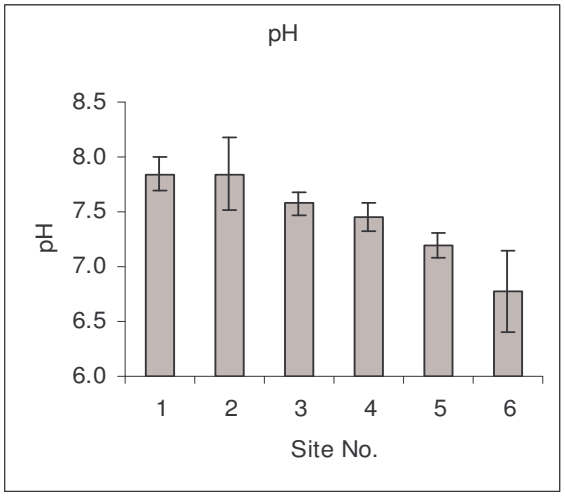


Figure 7: Background pH
Error bars represent standard deviation (n=12).

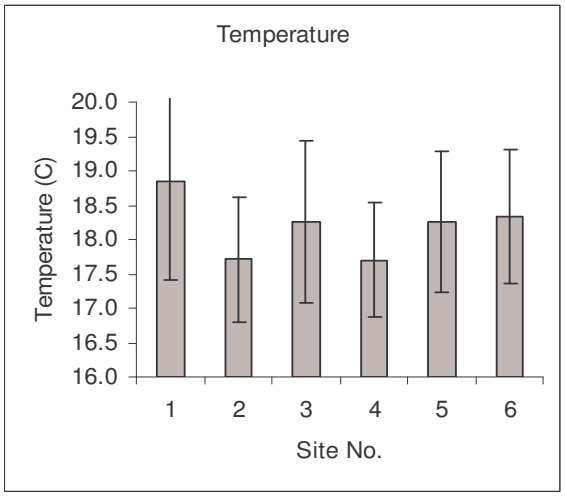


Figure 8: Background Water Temperature
Error bars represent standard deviation (n=12).

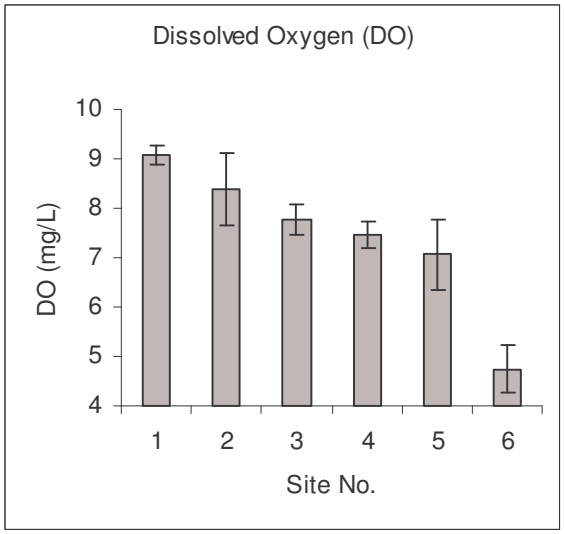


Figure 9: Background Dissolved Oxygen
Error bars represent standard deviation (n=12).

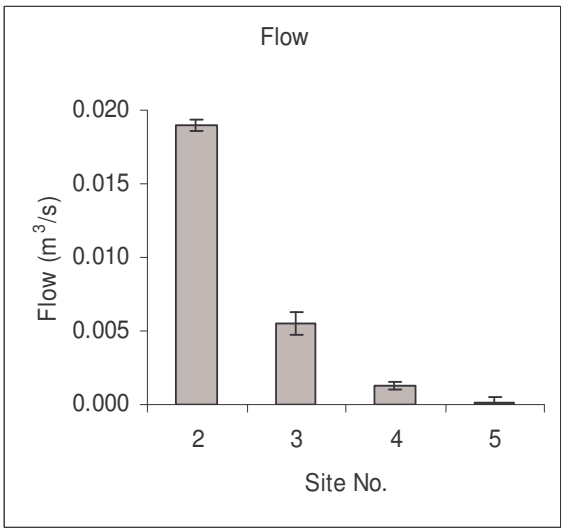


Figure 10: Background Flow
Error bars represent standard deviation (n=12).

Fecal Indicator Bacteria

Sample Quality Control

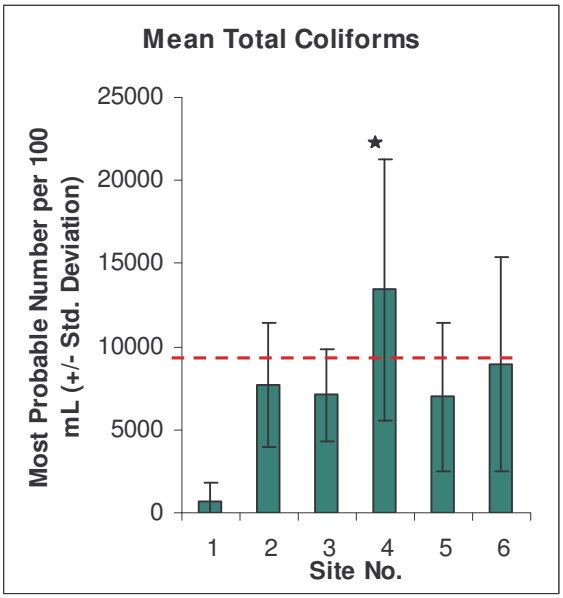
Surf-zone water samples taken 25 yards from where the creek enters the ocean by the County of Santa Barbara did not exceed AB 411 standards during the summer sampling period. However, the summer research group found that its samples from the surf zone site directly in front of the creek exceeded the standards on three separate occasions (see Appendix 4 for weekly exceedance data). Willie Brummett from the Santa Barbara County Department of Environmental Health Services independently analyzed one of the group's samples and received similar results. Specifically, Brummett quantified concentrations of 279, 41, and 161 for Total Coliform (TC), *E. coli*, and Enterococcus, respectively, while the group researchers found concentrations of 402, 97, and 199 for the same order of FIB counts. This general level of variability was anticipated due to natural variations between the individual laboratory settings (Noble *et al.* 2003a). The goal of this split, as achieved, was to demonstrate the development of credible sampling and analysis methods among members of the research team from the Bren school group, as well as preliminarily show that spatial proximity to the creek can affect water quality.

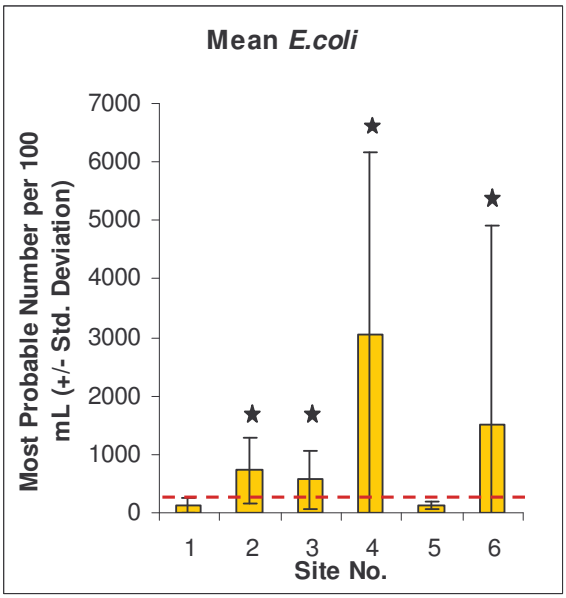
Summer Analysis

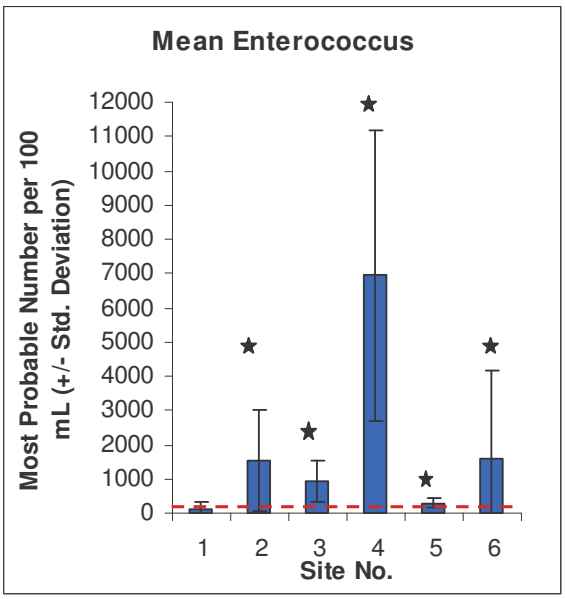
The raw data on FIB concentrations from the summer sampling phase are reported along with the AB 411 standards in Appendix 4.[1] The graphical representations of FIB concentrations in Figures 11-13 on the next page depict an average bacterial concentration for each of the 12 weeks and the associated standard deviation. To summarize, all sites had elevated levels of FIB, while Sites 4 and 6 appear to be the most contaminated areas. Site 4 was the only site with the average bacterial concentrations statistically at or above the AB 411 single sample ocean water quality standards for all three indicators. The ocean-creek mixing (surf) zone at Site 1 had the lowest concentrations of FIB, and though it exceeded the standards on three separate occasions, the FIB concentrations averaged over the summer did not statistically exceed the standards.

Site 5 exhibited frequently high levels of Enterococcus, but was otherwise in compliance with AB 411 standards; this site was the only creek site that did not exceed the *E. coli* or FC/TC ratio. On the other hand, in terms of percent exceedances, Site 4 exceeded the standards most frequently for all indicators (Figure 14).

[1] The FIB analyses for Sites 2-6 are based on the assumption that fresh water can be held to similar quality standards as ocean water.







Figures 11-13: Summer FIB Averages for Total Coliforms, *E. coli*, and Enterococcus with standard deviation bars. The dashed red line indicates the AB 411 marine water standard for each indicator. The stars indicate that the average is statistically at or above the AB 411 standard over the twelve weeks of sampling (Wilcoxon signed-rank, one-tailed, threshold p = 0.05, n=12). Error bars represent standard deviations.

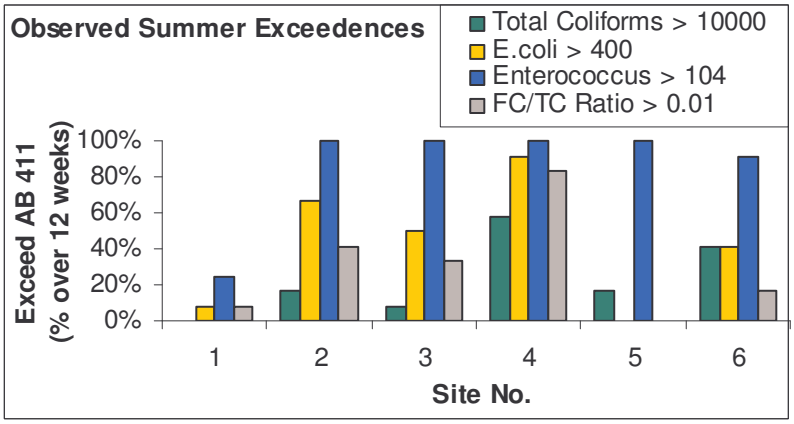


Figure 14: Percent of all summer sampling dates where Total Coliforms, *E. coli*, Enterococcus, and the Fecal Coliform to Total Coliform Ratio (FC/TC) exceeded the AB 411 standard.

Winter Analysis

Similar to analyses conducted with the summer samples, IDEXX reagents were used to quantify FIB concentrations for winter storms. As shown in Figure 15, the results of the FIB concentrations from the first flush indicate an increase of 3 orders of magnitude above the summer levels during the rising limb of the storm. Each block between the vertical dashed lines represents a day in order to convey the change in FIB concentrations with time. Total Coliform levels often approach 250,000/100mL in the graph, which represents the maximum level (241,960/100mL) IDEXX can quantify given the applied dilution. Actual bacterial concentrations for these specific dates and sites may have been at or above these measured concentrations. Omitting Total Coliform from the first flush data provides a clearer presentation of *E. coli* and Enterococcus levels, as shown in Figure 16. While Enterococcus levels show a decreasing trend from the rising limb to the falling limb, *E. coli* levels tend to remain the same or increase from the rising limb to the falling limb. A week after the storm, only Site 1 had all three indicators fall below their summer averages, while Sites 2 and 4 had Enterococcus concentrations that fell below their summer averages (Table 3).

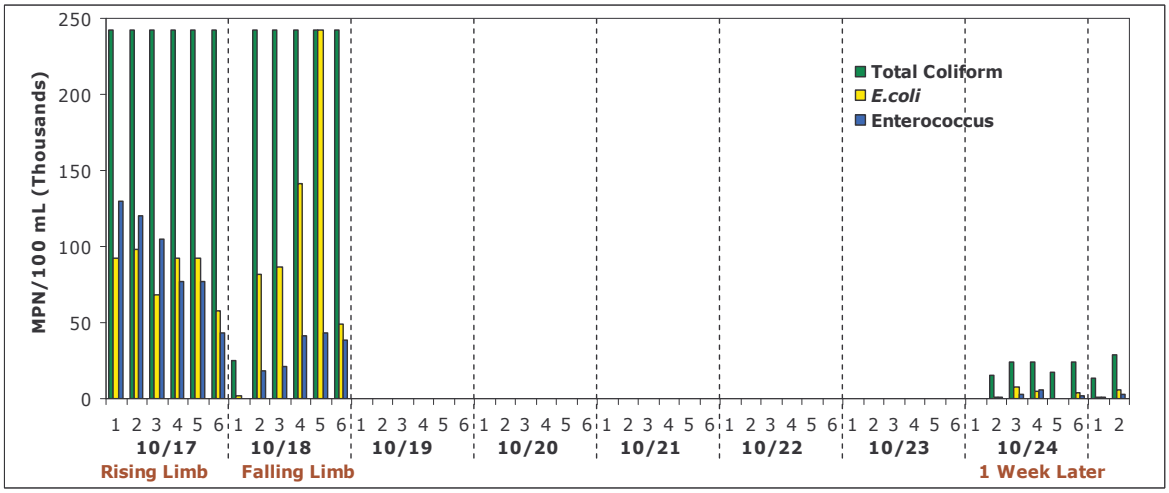


Figure 15: First Flush FIB Concentrations by Date

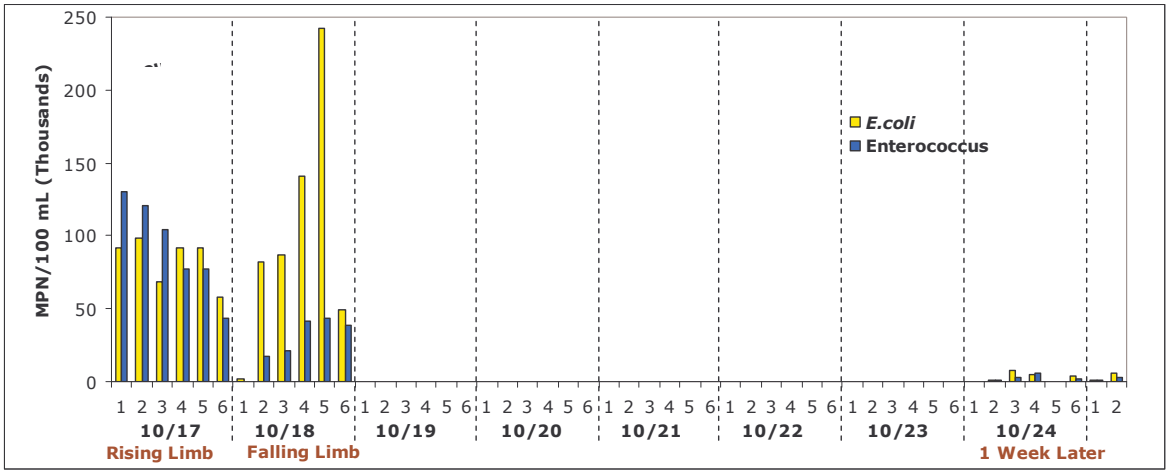


Figure 16: First Flush *E. coli* and Enterococcus Concentrations Only

Figure 17 presents the same data format as Figure 15, but for the numbers obtained in the second captured flush. Total Coliform levels for the rising limb were also too high to be quantified by IDEXX using a 1:100 dilution. However, *E. coli* and Enterococcus levels were about 10 to 20 times lower during this storm than they were at the first flush. Again, data for Total Coliforms were omitted from Figure 18 for a more clear illustration of *E. coli* and Enterococcus levels. As noted above, *E. coli* and Enterococcus rising limb levels were much lower than during the first flush, and the falling limb levels show an even more dramatic decrease compared to the falling limb of the first flush. This may be due in part to the storm, which lasted multiple days, and the falling limb sample was not taken until three days after the rising limb sample. Neither indicator shows similar trends as those seen during the first flush. Only Sites 2 and 4 had all three indicators return to summer averages a week after the storm, and all sites returned below the summer average for *E. coli* and Enterococcus (Table 3).

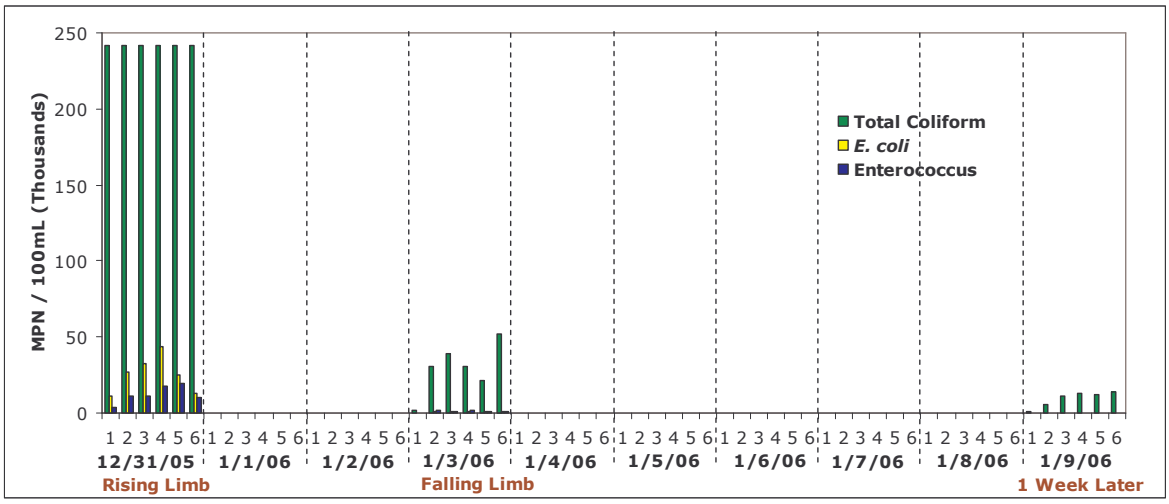


Figure 17: Second Flush FIB Concentrations by Date

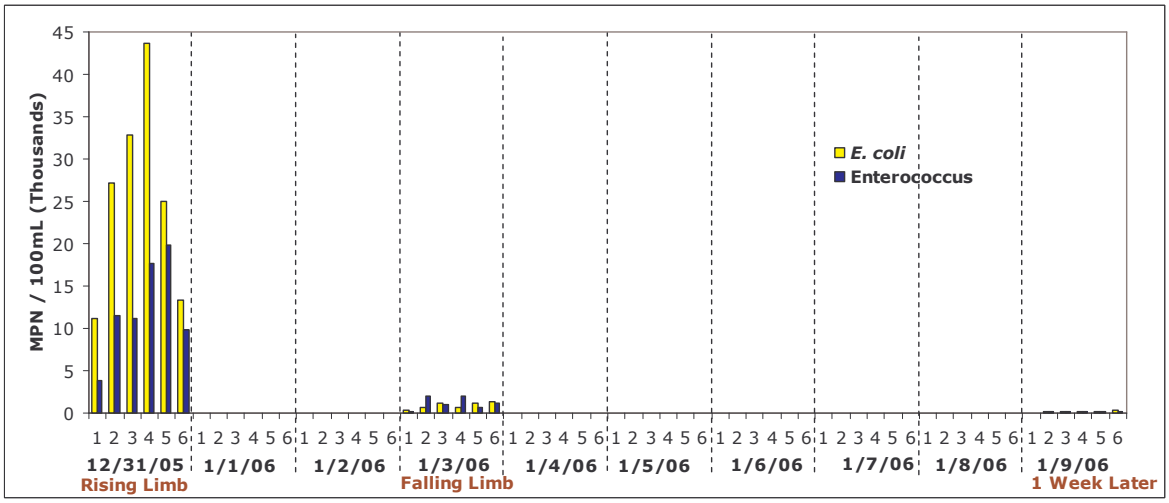


Figure 18: Second Flush *E. coli* and Enterococcus Concentrations Only

The third sampled storm event showed the same trend of elevated FIB during the storm and lower concentrations afterwards, shown in Figure 19 and 20. The Total Coliform concentrations of the sample taken within 4 hours of beginning of the storm were much lower than those of the start of the other two flushes, and the values actually increased during the falling limb. Enterococcus and *E. coli* demonstrated different trends than those seen in the first flush; during the third storm event Enterococcus levels stayed the same or increased during the falling limb, while *E. coli* levels decreased (Figure 20). None of the sites had all three indicators drop below the summer averages; however, all sites except Site 1 dropped below the summer mean concentrations of *E. coli* and Enterococcus (Table 3).

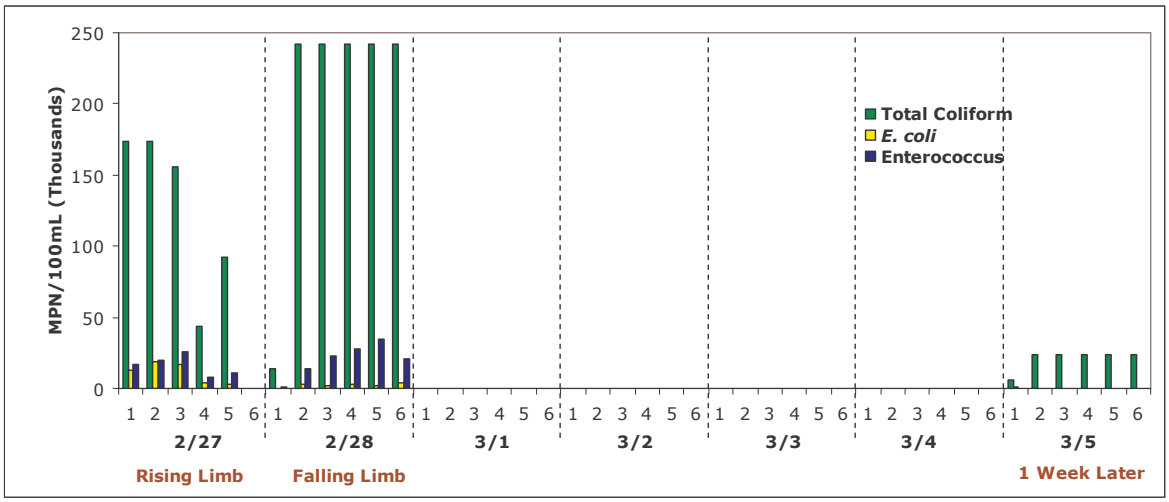


Figure 19: Third Flush FIB Concentrations by Date

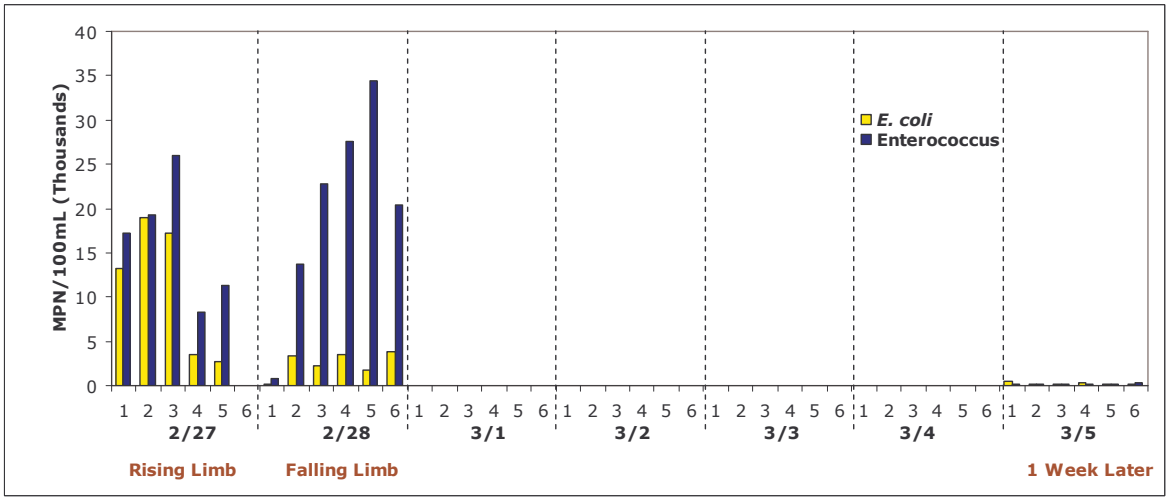


Figure 20: Third Flush *E. coli* and Enterococcus Concentrations Only

Flush	Site	TC	*E. coli*	Ent.
1st	1	*	*	*
	2			*
	3			
	4			*
	5			
	6			
2nd	1		*	*
	2	*	*	*
	3		*	*
	4	*	*	*
	5		*	*
	6		*	*
3rd	1			*
	2		*	*
	3		*	*
	4		*	*
	5		*	*
	6		*	*

Table 3: Post-Storm Samples Exhibiting FIB Levels Beneath Summer Means. An asterisk (*) indicates a sample taken 1 week following a storm that was lower than the respective summer mean concentration of that particular indicator bacterium.

Risk Calculations

Prior epidemiological studies have focused on two main questions: does contact with water increase the risk of adverse health effects, and if so, are the illnesses related to elevated FIB concentrations? While these organisms are not pathogenic, studies suggest increased concentrations of FIB are associated with elevated rates of gastrointestinal illness (Cabelli *et al.* 1979, Prüss 1998, Haile *et al.* 1999, and Wade *et al.* 2003). Potential symptoms associated with swimming in waters contaminated with FIB are fever, nausea, vomiting, diarrhea, stomach pain, earache, cough, runny nose, skin rash, and respiratory illnesses. Further investigations support the use of Enterococci species as the most appropriate indicator of water quality in marine environments, while in fresh water *E. coli* is a more consistent predictor of illness than other bacterial indicators (Prüss 1998, Wade *et al.* 2003). The data collected suggest that there measurable health effects exist associated with swimming in sewage polluted waters. Additionally, the rate of symptoms was higher among children, Hispanic Americans, and low-middle class socioeconomic groups.

Of further interest is the increased frequency of beach closures following storm events and the influence urban runoff has on these results. More than half of the beach water quality failures in Santa Monica Bay are associated with rainfall events, even though it typically rains less than 15 days per year (Schiff *et al.* 2003). Another study conducted in southern California found that 60% of the shoreline failed water quality standards after a storm, compared to only 6% during dry weather. The same study found that the failure of water to meet quality standards increased to more than 90% for shoreline areas adjacent to urban runoff outlets (Noble *et al.* 2003b). Haile *et al.* (1999) demonstrated illness rates more than double among swimmers at beaches near such outlets compared to swimmers at other beaches.

In order to more effectively extrapolate the project results to the concern of human health, a risk assessment was conducted in order to elucidate results from the FIB analysis using

previously published epidemiological studies. This risk assessment ultimately estimates the probability of contracting an illness after recreating in the water at the sampling sites, using published information on exposure to indicators and pathogens, as well as water quality data collected from June of 2005 until March of 2006.

Probabilistic Risk Assessment

The Cabelli research group obtained a linear relationship between swimming-associated human illness and the quality of ocean waters where swimmers recreated, with immersion of the head as the measure of exposure. Studies were conducted in New York from 1973 to 1975; Lake Pontchartrain, Louisiana from 1977 to 1978; and Boston, Massachusetts in 1978. The results indicated that for ocean water, Enterococcus concentrations showed highest correlation with gastrointestinal and highly credible gastrointestinal symptoms (Cabelli *et al.* 1979). After application of these regression equations to summer and rainy season FIB data for Hope Ranch Beach, the swimming-associated rates of gastrointestinal illness are presented in Tables 4 and 5, respectively.

Table 4: Swimming-Associated Rate of Gastrointestinal Symptoms / 1000 Swimmers

Site #	Summer Mean	First Flush			Second Flush			Third Flush		
		storm start	storm end	1 week after	storm start	storm end	1 week after	storm start	storm end	1 week after
1	46	119	0	34	106	43	0	97	66	45
2	72	118	98	69	117	75	47	99	95	50

Table 5: Swimming-Associated Rate of Highly Credible Gastrointestinal Symptoms/ 1000 Swimmers

Site #	Summer Mean	First Flush			Second Flush			Third Flush		
		storm start	storm end	1 week after	storm start	storm end	1 week after	storm start	storm end	1 week after
1	26	63	0	20	56	25	0	52	36	26
2	39	62	52	38	62	40	27	52	51	28

The significance of the probabilistic risk assessment is in its ability to put the health effects related to ocean and creek water recreation into terms of chance. According to the above regressions, while summer conditions suggest that 46 out of 1000 swimmers at the surf zone, and 72 out of 1000 at the creek mouth, will develop swimming-related gastrointestinal symptoms upon immersion in the water at the site, winter conditions can commit up to 119 out of 1000 ocean swimmers to gastrointestinal illness – over 10%. The probabilistic risks calculated one week after each storm event, however, are significantly reduced, and mirror the low risks calculated for the summer baseline water quality conditions.

Relative Risk Assessment

A supplement to the Cabelli study, Wade *et al.* (2003) conducted a systematic review on existing studies and quantified a relationship between gastrointestinal illnesses and two different microbial water quality indicators. According to this study, Enterococcus is a better marine water quality indicator, whereas *E. coli* is a more consistent indicator for the risks posed by fresh water quality issues. Out of 976 studies, Wade *et al.* included 27 based on similar types of water exposure, at least one microbial measurement for water quality, and at least one health outcome related to water quality. Regression analyses were performed by incorporating all 27 studies to determine the relationship between relative risk for people who

swim in the fecal polluted water to clean water and the concentration of the fecal indicators (Wade *et al.* 2003). The fecal indicator concentration data from this study were used in the regression equation developed by Wade *et al.* for determining relative risk based on Enterococcus (in marine water) and *E. coli* (in fresh water) as shown in Table 6 and 7, respectively.

Table 6: Relative Risk of Swimming in Fecal-Polluted Water versus Clean Water based on Enterococcus Concentrations. Relative risk for clean water = 1.

site	summer mean	First Flush			Second Flush			Third Flush		
		storm start	storm end	1 week after	storm start	storm end	1 week after	storm start	storm end	1 week after
1	2.07	5.12	0.00	1.79	4.36	2.01	0.00	3.93	2.66	2.06
2	2.87	5.07	3.95	2.76	5.04	2.97	2.12	3.99	3.82	2.18

Table 7: Relative Risk of Swimming in Fecal-Polluted Water versus Clean Water based on *E. coli* Concentrations. Relative risk for clean water = 1.

site	Summer mean	First Flush			Second Flush			Third Flush		
		storm start	storm end	1 week after	storm start	storm end	1 week after	storm start	storm end	1 week after
2	2.94	14.49	13.65	3.50	20.21	2.80	1.58	8.50	4.85	1.98
3	2.71	12.90	13.92	6.33	21.48	3.35	1.88	8.23	4.26	2.00
4	4.68	14.20	16.33	5.25	23.56	2.80	1.92	4.92	4.87	2.20
5	1.71	14.20	19.45	2.42	19.67	3.46	1.46	4.47	3.89	1.59
6	3.73	12.21	11.55	4.96	16.02	3.56	2.17		5.04	1.74

Enterococcus was used as an indicator of relative risk for recreation in marine water, which includes site 1 (ankle-deep in the ocean) and site 2 (where creek water empties into the ocean). Similarly, calculations that rely on *E. coli* concentrations, which serve primarily as a fresh water indicator of human health risk, were performed only on sites 2 through 6 as they progress up the creek (Wade *et al.* 2003).

Relative risks calculated from *E. coli* concentrations were typically similar to those from Enterococcus when compared across the duration of any storm event. However, the relative risks were notably higher for recreation in the creek during the first flush, even at the storm's end at sites 3, 4, and 5, and remained higher than those for the summer mean *E. coli* levels even one week afterwards.

In contrast, week-later data from both the second and third flushes indicates a relative risk beneath that which corresponds to the AB 411 standard, which, under the same model, is calculated as 2.41 (Wade *et al.* 2003). The same trend is true for the ocean sites – in particular, the surf zone, where recreation is most common. Upon application of the Enterococcus model to the surf zone data, the relative risks at the beginning of each storm clearly decrease from first to third flush.

From a risk assessment standpoint, sample sites in exceedance of AB 411 FIB standards, in both summer and winter, suggest that the potential for illness when in contact with ocean in creek water is present and in some cases, quite large. For example, immersion in freshwater at site 4 presents a relative risk of intestinal illness at the start of the first flush as high as nearly 25 times that of a swimmer in pristine water, based on the regression of risk on *E. coli*

concentrations (Wade et al 2003). In a more realistic scenario, immersion at the surf zone one week after a storm event still presents roughly double the risk of illness from swimming in clean water, based on the observed level of Enterococci.

Santa Barbara County Creek Comparison

In order to gauge the cleanliness of Las Palmas Creek and Hope Ranch Beach in comparison to other Santa Barbara creeks and beaches, a statistical analysis was conducted on Project Clean Water (PCW) data collected during two distinct periods by the County of Santa Barbara. Creek data collected at Las Palmas Creek and other coastal Santa Barbara area creeks during the rising limbs of the 1999-2000 winter storms were used to compare water quality in Las Palmas Creek to other County creeks during flushes (Figure 21 and 22). Ocean data collected weekly by the County's Ocean Water Monitoring Program from June 2005-January 2006 was used to compare ocean water quality at Hope Ranch Beach to other County beaches (Figure 23).

When comparing winter-season mean water quality at the mouth of Las Palmas Creek to mean water quality at the mouths of all other creeks sampled by PCW, there is only a marginal statistical difference between the mean values of Total Coliforms and *E. coli* (Figure 21). This signifies that most of the creeks in the County have comparable water quality during storms. Including only creeks in Goleta, Santa Barbara, and Carpinteria, the creeks behave similarly, where only the mean Total Coliform values are marginally statistically different from each other (Figure 22). This indicates that Las Palmas Creek water quality resembles that of other Santa Barbara creeks during storm events – which in turn implies that rainy conditions may impair creek water in Hope Ranch to the extent of contamination which urban creeks are typically subject to in incorporated downtown Santa Barbara.

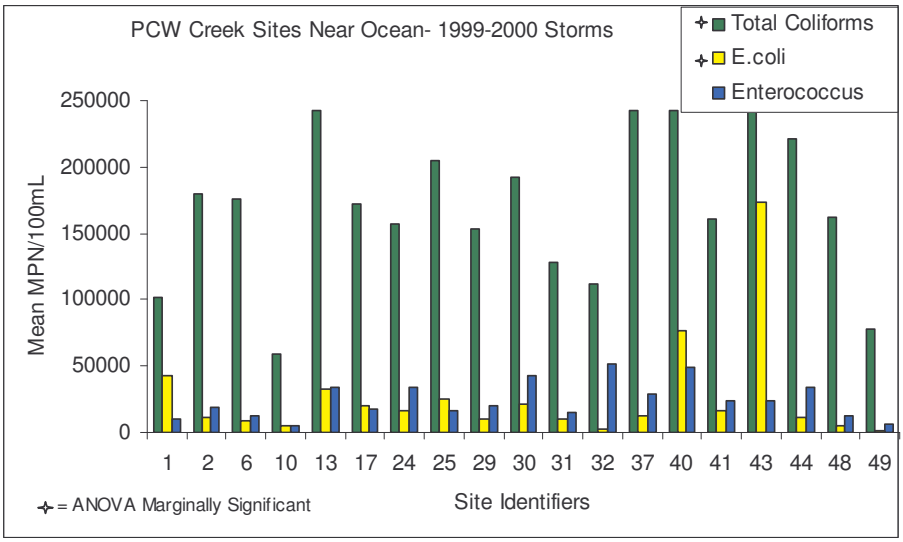


Figure 21: Mean Project Clean Water (PCW) Data from the 1999-2000 Storm Season. One-way ANOVA analysis to determine that the means of Total Coliforms and *E. coli* over all the sites are marginally statistically significant (one-way ANOVA, p=<0.05). (Site Identifiers are clarified with creek names in Table 8.)

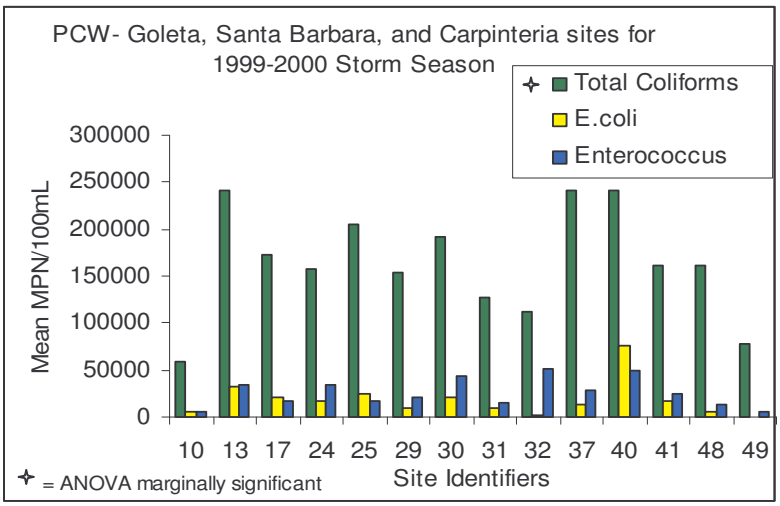


Figure 22: Mean PCW Data for Goleta, Santa Barbara, and Carpinteria Creek Mouths from the 1999-2000 Storm Season. One-way ANOVA analysis to indicate that the means for these sites are only marginally different for Total Coliforms (one-way ANOVA, p=<0.05).

Site Identifier	Creek Name
1	Tecolote Canyon
2	Bell Canyon
3	Devereux
6	Devereux
10	Tecolotito
13	San Pedro
17	Atascadero
24	Las Palmas
25	Arroyo Burro
29	Montecito
30	Oak
31	San Ysidro
32	Romero
37	Toro Canyon
40	Garrapata
41	Arroyo Paredon
43	Franklin
44	Franklin
48	Carpinteria
49	Rincon

Table 8: Creeks Associated with Site Identifiers for PCW Data

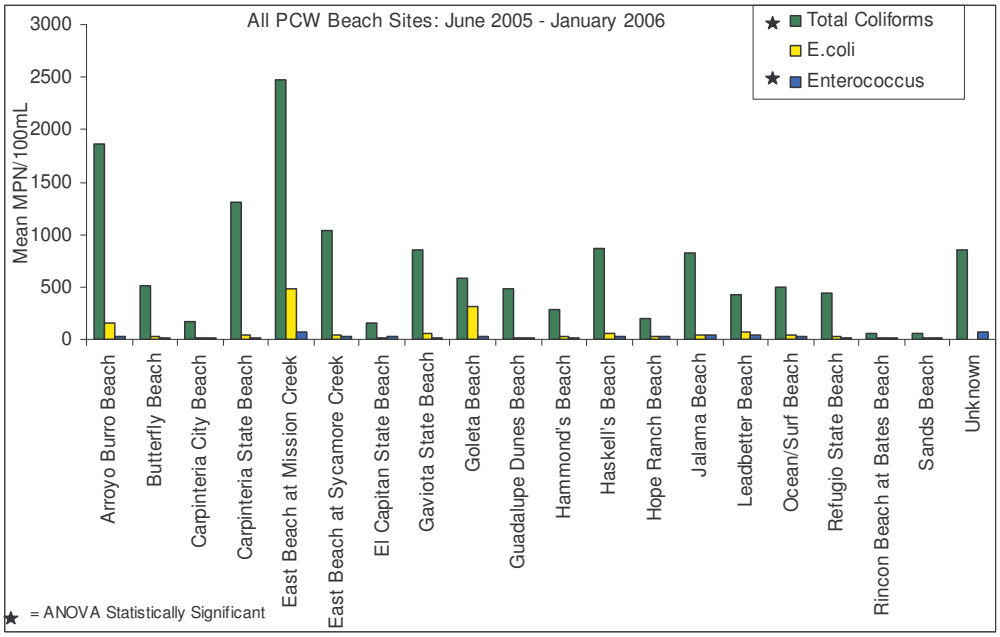


Figure 23: Mean PCW Weekly Beach Data from June 2005 – January 2006 for the South Coast. Mean Total Coliforms and Enterococcus values are significantly different over all sites.

Mean ocean water quality at all County beaches from June 2005-January 2006 is statistically different from that at Hope Ranch as well as other sites in terms of Total Coliform and Enterococcus concentrations (Figure 23). The same statistical difference appears when comparing mean ocean water quality at Goleta beaches with those in Santa Barbara and those in Carpinteria (Figure 24).

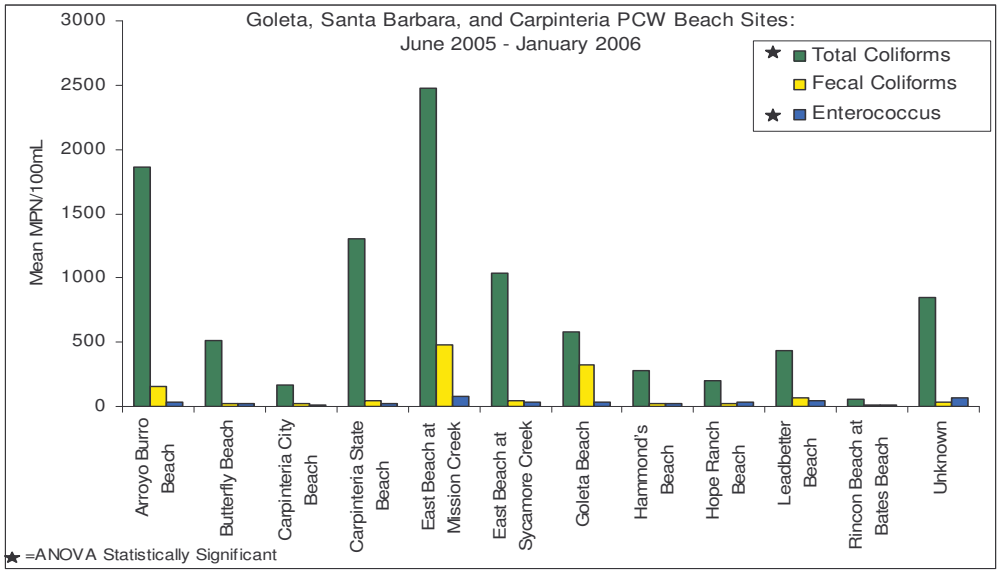


Figure 24: Mean PCW Weekly Beach Data for Goleta, Santa Barbara, and Carpinteria Beaches. One-way ANOVA analysis indicates that mean Total Coliform and Enterococcus values are significantly different.

Hope Ranch Beach was also compared with the beaches in Montecito, and water quality at Hope Ranch was found to not differ from that at such sites in a statistically significant way for levels of any FIB other than Total Coliforms (Figure 25).

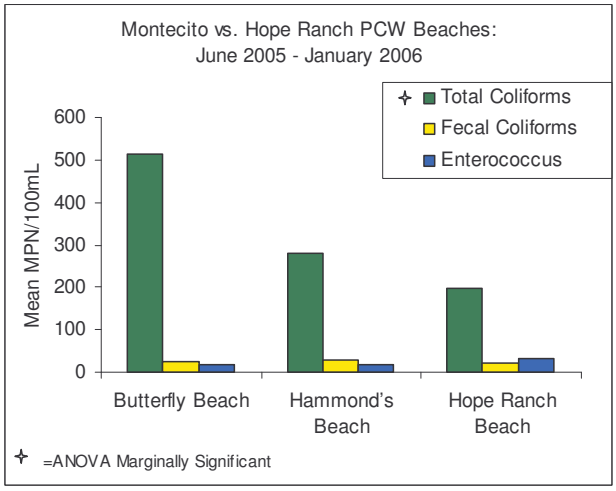


Figure 25: Mean PCW Weekly Data for Beaches in Montecito and Hope Ranch. One-way ANOVA analysis indicates that mean Total Coliforms are only marginally different between the three sites.

Overall, as Hope Ranch Beach has lower baseline (weekly) FIB concentrations compared to the other beaches in the analysis, it can be inferred that Hope Ranch Beach has better than average water quality among other beaches in the area during the dry-weather season.

Source-Tracking/PCR: Presence-Absence

Protocols for tracking the presence of human specific *B. prevotella*, *R. coprophilus*, horse specific *Bacteroides*, *Cryptosporidium* spp., and *G. lamblia* using PCR were developed at the project's initiation. Because trials for the *Giardia* protocol were never successful, the test for this

organism was eliminated from the scope of this investigation and replaced with one for a horse-specific marker. Ultimately, presence-absence results were compiled for human specific *B. prevotella*, *R. coprophilus*, horse specific *Bacteroides*, and *Cryptosporidium*.

These results were inferred from gel electrophoresis analysis. Typical images of gel electrophoresis contain two rows of PCR products, an example of which is shown in Figure 26. For first-flush data in particular, each row contains a ladder, a positive control for *R. coprophilus*, and amplified DNA from samples taken either September 9, 2005 (top row) or October 17, 2005 (bottom row). Sample sites range from 1 (left) to 6 (right) starting on the third column on the left. The last sample on the top row is a negative control for verifying that the run was performed without contamination, given that no positive result is visible at this well. Imaging results are based on the use of Kodak imaging software to locate the substantial fluorescence of a band that is spatially aligned with the positive control, defining a "presence" result. A positive signal is verified through two indicators: a band visually locates at the same position as the positive control and the presence of a peak in band intensity at the same location determined by the digital imagery software. Therefore, in Figure 26, sites 3, 4, and 5 on the top row and Site 1, 2, and 4 on the bottom row express positive signals and imply the presence of *R. coprophilus* in water collected at these sites.

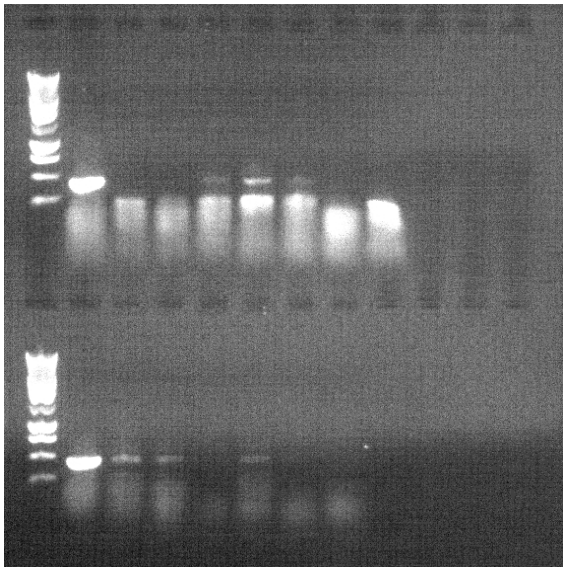


Figure 26: Gel Electrophoresis Image for *R. coprophilus* PCR Products, 9/6/05 and 10/17/05

Tables 9 through 12 on the next two pages present the presence-absence results of PCR- and gel electrophoresis-based tests on water samples preformed for human specific *B. prevotella*, *R. coprophilus*, horse specific *Bacteroides*, and *Cryptosporidium* spp., respectively, throughout the summer and the winter phases of the project. The complete array of gel images is provided in Appendix 8.

Markers specific to neither human waste nor horse waste were detected in the water during either season (Tables 9, 11). However, indication of an herbivore source of contamination was noted, given that *R. coprophilus* was first detected in water samples taken at the end of June and beginning of July 2005 (Table 10). It was not detected again until August of the same year. The most frequent signaling of herbivore contamination during the summer occurred at

the end of August and beginning of September, and at Sites 2 and 6. *R. coprophilus* was not detected in the ocean (Site 1) during the summer.

A *Cryptosporidium* species was potentially detected at Site 2 on October 18, 2005, but since the band did not line up with the positive control, it is unclear whether an unspecific *Cryptosporidium* species was detected or the result was a false positive. *Cryptosporidium* was not detected at Site 2 at any other sampling event (Table 12).

During the winter, the marker for herbivore fecal material was detected most frequently in water collected during the first flush. Notably, herbivore waste appeared to be present in the ocean throughout the duration of the storm and even one week later. Also, herbivore waste was present at all sites during the falling limb after the storm. Herbivore waste was detected most frequently at Sites 1 and 2 during the winter. No herbivore waste was detected at the ocean during the last flush.

Date	Site					
	1	2	3	4	5	6
06/27/05	-	-	-	-	-	-
07/05/05	-	-	-	-	-	-
07/11/05	-	-	-	-	-	-
07/18/05	-	-	-	-	-	-
07/25/05	-	-	-	-	-	-
08/01/05	-	-	-	-	-	-
08/09/05	-	-	-	-	-	-
08/15/05	-	-	-	-	-	-
08/22/05	-	-	-	-	-	-
08/30/05	-	-	-	-	-	-
09/06/05	-	-	-	-	-	-
10/17/05	-	-	-	-	-	-
10/18/05	-	-	-	-	-	-
10/24/05	-	-	-	-	-	-
12/31/05	-	-	-	-	-	-
01/03/06	-	-	-	-	-	-
01/09/06	-	-	-	-	-	-
02/27/06	-	-	-	-	-	-
02/28/06	-	-	-	-	-	-
03/05/06	-	-	-	-	-	-

Table 9: Presence-Absence of Human specific *Bacteroides* in Water
- Negative result: no Human *Bacteroides* detected in gel electrophoresis analysis.

Date	Site					
	1	2	3	4	5	6
06/27/05	-	-	-	+	+	+
07/05/05	-	+	-	-	-	-
07/11/05	-	-	-	-	-	-
07/18/05	-	-	-	-	-	-
07/25/05	-	-	-	-	-	-
08/01/05	-	-	-	-	-	-
08/09/05	-	+	-	-	-	-
08/15/05	-	-	-	-	-	+
08/22/05	-	+	+	-	-	+
08/30/05	-	+	+	-	-	+
09/06/05	-	-	+	+	+	-
10/17/05	+	+	-	+	-	-
10/18/05	+	+	+	+	+	+
10/24/05	+	-	-	-	+	-
12/31/05	-	-	+	-		
01/03/06	+	+	-	-	-	-
01/09/06	-	-	-	-	-	-
02/27/06	-	+	-	-	-	-
02/28/06	-	-	-	-	+	+
03/05/06	-	-	-	-	-	-

Table 10: Presence-Absence of *R. coprophilus* in Water + Positive hit: *R. coprophilus* detected.
- Negative result: no *R. coprophilus* detected in analysis.

Date	Site					
	1	2	3	4	5	6
06/27/05	N/A	N/A	N/A	-	-	-
07/05/05	N/A	N/A	N/A	N/A	N/A	N/A
07/11/05	N/A	N/A	N/A	N/A	N/A	N/A
07/18/05	N/A	N/A	N/A	N/A	N/A	N/A
07/25/05	N/A	N/A	N/A	N/A	N/A	N/A
08/01/05	N/A	N/A	N/A	N/A	N/A	N/A
08/09/05	N/A	-	N/A	N/A	N/A	N/A
08/15/05	N/A	N/A	N/A	N/A	N/A	-
08/22/05	N/A	-	-	N/A	N/A	-
08/30/05	N/A	-	-	-	N/A	-
09/06/05	N/A	N/A	-	-	-	N/A
10/17/05	-	-	N/A	-	N/A	N/A
10/18/05	-	-	-	-	-	-
10/24/05	-	N/A	N/A	N/A	-	N/A
12/31/05	N/A	N/A	-	N/A	N/A	N/A
01/03/06	-	-	N/A	N/A	N/A	N/A
01/09/06	N/A	N/A	N/A	N/A	N/A	N/A
02/27/06	N/A	-	N/A	N/A	N/A	N/A
02/28/06	N/A	N/A	N/A	N/A	N/A	-
03/05/06	N/A	N/A	N/A	N/A	N/A	N/A

Table 11: Presence-Absence of Horse *Bacteroides* in Water
N/A: samples that were not tested for this organism of interest.
* A positive hit was detected, but not for the DNA segment of interest

Date	Site 2
06/27/05	-
07/05/05	-
07/11/05	-
07/18/05	-
07/25/05	-
08/01/05	-
08/09/05	-
08/15/05	-
08/22/05	-
08/30/05	-
09/06/05	-
10/17/05	-
10/18/05	+
10/24/05	-
12/31/05	-
01/03/06	-
01/09/06	-
02/27/06	-
02/28/06	-
03/05/06	-

Table 12: *Cryptosporidium* spp. in Water
+ Positive hit: organism in genus *Cryptosporidium* detected (species unknown).

Soil and Sediment

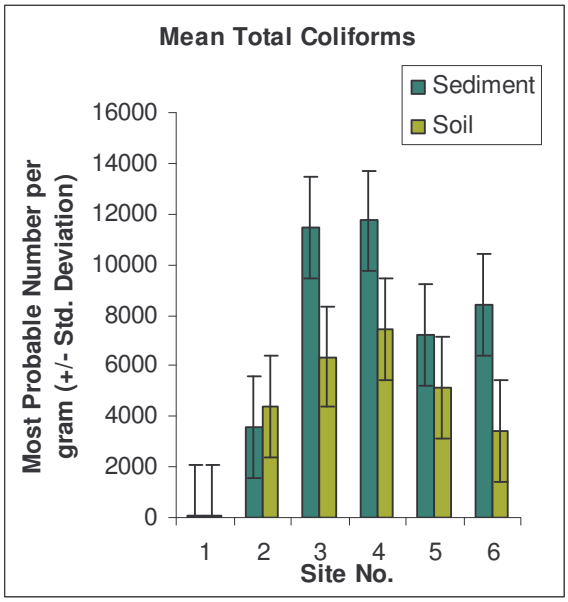
Fecal Indicator Bacteria

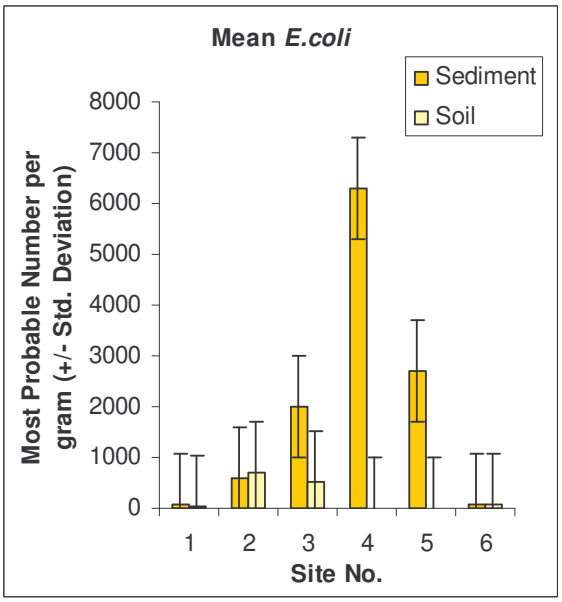
FIB concentrations in the soil and sediments at each site were assayed in order to provide information as to whether or not the elevated FIB found in the creek might be in part due to microbes residing in the soil of the stream bed and banks. Generally, the amount of FIB organisms found in the soil appears to be significant and is above what one would expect to find in uncontaminated soil. However, no statistical analyses were conducted to see if this is the case, as an "uncontaminated control site" was not analyzed in parallel with the experimental sites. Raw FIB data for soil and sediment is presented in Appendix 6.

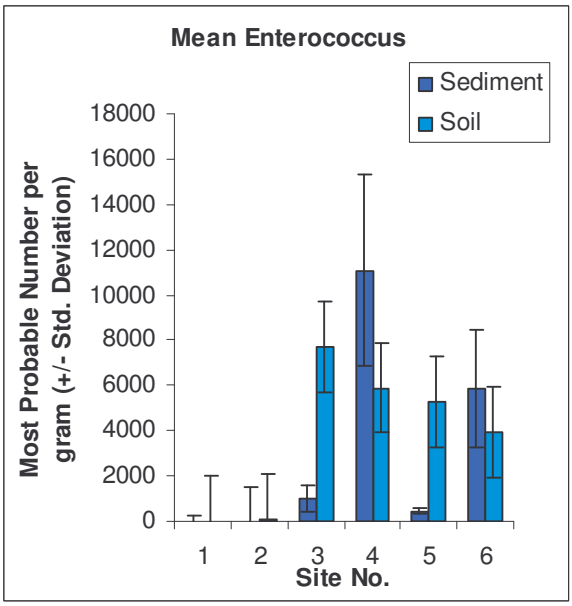
Summer Analysis

IDEXX results for Total Coliforms, *E. coli*, and Enterococcus in the summer samples are presented in Figures 27-29, using the same format as already seen in the FIB results for water. When comparing standard deviation bars, sediment appears to generally harbor more FIB than soil, with the exception of Entertococcus data from Sites 3 and 5. Site 4 tended to have the highest concentration of FIB in sediment for all three indicator organisms during the summer. Site 1 tended to have the lowest FIB concentrations in both soil and sediment

during the summer, with raw data from Site 2 also occasionally appearing to have below-normal levels of FIB compared to the rest of the creek. The quantification of FIB in soil and sediment follows roughly the same trend as that in water, where the creek sites have FIB concentrations higher than those exhibited by the surf zone samples.







Figures 27-29: Summer Mean Concentrations of Total Coliforms, *E. coli*, and Enterococcus in Sediment and Soil Samples, with Standard Deviation Bars (n=3)

Across time in the summer, the FIB concentrations also appear to increase from July to August, especially in the soil, and then decrease from August to September, as illustrated by the bar graphs in Figures 30 and 31. The validity of the latter trend is questionable, and may be due to an over-dilution of the samples recorded during analysis. A 1:1000 dilution was used instead of the 1:100 dilution used with the prior samples, which could have generated samples containing too few organisms to effectively conduct the Colilert and Enterolert assays – essentially diluting samples down to below their lower limits of detection.

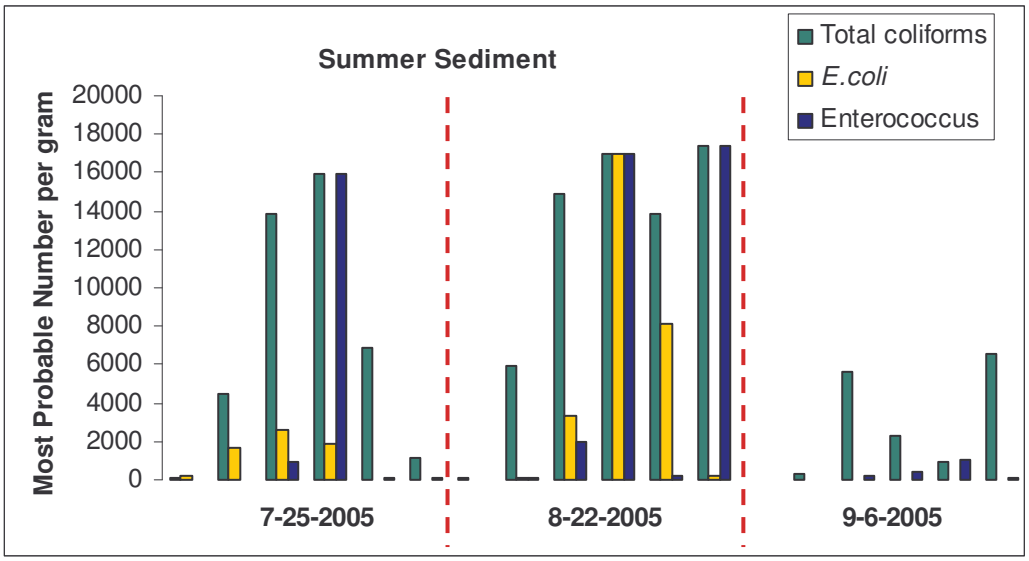


Figure 30: Levels of Total Coliforms, *E. coli*, and Enterococcus Found in Summer Sediment Samples

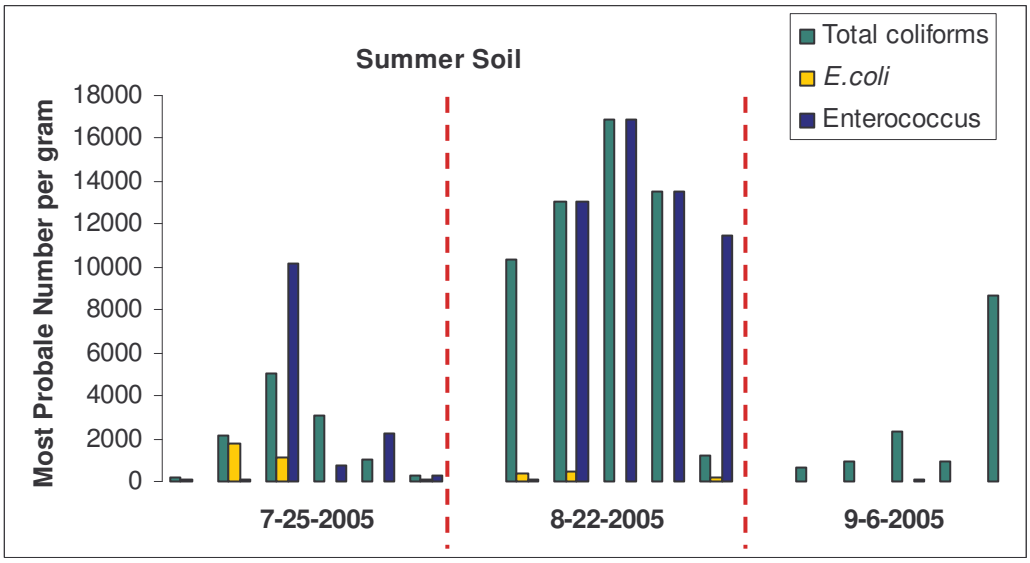
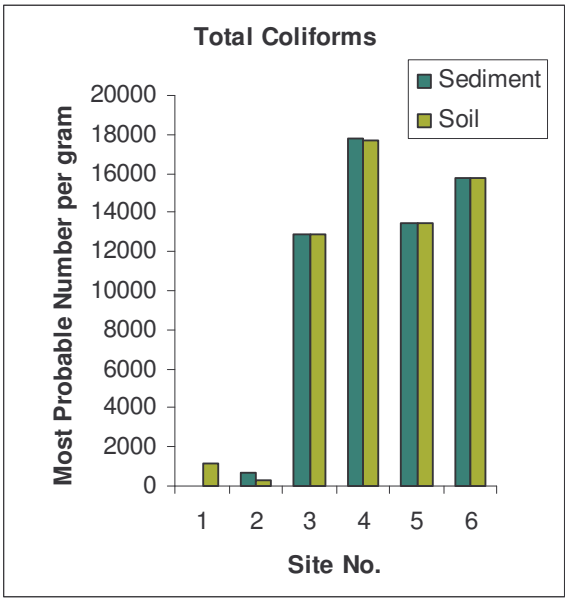
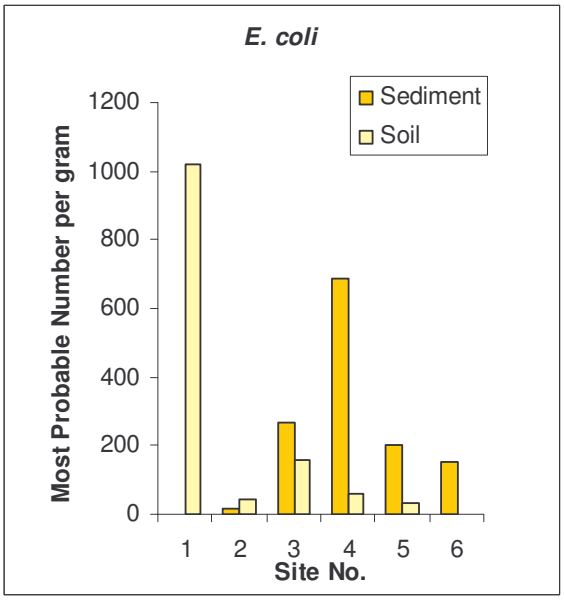


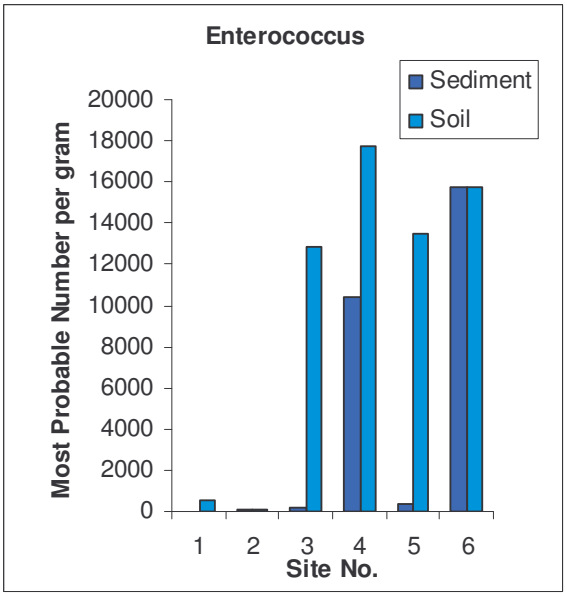
Figure 31: Levels of Total Coliforms, *E. coli*, and Enterococcus Found in Summer Soil Samples

Winter Analysis

As seen in the summer, sediment samples from Site 4 again yield the highest values in each of the indicator bacteria assays in the winter dry period, as shown in Figures 32-34. Winter dry period FIB concentrations are on the same order of magnitude as the July and August concentrations seen in Figures 30 and 31. With the exception of *E. coli* in the "soil" (i.e., sand) collected at Site 1 (the surf zone), Sites 1 and 2 exhibit the lowest levels of indicator bacteria in soil and sediment collected during the rainy season. The creek sites again tend to have higher concentrations of FIB than the surf zone, as seen in the results for water in the winter, with the exception of *E. coli* levels at Site 1.







Figures 32-34: Winter Summary of Total Coliform, *E.coli*, and Enterococcus Concentrations in Sediment and Soil Samples (n=1)

Source-Tracking/PCR: Presence-Absence

As with water samples, the presence or absence of source-tracking organisms in soil and sediment samples was determined using PCR to amplify a DNA marker, and gel electrophoresis to visualize the products. The results were compiled for human specific *Bacteroides prevotella*, *Rhodococcus coprophilus*, horse specific *Bacteroides* sp., and *Cryptosporidium* spp., and are summarized in Tables 13-16. As with water, soil and sediment samples revealed only the presence of *R. coprophilus*, the marker for herbivore waste. *R. coprophilus* appeared both at the end of the summer and in mid-winter, suggesting that soil and sediment could be acting as a reservoir for microbes in herbivore fecal material.

Soil	1	2	3	4	5	6
07/18/05	-	-	-	-	-	-
08/15/05	-	-	-	-	-	-
09/06/05	-	-	-		-	
02/01/06	-	-	-	-	-	-
Sediment	**1**	**2**	**3**	**4**	**5**	**6**
07/25/05	-	-	-	-	-	-
08/22/05	-	-	-	-	-	-
09/06/05	-	-	-	-	-	-
02/01/06	-	-	-	-	-	-

Table 13: Presence-Absence of Human Specific *B. prevotella* in Soil and Sediment.
- Negative result: no human *Bacteroides* detected.
N/A: samples that were not tested for this organism of interest

Soil	1	2	3	4	5	6
07/18/05	-	-	-	-	-	-
08/15/05	-	-	+	-	+	+
09/06/05	-	-	-		-	
02/01/06	-	-	+	-	-	+
Sediment	**1**	**2**	**3**	**4**	**5**	**6**
07/25/05	-	-	-	-	-	-
08/22/05	-	-	-	-	+	-
09/06/05	-	-	-	+	-	-
02/01/06	-	-	+	-	-	-

Table 14: Presence-Absence of *R. coprophilus* in Soil and Sediment
+ Positive hit: *R. coprophilus* detected.
- Negative Result: no *R. coprophilus* detected.

Soil	1	2	3	4	5	6
07/18/05	N/A	N/A	N/A	N/A	N/A	N/A
08/15/05	N/A	N/A	-	N/A	-	-
09/06/05	N/A	N/A	N/A	N/A	N/A	N/A
02/01/06	N/A	N/A	-	N/A	N/A	-
Sediment	**1**	**2**	**3**	**4**	**5**	**6**
07/25/05	N/A	N/A	N/A	N/A	N/A	N/A
08/22/05	N/A	N/A	N/A	N/A	-	N/A
09/06/05	N/A	N/A	N/A	*	N/A	N/A
02/01/06	N/A	N/A	-	N/A	-	-

Table 15: Presence-Absence of Horse Specific *Bacteroides* in Soil and Sediment
N/A: samples that were not tested for this organism of interest.
- Negative result: no Horse *Bacteroides* detected.
* Positive hit detected, but not for DNA segment of interest

Soil	2
07/18/05	-
08/15/05	-
09/06/05	-
2/1/2006	-
Sediment	**2**
07/25/05	-
08/22/05	-
09/06/05	-
2/1/2006	-

Table 16: Presence-Absence of *Cryptosporidium* in Soil and Sediment
- Negative result: no *Cryptosporidium* spp. detected.

Relationship between Soil Composition and FIB

The complete analysis of 6 sites along the creek during the dry season suggests that sites 4 and 6 – whose high levels of FIB, as detected in this investigation, confirm the results of prior studies by Dr. Hugo Loaiciga (Loaiciga and Renehan 2000) – are also the most clay-rich locations. Based on the silt/clay and sand analyses, soil classification for each sample site were determined as shown in Table 17. Percents organic matter for the sampled soils were also calculated. Detailed data are presented in Appendix 9. Creek sites (3-6) tended to have higher organic content than the sites at or near the ocean (sites 1-2), with the tops of the creek banks containing the largest amounts of organic matter.

Clay and organic matter were selected for regression analysis based on their known capacity to bind microorganisms in soil, which could explain some of the spatial variation of water FIB concentrations at the sites. The relationship between soil texture and FIB concentrations

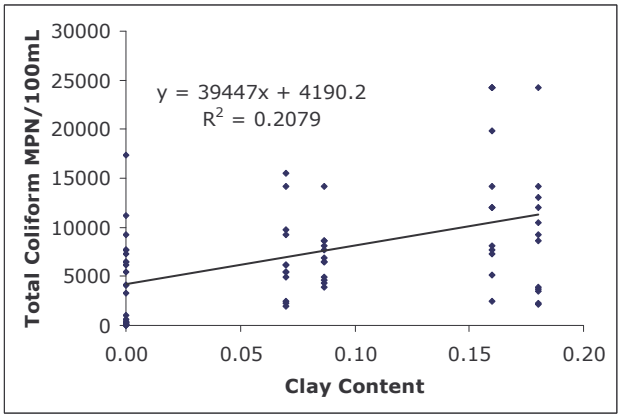
observed at the six sample sites was found to be statistically significant, based on a linear regression with replication.

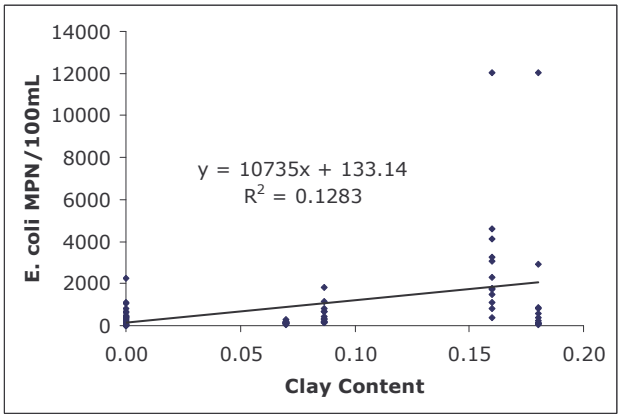
In order to use a linear regression analysis, multiple assumptions need to be met: (1) the data should be normally distributed, (2) each datum should be independent of the other data, and (3) the data should exhibit a linear relationship (Zar 1999). However, linear regression analyses are robust, and often this statistical analysis will interpret the data correctly even when one or more of these assumptions are violated. The data documented in Table 17 violate the assumption of normality and often the assumption of linearity. Extremely non-linear data sets were excluded from the analysis, or $p<0.01$. Since there are 12 weeks of FIB data for each soil texture value, replication is built into the analysis to satisfy the assumption of independence.

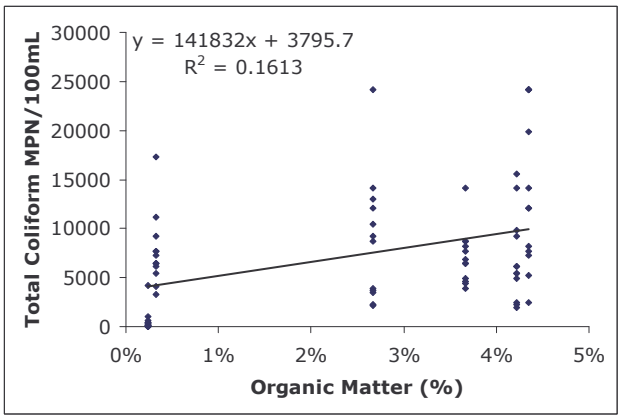
Table 17: Final Grain Size Distribution (percent by size) and Organic Matter ("OM," by percent).
For all samples, numbers indicate site numbers and letters indicate soil horizon according to the pictures in Appendix 9.

Sample	Sand	Silt	Clay	Classification	OM
1	100%	-	-	sand	0.24%
2	100%	-	-	sand	0.33%
3A	47%	38%	15%	loam	4.10%
3B	77%	19%	4%	loamy sand	2.94%
3C	62%	31%	7%	sandy loam	3.97%
4A	52%	35%	13%	sandy loam	6.67%
4B	20%	75%	5%	silt loam	2.74%
4C	30%	40%	30%	clay loam	3.63%
5A	57%	35%	8%	sandy loam	5.82%
5B	58%	36%	6%	sandy loam	2.63%
6A	31%	46%	23%	loam	3.40%
6B	55%	32%	13%	sandy loam	1.94%

Significant relationships were found to exist between clay content and Total Coliform and *E. coli* concentrations, as well as between organic matter and Total Coliforms, as detailed in Table 18. High concentrations of these indicator organisms were associated with both greater clay content (Figures 35-36) and organic content (Figure 37) in soils. This relationship is most notable at sites 4 and 6, which are the most clay-rich sampling sites and also have the poorest water quality based on samples analyzed in this study. No statistical relationship between concentrations of other FIB and soil content was found because the p-value for linearity is below the threshold of 0.01, which indicates that the data is extremely non-linear.







Figures 35-37: Linear Regression with Replication for Clay Content and Total Coliform, Clay Content and *E. coli*, and Organic Matter and Total Coliform

Table 18: P-values for soil texture linear regression analysis with replication. A p-value greater than 0.05 indicates that the relationship is linear and meets the assumption of linearity for the statistical analysis (Assumption of Linearity). A p-value less than 0.05 indicates that there is a statistically significant relationship between the concentration of indicator organism(s) and soil content (FIB Relationship).

	Clay			Organic Matter		
	Total Coliforms	*E. coli*	Enterococcus	Total Coliforms	*E. coli*	Enterococcus
Assumption of Linearity (p>0.05)	0.1158	0.0367	< 0.0001	0.0206	0.002	< 0.0001
FIB Relationship (p<0.05)	< 0.0001	0.002	0.0002	0.0005	0.0786	0.0059

DISCUSSION

The combinations of findings from FIB quantification and source-tracking methods suggest that Hope Ranch Beach and Las Palmas Creek are indeed impaired by some combination of point and non-point source pollution. The findings above expand upon the findings from Dr. Hugo Loaiciga's study (1999) on fecal coliform bacteria in Las Palmas Creek, and provide further understanding of the presence of pollution and its mobility during rain events in the creek and coastal environment. Although they did not succeed in specifying the potential source(s) of waste beyond that of herbivore(s), the source-tracking methods detailed in this report represent an analytical approach beyond culture-dependent FIB quantification to provide further evidence that horses in Hope Ranch are likely to be at least partial contributors to the problem of fecal contamination in the creek.

FIB analyses are useful as a proxy for contaminants entering the water, but they have their limitations. First, FIB must not be viewed as a cause or result of harmful pollution in the creek or ocean. There is no direct link between FIB assay results and human health. The field of epidemiology attempts to bridge this gap by developing models from case studies, such as those used in the above risk calculations, which describe risk as a function of observed FIB levels. Second, every FIB assay has a maximum limit of detection. In this study, that limit occurs when concentrations in the water sample exceed 24,196 MPN per 100mL water during the summer, and 241,960 MPN per 100mL water during the winter. When maximum FIB results are obtained during flush events, this procedure provides a qualitative rather than a quantitative observation that the creek or ocean water is highly polluted at the particular time and place where the sample was taken. Finally, FIB are not source-specific, may reside in the environment beyond the period of contamination, and in some cases are indigenous to the microbial community, particularly in tropical waters (Hernandezdelgado *et al.* 1991). In the latter cases, FIB are not suitable as indicators for fecal contamination.

From a management standpoint, the data from IDEXX assays are most informative when observed FIB levels are compared to the standards imposed by California state law AB 411 for water quality. The summer analysis confirms that FIB levels in the creek represent a significant pollution problem, as seen in Dr. Loaiciga's study. Occasionally this risk is present directly in front of the creek mouth in the ocean during the summer, which was not detectable at the County sampling site 25 yards away. While the County sampling may be representative of general water quality conditions at Hope Ranch Beach, these results suggest that recreating in close proximity to the creek mouth could pose a higher risk than recreating further away from the creek. The distance from the creek mouth required to ensure safe recreation was not established during this study. Nevertheless, swimmers would be advised to avoid contact, direct or indirect, with creek water.

Furthermore, as expected, storm events appear to cause a significant increase in FIB, which implies a dramatic increase in health risk posed by creek and ocean water during times of rain. Interestingly, FIB levels during the first flush remained higher than the summer averages even a week after the end of the storm, with the exception of the ocean site. It is generally understood in the water quality sciences that the first flush mobilizes contaminants that have been building up on the hillsides throughout the summer, and subsequently results in notably

diminished water quality when these contaminants are mobilized (personal communication, Rob Almy, Santa Barbara County Water Agency). The flushes appear to have a cleansing effect during the storms that follow: this study found that FIB concentrations typically drop below the summer averages by the time one week has passed since a mid-winter storm. Thus, while the first flush may increase recreational health risks even up to a week after the storm, the following storms could marginally improve water quality. The degree to which this occurs was not determined by the water quality investigation discussed herein, although the time necessary for water quality to return to acceptable levels of risk would be an interesting topic for further study.

Regardless of their duration, that human health risks quantified in this study provide a preliminary understanding of the link between the environmental conditions and health of swimmers at Hope Ranch Beach. It should be noted that these calculated numbers are the result of two of many epidemiological studies published on this subject, most of which are in disagreement on the relationship between FIB and risk to human health (Prüss 1998, Colford *et al.* 2005). The study by Wade *et al.* (2003) alone used 27 different studies to derive the relative risk relationships between Enterococcus and marine water and *E. coli* and freshwater. It is also important to note that the EPA has recommended a shift away from the use of fecal coliforms as an indicator of risk of gastrointestinal illness (Wade *et al.* 2003), which suggests the need for more specific indicator organisms to guide watershed assessment and management actions.

However, given the wide variety of indicators used to calculate human health risks, as well as the environmental and recreational distinctions between ocean and creek water and their likelihood of transmission of illness, it is necessary to consider a spectrum of exposure scenarios in order to develop the broader risk assessment picture. The same need is illustrated in a brief comparison of the calculated probabilistic and relative risks, and of the risks posed by fresh compared to marine water. For one, the progression of decreasing relative risk of gastrointestinal illness through the duration of a storm is of particularly greater importance for marine water, and its popularity for recreation, than for fresh water at this study area. In sum, there is risk present under a number of definitions; different quantifications across time and space do not counter the fact that FIB levels such as those observed along Las Palmas Creek can, and do, represent recreational waters that threaten human health.

Despite the apparent health risks posed at several observed exceedances during both the winter and the summer sampling periods, it is also worth noting that other Santa Barbara area creeks exhibit similar, if not less favorable, FIB counts. The PCW analysis indicates that Las Palmas Creek is relatively clean compared to other Santa Barbara creeks during the summer months, but its water quality is affected like that of an urban creek during storm events. Indeed, it is not unusual for winter storms to cause such sharp increases in FIB levels in steep Santa Barbara watersheds (personal communication, Rob Almy, Santa Barbara County Water Agency). The result of these analyses indicate that the Beach Committee should recognize the often elevated levels of pollution in the creek and ocean, but the data should be considered in the context of the additional finding that during the summer, Hope Ranch Beach is a notably clean recreation area when compared to the rest of the Santa Barbara County coastline. Nonetheless, during the winter storms, Hope Ranch Beach should be given the same management considerations as other locations of creek discharge in the Santa Barbara area.

One such consideration might be to call further upon microbial source-tracking, which helps elucidate what are, or are not, the probable sources of FIB contamination in the watershed. While source-tracking is becoming an extremely valuable tool in watershed management, this technique has limitations that must be considered to help understand the implications of these results. First, microbes are temporally limited. Microbes will only persist for a certain length of time once removed from their optimal environments, and the longevity of each signal differs depending on the organism and the environment. Second, DNA-based analyses require a certain amount of DNA to successfully amplify the DNA marker; a sensitivity analysis can indicate a minimum amount of DNA necessary for successful PCR. Finally, it is impossible to recover all potentially important DNA during sampling and extraction. Sampling protocols require that small quantities of water are collected to represent the entire creek, and even DNA that is actually captured in a grab sample may not be effectively recovered through each step of the extraction process (personal communication, Dr. Patricia Holden, UC Santa Barbara). Hence, not every signal present in a creek such as Las Palmas will be observed through source-tracking studies because it might not make it into the water sample, or alternatively, the DNA marker concentration may have been too low to be recovered during the extraction process.

Because analysis for DNA-based markers included a lack of positive hits for the human specific *Bacteroides prevotella* sequence, it is probable that no significant amount of fresh human waste was present in the water at the time of sampling. The PCR sensitivity analysis conducted during this study was able to recover DNA from roughly the AB 411 benchmarks for human specific *B. prevotella*, which indicates that the PCR assay should have been able to recover a signal from human waste. The Holden laboratory independently confirmed the sensitivity of this protocol as well during a separate research project (personal communication, Dr. Patricia Holden, UC Santa Barbara). However, without a more detailed sensitivity analysis it is not possible to deduce whether or not there were other human-waste signals not detectable below the AB 411 standards. Furthermore, because the human specific *Bacteroides* organism may not persist beyond a few days up to a week, it may be missed during a weekly (or more intermittent) sampling regime.

Horse specific *Bacteroides* was also not detected during PCR analysis, but due to the presence of the herbivore marker in the water and visual observations of horse manure on the creek trail, it remains a viable possibility that horse waste was present in the water during sampling. The sensitivity analysis indicated that a signal should have been recovered when 0.15 grams of fresh horse waste are present in 2 liters of water. However, below this threshold it is unknown whether the PCR assay will recover a horse contamination signal. As with human specific *B. prevotella*, the horse specific *Bacteroides* may not persist beyond a few days to a week in the environment. Also, the horse specific *Bacteroides* assay was only performed on samples that generated a positive *R. coprophilus* hit, and it is unknown as to whether or not the signal was present in other samples collected during the project. The variable nature of the freshness of the waste and the viability of the horse-specific *Bacteroides* signal in the water can all affect the predictive power of a hit or miss for this particular marker. A longevity study would need to be conducted in order to determine how long horse specific *Bacteroides* survives in Las Palmas Creek before any further conclusions are made on its absence in the water sampled during this project.

Of course, the presence of the *R. coprophilus* marker in both summer and winter water samples confirms that there is some herbivore waste in the water, but requires additional evidence in order to identify a more specific source. *R. coprophilus* is a useful test with which to begin sorting out the potential sources of contamination in the watershed; this organism is a less ephemeral marker compared to *Bacteroides*, as it will persist in the aquatic environment for up to two weeks (Long 2003). The sensitivity of *R. coprophilus* is also significant, with one gram of horse waste carrying up to 76,000 cells of *R. coprophilus*, and 0.15 gram of horse manure (about the size of half a pea) in two liters of water gives a potential signal of 11,400 cells. The published literature has found that this protocol is able to recover DNA from 40 of these cells (Savill et al. 2001). However, especially compared to the other markers used in this study, *R. coprophilus* is also less specific to the precise source of fecal matter: PCR analysis will highlight the expression of the *R. coprophilus* marker as an indicator for cattle, sheep, and deer, as well as horse (Savill *et al.* 2001). Human, pig, possum, rabbit, and duck feces did not register a positive signal in this study. Although they have not been shown to "hit" in previous PCR-based studies, hens, geese, and seagulls are additional animals also known to carry *Rhodococcus coprophilus* (Mara 1981).

The nonspecific hit for *Cryptosporidium* spp. at site 2 (the creek mouth) on Oct. 18, 2005 provides additional information on the microbiological conditions at Las Palmas Creek. While in some cases, such a hit may be due to nonspecific amplification – the erroneous amplification of a sequence of DNA other than the segment of interest - it is also possible that a non-pathogenic species of *Cryptosporidium* was amplified during PCR. The source-tracking primers selected for *Cryptosporidium* are only specific to the genus level, and the restriction-fragment length polymorphism (RFLP) method provided by Guyot *et al.* 2002 is necessary to determine which species was found. This analysis was not conducted during this study. As with the source-tracking organisms discussed herein, a positive result for a *Cryptosporidium* species related to the pathogenic species may not necessarily be attributed to fresh waste deposits – instead, the release of these microbes stored in watershed reservoirs such as clays, soils, and sediments, can induce signals that were introduced into the environment at an earlier time, before the advent of a storm (Walker *et al.* 1998).

In fact, the soil and sediment studies explained herein suggest that clay-like soils, such as those present at site 4, are especially good reservoirs for FIB, and potentially other contaminants. A variety of soil and sediment studies have investigated the effects of contaminants in soils and sediments on water quality. As soil and sediments have the capability of harboring FIB in concentrations as many as 1-4 orders of magnitude greater than levels present in water, and as suspended solids can carry about 20% of the fecal coliform bacteria contained in stormwater, a plan of analysis for these media became an integral part of the project (Bai and Lung 2005). The storage of bacteria in soil and sediment serves as a major reservoir, which can be released during/following storm events. Additionally, since FIB are much more persistent in soils than in water, investigation of such sources is imperative. Sherer (1992) found that FIB can persist in soil/sediment for months, compared to a few days when suspended in the water column (Sherer 1992).

Furthermore, the literature suggests that generally, certain soils are more likely reservoirs than others. Specifically, soils with high clay and organic content tend to harbor the highest concentrations of FIB. Consequently, clay particles can harbor the highest concentrations of FIB, while rocky/sandy soils display the lowest. The organic-rich fine particles have been

shown to support populations three times greater than in those of course particles (Gerba 1984, Burton 1987).

These findings are consistent with some of the standard properties of clay minerals. This can attributed to (1) the greater porosity associated with clays compared to other particles, (2) greater nutrient holding capacity, (3) greater water holding capacity, (4) the charge associated with clays, thereby facilitating binding to nutrients, as well as negatively charged bacteria, that are in solution, and (5) the immense surface area of these sediments (Tate 1978). An additional trend is a decreased concentration of Total Coliforms and *E. coli* with increased distance upland from the water's edge, while Enterococcus concentrations remain relatively constant.

The possibility that fecal indicator microbes are binding to soils in Las Palmas Creek affects the implications for source-tracking results, and the best management practices used to address those results. Clay and organic content could explain part of the high FIB concentrations in the creek. Thus, at sites where high *E. coli* and Total Coliforms are associated with high clay content, the levels of FIB observed in water are likely attributable, in part, to FIB released from these bound conditions. Also, the FIB concentrations in soil and sediment appear to follow the same trend as the water data, where higher FIB concentrations are found in the creek sites instead of the ocean sites. These results support the hypothesis that contamination from soil and sediments could impair water quality in Las Palmas Creek. However, the full extent of the relationship soil texture and water FIB concentrations could not be determined because some of the data was extremely non-linear, and a nonlinear regression analysis with replication was not located during this analysis.

Soil-bound bacteria may extend an FIB signal that persists for some time beyond the contamination event, but this possibility will not eliminate the need for management to address potential enduring health risks. Nonetheless, the difficulty of knowing when and how these FIB were introduced into the various media within the creek affects the resulting management recommendations. If signals recovered in this data set were introduced into the environment many months before, the urgency of addressing sources of fecal contamination may be lower than if these FIB were deposited into water and bound to clays much more recently. Future studies might be useful if they pair the timing and number of source-tracking studies for soil with those for water samples, in order to test a hypothesis on whether or not the soil also harbors source-tracking markers or pathogens, deposited in earlier contamination event(s), which contribute to present signals observed in creek and ocean water.

At this time, a review of the frequency of the presence of herbivore waste in multiple media combined with the magnitude of FIB concentrations in Las Palmas Creek during the winter flushes suggests that the first flush is a significant event for water quality in this area. The highest FIB concentrations throughout the study and the most frequent hits for the herbivore waste marker occurred during the first flush. The presence of herbivore waste at all sites the day following the storm, including the ocean, indicates that people recreating in the ocean could be exposed to pathogens potentially associated with herbivore waste, even if they do so a full day or two after a storm. Also, the presence of herbivore waste in the ocean-creek mixing zone one week after the first flush suggests that this elevated health risk, as calculated above, could be sustained even a week after the first rains of the season.

Along a greater time scale, flushes may have a cleansing effect by scouring away contamination that otherwise remains latent in the watershed. While 61% of the 18 samples from the first flush revealed the presence of herbivore waste marker, the following flushes appear to have less fecal contamination in the water – this signal was recovered in only 17% of 18 samples for each flush. Such news may serve as a reminder to beachgoers at Hope Ranch that while precautions must be taken near creek water, even seasonal changes suggest that water quality can be easily improved. The implementation of a few small but significant changes in management practices can help insure safety in ocean recreation, year-round.

CONCLUSIONS AND RECOMMENDATIONS

Samples taken monthly in the dry season, and during significant storm events in the wet season, have served to track the changes in FIB levels as well as search for identifier organisms that can provide preliminary information on the sources of such contamination in creek water. Where little data has been collected and many problems with water quality and human health have been observed, the baseline data for fecal indicator bacteria (FIB) levels provide information on the status and changes of pollution, which supplements an overarching objective in watershed management: promoting a safe and clean environment for living and recreating. Source-tracking begins the next step in remediation of observed pollution, and is available to preliminarily suggest the presence of herbivore, and potentially horse, waste, as well as its associated pathogens in the waters of Las Palmas Creek. To address the finding that herbivore waste is a contributor to fecal contamination in the creek as well as in the surf zone, the following management recommendations are offered:

- **Restrict recreation in water while promoting stewardship**: Based on the results of this water quality investigation, County-mandated beach closures do not provide sufficient protection of human health at Las Palmas Creek, which is contaminated year-round by state standards for marine water quality. Furthermore, indicator bacteria levels appear highest at the first flush event, which confirms yet another temporal component to water quality characteristics at Hope Ranch. A warning sign near the creek mouth can help advise residents of where not to swim, too, rather than simply when not to swim. This sign could be part of a larger signage campaign to educate residents beyond the concerns of human health to those of watershed health, including a Las Palmas Creek stewardship program that follows in the footsteps of Friends of Arroyo Burro, a working group created in the 1990s to support the health of the watershed just south of Hope Ranch (City of Santa Barbara Creeks Division 2004). Some form of community organization and involvement in the watershed would assist in developing and improving upon a general understanding of how waste on home sites or on trails will, and does, make its way downstream to Hope Ranch Beach. If necessary, the Hope Ranch Security Patrol may be utilized to observe any potential recreation occurring upstream and to immediately advise against it.

- **Educate local residents through County programs and private efforts**: The rather close-knit nature of the Hope Ranch community and the presence of a private primary school, Laguna Blanca School, on-site suggest that efforts to improve water quality through education should not be underestimated. There are already school education programs implemented at public schools by the Santa Barbara Public Works Department, including intensive curricula on watershed health from the fourth through eighth grades (SB Water). Other watershed-education prototypes can be found in the programs put on by the local Community Environmental Council at the Watershed Resource Center at Arroyo Burro Beach. If these lessons can be adapted to the specific issues affecting a local watershed – such as suspected horse waste entering the creek and traveling down to the ocean – schoolchildren can become a valuable mode of communication to Hope Ranch families who may not be aware of

the potential harms posed by horse waste in the water, or of the potential risk of recreation at the surf zone.

- **Assess point-sources from private homes**: Before extensive horse trail management actions are taken, it will be helpful to estimate the relative fecal waste inputs from horse trails and private stables. If the majority of fecal waste identified through the span of this project is coming from a point further up in the watershed – such as manure from an on-site stable – it will be necessary to evaluate the impact of this waste, and subsequently work with individual horse owners to encourage more meticulous and frequent stable sweeping and thereby prevent the entrance of large amounts of horse manure into the creek. The many pipes that discharge into Las Palmas from the individual properties along the creek would form a useful set of supplemental sampling sites to better understand the geographical source of herbivore-linked and other FIB. Alternatively, surveying homeowners residing adjacent to the creek will provide additional information on the resident horse populations and status-quo stable upkeep at Hope Ranch, and at a lower cost.

- **Adopt County ordinance on waste pick-up**: The County of Santa Barbara already requires horseback riders as well as dog owners to clean up after their pets, per Section 17-10 of the County Solid Waste Services Code (Project Clean Water).[2] Water quality management at Hope Ranch Beach stands to benefit from enforcement of this code, which might be "enforced" through community cooperation rather than government intervention. Information posted at homeowners meetings, in neighborhood newsletters, and on posted along the trail can assist in community-wide compliance and cleaner trails, reducing the load of fecal waste into the water.

- **Increase frequency and thoroughness of trail maintenance and cleanings**: The input of fecal matter from the horse trail into the creek can be further reduced by increased attention and response to trail conditions. Horse waste should be regularly removed, if not by horseback riders themselves, then by a supplemental sweeping program. If the neighborhood is made aware of the program, it would be feasible to base sweepings on a call-in basis rather than a regular schedule, so that observed waste is taken care of promptly in response to community concerns. This "neighborhood watch"-style program for horse waste removal would secondarily foster a unified effort towards a cleaner watershed for horseback riders and other residents alike.

It is important to conclude any study involving source-tracking methods with another word of caution that there is no foolproof way to identify the source of a contaminant. The degree of uncertainty associated with the presence of *Rhodococcus coprophilus*, for example, suggests that despite the valuable information on the presence of pollution that is likely entering the creek by way of an herbivorous animal, it is best complemented with an appropriate change in management practices along the creek and a follow-up study to determine if indeed, pollution can be ameliorated though such actions.

[2] The section regulating animal waste reads "It shall be unlawful for any person to place keep or bury any solid waste.."

For this reason as well as limitations that were recognized in the scope of this project, future water quality research is recommended if the Hope Ranch community desires further information on the sources of contamination in Las Palmas Creek. Quantitative or real-time PCR (qPCR) is one tool that would help not only to identify sources of pollution, but also to determine the likely amount of any one contaminant. qPCR is also more sensitive than conventional PCR, and requires fewer copies of DNA marker(s) to return a positive signal. Although at a higher cost than previous studies, this would improve the ability of managers at Las Palmas Creek to better estimate risks and take action in response to such risks. A future analysis could also investigate the presence or absence of chemical signals of contamination to broaden the predictive capability of the source tracking analysis. This project has provided a substantial baseline picture of water quality that can only be made more useful if such study continues through time and incorporates assays for additional markers for specific types of aquatic pollution. However, the current analysis provides sufficient evidence that Las Palmas Creek is polluted, and probable as well as obvious sources of contamination should be addressed through a selection of the management actions listed above.

ACKNOWLEDGEMENTS

Members of the Hope Ranch Beach Committee – Ken Richards, Neal Rabin, Laird Riffle, Ken Young, and Mark Harris – and Jim Trebbin were invaluable during the entire project. Upon introductions in April 2005, they shared with us their knowledge on the land-use and hydrological characteristics of Las Palmas Creek and related documentation. As stakeholders they provided a great deal of support to past, present, and upcoming research and maintained open and willing communication with our group. Moreover, they raised the awareness and financial support within their community that made it possible for this study to happen throughout the past year. On this note we extend our sincere gratitude, in particular, to the twelve Hope Ranch residents who collectively funded this mutually beneficial endeavor.

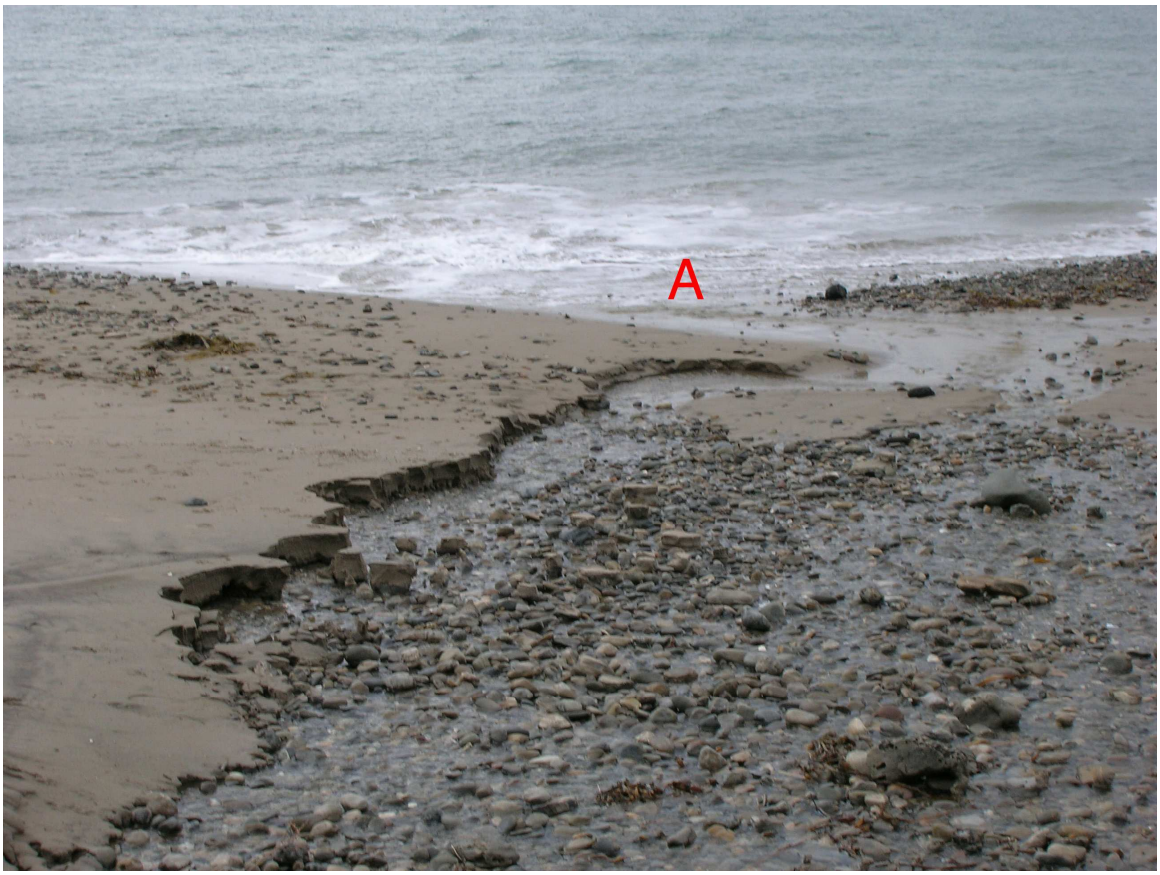
As anticipated, the project provided opportunities for working with a network of advisors external to the group, including academic researchers from the UC system and water quality experts from the County of Santa Barbara, as well as representatives from the Hope Ranch Beach Committee. The group's successful cooperation with such advisors was demonstrated in a series of presentations and feedback given during the Progress Reviews on June 3 and November 10, 2005, and the Project Defense on February 27, 2006, at the Donald Bren School of Environmental Science and Management at UC Santa Barbara.

As our Bren School Group Project Advisor, Dr. Patricia Holden was indispensable for the group management and laboratory guidance she offered. In particular, her lab methods on PCR for *Bacteroides prevotella* were transferred over to this project. Dr. Holden's lab technician, Laurie Van de Werfhorst, also worked with our group to provide advice on protocols, primer selection, and general lab upkeep. Dr. Arturo Keller, Associate Professor at the Bren School, provided the project with professional knowledge on the scientific components and management of a watershed such as Las Palmas. Specifically, he assisted project members with modeling the effects of various contaminant inputs and FIB levels on watershed health, and provided feedback on human health risk assessment calculation. Dr. Hugo Loaiciga, Professor in the Geography department at UCSB, who conducted the pilot water quality study at Las Palmas Creek in 1999 and 2000, provided us with a baseline understanding of contaminant levels as distributed throughout the length of Las Palmas Creek when this project was proposed. Finally, Bren Ph.D. candidate Peng Wang deserves our thanks for sharing his extensive knowledge on soil and sediment analysis. Technical assistance from all of the above was essential for the completion of the project, as was the aid and support of Bren Hall building engineer Jeff Kirby.

The County of Santa Barbara also provided valuable resources to the public as well as to this project. Rob Almy, who directs the County's Project Clean Water, provided professional information on county water sampling objectives and methods. Willie Brummett, from the County's Department of Environmental Health Services, assisted in data acquisition and interpretation by sharing the measurements and analysis obtained from weekly sampling at Hope Ranch Beach, which served as a benchmark data set for the surf zone sample site in this project. As discussed above, he also was instrumental in establishing quality assurance for the FIB analysis methods used in our Bren Hall laboratory, providing verification of and confidence in the techniques that ultimately formed this water quality investigation.

APPENDIX 1: SOIL SAMPLE LOCATION

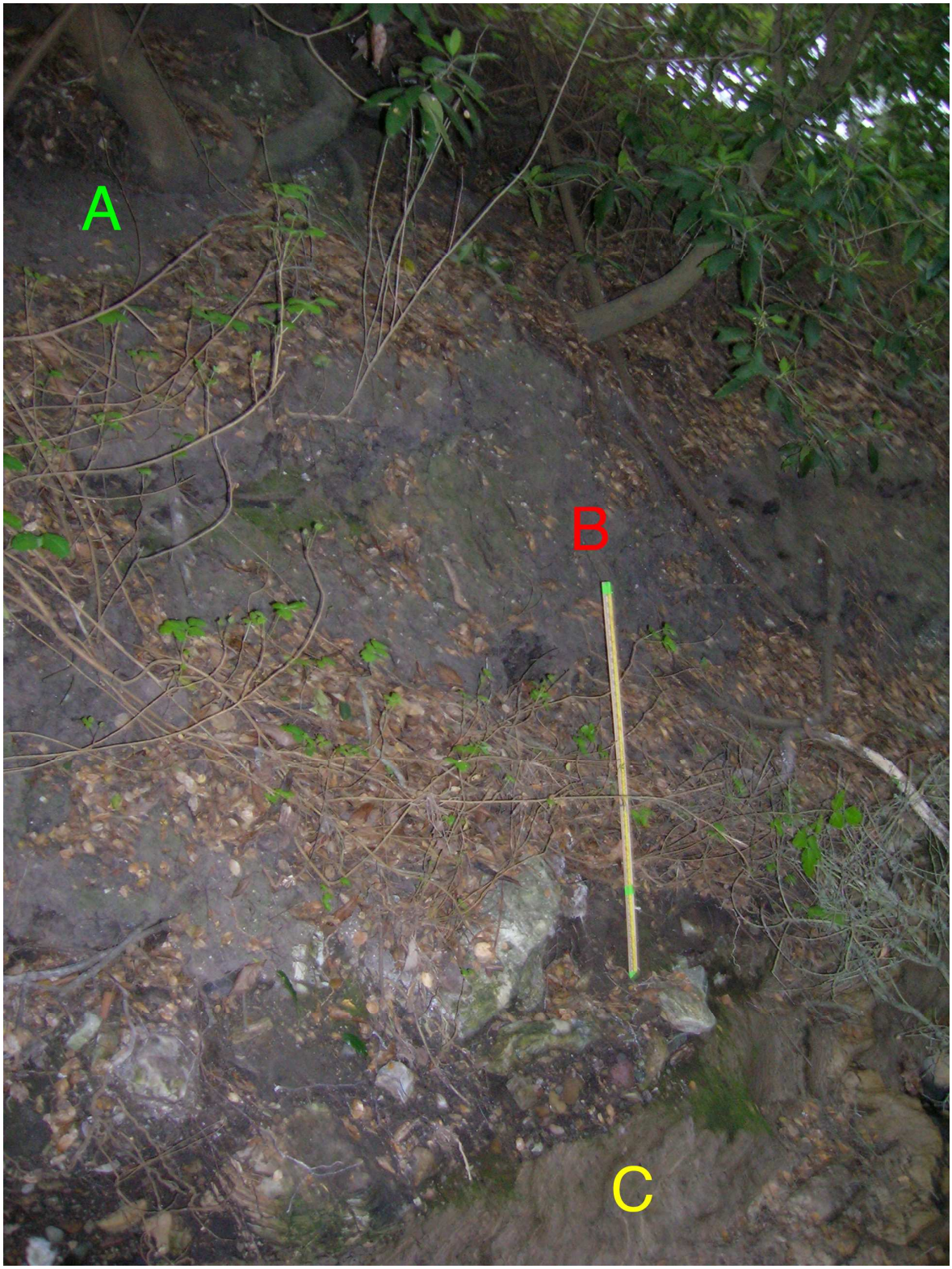
NOTE: sites 3-6 display a meter stick as scale reference



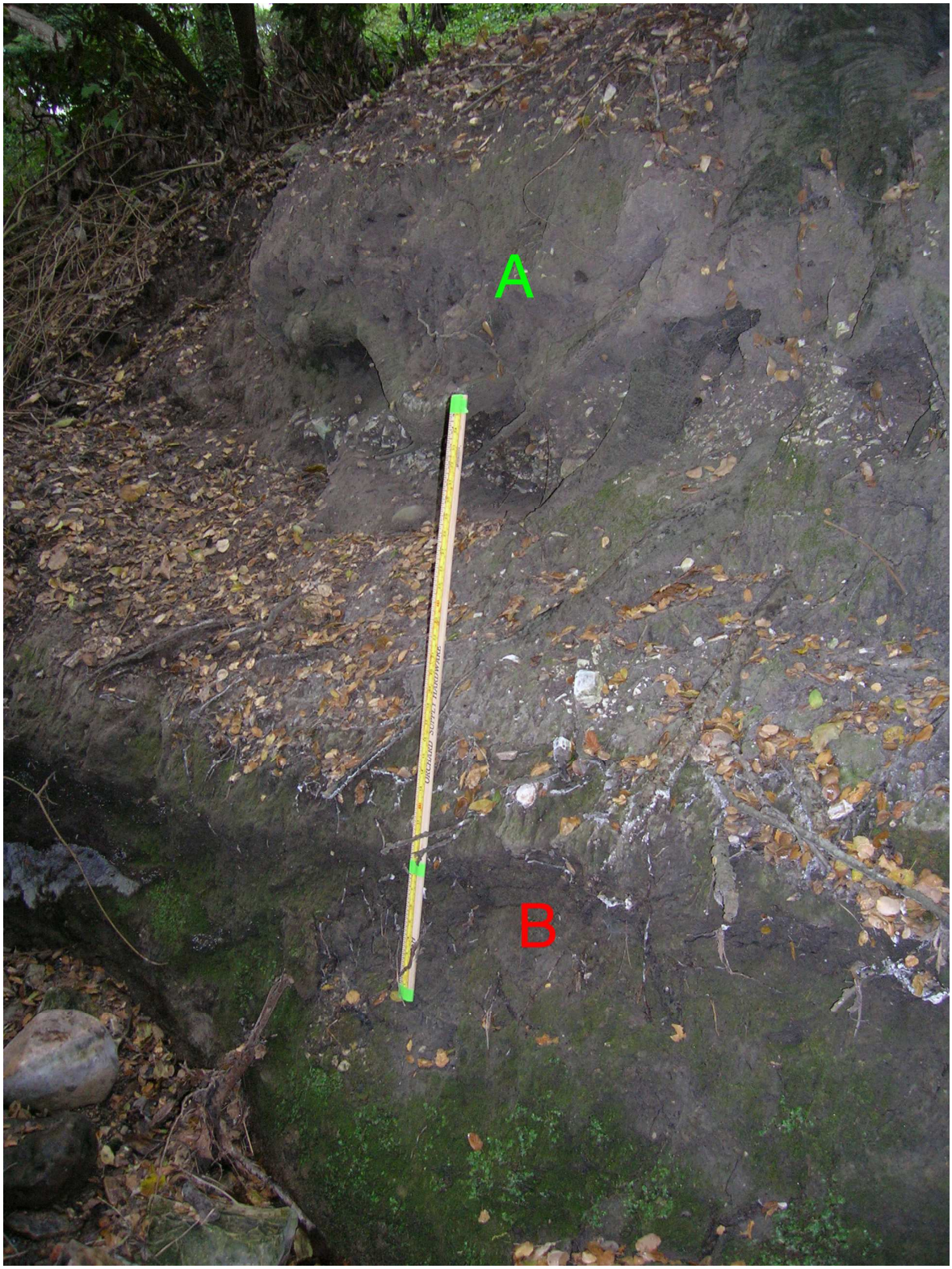
Site 1

Site 2

Site 3



Site 4



Site 5

Site 6

APPENDIX 2: PRIMER SELECTION

2.1 Bacteroides prevotella (human specific)

The methods of Bernhard and Field (2000) have been used in multiple source-tracking studies to identify *B. prevotella* in surface waters contaminated with fresh human sewage or septage. The Holden Laboratory at UCSB has optimized this method, and the group has adopted their procedures as standard laboratory routine.

2.2 Rhodococcus coprophilus

Rhodococcus coprophilus has been used as an indicator for herbivore waste (Savill *et al.* 2001). This marker is desirable since it is able to persist in the environment for two weeks (Long *et al.* 2003), which is within the sampling time frames of the study. However, Mara and Orugui (1981) were able to culture this organism from seagull and duck feces. Given the proximity to Laguna Blanca, and more importantly, the ocean, a positive hit in the creek could potentially result from an avian source. The marker is still useful for distinguishing between animal waste and human waste, even if it lacks specificity, and can be combined with field observations to pose a reasonable set of conclusions and management recommendations.

2.3 Bacteroides sp. (horse specific)

Currently, the DNA marker published by Dick *et al.* 2005 is the only marker shown to specifically identify equine waste. The protocol for human specific *B. prevotella* is almost identical to the protocol for horse specific *Bacteroides*, so the methods of the Holden Laboratory were again adopted as standard laboratory routine.

2.4 Cryptosporidium spp.

C. parvum and *G. lamblia* primers were chosen based on their sensitivity and specificity, as related to human health. Sensitivity is based on the infectious dose of cysts for humans, while specificity refers to the particular species that cause human illness.

C.parvum is the particular species that causes human illness, with an infectious dose of about 132 cysts (Dupont *et al.* 1995). The primers originally designed by Laxer (1991) have been used in multiple studies and would provide enough specificity to show whether or not *Cryptosporidium* is present in the water (Rochelle *et al.* 1997, Champliaud 1998, Hallier-Soulier and Guillot 1999, Guyot *et al.* 2002). Rochelle *et al.* 1997 conducted an optimization analysis on the PCR parameters, which makes this a desirable protocol for the "Laxer" primer.

However, this primer is not able to distinguish between *C. parvum* and *C. meleagridis* (Champliaud *et al.* 1998, Wiedenmann *et al.* 1998, Fontaine and Guillot 2002, Guyot *et al.* 2002). *C. meleagridis* is not a threat to human health. Multiple analyses have been developed to distinguish *C. parvum* from *C. meleagridis* and other *Cryptosporidium* species, including restriction fragment length polymorphism (RFLP) analyses (Guyot *et al.* 2002, Coupe *et al.* 2005). The Guyot *et al.* 2002 article used RFLP on the "Laxer" primer. Although Guyot *et al.* 2002 and other studies suggested that the original "Laxer" primer may have errors, they did not suggest that this appears to have reduced their ability to detect *C. parvum* with RFLP.

The "Laxer" primers was used according to the methods of Rochelle *et al.* (1997), and then the Guyot *et al.* 2002 RFLP analysis was used on any positive hits for *Cryptosporidium* to determine which species is present in the water.

2.5 Giardia lamblia

G. lamblia primers were chosen based on their sensitivity and specificity (Table 1). The infectious dose for *Giardia* is 10 cysts (Adam 1991, Adam 2001), while *G. lamblia* Assemblages A and B are the organisms that cause disease in humans (Lane 2002). The Caccio (2002) method provides the best combination of sensitivity and specificity for the project goals.

Caccio reported 0.5pg sensitivity with the b-Giardin gene (Caccio *et al.* 2002), which translates to 10-20 cysts (Table 2). The method was to be used without RFLP unless a positive result occurs. The "Caccio" method is desirable since it is recent, specific, and has been used in an environmental study. However, more sensitivity would be advantageous.

The other primers reviewed did not meet the project's criteria for both sensitivity and specificity. The "Mahbubani 171bp" primers are unable to differentiate between multiple strains of *Giardia* (Mahbubani *et al.* 1992). The "Mahbubani 218bp" primers are more specific than the "Mahbubani 171bp" primers, but are not as sensitive. Mahbubani originally reported 1 cyst sensitivity for the 218bp primers, but Rochelle (1997) later reported 10 cyst sensitivity. The "Mahbubani 218bp" primers are about as sensitive as the "Caccio 2002" primers, and do not require RFLP. However, Rochelle (1997) did not test the specificity of primers with a suite of species and assemblages, so it is only known that it discriminated between *G. lamblia* and *G. muris* (Rochelle *et al.* 1997).

Other investigated primers target the TPI or GDH genes (Amar 2002, Rimhanen-Finne 2002, Read 2004). None of these primers had the desired combination of sensitivity and specificity for the project's needs. The TPI primers were not sensitive, requiring 1 nanogram DNA per microliter (Amar 2002). This paper has a RFLP protocol that only discriminates between Assemblage A from B, and does not incorporate other *Giardia* species. The Rimhanen-Finne (2002) primers targeting the GDH gene also were not specific enough (50-100 cysts). Finally, the Read (2004) primers targeted the GDH gene with a semi-nested PCR, and had excellent specificity within *G.lamblia* Assemblages (AI, AII, B, C, D, E). However, this assay only has 2pg DNA sensitivity, or 50 cysts (Table 2).

Table 19: Protocol review for *Giardia* primers

Primers	Gene	Sensitivity	Specificity	RFLP?	Citations
Mahbubani 171 bp	B-giardin	1 cyst	*Giardia*	IP	Mahbubani 1992, Rochelle 1997
Mahbubani 218 bp	B-giardin	10 cysts	*G. lamblia*	No	Mahbubani 1992, Rochelle 1997
Caccio	B-giardin	12-25* cysts (0.5 pg/ul)	Assem. A,B	Yes	Caccio 2002, Caccio 2003, Matsubayashi 2005
Rimhanen-Finne	GDH	50-100 cysts	*Giardia*	No	Rimhanen-Finne 2002
Read	GDH	50-100* cysts (2 pg/ul)	Assem. AI, AII, B, C, D, E	Yes	Read 2004, Miller 2005
Amar	TPI	25,000* cysts (1 ng/ul)	Assem. A,B	Yes	Amar 2002

Table 20: Conversion from cyst DNA weight to number of cysts

*Calculations
According to the parameters below, the Giardia genome weighs approximately 0.01pg (0.013pg). • 1.2*10^7 base pairs (Adam 2000) • 660 Daltons = approx. weight of a base pair (DOE 2003) • 1 Dalton (amu) = 1.66*10^-24 g • 2-4 nuclei per cyst (NCBI)

APPENDIX 3: BACKGROUND DATA

Date	SiteNum	pH	Temp	DO	Salinity	Flow (m^3/s)
6/22/2005	1	7.75	19.1	8.90		
6/22/2005	2	7.98	17.9	9.88	4.8	0.01814
6/22/2005	3	7.76	21.3	7.80	1.9	0.00606
6/22/2005	4	7.60	17.8	7.53		0.00135
6/22/2005	5	7.36	20.5	8.40	33.5	0.00073
6/22/2005	6	7.11	20.0	4.30	7.9	
6/27/2005	1	7.76	16.0	9.20		
6/27/2005	2	7.91	16.5	8.50	150.2	0.01772
6/27/2005	3	7.55	18.0	8.40	32.3	0.00716
6/27/2005	4	7.42	16.8	7.68	25.6	0.00178
6/27/2005	5	7.28	17.7	7.88	7.7	0.00056
6/27/2005	6	6.85	17.3	5.23	14.6	
7/5/2005	1	8.05	17.5	9.30		
7/5/2005	2	8.11	16.5	8.21	19.0	0.01780
7/5/2005	3	7.59	18.0	7.51	9.8	0.00711
7/5/2005	4	7.46	16.9	7.50	6.5	0.00127
7/5/2005	5	7.32	17.4	8.08	3.9	0.00024
7/5/2005	6	5.91	17.2	5.52	5.7	
7/11/2005	1	7.85	19.6	9.33		
7/11/2005	2	7.00	17.5	9.20	14.4	0.01847
7/11/2005	3	7.55	19.6	7.50	8.5	0.00563
7/11/2005	4	7.43	18.6	7.81	8.2	0.00142
7/11/2005	5	7.17	18.2	6.80	2.2	-0.00024
7/11/2005	6	6.88	18.9	4.99	7.0	
7/18/2005	1	7.67	17.4	8.98		
7/18/2005	2	7.75	17.5	7.35	38.5	0.01884
7/18/2005	3	7.58	18.9	7.70	8.1	0.00570
7/18/2005	4	7.53	18.1	7.45	2.4	0.00110
7/18/2005	5	7.06	19.2	6.35	4.9	0.00021
7/18/2005	6	7.01	18.6	5.26	2.6	
7/25/2005	1	7.88	20.2	9.06		
7/25/2005	2	7.76	18.8	8.62	30.2	0.01849
7/25/2005	3	7.41	18.8	7.50	31.4	0.00534
7/25/2005	4	7.16	18.6	6.90	18.6	0.00105
7/25/2005	5	7.16	19.6	7.75	8.4	-0.00017
7/25/2005	6	6.76	19.0	4.29	16.0	
8/1/2005	1	7.57	19.7	9.48		
8/1/2005	2	7.78	18.7	8.75	24.2	0.01886
8/1/2005	3	7.49	18.0	7.97	6.3	0.00565
8/1/2005	4	7.36	19.2	7.49	18.2	0.00095
8/1/2005	5	7.18	18.5	7.12	14.7	0.00020
8/1/2005	6	6.79	19.4	4.44	16.6	
8/9/2005	1	7.65	18.9	8.99		
8/9/2005	2	7.75	18.5	8.72	34.2	0.01816

Date	SiteNum	pH	Temp	DO	Salinity	Flow (m^3/s)
8/9/2005	3	7.53	17.9	7.63	7.4	
8/9/2005	4	7.44	18.3	7.25	10.3	
8/9/2005	5	7.10	18.9	6.97	11.4	
8/9/2005	6	6.53	18.9	5.03	3.2	
8/15/2005	1	7.93	17.8	9.18		
8/15/2005	2	8.00	17.3	9.01	28.4	0.01717
8/15/2005	3	7.51	16.7	8.14	11.5	0.00453
8/15/2005	4	7.43	16.5	7.90	13.1	
8/15/2005	5	7.11	16.5	6.32	12.9	
8/15/2005	6	6.96	17.3	4.87	12.1	
8/22/2005	1	8.10	20.6	8.34		
8/22/2005	2	8.11	18.1	7.12	125.8	0.01890
8/22/2005	3	7.63	17.7	7.21	2.8	0.00440
8/22/2005	4	7.50	17.1	6.50	1.4	0.00131
8/22/2005	5	7.18	17.5	6.48	15.1	0.00010
8/22/2005	6	7.08	18.2	3.39	13.3	
8/30/2005	1	7.98	20.6	8.94		
8/30/2005	2	8.15	19.0	7.40	39.5	0.02316
8/30/2005	3	7.62	18.0	7.68	12.3	0.00444
8/30/2005	4	7.48	18.2	7.76	14.7	0.00191
8/30/2005	5	7.25	18.4	6.50	10.3	-0.00010
8/30/2005	6	7.01	18.0	5.36	9.2	
9/6/2005	1	7.92	18.9	9.21		
9/6/2005	2	7.82	16.2	7.73	67.4	0.02199
9/6/2005	3	7.68	16.2	8.20	38.8	0.00480
9/6/2005	4	7.53	16.3	7.78	19.0	0.00075
9/6/2005	5	7.15	16.7	6.09	5.5	-0.00020
9/6/2005	6	6.36	17.2	4.22	14.8	
FIRST FLUSH						
10/17/2005	1	7.43	15.5	9.98		
10/17/2005	2	6.65	15.6	9.52	26.5	0.23424
10/17/2005	3	6.80	15.7	6.58	4.9	0.12749
10/17/2005	4					
10/17/2005	5					
10/17/2005	6					
10/18/2005	1	7.57	13.9	9.60		
10/18/2005	2	7.75	13.7	8.88	19.3	0.00525
10/18/2005	3	7.27	14.2	7.61	22.4	0.00695
10/18/2005	4	7.01	14.0	8.23	0.03	0.00182
10/18/2005	5	6.84	18.9	5.36	0.04	0.00005
10/18/2005	6	6.65	?	3.25	0.1	
10/24/2005	1	7.52	15.0	9.50		
10/24/2005	2	7.87	14.9	9.19	58.8	0.00461
10/24/2005	3	7.36	15.0	7.71	46.7	0.00493
10/24/2005	4	7.32	15.0	7.42	13.8	0.00541
10/24/2005	5	7.17	15.0	6.48	14.6	0.00077

Date	SiteNum	pH	Temp	DO	Salinity	Flow (m^3/s)
10/24/2005	6	6.59	17.7	2.82	21.0	
10/24/2005	1	PM sampling event due to rainfall				
10/24/2005	2	(IDEXX only, no DNA or field measurements)				
Second Flush						
12/31/2005	1	7.01	15.1	9.00		
12/31/2005	2	7.03	15.4	8.73	8.08	0.03050
12/31/2005	3	7.13	15.0	8.75	10.05	
12/31/2005	4	7.33	14.8	8.60		
12/31/2005	5					
12/31/2005	6					
1/3/2006	1	7.54	17.1	9.72		
1/3/2006	2	7.77	15.7	9.80	51.3	0.00913
1/3/2006	3	7.08	17.1	8.87	14.8	0.00531
1/3/2006	4	6.76	15.0	8.98	10.0	0.00332
1/3/2006	5	6.55	14.0	8.75	11.1	0.00240
1/3/2006	6	6.83	14.2	8.07	14.8	
1/9/2006	1	7.92	13.5	10.77		
1/9/2006	2	7.92	9.1	11	175.4	0.0047118
1/9/2006	3	7.45	10.3	10.7	60.3	0.003128
1/9/2006	4	7.29	10.2	10.3	17.1	0.0015533
1/9/2006	5	7.03	9.7	6.55	17.8	0.000924
1/9/2006	6	6.6	10.6	5.63	15.2	
Third Flush						
2/27/2006	1	7.63	13.2			
2/27/2006	2	7.45	12.2	10.7	25.2	
2/27/2006	3	6.84	12.3	9.6	3.1	0.18498
2/27/2006	4	6.74	11.9	9.6	4.3	0.119952
2/27/2006	5	6.66	12	9.4	1.2	0.0797355
2/27/2006	6					
2/28/2006	1	8.25	15.5	9.8		
2/28/2006	2	8.15	16	8.75	31.8	0.01597
2/28/2006	3	7.53	15.3	6.93	18.8	0.013126
2/28/2006	4	7.48	14.2	8.23	7.8	0.00565
2/28/2006	5	6.51	13.5	7.48	5.1	0.0032634
2/28/2006	6	6.97	14.6	7.89	11.9	
3/5/2006	1	8.00	19.3	10.2	83.7	
3/5/2006	2	8.29	16.9	8.49	13.5	0.00656
3/5/2006	3	7.70	16.0	8.11	18.8	0.00394
3/5/2006	4	7.60	14.5	7.36	7.9	0.00279
3/5/2006	5	7.32	13.0	7.59	3.1	0.00097
3/5/2006	6	6.98	12.1	6.30	12	

APPENDIX 4: SUMMER FIB DATA AS MPN/100ML

The number "1" in the last four columns indicates that the AB 411 standard for marine water was exceeded, while "0" indicates that the sample was below the standard (AB 411: Total Coliforms = 10000, Fecal Coliforms (*E. coli*) = 400, Enterococcus = 104, FC:TC = Fecal to Total Coliform Ratio).

Date	Site #	Total Coliforms	*E. coli*	Enterococcus	FC: TC	> 10000	> 400	> 104	>FC:TC, >1000 TC
6/22/2005	1	153	41	0	0.27	0	0	0	0
6/22/2005	2	3255	420	435	0.13	0	1	1	1
6/22/2005	3	4611	663	459	0.14	0	1	1	1
6/22/2005	4	14136	4611	2489	0.33	1	1	1	1
6/22/2005	5	4884	292	187	0.06	0	0	1	0
6/22/2005	6	2142	41	171	0.02	0	0	1	0
6/27/2005	1	75	20	0	0.27	0	0	0	0
6/27/2005	2	5475	365	444	0.07	0	0	1	0
6/27/2005	3	8664	432	412	0.05	0	1	1	0
6/27/2005	4	24196	12033	15331	0.50	1	1	1	1
6/27/2005	5	2481	100	206	0.04	0	0	1	0
6/27/2005	6	2282	156	487	0.07	0	0	1	0
7/5/2005	1	40	20	52	0.50	0	0	0	0
7/5/2005	2	7270	202	2851	0.03	0	0	1	0
7/5/2005	3	4352	143	1100	0.03	0	0	1	0
7/5/2005	4	2430	800	5794	0.33	0	1	1	1
7/5/2005	5	2282	133	210	0.06	0	0	1	0
7/5/2005	6	3448	80	202	0.02	0	0	1	0
7/11/2005	1	359	41	10	0.11	0	0	0	0
7/11/2005	2	7701	464	1314	0.06	0	1	1	0
7/11/2005	3	6488	836	2098	0.13	0	1	1	1
7/11/2005	4	8164	3255	11199	0.40	0	1	1	1
7/11/2005	5	1989	142	204	0.07	0	0	1	0
7/11/2005	6	3873	73	80	0.02	0	0	0	0
7/18/2005	1	41	20	10	0.49	0	0	0	0
7/18/2005	2	4106	384	1467	0.09	0	0	1	0
7/18/2005	3	3873	171	1670	0.04	0	0	1	0
7/18/2005	4	5172	1789	5475	0.35	0	1	1	1
7/18/2005	5	5475	203	465	0.04	0	0	1	0
7/18/2005	6	3654	259	689	0.07	0	0	1	0
7/25/2005	1	153	63	0	0.41	0	0	0	0
7/25/2005	2	7701	359	689	0.05	0	0	1	0
7/25/2005	3	8164	275	556	0.03	0	0	1	0
7/25/2005	4	12033	393	7270	0.03	1	0	1	0
7/25/2005	5	9804	41	355	0.00	0	0	1	0
7/25/2005	6	12997	857	318	0.07	1	1	1	0

Date	Site #	Total Coliforms	*E. coli*	Enterococcus	FC: TC	> 10000	> 400	> 104	>FC:TC, >1000 TC
8/1/2005	1	325	134	20	0.41	0	0	0	0
8/1/2005	2	17329	2247	813	0.13	1	1	1	1
8/1/2005	3	14136	148	420	0.01	1	0	1	0
8/1/2005	4	24196	3076	2851	0.13	1	1	1	1
8/1/2005	5	15531	97	422	0.01	1	0	1	0
8/1/2005	6	10462	74	448	0.01	1	0	1	0
8/9/2005	1	6488	836	1529	0.13	0	1	1	1
8/9/2005	2	6867	1801	1169	0.26	0	1	1	1
8/9/2005	3	6867	1801	1169	0.26	0	1	1	1
8/9/2005	4	24196	2282	1012	0.09	1	1	1	0
8/9/2005	5	9208	161	521	0.02	0	0	1	0
8/9/2005	6	8664	816	1421	0.09	0	1	1	0
8/15/2005	1	4160	275	404	0.07	0	0	1	0
8/15/2005	2	9208	1076	987	0.12	0	1	1	1
8/15/2005	3	7701	683	712	0.09	0	1	1	0
8/15/2005	4	19863	4106	3448	0.21	1	1	1	1
8/15/2005	5	6131	97	359	0.02	0	0	1	0
8/15/2005	6	24196	12033	6867	0.50	1	1	1	1
8/22/2005	1	1008	414	749	0.41	0	1	1	1
8/22/2005	2	11199	1112	5794	0.10	1	1	1	0
8/22/2005	3	8664	350	1726	0.04	0	0	1	0
8/22/2005	4	7701	1722	9804	0.22	0	1	1	1
8/22/2005	5	14136	197	323	0.01	1	0	1	0
8/22/2005	6	14136	383	717	0.03	1	0	1	0
8/30/2005	1	581	231	52	0.40	0	0	0	0
8/30/2005	2	6131	657	1043	0.11	0	1	1	1
8/30/2005	3	4884	206	368	0.04	0	0	1	0
8/30/2005	4	12033	1497	8164	0.12	1	1	1	1
8/30/2005	5	6131	73	110	0.01	0	0	1	0
8/30/2005	6	9208	569	173	0.06	0	1	1	0
9/6/2005	1	402	97	199	0.24	0	0	1	0
9/6/2005	2	6488	620	934	0.10	0	1	1	0
9/6/2005	3	6488	1145	712	0.18	0	1	1	1
9/6/2005	4	7270	1112	10462	0.15	0	1	1	1
9/6/2005	5	5475	135	185	0.02	0	0	1	0
9/6/2005	6	12033	2909	7270	0.24	1	1	1	1

APPENDIX 5: WINTER FIB DATA AS MPN/100ML

The number "1" in the last four columns indicates that the AB 411 standard for marine water was exceeded, while "0" indicates that the sample was below the standard (AB 411: Total Coliforms = 10000, Fecal Coliforms (*E. coli*) = 400, Enterococcus = 104, FC:TC = Fecal to Total Coliform Ratio).

Date	Site #	Total Coliforms	*E. coli*	Entero coccus	FC: TC	> 10000	> 400	> 104	>FC:TC, >1000 TC
First Flush									
10/17/2005	1	241960	92080	129970	0.38	1	1	1	1
10/17/2005	2	241960	98040	120330	0.41	1	1	1	1
10/17/2005	3	241960	68670	104620	0.28	1	1	1	1
10/17/2005	4	241960	92080	77010	0.38	1	1	1	1
10/17/2005	5	241960	92080	77010	0.38	1	1	1	1
10/17/2005	6	241960	57940	43520	0.24	1	1	1	1
10/18/2005	1	24810	1710	0	0.07	1	1	0	0
10/18/2005	2	241960	81640	17820	0.34	1	1	1	1
10/18/2005	3	241960	86640	20980	0.36	1	1	1	1
10/18/2005	4	241960	141360	41060	0.58	1	1	1	1
10/18/2005	5	241960	241960	43520	1.00	1	1	1	1
10/18/2005	6	241960	48840	38730	0.20	1	1	1	1
10/24/2005	1	421	73	41	0.17	0	0	0	0
10/24/2005	2	15331	1250	1145	0.08	1	1	1	0
10/24/2005	3	24196	7701	3255	0.32	1	1	1	1
10/24/2005	4	24196	4352	5794	0.18	1	1	1	1
10/24/2005	5	17329	404	318	0.02	1	1	1	0
10/24/2005	6	24196	3654	2187	0.15	1	1	1	1
10/24/2005	1	13760	1440	850	0.10	1	1	1	1
10/24/2005	2	29090	6130	3010	0.21	1	1	1	1
Second Flush									
12/31/2005	1	241960	112400	38100	0.46	1	1	1	1
12/31/2005	2	241960	272300	115300	1.13	1	1	1	1
12/31/2005	3	241960	328200	112200	1.36	1	1	1	1
12/31/2005	4	241960	436000	177500	1.80	1	1	1	1
12/31/2005	5	241960	250400	198900	1.03	1	1	1	1
12/31/2005	6	241960	133400	98500	0.55	1	1	1	1
1/3/2006	1	1730	310	100	0.18	0	0	0	1
1/3/2006	2	30760	630	1970	0.02	1	1	1	0
1/3/2006	3	38730	1090	1080	0.03	1	1	1	0
1/3/2006	4	30760	630	1990	0.02	1	1	1	0
1/3/2006	5	20980	1210	740	0.06	1	1	1	0
1/3/2006	6	51720	1320	1220	0.03	1	1	1	0
1/9/2006	1	617	10	0	0.02	0	0	0	0
1/9/2006	2	6015	109	148	0.02	0	0	1	0
1/9/2006	3	11199	187	216	0.02	1	0	1	0
1/9/2006	4	12997	199	153	0.02	1	0	1	0
1/9/2006	5	12033	85	173	0.01	1	0	1	0
1/9/2006	6	14136	288	187	0.02	1	0	1	0

Date	Site #	Total Coliforms	*E. coli*	Entero coccus	FC: TC	> 10000	> 400	> 104	>FC:TC, >1000 TC
Third Flush									
2/27/2006	1	173290	13170	17230	0.08	1	1	1	0
2/27/2006	2	173290	19040	19350	0.11	1	1	1	0
2/27/2006	3	155310	17250	25950	0.11	1	1	1	0
2/27/2006	4	43520	3550	8300	0.08	1	1	1	0
2/27/2006	5	92080	2650	11340	0.03	1	1	1	0
2/27/2006	6								
2/28/2006	1	13760	100	860	0.01	1	0	1	0
2/28/2006	2	241960	3410	13760	0.01	1	1	1	0
2/28/2006	3	241960	2280	22820	0.01	1	1	1	0
2/28/2006	4	241960	3450	27550	0.01	1	1	1	0
2/28/2006	5	241960	1730	34480	0.01	1	1	1	0
2/28/2006	6	241960	3840	20460	0.02	1	1	1	0
3/5/2006	1	5475	520	121	0.09	0	1	0	0
3/5/2006	2	24196	216	185	0.01	1	0	0	0
3/5/2006	3	24196	223	160	0.01	1	0	0	0
3/5/2006	4	24196	301	238	0.01	1	0	0	0
3/5/2006	5	24196	110	209	0.00	1	0	0	0
3/5/2006	6	24196	146	309	0.01	1	0	0	0

APPENDIX 6: SUMMER AND WINTER SOIL AND SEDIMENT FIB DATA AS MPN/GRAM

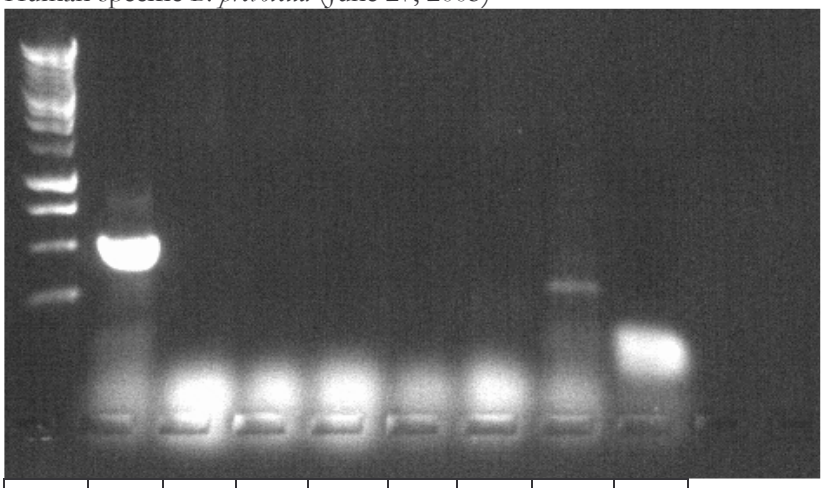
Date	Type	Site	Total Coliforms	*E. coli*	Enterococcus
25-Jul	sed	1	131	223	6
25-Jul	sed	2	4477	1657	12
25-Jul	sed	3	13906	2650	899
25-Jul	sed	4	15922	1914	15922
25-Jul	sed	5	6839	45	71
25-Jul	sed	6	1110	23	113
22-Aug	sed	1	73	32	25
22-Aug	sed	2	5929	113	105
22-Aug	sed	3	14914	3375	2006
22-Aug	sed	4	16931	16931	16931
22-Aug	sed	5	13876	8107	188
22-Aug	sed	6	17431	168	17431
6-Sep	sed	1	0	0	0
6-Sep	sed	2	313	17	6
6-Sep	sed	3	5583	0	196
6-Sep	sed	4	2333	0	377
6-Sep	sed	5	989	0	1022
6-Sep	sed	6	6608	6	140
30-Jan	sed	1	26	0	0
30-Jan	sed	2	638	18	117
30-Jan	sed	3	12895	269	189
30-Jan	sed	4	17769	686	10381
30-Jan	sed	5	13484	203	348
30-Jan	sed	6	15772	153	15772
18-Jul	soil	1	141	131	5
18-Jul	soil	2	2120	1782	94
18-Jul	soil	3	5066	1121	10132
18-Jul	soil	4	3122	16	700
18-Jul	soil	5	1011	0	2277
18-Jul	soil	6	302	63	304
15-Aug	soil	1	18	6	0
15-Aug	soil	2	10395	365	125
15-Aug	soil	3	13067	456	13067
15-Aug	soil	4	16861	0	16861
15-Aug	soil	5	13513	6	13513
15-Aug	soil	6	1187	207	11514
6-Sep	soil	1	0	0	0
6-Sep	soil	2	691	14	0
6-Sep	soil	3	934	0	6
6-Sep	soil	4	2377	0	134
6-Sep	soil	5	951	0	43
6-Sep	soil	6	8702	5	21
30-Jan	soil	1	1109	1021	584
30-Jan	soil	2	304	43	85
30-Jan	soil	3	12886	160	12886
30-Jan	soil	4	17700	62	17700
30-Jan	soil	5	13467	35	13467
30-Jan	soil	6	15768	0	15768

APPENDIX 7: SOIL SURVEY MAP

The descriptions of the soil types represented below are found in the Soil Survey document (see References*).*

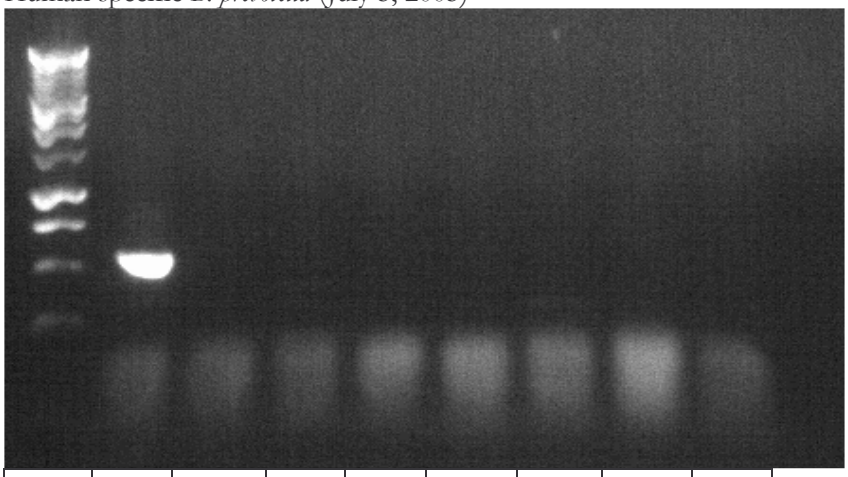
APPENDIX 8: GEL IMAGES

Human specific *B. prevotella* (June 27, 2005)



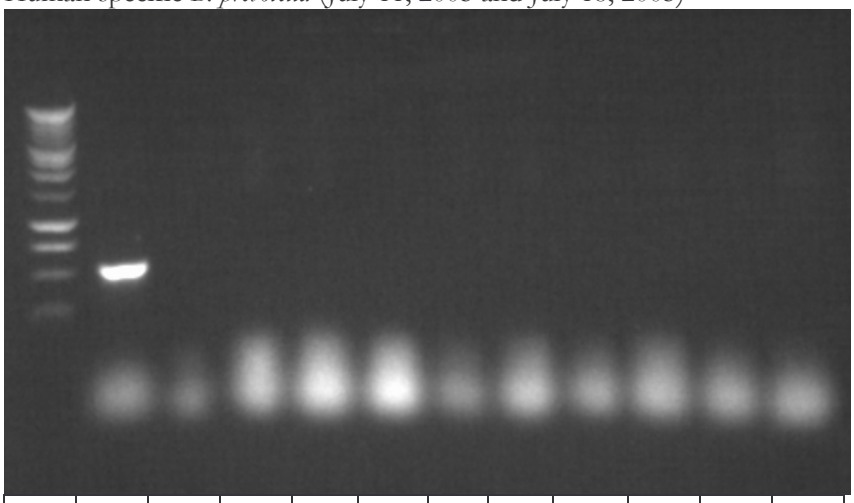
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* (July 5, 2005)



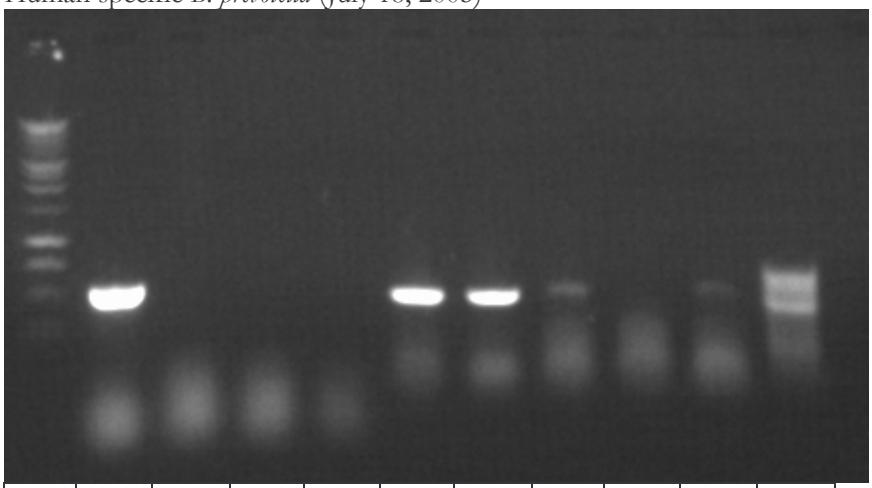
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* (July 11, 2005 and July 11, 2005)



1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 1	2	3	4	5	6	site 1	2	3	(-)
		7-11-05						7-18-05			

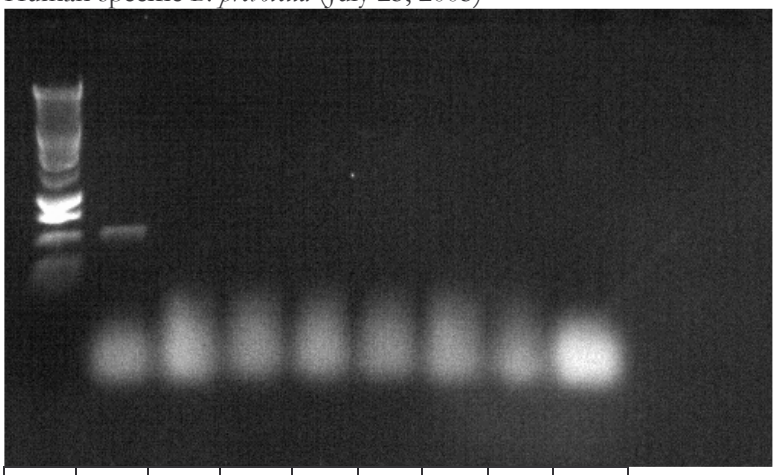
Human specific *B. prevotella* (July 18, 2005)



1	2	3	4	5	6	7	8	9	10	11
LADDER	(+)	Site 4	5	6	RC 1	RC 2	RC 3	RC (-)	RC (+)	50b
		7-18-05								

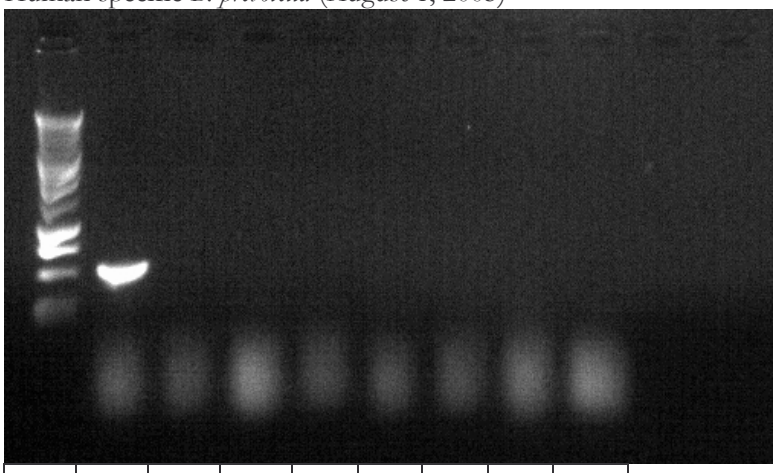
Note: RC = *Rhodococcus coprophilus,* 1, 2, and 3 refer to different known concentrations of *R. coprophilus*. 50b is a testing DNA ladder.

Human specific *B. prevotella* (July 25, 2005)



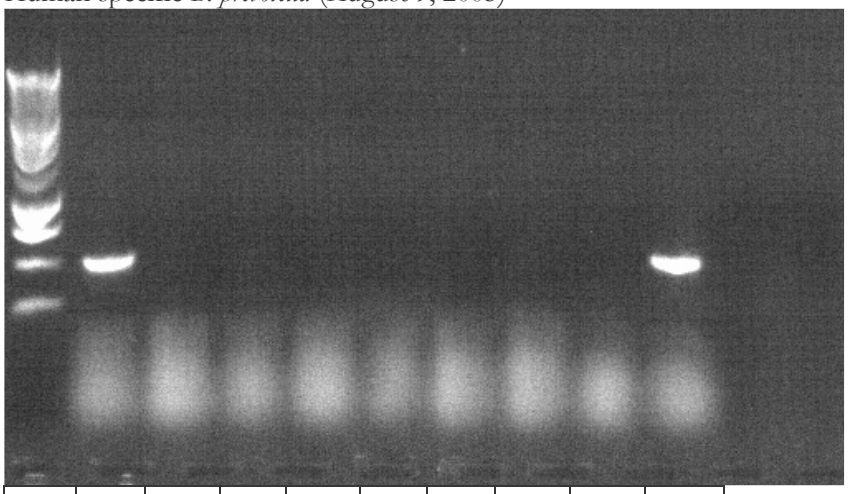
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* (August 1, 2005)



1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* (August 9, 2005)



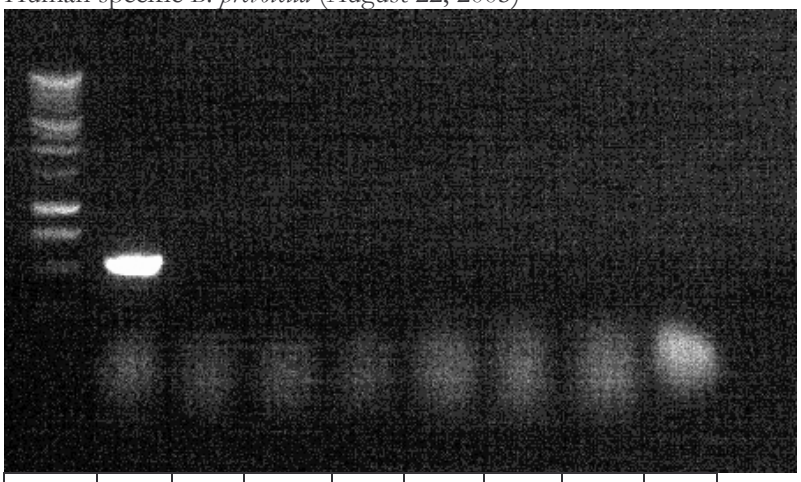
1	2	3	4	5	6	7	8	9	10
LADDER	(+)	site 1	2	3	4	5	6	(-)	H FT

Note: H FT refers to Human waste tested with Freeze-Thaw method

Human specific *B. prevotella* (August 15, 2005)

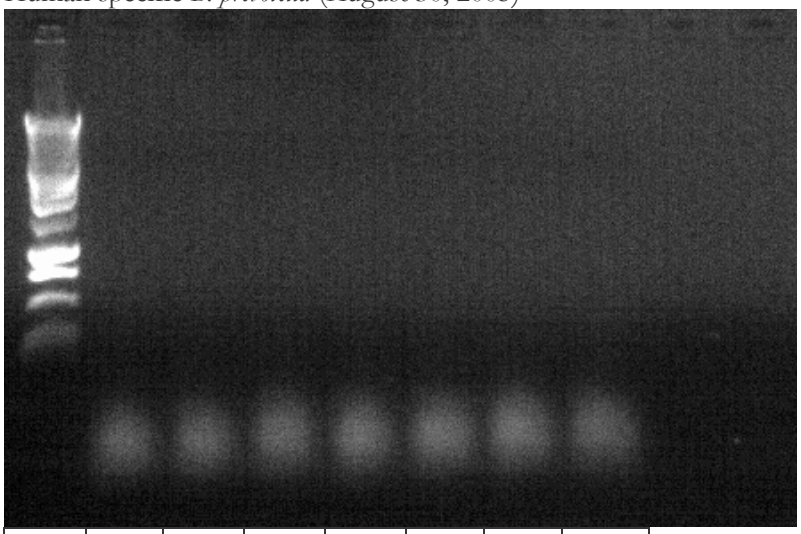
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* (August 22, 2005)



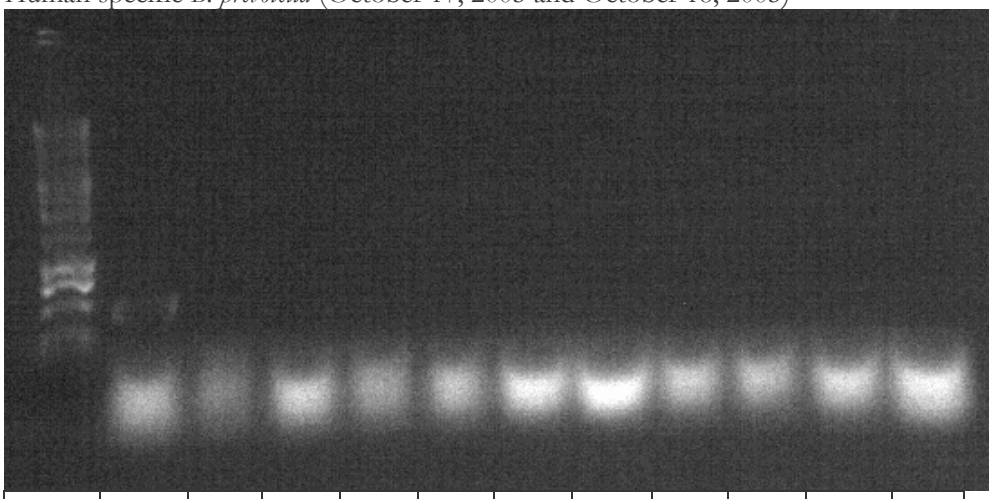
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* (August 30, 2005)



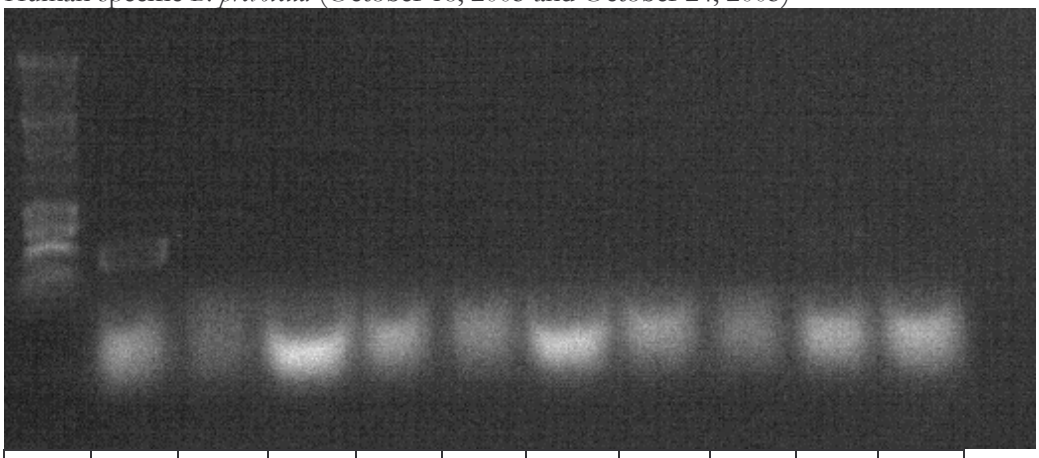
1	2	3	4	5	6	7	8
LADDER	(+)	site 1	2	3	4	5	6

Human specific *B. prevotella* (October 17, 2005 and October 18, 2005)



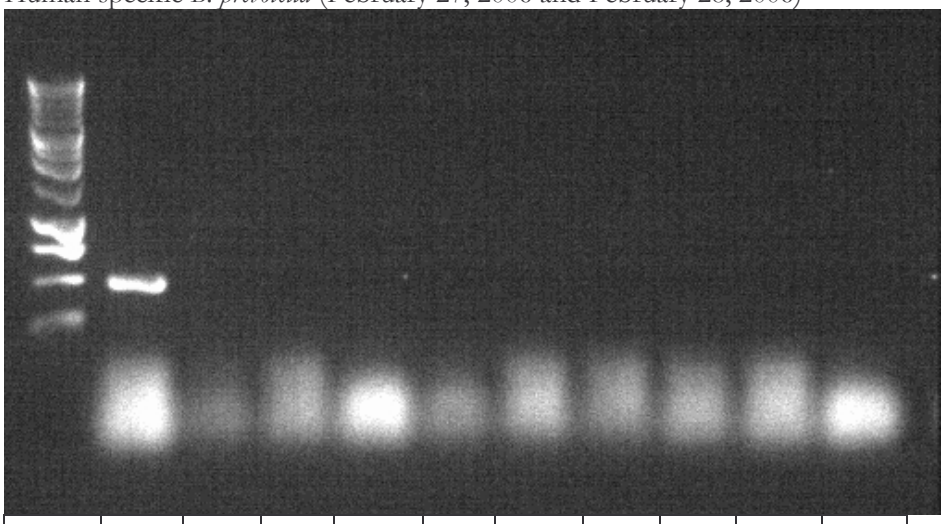
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 1	2	3	4	5	6	site 1	2	3	4
		10-17-05						10-18-05			

Human specific *B. prevotella* (October 18, 2005 and October 24, 2005)



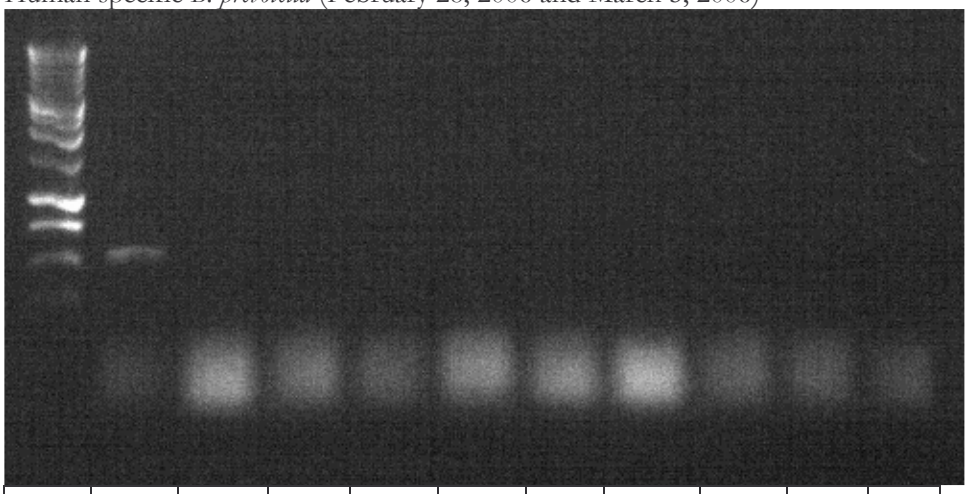
1	2	3	4	5	6	7	8	9	10	11
LADDER	(+)	site 5	6	site 1	2	3	4	5	6	(-)
		10-18-05		10-24-05						

Human specific *B. prevotella* (February 27, 2006 and February 28, 2006)



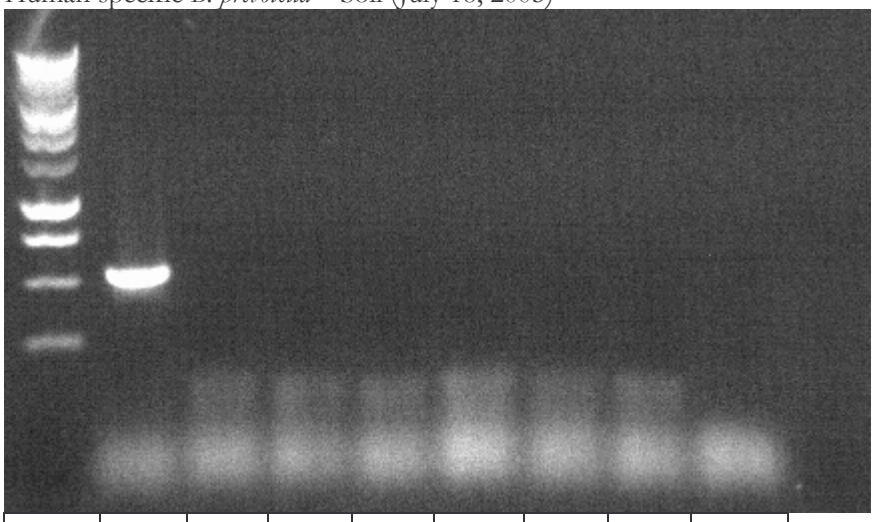
1	2	3	4	5	6	7	8	9	10	11
LADDER	(+)	site 1	2	3	4	5	site 1	2	3	4
		2-27-06					2-28-06			

Human specific *B. prevotella* (February 28, 2006 and March 5, 2006)



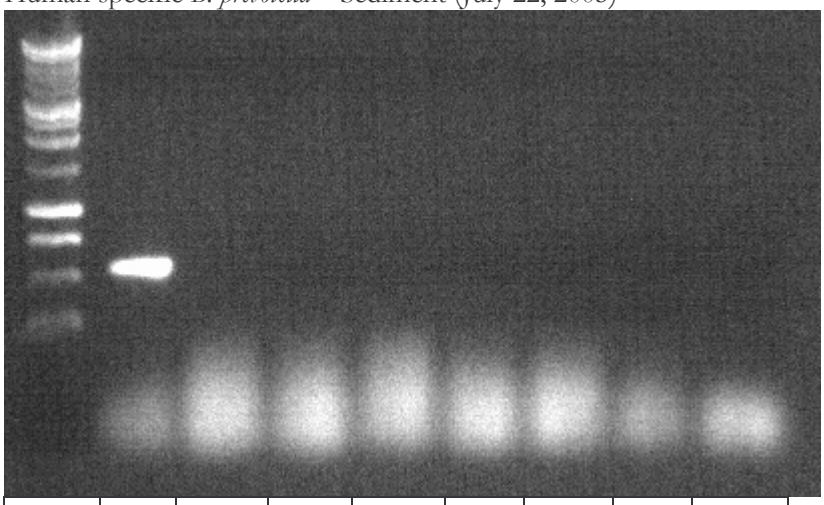
1	2	3	4	5	6	7	8	9	10	11
LADDER	(+)	site 5	6	site 1	2	3	4	5	6	(-)
		2-28-06		3-5-06						

Human specific *B. prevotella* – Soil (July 18, 2005)



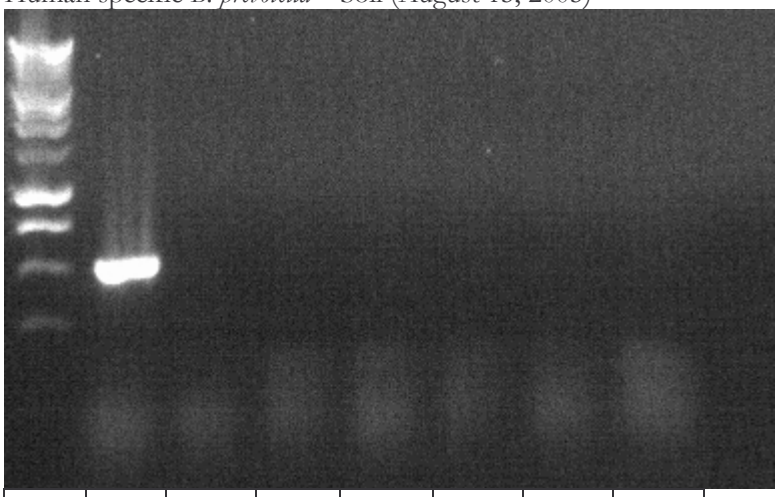
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* – Sediment (July 22, 2005)



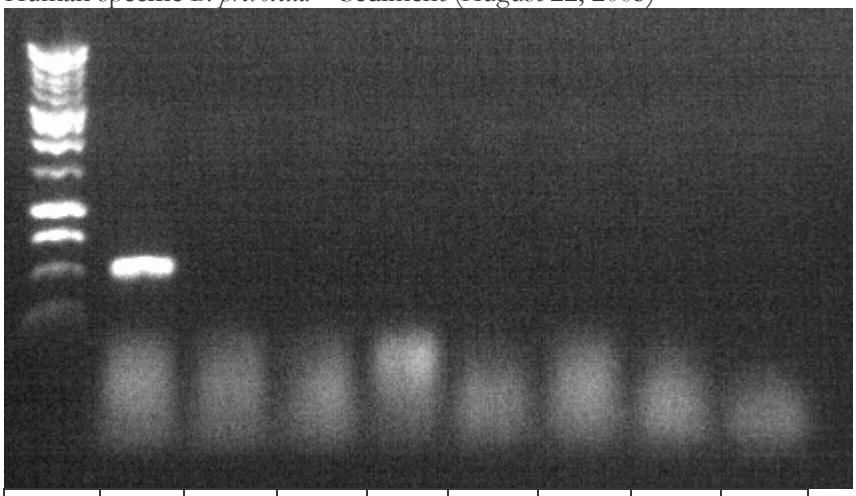
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* – Soil (August 15, 2005)



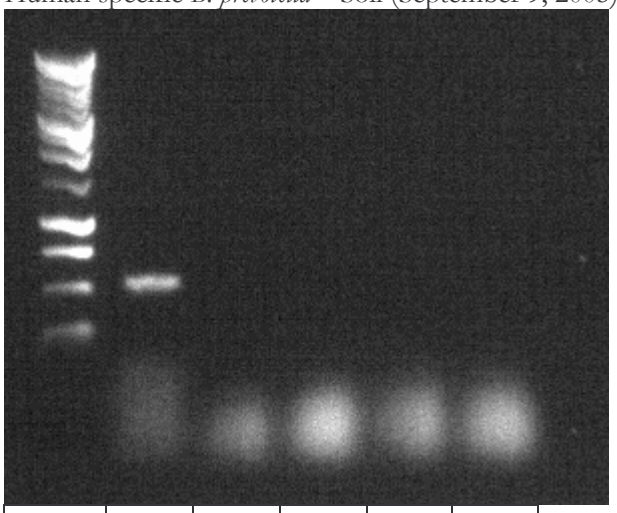
1	2	3	4	5	6	7	8
LADDER	(+)	site 1	2	3	4	5	6

Human specific *B. prevotella* – Sediment (August 22, 2005)



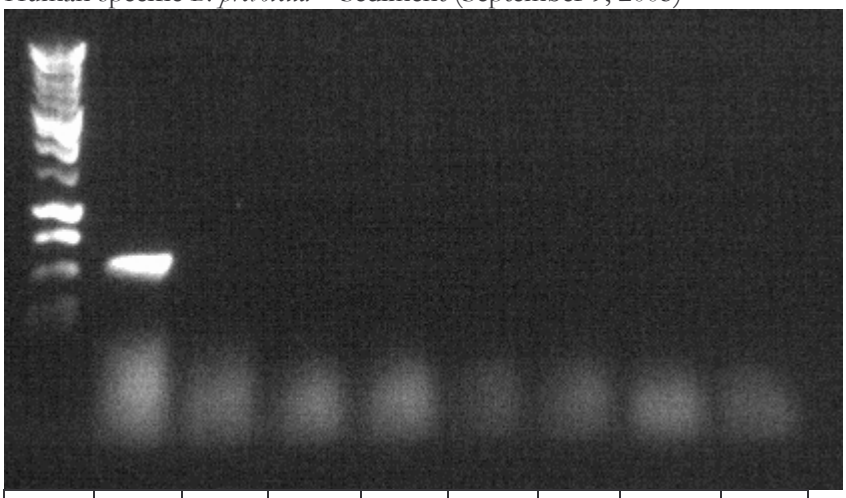
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* – Soil (September 9, 2005)



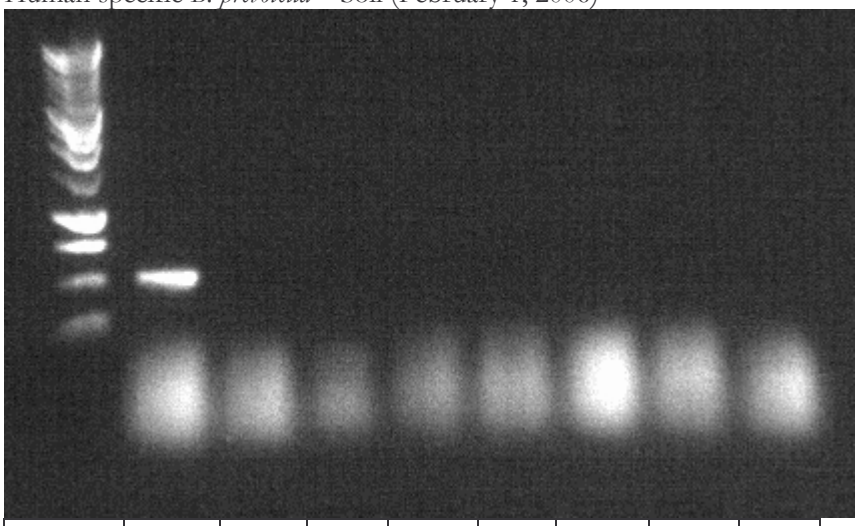
1	2	3	4	5	6
LADDER	(+)	site 1	2	3	5

Human specific *B. prevotella* – Sediment (September 9, 2005)



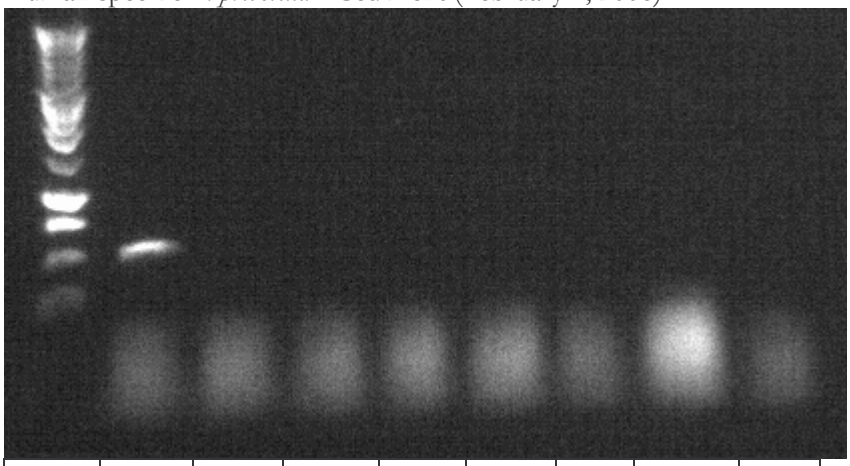
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* – Soil (February 1, 2006)



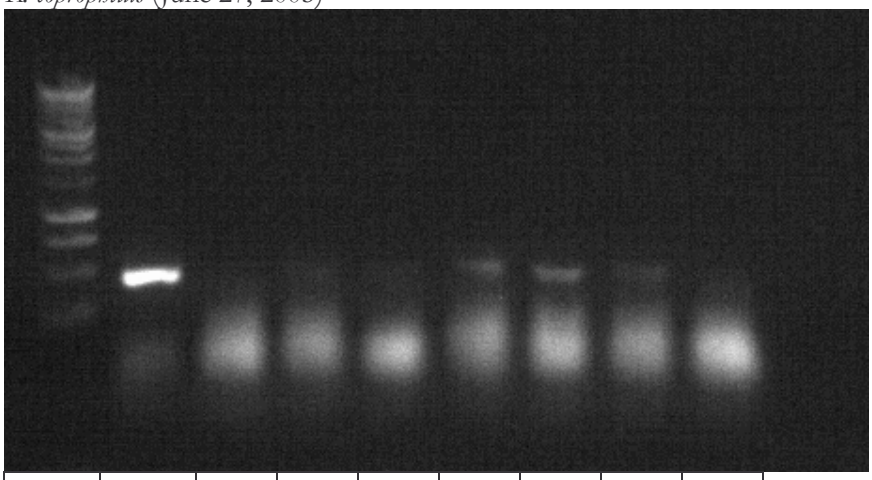
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Human specific *B. prevotella* – Sediment (February 1, 2006)



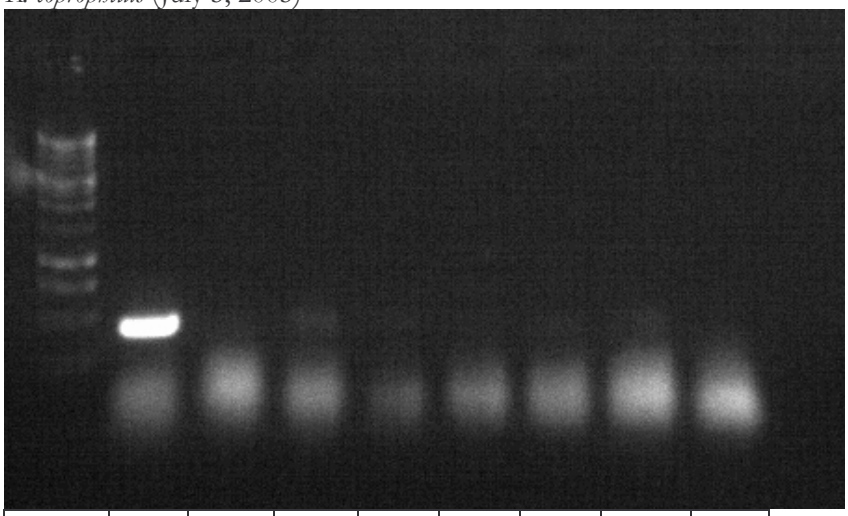
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

R. coprophilus (June 27, 2005)



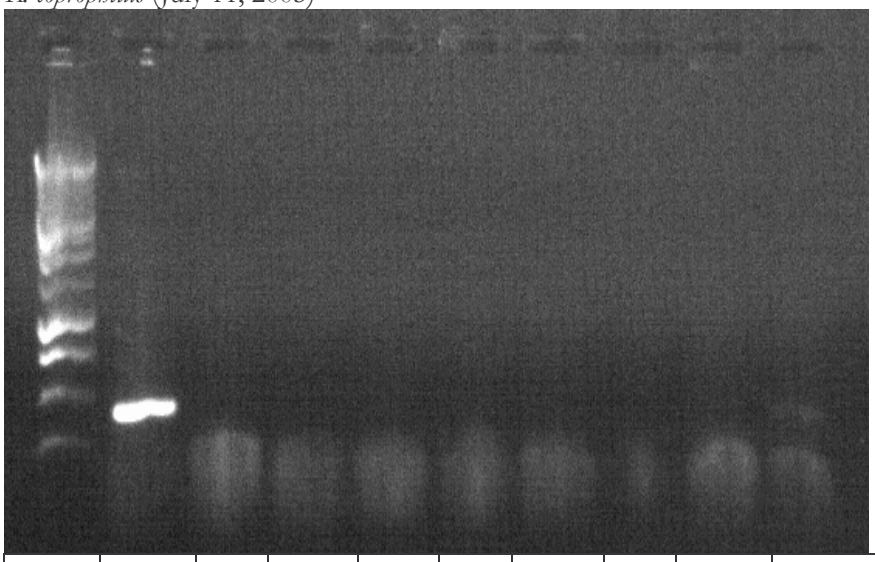
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

R. coprophilus (July 5, 2005)



1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

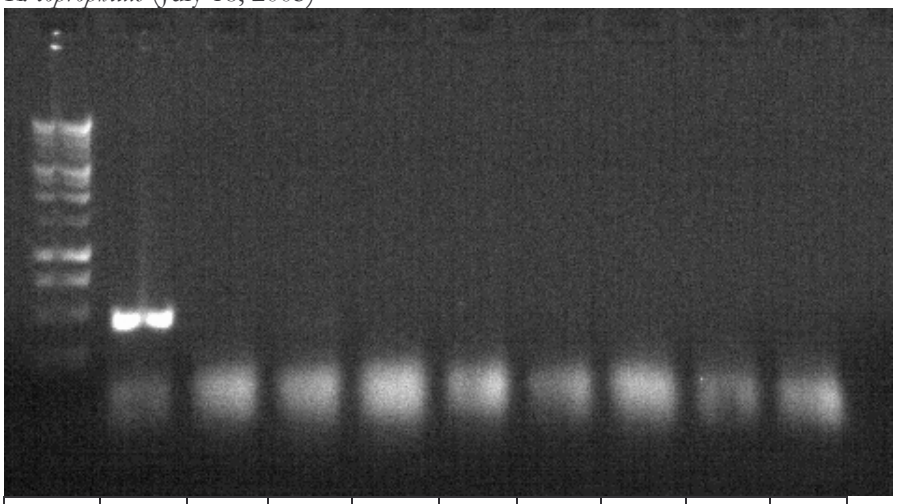
R. coprophilus (July 11, 2005)



1	2	3	4	5	6	7	8	9	10
LADDER	(+)	site 1	2	3	4	5	6	(-)	Horse FT 2/6

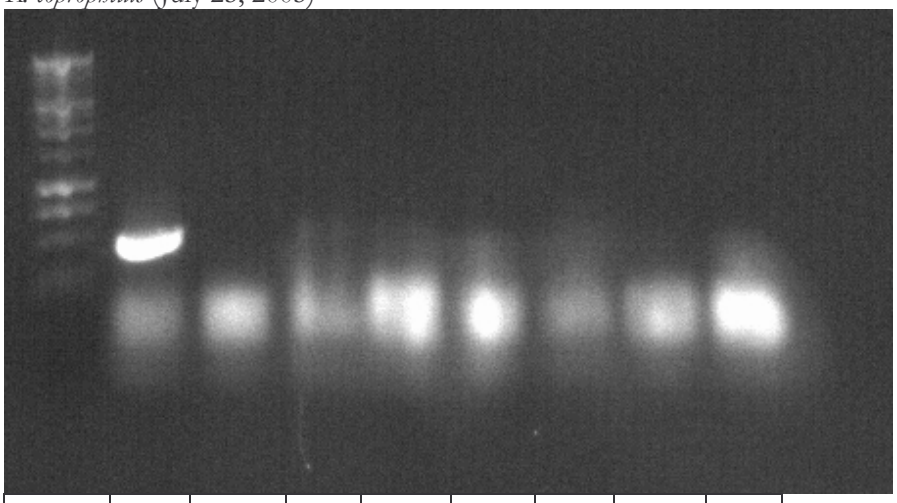
Note: Horse FT refers to Horse waste tested for Freeze-Thaw method

R. coprophilus (July 18, 2005)



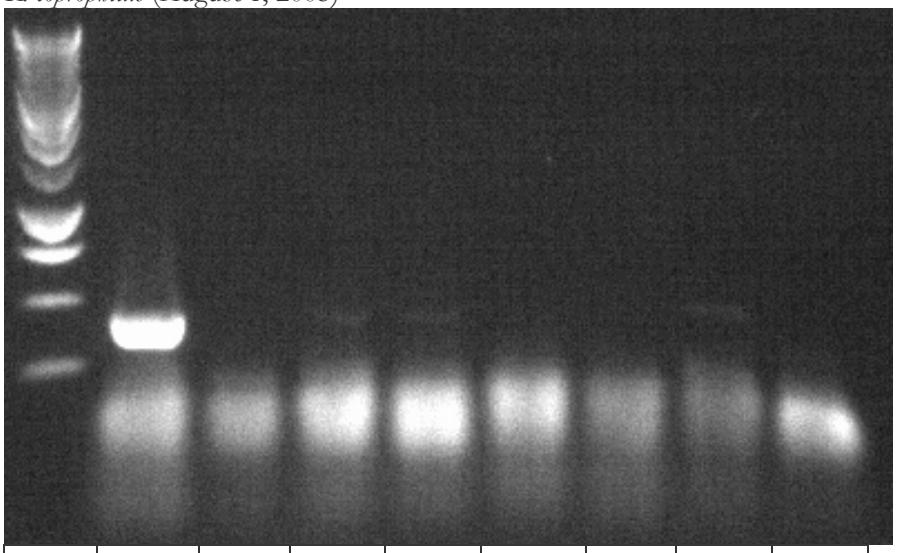
1	2	3	4	5	6	7	8	9	10
LADDER	(+)	site 1	1	2	3	4	5	6	(-)

R. coprophilus (July 25, 2005)



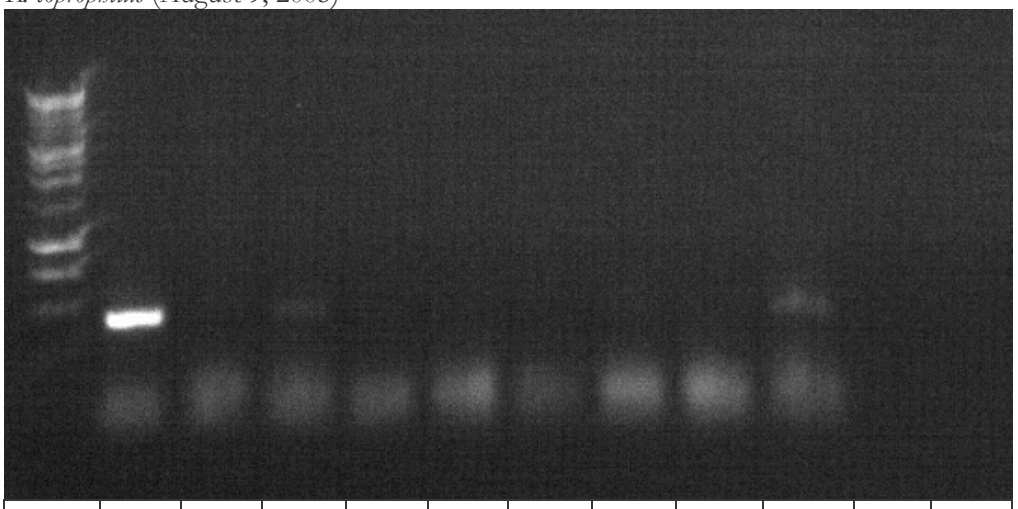
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

R. coprophilus (August 1, 2005)



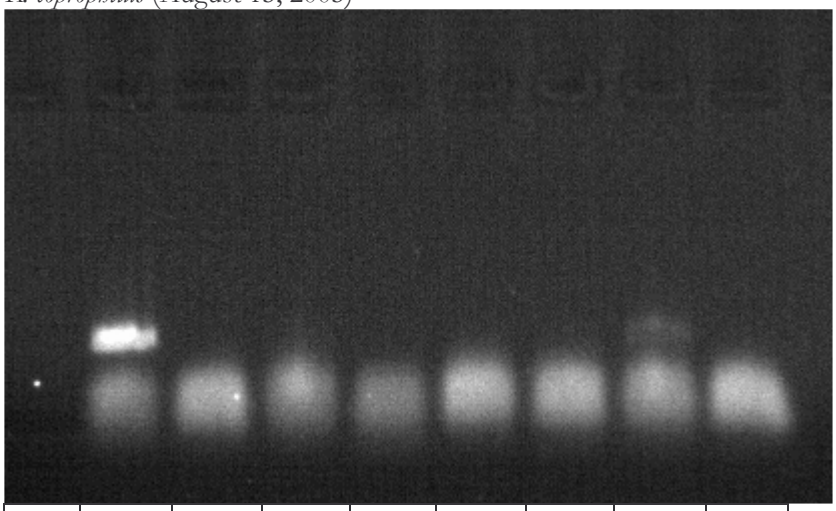
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

R. coprophilus (August 9, 2005)



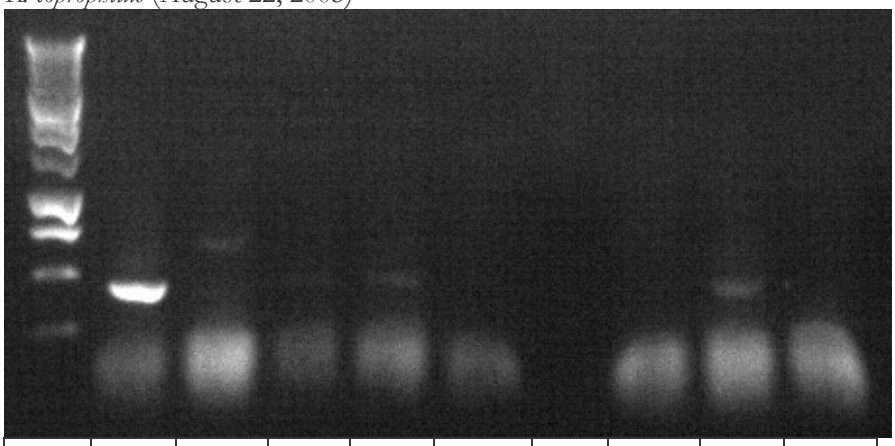
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 1	2	3	4	5	6	(-)	site 6		(+)
									8-15		7-15

R. coprophilus (August 15, 2005)



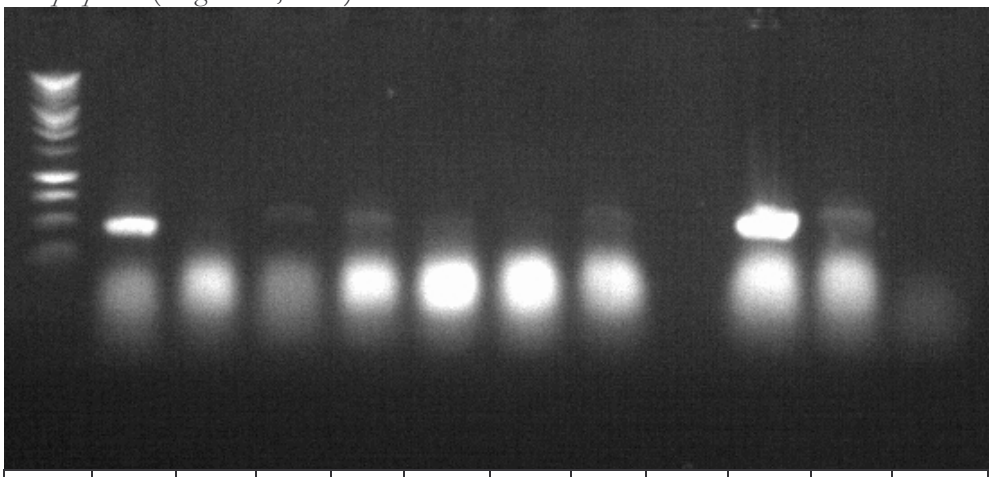
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

R. coprophilus (August 22, 2005)



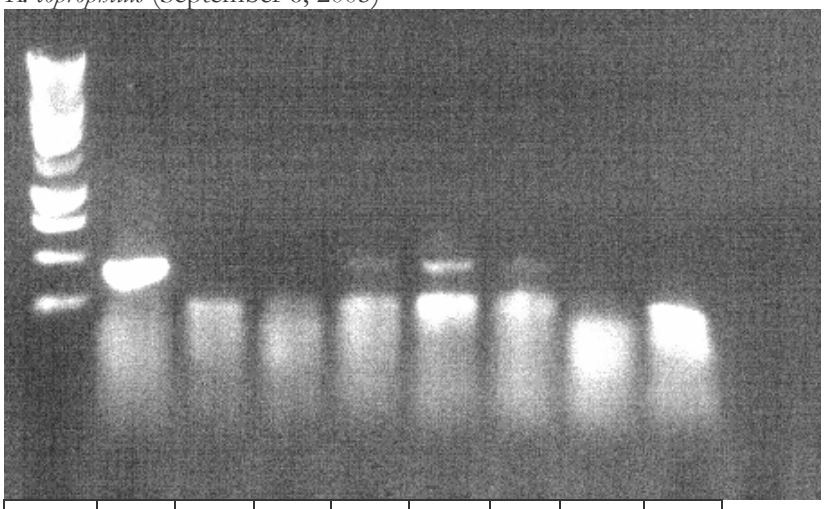
1	2	3	4	5	6	7	8	9	10
LADDER	(+)	site 1	2	3	4		5	6	(-)

R. coprophilus (August 30, 2005)



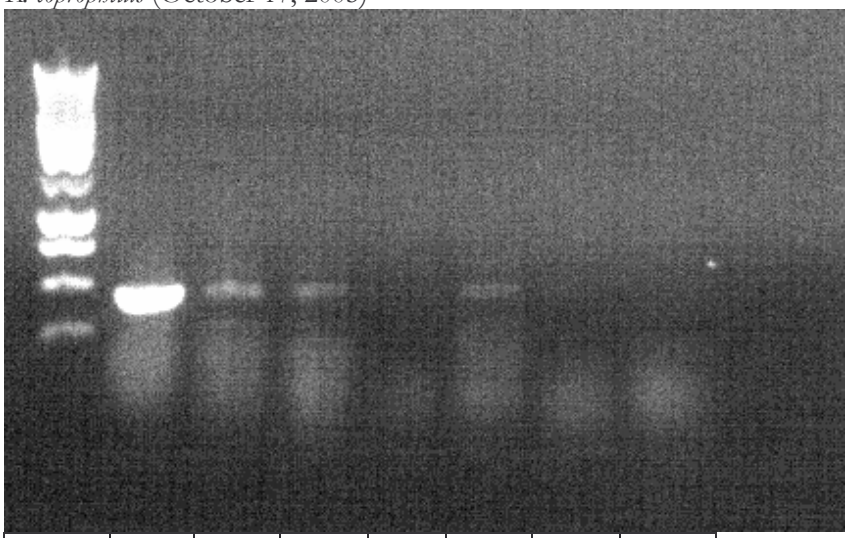
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 1	2	3	4	5	6		(+)	site 6	(+)
									8-9	8-15	7-11

R. coprophilus (September 6, 2005)



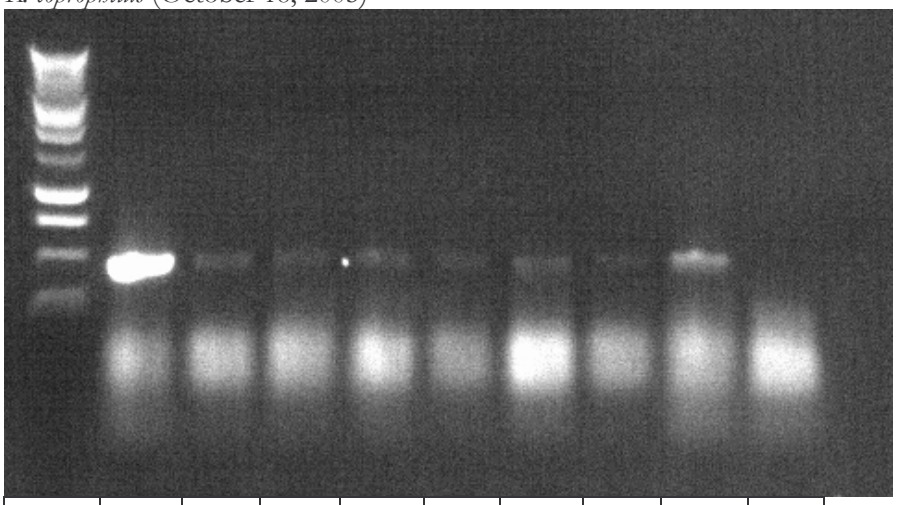
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

R. coprophilus (October 17, 2005)



1	2	3	4	5	6	7	8
LADDER	(+)	site 1	2	3	4	5	6

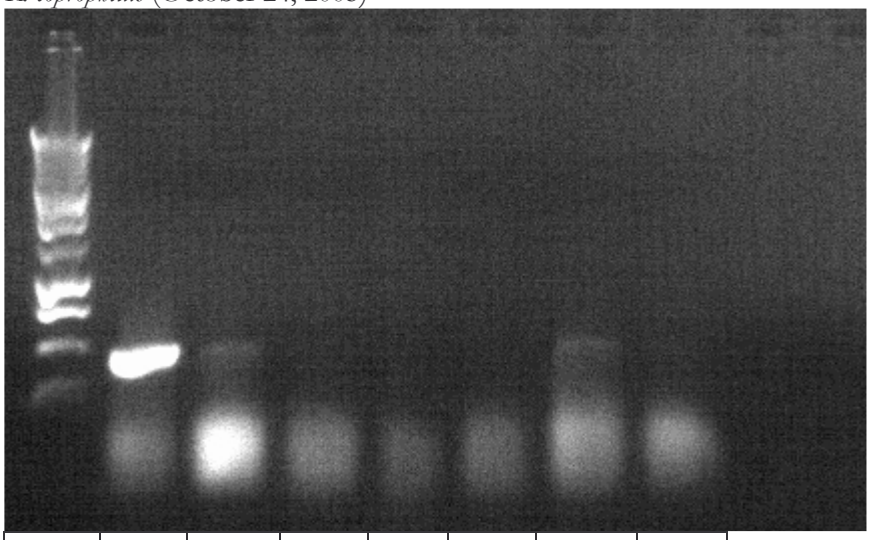
R. coprophilus (October 18, 2005)



1	2	3	4	5	6	7	8	9	10
LADDER	(+)	site 1	2	3	4	5	6	H FT 2	(-)

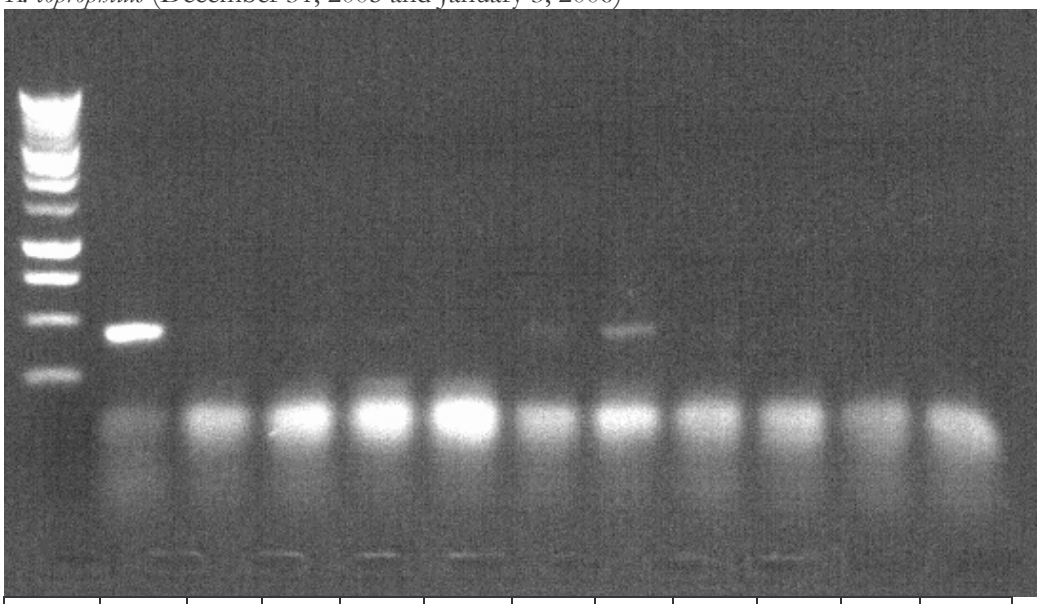
Note: H FT refers to horse waste tested for Freeze-Thaw method

R. coprophilus (October 24, 2005)



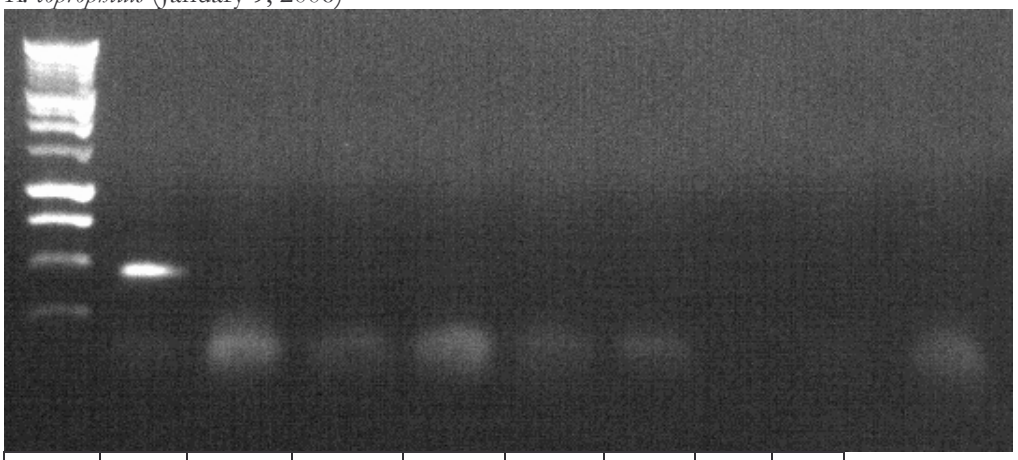
1	2	3	4	5	6	7	8
LADDER	(+)	site 1	2	3	4	5	6

R. coprophilus (December 31, 2005 and January 3, 2006)



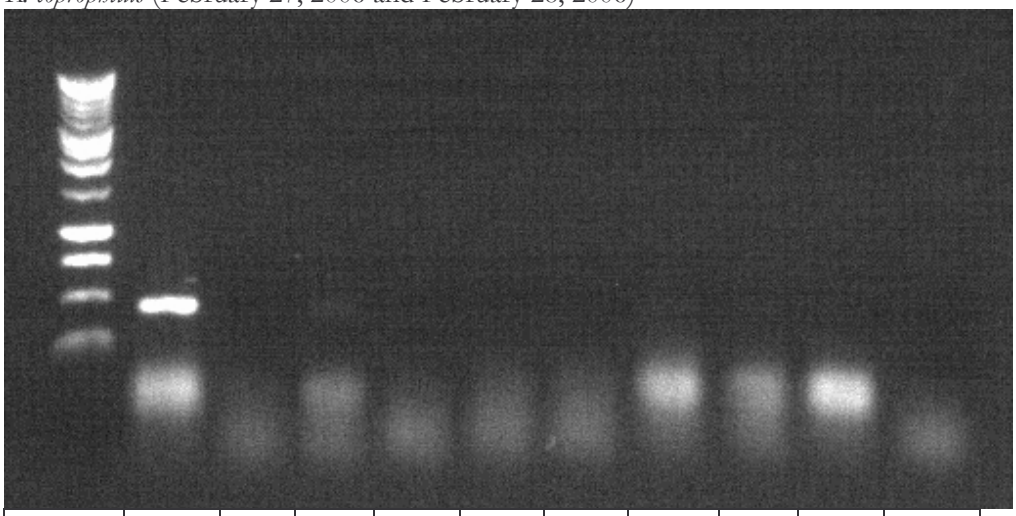
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 1	2	3	4	site 1	2	3	4	5	6
		12-31-05				1-3-06					

R. coprophilus (January 9, 2006)



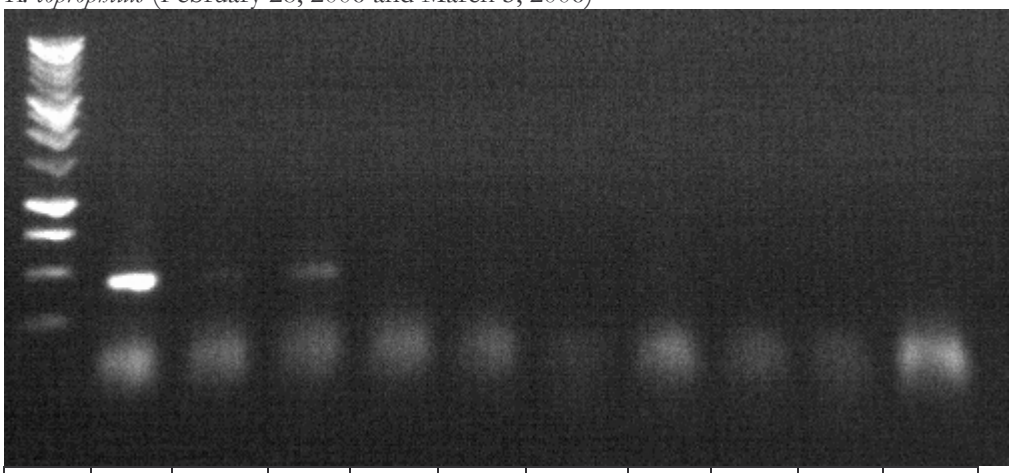
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

R. coprophilus (February 27, 2006 and February 28, 2006)



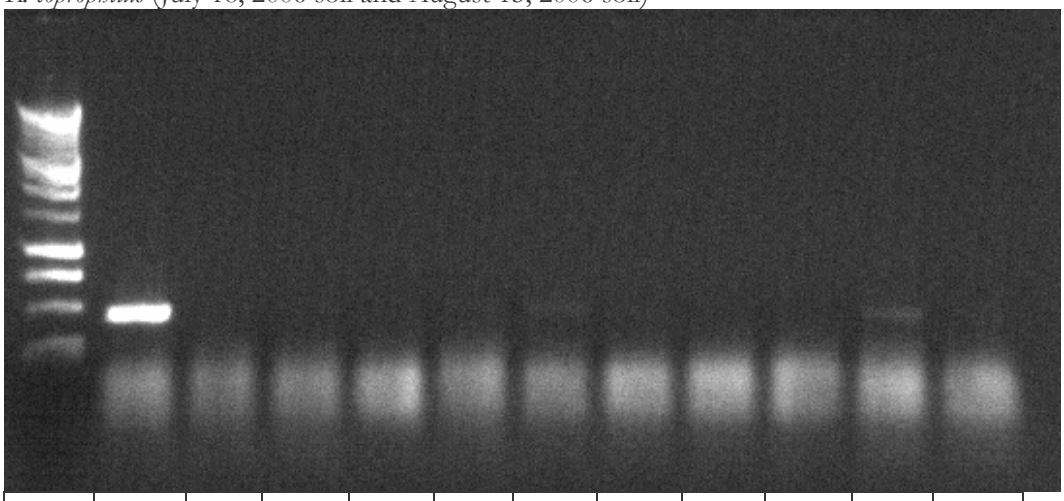
1	2	3	4	5	6	7	8	9	10	11
LADDER	(+)	site 1	2	3	4	5	site 1	2	3	4
		2-27-06					2-28-06			

R. coprophilus (February 28, 2006 and March 5, 2006)



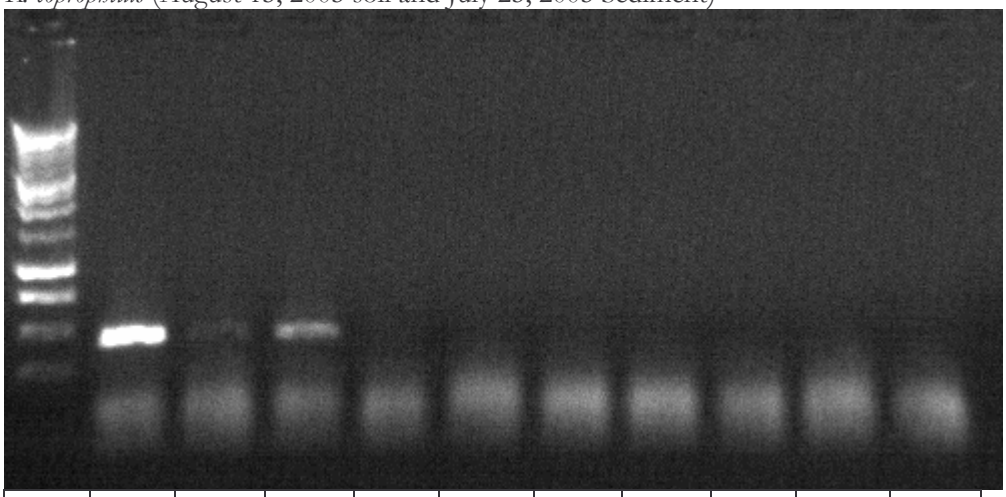
1	2	3	4	5	6	7	8	9	10	11
LADDER	(+)	site 5	6	site 1	2	3	4	5	6	(-)
		2-28-06		3-5-06						

R. coprophilus (July 18, 2006 soil and August 15, 2006 soil)



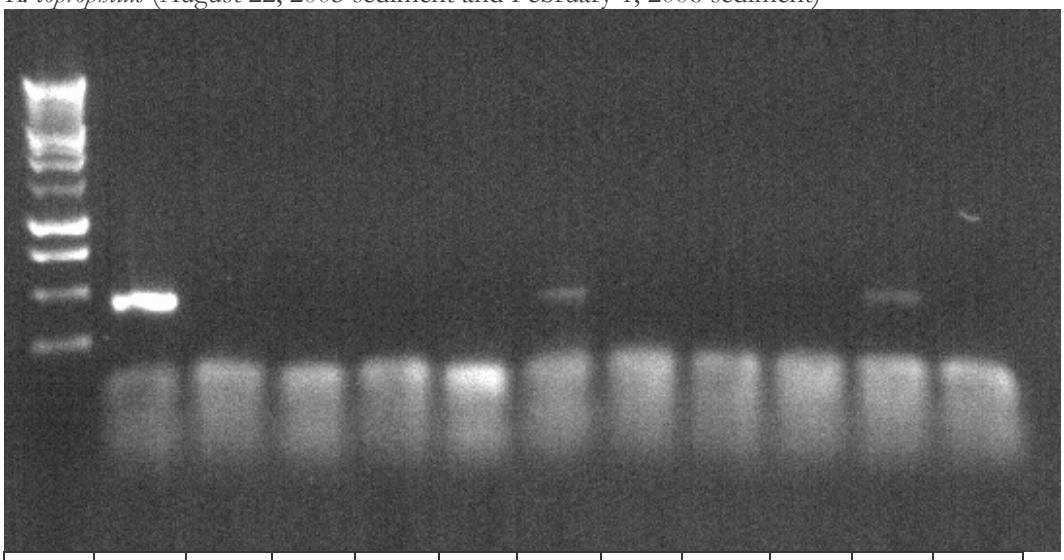
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 1	2	3	4	5	6	site 1	2	3	4
		7-18-06 soil						8-15-06 soil			

R. coprophilus (August 15, 2005 soil and July 25, 2005 Sediment)



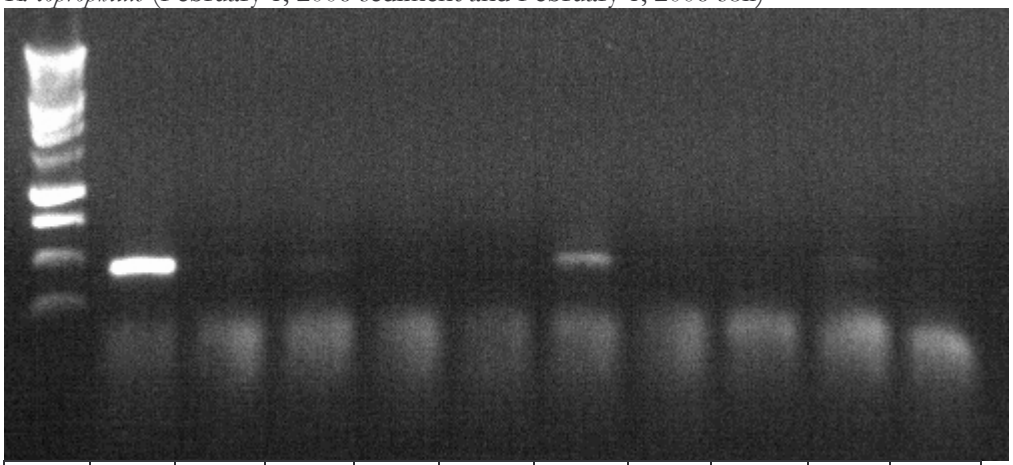
1	2	3	4	5	6	7	8	9	10	11
LADDER	(+)	site 5	6	site 1	2	3	4	5	6	(-)
		8-15-06 soil		7-25-06 sediment						

R. coprophilus (August 22, 2005 sediment and February 1, 2006 sediment)



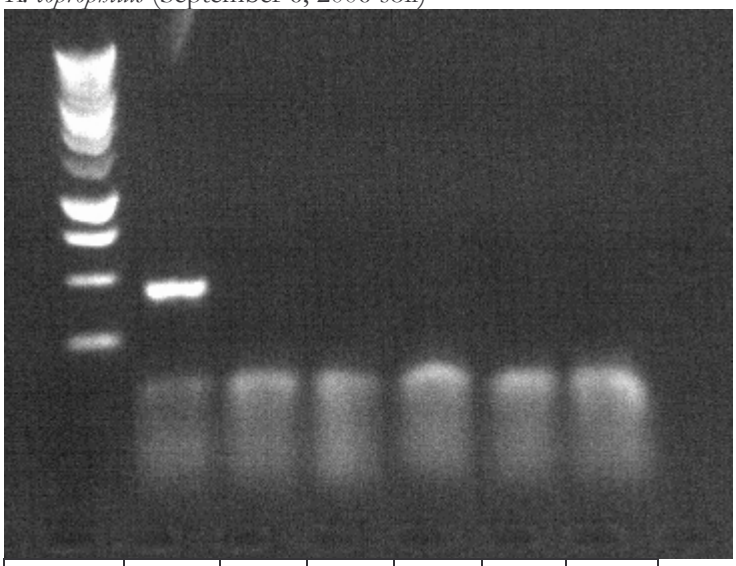
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 1	2	3	4	5	6	site 1	2	3	4
		8-22-05 sediment						2-1-06 sediment			

R. coprophilus (February 1, 2006 sediment and February 1, 2006 soil)



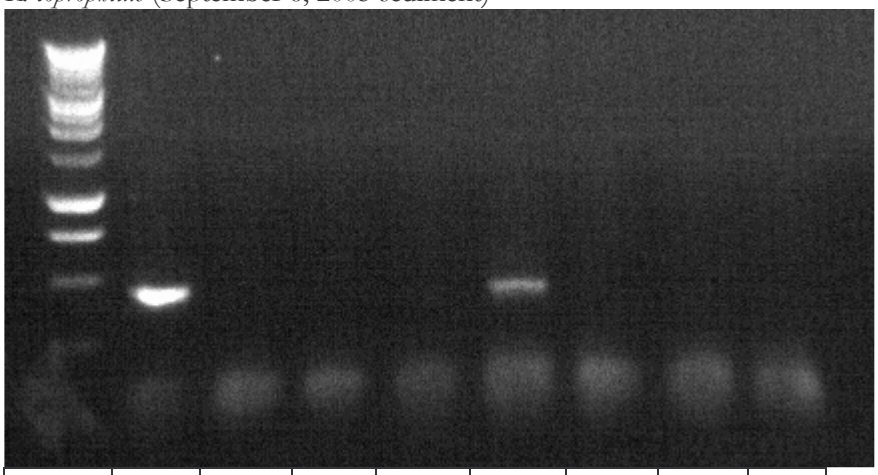
1	2	3	4	5	6	7	8	9	10	11
LADDER	(+)	site 5	6	site 1	2	3	4	5	6	(-)
		2-1-06 sediment		2-1-06 soil						

R. coprophilus (September 6, 2006 soil)



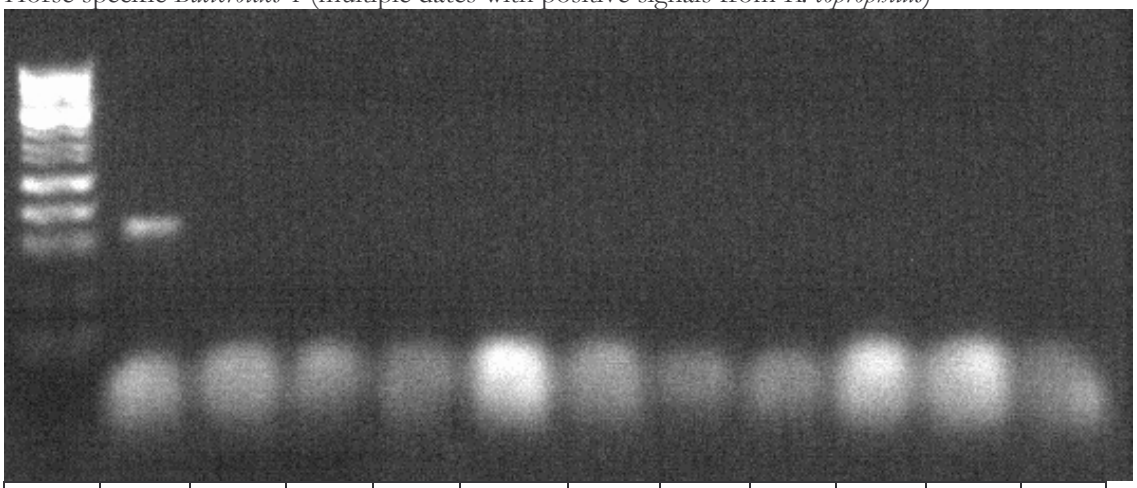
1	2	3	4	5	6	7
LADDER	(+)	site 1	2	3	5	(-)

R. coprophilus (September 6, 2005 sediment)



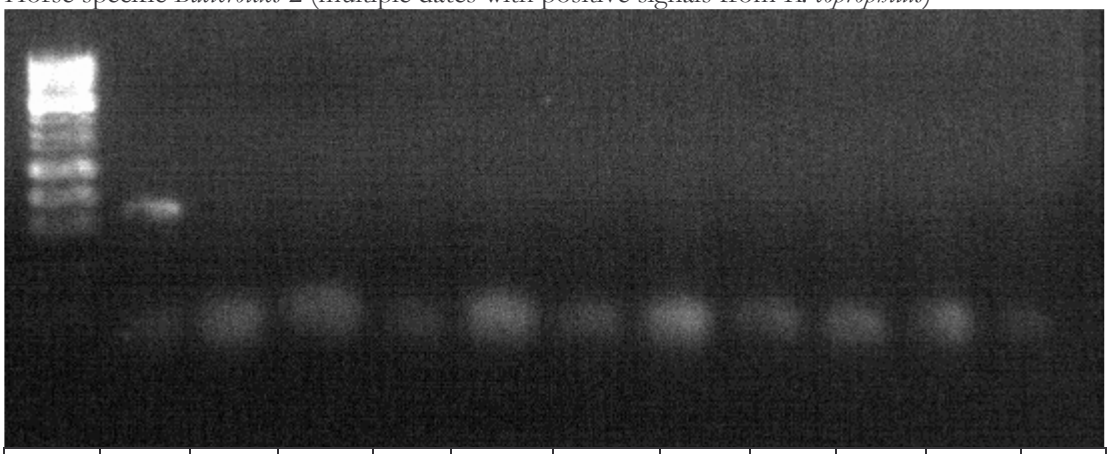
1	2	3	4	5	6	7	8	9
LADDER	(+)	site 1	2	3	4	5	6	(-)

Horse specific *Bacteroides* 1 (multiple dates with positive signals from *R. coprophilus*)



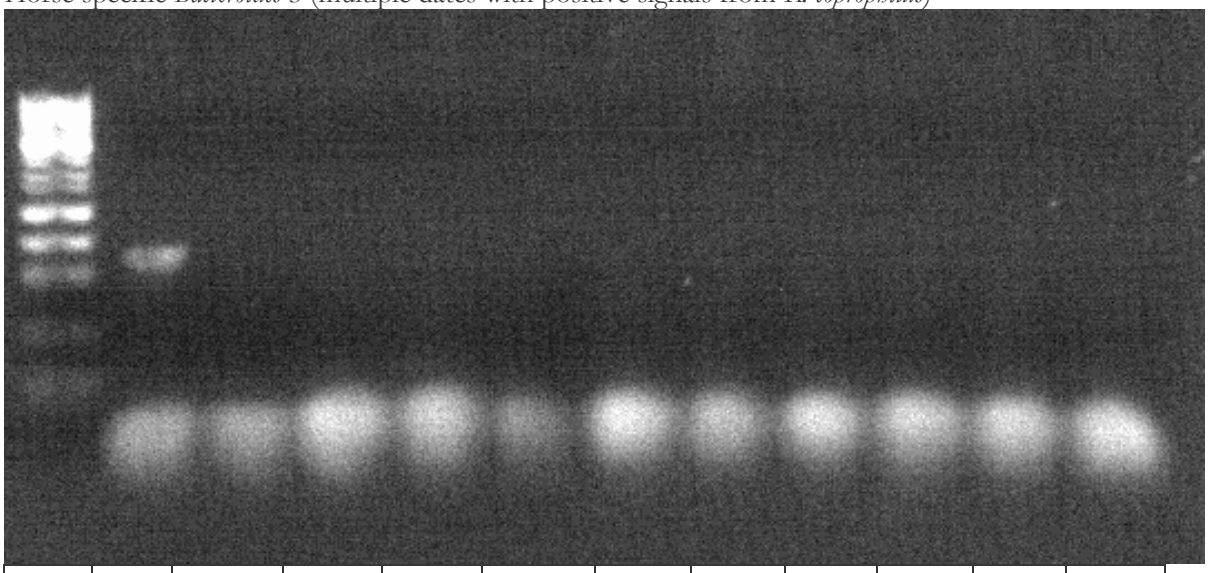
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 4	5	6	2	6	2	3	4	6	(-)
		6-27	6-27	6-27	8-9	8-15	8-22	8-30	8-30	8-30	

Horse specific *Bacteroides* 2 (multiple dates with positive signals from *R. coprophilus*)



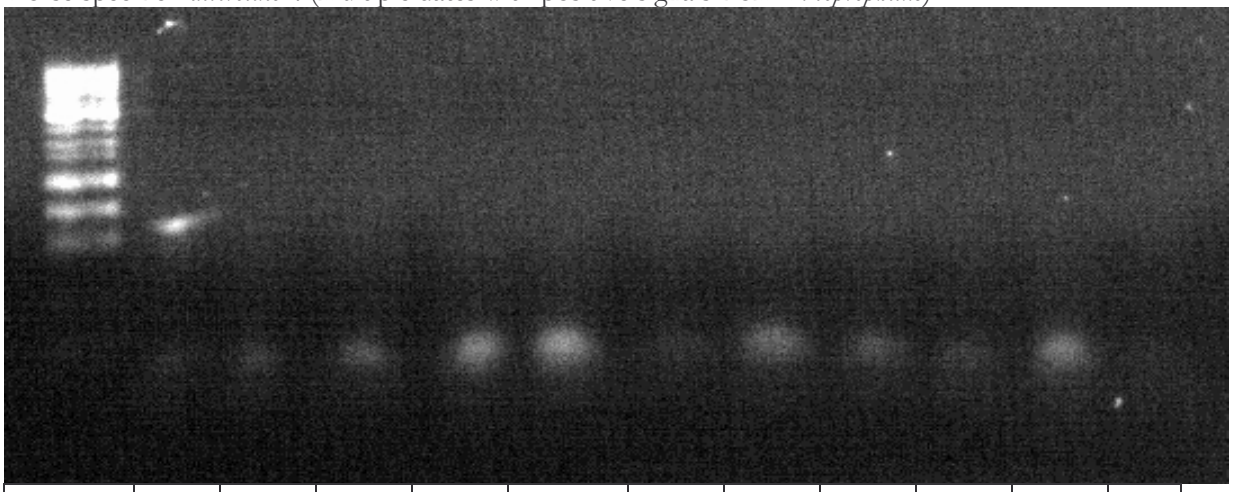
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 3	4	5	1	2	4	1	2	3	(-)
		9-6	9-6	9-6	10-17	10-17	10-17	10-18	10-18	10-18	

Horse specific *Bacteroides* 3 (multiple dates with positive signals from *R. coprophilus*)



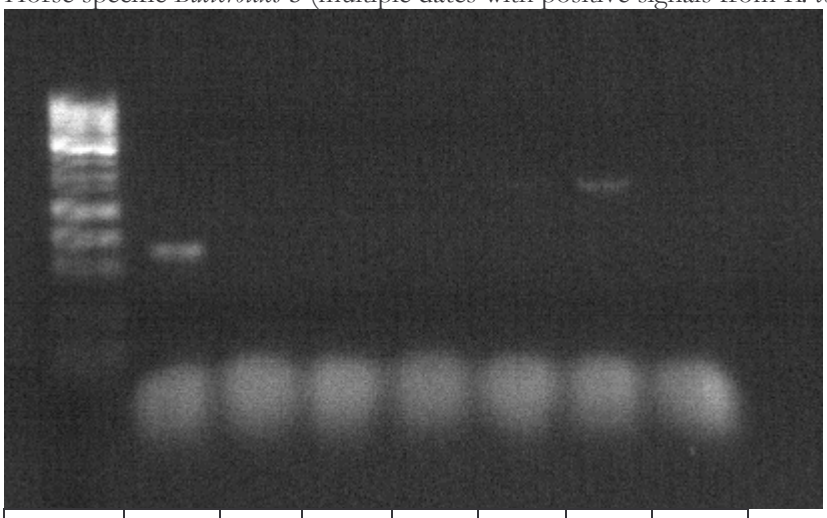
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 4	5	6	1	5	3	1	2	2	(-)
		10-18	10-18	10-18	10-24	10-24	12-31	1-3	1-3	2-27	

Horse specific *Bacteroides* 4 (multiple dates with positive signals from *R. coprophilus*)



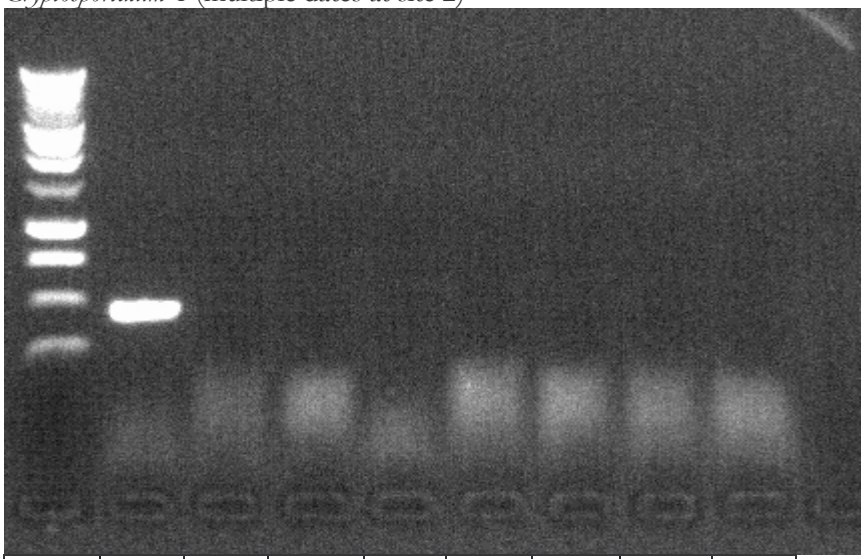
1	2	3	4	5	6	7	8	9	10	11	12
LADDER	(+)	site 5	6	3 soil	5 soil	6 soil	3 soil	6 soil	3 sed.	5 sed.	6 sed.
		2-28	2-28	8-15	8-15	8-15	2-1	2-1	2-1	2-1	2-1

Horse specific *Bacteroides* 5 (multiple dates with positive signals from *R. coprophilus*)



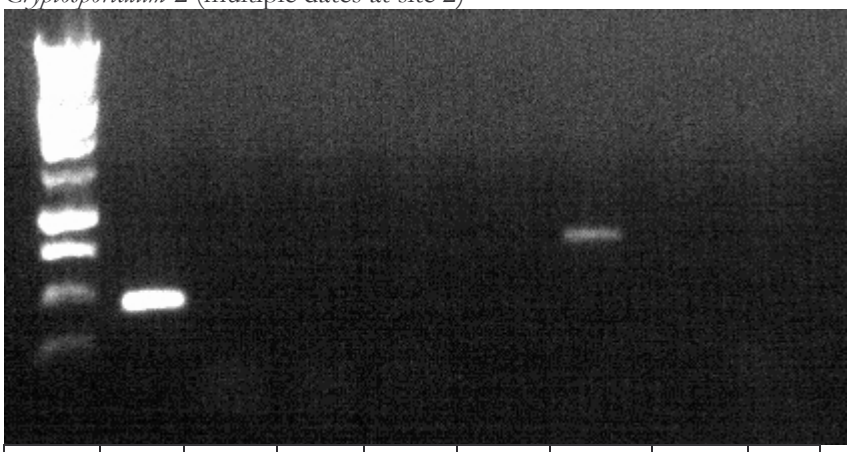
1	2	3	4	5	6	7	8
LADDER	(+)	site 3	6	2	5 sed.	4 sed.	(-)
		8-22	8-22	8-30	8-22	9-6	

Cryptosporidium 1 (multiple dates at site 2)



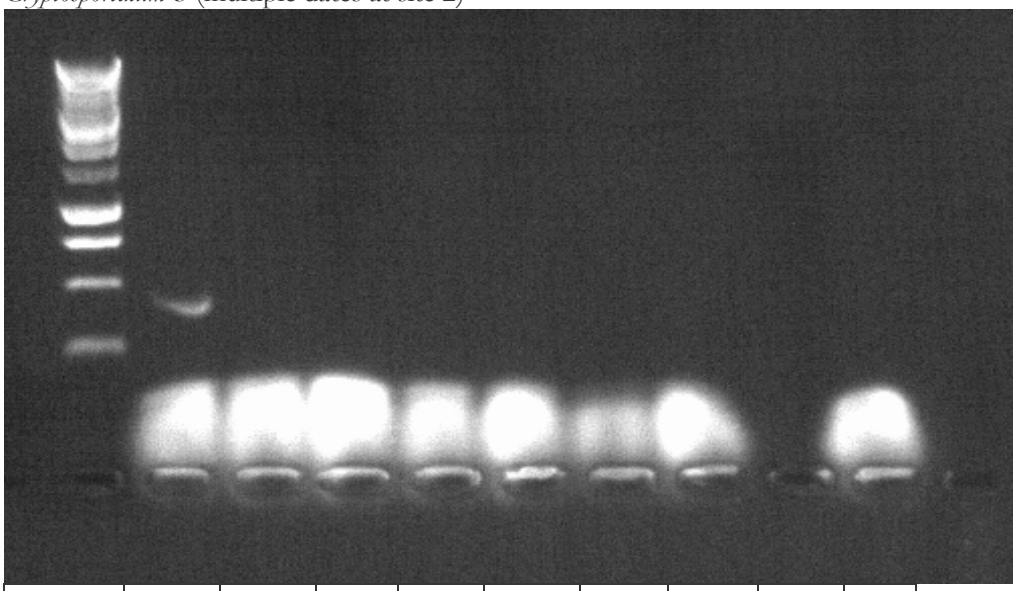
1	2	3	4	5	6	7	8	9
LADDER	(+)	6-27	7-5	7-18	7-25	8-1	8-9	(-)

Cryptosporidium 2 (multiple dates at site 2)



1	2	3	4	5	6	7	8	9
LADDER	(+)	8-15	8-22	9-6	10-17	10-18	10-24	(-)

Cryptosporidium 3 (multiple dates at site 2)



1	2	3	4	5	6	7	8	9	10
LADDER	(+)	12-31	1-3	1-9	2-27	2-28	3-5		(-)

APPENDIX 9: SOIL TEXTURE DATA

Sieve Data		SAND (μm)						
Sample	**O.D. wt (g)**	**O.D. wt.(g)**	**500**	**250**	**180**	**125**	**Remainder**	**silt/clay (g)**
1	40.37	38.69	3.61	26.21	7.57	1.03	0.28	1.68
			91%	***26%***	***7%***	***5%***	***4%***	
2	41.47	39.32	1.94	11.55	17.55	7.58	0.71	2.15
			95%	***67%***	***25%***	***7%***	***5%***	
3A	41.45	20.37	1.1	4.35	4.4	3.58	5.98	21.08
			97%	***87%***	***76%***	***68%***	***53%***	
3B	40.54	32.02	0.34	3.99	10.45	9.45	6.83	8.52
			99%	***89%***	***64%***	***40%***	***23%***	
3C	40.24	26.02	0.42	2.34	4.82	6.17	11.3	14.22
			99%	***93%***	***81%***	***66%***	***38%***	
4A	40.02	21.97	0.68	3.24	3.81	4.18	9.1	18.05
			98%	***90%***	***81%***	***70%***	***48%***	
4B	40.81	9.26	0.92	1.76	0.97	1.08	3.57	31.55
			98%	***93%***	***91%***	***88%***	***80%***	
4C	40.78	13.08	0.52	2.24	2.15	2.93	4.28	27.7
			99%	***93%***	***88%***	***81%***	***70%***	
5A	37.92	22.55	0.75	3.3	4.33	4.62	8.59	15.37
			98%	***89%***	***78%***	***66%***	***43%***	
5B	37.55	22.82	1.82	2.11	3.1	4.78	10.05	14.73
			95%	***90%***	***81%***	***69%***	***42%***	
6A	40.02	13.37	0.62	1.25	1.13	1.37	8.04	26.65
			98%	***95%***	***93%***	***89%***	***69%***	
6B	40.04	23.02	1.3	3.63	4.22	4.85	8.06	17.02
			97%	***88%***	***77%***	***65%***	***45%***	

Note: Sample number refers to the sample site 1-6 and the letter A, B, or C refers to the soil horizon (Appendix 8). Samples were acquired on January 30, 2006.

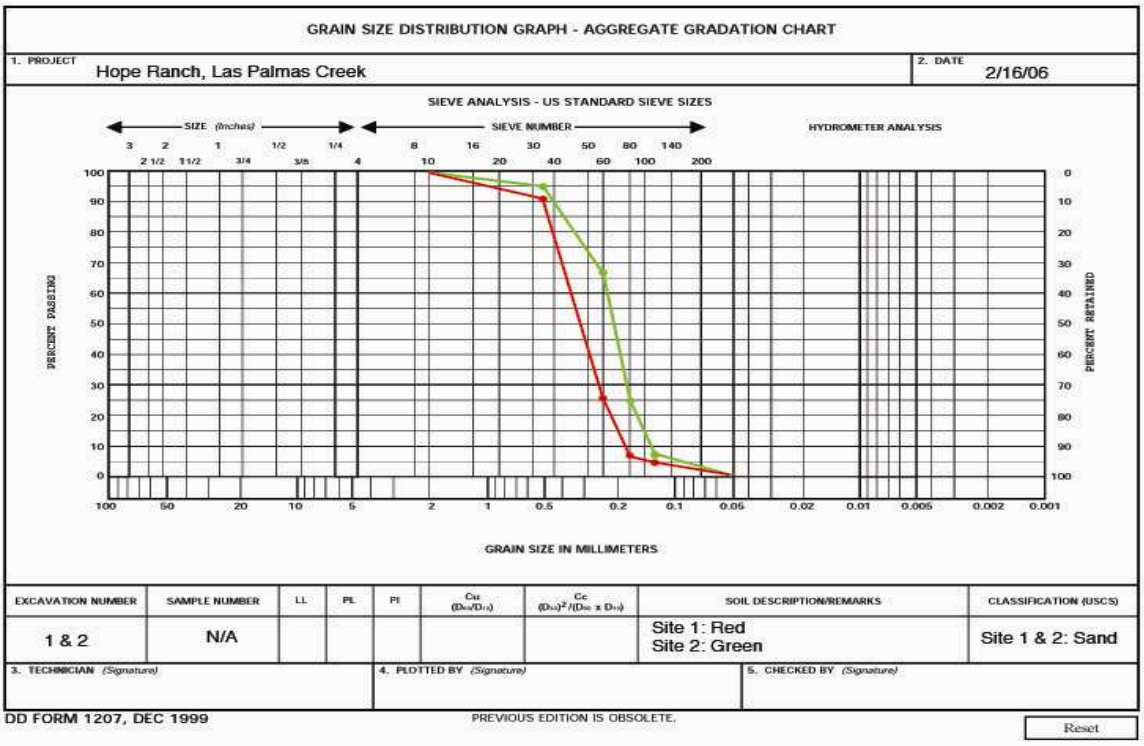
Hydrometer Analysis Data:

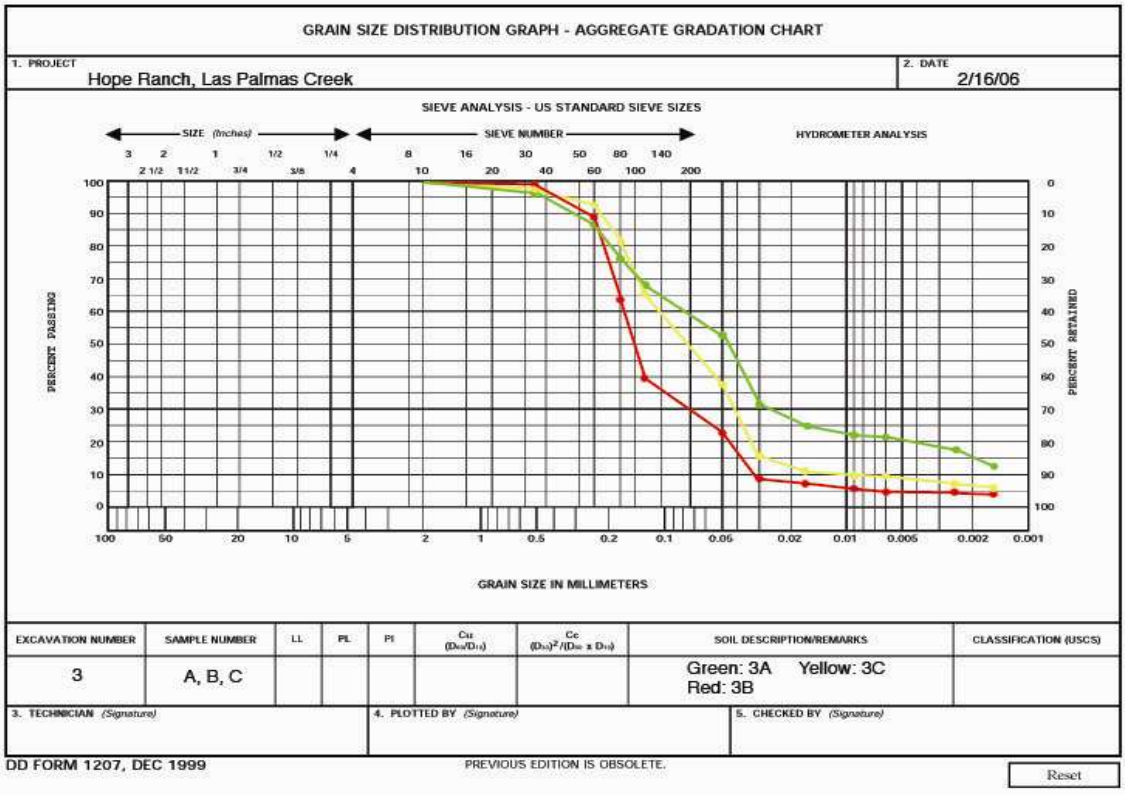
3A	Initial Sample Mass (g)		41.45		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	14	8.204	13.99896	0.05893	34%
3	13	8.368	14.16296	0.02794	31%
10	10.5	8.778	14.57296	0.01552	25%
30	9.5	8.942	14.73696	0.00901	23%
60	9	9.024	14.81896	0.00639	22%
420	7	9.352	15.14696	0.00244	17%
1260	6	9.516	15.31096	0.00142	14%
3B	Initial Sample Mass (g)		40.54		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	3.5	9.926	15.72096	0.062449	9%
3	3.5	9.926	15.72096	0.029439	9%
10	3	10.008	15.80296	0.016166	7%
30	2.5	10.09	15.88496	0.009358	6%
60	2	10.172	15.96696	0.006634	5%
420	2	10.172	15.96696	0.002507	5%
1260	1.5	10.254	16.04896	0.001451	4%

3C	Initial Sample Mass (g)		40.24		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	8	9.188	14.98296	0.060966	20%
3	6.5	9.434	15.22896	0.028974	16%
10	5	9.68	15.47496	0.015998	12%
30	4	9.844	15.63896	0.009285	10%
60	4	9.844	15.63896	0.006566	10%
420	3	10.008	15.80296	0.002495	7%
1255	2.5	10.09	15.88496	0.001447	6%
4A	Initial Sample Mass (g)		40.02		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	12.5	8.45	14.24496	0.059445	31%
3	11	8.696	14.49096	0.028264	27%
10	10	8.86	14.65496	0.015568	25%
30	8.5	9.106	14.90096	0.009063	21%
60	7	9.352	15.14696	0.006461	17%
420	5.5	9.598	15.39296	0.002462	14%
1255	5	9.68	15.47496	0.001428	12%
4B	Initial Sample Mass (g)		40.81		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	25	6.4	12.19496	0.055002	61%
3	24	6.564	12.35896	0.026102	59%
10	22	6.892	12.68696	0.014485	54%
30	19	7.384	13.17896	0.008524	47%
60	17.5	7.63	13.42496	0.006083	43%
420	5	9.68	15.47496	0.002468	12%
1290	0	10.5	16.29496	0.001445	0%
4C	Initial Sample Mass (g)		40.78		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	21	7.056	12.85096	0.056462	51%
3	20	7.22	13.01496	0.026786	49%
10	19	7.384	13.17896	0.014763	47%
30	17	7.712	13.50696	0.008629	42%
60	15.5	7.958	13.75296	0.006157	38%
420	12.5	8.45	14.24496	0.002368	31%
1290	6	9.516	15.31096	0.001401	15%
5A	Initial Sample Mass (g)		37.92		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	9	9.024	14.81896	0.060631	24%
3	8	9.188	14.98296	0.02874	21%
10	6.5	9.434	15.22896	0.01587	17%
30	5	9.68	15.47496	0.009236	13%
60	4.5	9.762	15.55696	0.006548	12%
1200	3	10.008	15.80296	0.001476	8%

5B	Initial Sample Mass (g)		37.55		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	10	8.86	14.65496	0.060295	27%
3	10	8.86	14.65496	0.028423	27%
10	8	9.188	14.98296	0.015741	21%
30	5.5	9.598	15.39296	0.009212	15%
60	5	9.68	15.47496	0.006531	13%
1190	2	10.172	15.96696	0.00149	5%
6A	Initial Sample Mass (g)		40.02		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	20	7.22	13.01496	0.056821	50%
3	19	7.384	13.17896	0.026954	47%
10	17	7.712	13.50696	0.014946	42%
30	15	8.04	13.83496	0.008733	37%
60	13	8.368	14.16296	0.006248	32%
1190	6.5	9.434	15.22896	0.001455	16%
6B	Initial Sample Mass (g)		40.04		
Time (min)	**R**	**L1 (cm)**	**L (cm)**	**D (cm)**	**Percent Finer**
0.66666667	12	8.532	14.32696	0.059616	30%
3	10	8.86	14.65496	0.028423	25%
10	8.5	9.106	14.90096	0.015698	21%
30	7	9.352	15.14696	0.009138	17%
60	6	9.516	15.31096	0.006496	15%
1200	5	9.68	15.47496	0.00146	12%

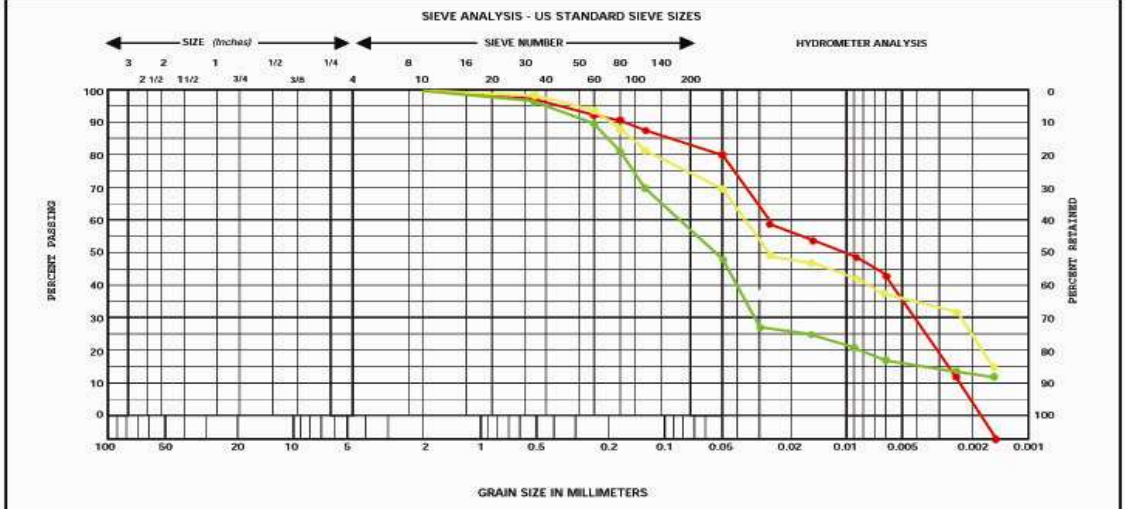
Percent Finer Graphs:





GRAIN SIZE DISTRIBUTION GRAPH - AGGREGATE GRADATION CHART

1. PROJECT	2. DATE
Hope Ranch, Las Palmas Creek	2/16/06



EXCAVATION NUMBER	SAMPLE NUMBER	LL	PL	PI	C_U (D_{60}/D_{10})	C_C $(D_{30})^2/(D_{60} \times D_{10})$	SOIL DESCRIPTION/REMARKS	CLASSIFICATION (USCS)
4	A, B, C						Green: 4A Yellow: 4C Red: 4B	

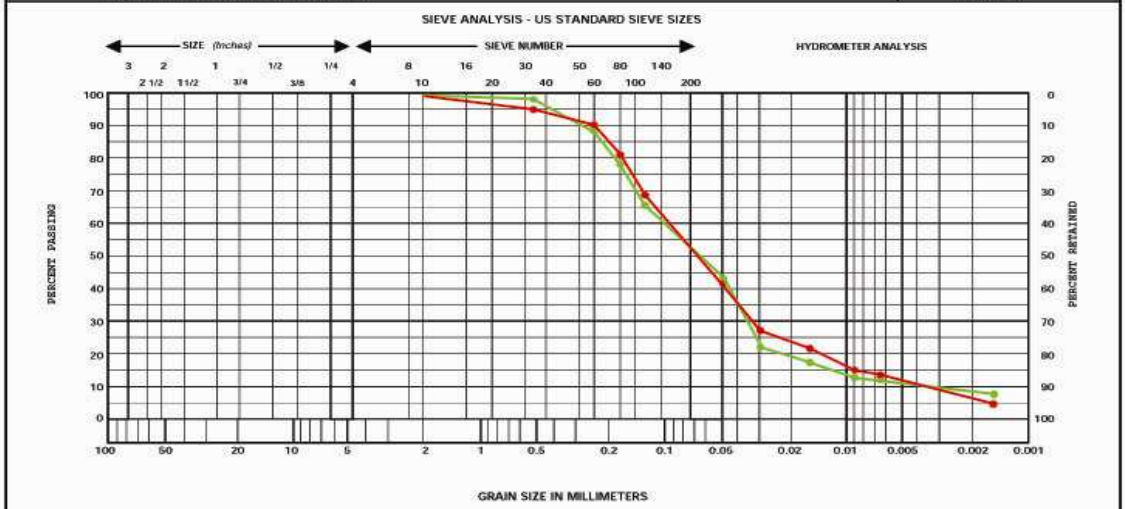
3. TECHNICIAN (Signature)	4. PLOTTED BY (Signature)	5. CHECKED BY (Signature)

DD FORM 1207, DEC 1999 PREVIOUS EDITION IS OBSOLETE.

Reset

GRAIN SIZE DISTRIBUTION GRAPH - AGGREGATE GRADATION CHART

1. PROJECT	2. DATE
Hope Ranch, Las Palmas Creek	2/16/06



EXCAVATION NUMBER	SAMPLE NUMBER	LL	PL	PI	C_U (D_{60}/D_{10})	C_C $(D_{30})^2/(D_{60} \times D_{10})$	SOIL DESCRIPTION/REMARKS	CLASSIFICATION (USCS)
5	A, B						Green: 5A Red: 5B	

3. TECHNICIAN (Signature)	4. PLOTTED BY (Signature)	5. CHECKED BY (Signature)

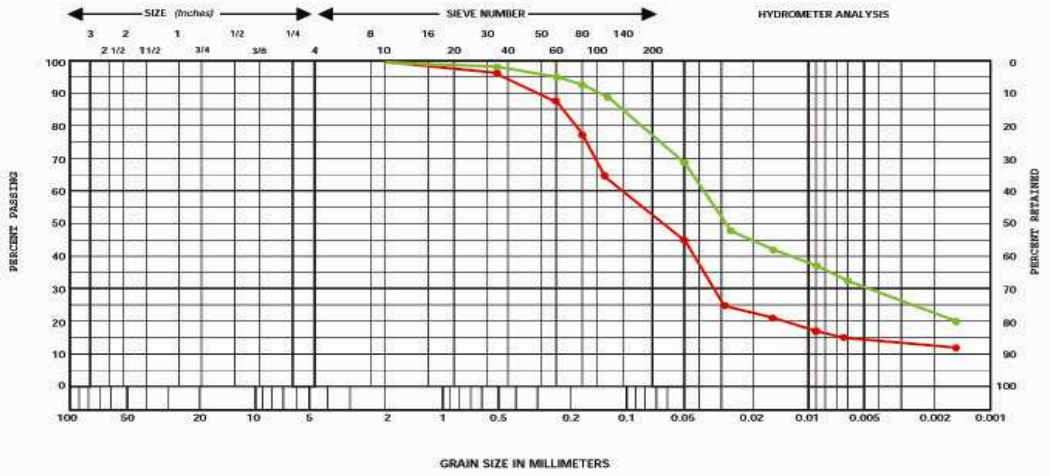
DD FORM 1207, DEC 1999 PREVIOUS EDITION IS OBSOLETE.

Reset

GRAIN SIZE DISTRIBUTION GRAPH - AGGREGATE GRADATION CHART

1. PROJECT	2. DATE
Hope Ranch, Las Palmas Creek	2/16/06

SIEVE ANALYSIS - US STANDARD SIEVE SIZES



EXCAVATION NUMBER	SAMPLE NUMBER	LL	PL	PI	C_U (D_{60}/D_{10})	C_C $(D_{30})^2/(D_{60} \times D_{10})$	SOIL DESCRIPTION/REMARKS	CLASSIFICATION (USCS)
6	A, B						Green: 6A Red: 6B	

3. TECHNICIAN *(Signature)*	4. PLOTTED BY *(Signature)*	5. CHECKED BY *(Signature)*

DD FORM 1207, DEC 1999 PREVIOUS EDITION IS OBSOLETE. Reset

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