Fecal Indicator Bacteria (FIB) Levels During Dry Weather from Southern California Reference Streams

Liesl L. Tiefenthaler
Eric D. Stein
Gregory S. Lyon

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Southern California Coastal Water Research Project
3535 Harbor Boulevard, Suite 110
Costa Mesa, California 92626, USA
www.sccwrp.org

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ABSTRACT

High levels of fecal indicator bacteria (FIB) in surface waters is a common problem in urban areas that often leads to impairment of beneficial uses such as swimming or other contact recreation. Once impaired, common management and regulatory solutions include development of Total Maximum Daily Loads (TMDLs) and other water quality management plans. A critical element of these plans is establishment of a "reference" level of exceedances against which to assess management goals and TMDL compliance. Unfortunately, existing "background" or reference data on contributions of FIB from undeveloped catchments during dry weather is limited to a small number of locations measured at few time points. The goal of this study was to provide information on indicator bacteria contributions from natural streams in undeveloped catchments throughout southern California during dry weather, non-storm conditions. Specific questions addressed were: a) What are the "background" ranges of concentrations of FIB associated with dry weather flow from reference areas? b) What is the frequency with which reference FIB levels exceed relevant water quality standards? c) How does seasonality influence stream FIB levels associated with reference areas? and d) How do the ranges of FIB concentrations associated with reference areas compare with those associated with urban (developed) areas? To help establish a regional reference data set, bacteria levels (i.e. *Escherichia coli* (*E. coli),* enterococci and total coliforms)) were measured from 15 unimpaired streams in 11 southern California watersheds weekly for one full year. A total of 590 water samples were collected from spring 2006 through spring 2007. Results were compared with data from the developed Ballona Creek watershed and to established State of California bacteria standards. Concentrations measured from reference areas were typically between one to two orders of magnitude lower than levels found in developed watersheds. The absence of *B. thetaiotaomicron* indicated that the FIB in reference streams were likely of non-human origin. Nearly 82% of the time, samples did not exceed daily and monthly bacterial indicator thresholds, demonstrating good bacteriological water quality in natural streams throughout southern California. *E. coli* had the lowest daily percent exceedance (1.5%). A total of 13.7% of enterococci exceeded daily thresholds. The average measured enterococci levels of these exceedances was 292 MPN/100 ml, with a maximum of 2098 MPN/100 ml and a minimum of 160 MPN/100 ml. Indicator bacteria levels fluctuated seasonally with an average of 79% of both enterococci and total coliforms exceedances occurring during summer months (June-August). Temperature, at all sites, explained about one-half the variation in total coliforms density suggesting that stream temperatures regulated bacterial populations. Studies of human health risk associated with natural bacteria levels have not been conducted, but the levels observed in this study are below those reported to cause risk in freshwater systems with known human sources of FIB. Accounting for natural background levels will allow for management targets that are more reflective of the contributions from natural sources. Additional monitoring during wet weather is warranted to further characterize background bacterial contamination in southern California reference waterbodies.

Keywords: Dry Weather Water Quality, Indicator Bacteria, Reference Condition, Background Water Quality, TMDL

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INTRODUCTION

The presence of fecal indicator bacteria (FIB) in surface waters is a prevalent concern for many municipalities, health departments, and regulatory agencies. Persistent or excessive bacteria levels often result in reduced opportunities for beneficial uses such as swimming, and may lead to waterbodies being listed as impaired under Section 303(d) of the Clean Water Act. Approximately 280 waterbodies are listed as impaired in the Los Angeles, Santa Ana, and San Diego regions (http://www.swrcb.ca.gov/tmdl/303d_lists.html). Management of impaired water bodies may involve development of Total Maximum Daily Loads (TMDLs), issuance of National Pollutant Discharge Elimination System (NPDES) permits, or development of water quality plans that are intended to reduce bacteria levels to a point where water quality standards are met and beneficial uses are protected. An important step in the development of TMDLs and other water quality management plans is to identify all sources of the constituent(s) of concern in order to accurately quantify loads and set appropriate management or regulatory targets. One of the challenges in developing appropriate targets is accounting for biogenic inputs, or the natural contribution from undeveloped catchments.

Most watersheds consist of both developed and undeveloped areas, both of which can contribute bacteria to streams via surface runoff. Bacteria associated with runoff from urban surfaces are well documented (Gore & Storrie Ltd. and Proctor & Redfern Ltd. 1981, USEPA 1993). For example, (Stein *et al.* 2007) observed that recreational (horse) and agricultural land uses in Los Angeles, CA contributed substantially higher storm fluxes for *Escherichia coli* (*E. coli*). Additional investigations by Bay and Schiff (1998), Noble *et al.* (2000) and Stein and Tiefenthaler (2005) found freshwater outlets such as storm drains to be especially high contributors of dry-weather FIB contamination.

Natural areas can also be a source of bacteria originating from wildlife, including birds and mammals, pets, and livestock (Griffith *et al.* 2006). Grant *et al*. (2001) found that enterococci bacteria generated in a restored wetland had greater effect on coastal water quality than dry season urban runoff. The presumed sources of these bacteria were birds that used the tidal salt marsh as habitat. (Ahn *et al.* 2005) also recognized that natural sources could be significant contributors to total bacteria levels in urban storm water in southern California. However, most previous studies have focused on either short measurements during or immediately following storm water runoff or on bacteria in coastal waters (beaches). Few studies have attempted to quantify naturally occurring background levels of bacteria in streams during baseflow (i.e. non-storm) conditions over an extended period of time. This data gap is critical because the non-storm period is when streams and the coastal waters they drain to receive the most human use and thus the potential risk is highest.

The goal of this study is to establish a “reference” level of bacteria that can be used to set appropriate water quality management targets. More specifically, we address the following questions: a) What are the “background” ranges of concentrations of FIB associated with dry-weather runoff from natural areas? b) What is the frequency with which reference FIB levels exceed relevant water quality standards? c) How does seasonality influence stream FIB levels associated with reference areas? and d) How do the ranges of FIB concentrations associated with reference areas compare with those associated with urban (developed) areas?

METHODS

The overall approach to the study was to characterize dry weather bacteria levels at a set of sites that is representative of existing natural conditions in southern California. The specific study design consisted of an intensive sampling regime with collection of weekly dry weather bacteria data for an entire year.

Sampling Sites

Fifteen sites were selected for inclusion in the study based on criteria developed by Stein and Yoon (2007, Stein and Yoon In press). Criteria were designed to ensure that sampling would capture natural conditions without influence from any land-based anthropogenic input. The criteria included: 1) contributing drainage area should be at least 95% undeveloped. 2) sites should be in a relatively homogenous setting in terms of underlying geology and landcover, 3) sites should have either year-round or prolonged dry-weather flow to allow sampling during at least a portion of the dry season, and 4) sites should not be within watersheds that have burned during the previous three years. Although fire can be a natural occurrence, inclusion of sites in burned catchments would have added a confounding factor and, therefore, were excluded. Catchment land use was determined by plotting watershed boundaries over (year 2003) land cover maps from the (National Oceanographic Administration (NOAA) 2003) Coastal Change Analysis Program (CCAP) - http://www.csc.noaa.gov/crs/lca/ccap.html. The 15 selected sites are located across five counties (Los Angeles, Orange, Riverside, San Bernardino and San Diego) and ten different watersheds: Los Angeles River, Los Alisos Canyon, Malibu Creek, Soltice Canyon, San Juan Creek, Santa Ana River, San Jacinto, Cucamonga, Santa Margarita, and San Dieguito (Figure 1, Table 1, and Appendix A).

Sampling

Weekly dry-season sampling was conducted at all 15 sites from May 15, 2006 through May 31, 2007. A site was eligible for sampling if it had not received measurable rainfall for at least 24 h and flow was no more than 20% above baseflow. Weekly sampling continued as long as there was measurable stream flow. For intermittent streams, sampling was suspended once the stream was too low to sample. Based on these criteria, the duration of sampling ranged from 9 to 55 weeks (Table 1). Water samples were collected as composite grab samples, with equivalent volumes collected from three different points across the stream (approximately 10, 50, and 90% distance across). These samples were taken from the flowing portion of the streams at a depth sufficient to exclude surface scum without introducing bottom sediment. A replicate water sample was collected in the same way after completion of the initial water sample for approximately 25% of the samples. A field blank sample was also collected at each site once a month. All water samples were collected in presterilized 125 ml high-density polyethylene (HDPE) sample bottles. Collected water samples were immediately placed on ice and transported to the laboratories within 6 h of sample collection for subsequent analyses.

At each sampling location and during each round of sample collection, water quality readings (i.e. temperature (°C), dissolved oxygen (DO) mg/L, pH, turbidity, and conductivity (μS/cm)) were measured using hand held field probes (i.e. Orion 125, YSI 63 and Horiba U-10). Measurements were taken in triplicate at each transect. In addition, physical and biological parameters of the site and general climatic conditions were recorded and documented (using both data forms and photo documentation). Stream discharge was measured as the product of the channel cross-sectional area and flow velocity. Channel cross sectional area was measured in the field. At each sampling event, velocity was measured using a Marsh-McBirney Model 2000 flow meter (Frederick, MD). The velocity, width, and depth were measured at three points along each transect. Flow for each transect subsection was computed and summed for a total flow for the transect. Values from three transects were averaged to estimate overall flow at each site (Rantz 1982).

Laboratory Analysis

Water quality samples were analyzed for four bacteria indicators; *E. coli*, enterococci, total coliforms and *Bacteroides thetaiotaomicron*. Enterococci, total coliforms and *E. coli* were measured by the chromogenic substrate method using Enterolert® for enterococci and Colilert® for *E. Coli* and total coliforms (Idexx 24 h, Inc.). This commercially available product uses a Multiple Tube Fermentation (MTF) type format with defined substrate technology to detect the presence or absence of bacteria indicator density in a water sample. In this medium, the detection of coliform densities is based upon a color change caused by the reaction of a fluorogen with a bacterial enzyme. This assay is read within 24 hours and coliform densities are reported as most probable number (MPN)/100 milliters (ml). Given the large geography covered by the study and the short holding time required for bacterial analysis, eight laboratories cooperated on sample analysis. Laboratory intercalibration studies were completed to ensure consistent methodology, data quality, and repeatability between laboratories. All laboratories had had good repeatability for all three bacterial indicators and all results fell within the median log comparability criteria. The low variability between labs indicated that interlab differences should not be a confounding factor in interpreting the results of the study. Details of the laboratory intercalibration study are provided in Appendix C.

*Bacteroide*s *thetaiotaomicron* are anaerobic bacteria that comprise the majority of microorganisms that inhabit the human digestive tract. As such, they may be a more reliable measure of human fecal matter or pathogens than *E. coli* (Bernhard and Field 2000a,b). Samples were analyzed for either presence or absence of *B. thetaiotaomicron* as a negative control for human bacteria sources. This analysis was initiated at a sampling site when the State of CA single-sample water quality thresholds for both *E. coli* and enterococci were exceeded for two consecutive weeks. The presence of *B. thetaiotaomicron* would suggest that bacteria observed in the surface waters were predominantly of human origin. *B. thetaiotaomicron* was measured by DNA extraction followed by polymerase chain reaction (PCR) as described by (Brinkman *et al.* 2003).

Data Analysis

Three analyses were used to characterize FIB levels from natural streams. First the 30-d geomeans, variances, and ranges of concentrations, and fluxes were calculated to provide an estimate of expected baseline bacterial levels. Flux estimates facilitated region wide comparisons among watersheds of varying sizes. Flux was calculated as the ratio of the 30-d geomean or mean yearly bacterial concentration (MPN/100 ml) and contributing watershed area (km^2) at a specific site. Second, dry weather FIB concentrations were compared with the state of CA standards for single-sample and 30-d geomean maximum allowable densities (Table 2).

Cumulative density frequency plots (CDFs) were produced to compare observed bacterial concentrations to the CA quantitative standards and to calculate accumulated relative exceedance percentages. Third, water quality statistics from natural sites were compared with previous data collected from watercourses draining developed areas of the greater Los Angeles basin to determine if significant differences existed between natural and developed areas (Stein *et al.* 2007, Stein and Yoon 2007).

Bacteria data were analyzed for differences between perennial vs. intermittent streams, between developed and undeveloped watersheds, and to assess temporal patterns. Differences in concentration or flux were tested using a one-way analysis of variance (ANOVA), with a significance level $p < 0.05$ (Sokal and Rohlf 1995). Differences based on flow regime were assessed using a Tukey-Kramer post-hoc test for multiple comparisons; differences between developed and undeveloped sites were investigated by comparing median values using a Kruskal-Wallis one-way ANOVA on ranks.

Spatial and temporal patterns were also investigated using Pearson's r correlation coefficient to determine if there were strong associations between FIB concentrations and continuous variables (i.e. temperature and flow; Helsel and Hirsch 2002); the null hypothesis, in this case, is that the correlation coefficient is zero.

RESULTS

Background Bacteria Concentrations and Fluxes

Annual median bacteria fluxes from the natural sites were 2 ± 1.4 MPN/100 ml/km^2, 3 ± 1.7 MPN/100 ml/km^2, and 106 ± 61.4 MPN/100 ml/km^2 for *E. coli*, enterococci, and total coliforms, respectively. *E. coli* and enterococci, median density values at the natural sites (based on single-sample measurements) were 10 MPN/100 ml and 20 MPN/100 ml respectively, while median density values in Ballona Creek are typically in the 10^3 range. Densities and fluxes were significantly lower for all indicator bacteria at the natural sites relative to data from developed areas ($p < 0.001$, Figure 2).

Only two sites exceeded State water quality standards for both *E. coli* and enterococci for two or more weeks during the yearlong study. During the period of exceedance, *E. coli* levels ranged from 327 to 9804 MPN/100 ml while enterococci ranged from 388 to 7270 MPN/100 ml. Repeat exceedances were seen most commonly for enterococci. In both cases, the *B. thetaiotaomicron* samples were negative, suggesting that the bacterial populations represented by the FIB were probably derived from non-human sources.

Frequency of Exceedance of Bacteria Standards at Natural Sites

A total of 18.2% of the indicator bacteria samples (for all three indicators) from the natural sites exceeded daily (single sample) water quality standards. Approximately 14% of enterococci exceeded the daily threshold of 104 MPN/100 ml (Figure 3). The average enterococci level of these exceedances was 292 MPN/100 ml, with a maximum of 2098 MPN/100 ml (Orange County) and a minimum of 160 MPN/100 ml (San Bernardino County). For *E. coli*, 1.5% of the measurements exceeded the single sample standard of 235 MPN/100 ml with a maximum and a minimum of 5500 MPN/100 ml and 241 MPN/100 ml, respectively (Orange County). For total coliforms, 3% exceeded the single sample standard of 10,000 MPN/100 ml.

A total of 39% of enterococci samples from the natural sites exceeded the 30-d geomean water quality standard of 33 MPN/100 ml. The average enterococci level of these exceedances was 47 MPN/100 ml, with a maximum of 744 MPN/100 ml and a minimum of 3 MPN/100 ml. For *E. coli,* approximately 1% exceeded the 30-d geomean threshold of 126 MPN/100 ml with a maximum and a minimum of 146 MPN/100 ml and 1 MPN/100 ml, respectively (Orange County). For total coliforms, 45% exceeded the 30-d geomean of 1000 MPN/100 ml with a maximum and a minimum of 5040 MPN/100 ml and 23 MPN/100 ml, respectively.

Seventy-five percent of enterococci and 83% of total coliforms exceedances occurred during the summer months (June-August, Table 4). In August all indicator thresholds were exceeded with 12.5%, 62.5% and 75% of *E. coli*, enterococci and total coliforms samples exceeding monthly thresholds, respectively (Table 4).

Temporal and Spatial Patterns in FIB Levels

Bacteria levels for all three indicators were significantly higher during the summer than during all other seasons (Table 4, $p < 0.01$). For example, 30-d geomeans for total coliforms were near the water quality standard in May 2006 with levels approximately 878 MPN/100 ml ± 3.2 SD, increased substantially during the summer, exceeding the criterion, peaking in July at 2586 MPN/100 ml ± 3.1 SD (Figure 4b). Total coliform geomeans decreased gradually throughout the winter nearing zero in February, 2007 (289 MPN/100 ml ± 4.2 SD), as stream temperatures fell below 10°C, before gradually returning to baseline geomeans throughout spring, 2007 (Figure 4a and b). Similar seasonal patterns were observed for *E. coli* and enterococci (Figure 5a and b).

Orange County had the highest daily and monthly water quality exceedances for both *E. coli* and total coliforms (12.9%; 25% and 3.2%; 100%, respectively, Table 3). For enterococci, approximately 47% of the San Diego County samples exceeded the daily threshold and 100% exceeded the monthly standard (Table 3). However, the Orange County and San Diego County streams had no flow in winter due to an unusually low 2006-2007 rainfall season, so the results are from only the spring and early summer months and do not represent annual averages that may occur in perennially flowing streams.

Perennial vs. Non-perennial Streams

Background bacteria levels differed based on the duration of stream flow (Table 1, Appendix A). *E. coli* and enterococci densities were significantly different in perennial vs. intermittent streams ($p < 0.05$, Figure 6). Mean $\log_{10}$ concentrations for *E. coli* and enterococci at perennial streams were 1.0 ± 0.4 and 1.3 ± 0.5, respectively. Intermittent streams had higher mean $\log_{10}$ concentrations for *E. coli* and enterococci (1.6 ± 0.5 and 1.8 ± 0.6, respectively). There were no statistical differences between stream types for total coliform densities (mean 2.7 ± 0.6 vs. 3.3 ± 0.4).

Relationship of Bacteria Levels to Environmental Variables

Of the five environmental variables measured (temperature, conductivity, dissolved oxygen, pH, turbidity), only stream temperature exhibited a significant correlation with seasonal FIB levels. Water temperature varied by about 5-10°C at each of the sites, reaching a maximum of 28°C on warm sunny afternoons. Streams located in the foothills (Mill Creek, San Bernardino Co.) or where the creek was significantly shaded had the lowest average temperatures (Table 1, Appendix B). For example streams in San Bernardino County ranged from 650 m to 1200 m in elevation and averaged 12.7°C. The highest monthly average water temperatures (20.4 °C) were recorded in Orange County where streams were approximately 200 m in altitude. Stream temperature and total coliforms were significantly positively correlated (Table 5, $p < 0.001$, $r^2 = 0.48$). A weaker, but still significant, positive correlation existed between stream temperature and *E. coli* or enteroccoci ($p < 0.04$, $r^2 = 0.20$ and $p < 0.04$, $r^2 = 0.26$, respectively). The Pearson's r for these two correlations was between 0.2 and 0.3 suggesting that similar processes may have controlled the relationship between stream temperature and FIB. A strong negative correlation existed between dissolved oxygen and both conductivity or stream temperature (Table 5, $p < 0.05$, $r^2 = -0.5$; $p < 0.001$, $r^2 = -0.84$, respectively). However, few statistically significant relationships existed among the other physical variables.

Total coliform densities increased exponentially at temperatures above 10°C (Figure 7, $r^2 = 0.48$). Dissolved oxygen concentrations varied inversely with stream temperatures throughout the study (Figure 4a). Monthly mean DO concentrations decreased sharply to approximately 8 mg/L at stream temperatures above 15°C, and concentrations increased to approximately 11 mg/L at stream temperatures below 10°C.

DISCUSSION

Enterococci, *E. coli* and total coliforms (FIB) are commonly used indicators of the possible presence of pathogenic (disease-causing) microorganisms in streams and the ocean. As shown in this study, these FIB can be found in natural streams, with populations increasing during warm summer months and persisting through winter. However, the densities observed in natural streams were usually below State water quality objectives, which are set below levels typically thought to impair beneficial uses (Geldreich 1978, Toranzos 2007). Furthermore, the absence of *B. thetaiotaomicron* indicated that the FIB in reference streams were likely of non-human origin (Carson *et al.* 2005). There are three possible sources of FIB observed in natural streams: External inputs from sources such as waterfowl, animals, or soil erosion; internal sources of bacterial growth and colonization within the stream associated with decomposition of organic matter; or a combination of the two (Byappanahalli *et al.* 2003, Toranzos 2007).

Higher bacteria levels observed during the summer suggest that factors existed which promote bacteria growth and regrowth in streams. The positive relationship between temperature and bacteria levels suggests that heat induced growth may be a contributing factor to seasonally high bacteria levels. In addition, warmer temperatures influence the dissolved oxygen content of the water. Decreased oxygen solubility associated with higher temperature may combine with lower dissolved oxygen levels producing algal blooms, which have been shown in previous studies to support growth of *E. coli* and enterococci in freshwater (Byappanahalli *et al.* 2003, Byappanahalli *et al.* 2007). These conditions may in turn accelerate death and decomposition of organic matter in the stream, further enhancing in situ bacterial growth. Increases in organic decomposition have been shown to increase survival and regrowth of enteric bacteria and viruses (Novotny and Olem 1994). This hypothesis is further supported by the negative correlation observed between conductivity and dissolved oxygen. Conductivity is closely correlated with total dissolved solids, which are typically comprised of inorganic and organic substances, a potential source of biological oxygen demand (BOD).

Higher FIB densities and incidence of water quality standard exceedences during the summer is consistent with the observations of others such as Noble *et al.* (2000) and Sieracki (1980). Nuzzi and Burhans (1998) compared the responses among indicator bacteria at 143 New York beach sites and found that survival was longer in the summer, but that the duration could be mediated by exposure to UV radiation from sunlight. More recently, growth or regrowth of fecal indicator bacteria in tropical and temperate soils during the summer months has also been reported (USEPA 2000, Ishii *et al.* 2006). Whitman *et al.* (1999) attributed a gradual increase of *E. coli* bacteria in water and sand at beaches during summer to higher survival and growth at warmer temperatures.

Another explanation for higher FIB levels during the summer could be higher external sources due to different patterns of use by wildlife and birds. A number of studies have shown that wildlife and other animals can be sources of bacteria in run-off (Baxter-Potter and Gilliland 1988, Bagshaw 2002, Stein *et al.* 2007). Previous studies have quantified that wildlife and bird feces contain high levels of FIB. Cox *et al*. (2005) measured fecal coliform levels of 10^3 - 10^5 CFU/g from native wildlife in Australian watersheds. Ricca and Cooney (1998) reported that droppings from feral populations of pigeons, geese and herring gulls from the environment around Boston Harbor, MA, USA contained up to 10^8 CFU/100 ml of enterococci. Bacteria from wildlife and birds can be associated with FIB levels in streams used by these animals. Noblet *et al.* (2004) found that birds were a likely source of intermittently high levels of FIB observed in the lower Santa Ana River watershed and the nearby surf zone in southern California. Similarly, Harwood *et al.* (2000) reported that animals were the dominant sources of indicator bacteria at Florida sample sites with relatively low anthropogenic impact. Bacterial source tracking studies conducted in Michigan suggested that feces from pets and raccoons were important contributors to FIB levels in streams and storm sewers (Ram *et al.* 2007). Moreover, levels increased in the late summer and fall coincident with increased raccoon den mobility following breeding.

Decreased stream flow may have also contributed to higher bacteria levels during the summer months. Although there was no statistically significant relationship between flow and bacterial densities, in all cases densities increased exponentially when stream flow decreased below approximately 0.5 m^3/s (2 cubic feet/sec). In addition, median annual bacterial densities were higher in intermittent streams than in perennial, with the differences being mainly due to high levels in the period immediately prior to streams drying up. Despite the differences between perennial and intermittent streams, the annual ranges of observed bacteria levels overlapped substantially. Therefore, the combined range of bacteria levels for perennial and intermittent streams observed in this study should reflect expected levels in natural streams throughout southern CA.

Relatively minor perturbations in the contributing watershed can cause sites to quickly deviate from background conditions. Four sites originally considered, but later rejected from the study had bacteria levels 2-3 log units greater than the natural sites retained, but significantly lower than levels observed in the developed Ballona Creek watershed (Figure 8). The watersheds of these four sites were almost entirely natural open space, but had small portions subject to agricultural or transportation related runoff. In one instance, a portion of the contributing watershed was affected by a recent fire. These small perturbations in the watershed led to dramatic changes in bacteria levels that moved sites away from reference conditions. Although these sites were not included in the analysis of background conditions, they provide valuable insight into the sensitivity of natural watersheds to small increases in anthropogenic sources.

Although this study focused on background FIB levels during dry weather (non-storm) conditions, comparison of these results to background levels in storm water is important because FIB are major constituents of concern in storm water runoff that can result in impairment of receiving waters (Noble *et al.* 2003, Schiff *et al.* 2003, Stein and Tiefenthaler 2005). Stein and Yoon (2007) reported geometric mean FIB levels from natural streams during storms of 125, 140, and 4,460 MPN/100 ml for *E. coli*, enterococci and total coliforms, respectively. These levels are generally 1.5 - 2 log units higher than geomean levels observed in this study during dry weather conditions (Figure 9). As is the case in urban areas, bacteria levels in natural systems are significantly lower during dry weather conditions than during storms, although the higher levels observed during storms are much more transient in nature. Griffith *et al.* (2006) reported that one-fifth of all samples collected within three days of rainfall from beaches at the bottom of natural catchments exceeded water quality thresholds for at least one bacterial indicator.

Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize "background" bacterial contamination in southern California reference waters following storms.

The results of this study indicated that streams in undeveloped watersheds contain low levels of FIB of non-human origin. An important management question is whether the levels observed pose a potential health risk. Wade *et al.* (2003) reviewed 27 studies and concluded that *E. coli* levels between 45 and 170 CFU/100 ml in freshwater pose a relative human health risk level of 1.22 (i.e. low level risk). We observed 30-day geomean *E. coli* levels ranging from 2 – 138 MPN/100 ml, with an overall 30-day geometric mean of 41 ±20 MPN/100 ml. Because the mean levels observed in this study were below the "low risk" range reported by Wade *et al.* (2003), it could be concluded that background levels in natural streams have a low likelihood of posing a human health risk. However, this conclusion should be made with caution because previous exposure and risk studies were conducted in areas known to receive wastewater or storm water discharges containing human fecal sources. In contrast, the FIB levels observed in this study were of non-human origin, so the actual risk is unknown.

Conclusion and Future Research

This study yielded the following conclusions about FIB levels in natural streams during dry weather conditions:

1. ***Fecal indicator bacteria typically occur in natural streams during dry weather conditions at levels below State water quality standards***. Annual mean concentrations (both single sample and 30-day geometric mean) were below established water quality criteria for all three indicators. A total of 18.2% of the indicator bacteria samples (for all three indicators) from the natural sites exceeded daily (single sample) water quality standards. Approximately 1.5%, 14%, and 3% of *E. coli*, enterococci, and total coliforms, respectively, exceeded single sample water quality criteria.

2. ***Fecal indicator bacteria in natural streams are most likely of non-human origin***. All samples tested for the presence of *B. thetaiotaomicron* were negative, indicating non-human sources in natural streams. FIB levels in natural streams likely result from a combination of natural inputs, such as wildlife, birds, and soil erosion and instream bacterial growth facilitated by high summer temperatures and presence of decaying organic matter.

3. ***Dry weather fecal indicator bacteria in natural streams are typically two orders of magnitude lower than those observed in streams draining developed watersheds***. Data from the developed Ballona Creek watershed were typically in the 10^3 MPN/100 ml range for *E. coli* and enterococci. Even slight watershed modifications appear to result in a relatively rapid departure from background FIB levels.

4. ***Fecal indicator bacteria levels exhibit seasonal patterns.*** Mean bacteria levels and frequency of exceedance of water quality standards were higher during the warmer summer months for all three bacteria indicators. This suggests that summer is a critical period for assessing background bacteria levels. Past studies indicate that fecal indicator

bacteria levels in natural streams during storms are one to two orders of magnitude higher than those observed during dry weather conditions; however, the duration of these elevated levels is unknown. Studies of water quality at beaches at the bottom of natural watersheds indicate that high bacteria levels may persist for up to three days following storms. Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize the persistence of "background" bacterial contamination in southern California reference waters following storms.

5. ***Bacteria levels in natural streams were generally higher during lower flow conditions.*** For all three indicators, densities increased exponentially when stream flow decreased below approximately 0.5 m^3/s (2 cubic feet/sec). In addition, median annual bacterial densities were higher in intermittent streams than in perennial, with the differences being mainly due to high levels in the period immediately prior to streams drying up. Despite the differences between perennial and intermittent streams, the annual ranges of observed bacteria levels overlapped substantially.

6. ***Dry weather fecal indicator bacteria levels were one to two orders of magnitude lower than those observed in natural streams during storm conditions.*** Past studies of water quality at beaches at the bottom of natural watersheds indicate that high bacteria levels may persist for up to three days following storms. Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize the persistence of "background" bacterial contamination in southern California reference waters following storms.

7. ***Fecal indicator bacteria in natural streams occurred at levels below those reported to pose health risks due to freshwater contact recreation***. However, past risk assessments have all occurred in waters that are known to receive bacteria inputs of human origin. No epidemiology studies have been conducted on FIB of non-human origin, so the precise risk is unknown.

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Table 1. List of natural stream sampling sites, characteristics and their median monthly fecal indicator bacteria densities (MPN/100 ml).

			Catchment	Number	Mean	Geomean (30-d)					
			Size	Sampling	Flow	*E. coli*		Enterococci		Total coliforms	
Site Name	Watershed	County	(km^2)	Weeks/Yr	(m^3/sec)	(MPN/100 ml)	SD	(MPN/100 ml)	SD	(MPN/100 ml)	SD
Arroyo Seco	LA River	Los Angeles	41.50	47	0.04	15.24	2.22	20.48	2.45	1291.90	2.85
Cold Creek	Malibu Creek		1.43	49	0.00	13.59	1.89	15.33	2.42	443.30	4.33
Lachusa Canyon	Los Alisos Canyon		3.86	49	0.01	16.08	2.24	20.55	2.26	1486.50	2.14
Solstice Canyon	Solstice Canyon		8.74	49	0.01	16.97	2.28	20.64	2.43	1109.21	2.68
Chesebro Creek	Malibu Creek		7.55	49	0.00	90.30	5.49	68.25	4.24	2940.41	2.88
Bell Creek	San Juan	Orange	17.97	12[a]	0.02	80.45	4.30	164.60	5.48	2008.67	3.16
San Juan Creek	San Juan		99.94	9[a]	0.03	74.66	2.46	25.25	3.29	2848.15	1.66
Santiago Creek	Santa Ana		17.02	10[a]	0.02	22.99	2.84	34.75	3.06	1869.15	1.98
Hurkey Creek	San Jacinto	Riverside	29.73	29	0.01	18.89	4.38	36.92	4.75	688.57	3.33
Mill Creek	Santa Ana	San Bernardino	15.21	55	0.08	2.06	2.68	12.74	3.32	75.00	2.98
Cucamonga Creek	Cucamonga		24.10	52	0.14	11.14	1.66	26.35	3.33	399.64	2.39
Day Creek	Santa Ana		11.70	55	0.32	11.02	1.58	25.18	2.87	545.71	2.41
Cajon Creek	Santa Ana		82.82	52	0.08	54.98	3.18	159.21	2.49	4794.47	2.04
Stone Creek	Santa Margarita	San Diego	7.00	50	0.00	138.18	3.86	52.72	3.58	1728.44	3.21
Boden Creek	San Dieguito		19.81	18[a]	0.01	45.33	6.14	98.26	2.86	1658.46	2.54
		Mean	25.89	39	0.05	40.79	3.15	52.08	3.26	1592.51	2.70
		SD	14.54	9	0.04	19.84	0.71	25.32	0.47	622.94	0.34

[a] Intermittent stream

Table 2. State of California marine water quality standards for fecal indicator bacteria as established in Assembly Bill 411. Currently a freshwater quality standard for total coliforms does not exist.

	CA Maximum Allowable Density (MPN/100 ml)	
Fecal Indictor Bacteria	**single-sample**	**30-day geometric mean**
Enterococci	104	33
E. coli	235	126
Total coliforms	10,000	1000

Additional Indicator	
Bacteroides thetaiotaomicron	Presence / absence of a human source

Table 3. Assessment of percent exceedances between counties in southern California during the present study. A [1] represents those counties in which samples were collected only during spring and/or summer due to intermittent streams with less stable flow regimes.

	Exceedance (%)		
	E. coli	Enterococci	Total Coliforms
Daily			
Los Angeles County	0.0	6.3	0.0
Orange County[1]	12.9	38.7	3.2
San Bernardino	0.0	13.1	0.0
San Diego[1]	5.3	47.4	0.0
Monthly			
Los Angeles County	0.0	7.7	46.2
Orange County[1]	25.0	75.0	100.0
San Bernardino	0.0	23.1	0.0
San Diego[1]	0.0	100.0	80.0

Table 4. Percent single-sample exceedance of fecal indicator bacteria (FIB) levels in natural streams during dry weather from May 2006-May 2007. Numbers in bold are significantly different (*p* <0.01).

	Exceedance (%)		
	E. coli	**Enterococci**	**Total coliforms**
Season			
Spring 06	0.0	41.7	75.0
Summer	**12.5**	**75.0**	**83.3**
Fall	0.0	0.0	28.6
Winter	0.0	0.0	11.1
Spring 07	0.0	22.2	44.4
Month			
May 2006	0.0	27.3	45.5
June 2006	0.0	66.7	75.0
July 2006	0.0	**72.7**	**90.9**
August 2006	**12.5**	**62.5**	**75.0**
September 2006	0.0	42.9	57.1
October 2006	0.0	0.0	14.3
November 2006	0.0	0.0	28.6
December 2006	0.0	0.0	14.3
January 2007	0.0	0.0	0.0
Febuary 2007	0.0	12.5	25.0
March 2007	0.0	22.2	11.1
April 2007	0.0	11.1	44.4
May 2007	0.0	25.0	62.5
Annual	1.0	26.4	41.8

Table 5. Correlation table (r^2 values) between water quality variables and fecal indicator bacteria (FIB) during dry weather in natural streams in southern California between May 2006-May 2007. Significant correlations (*p*<0.04) are shown in bold, while significant correlations (*p*<0.001) are both bolded and in italics.

			Pearson r^2-values		
	DO	**Flow**	***E. coli***	**Enterococci**	**Total Coliform**
Parameter	(mg/L)	(m^3/s)	(MPN/100 ml)		
Conductivity	**-0.50**	***0.48***	***0.22***	**0.01**	***0.19***
Dissolved Oxygen	-	0.12	***0.18***	**0.21**	***0.16***
pH	0.32	***0.09***	***0.11***	**0.02**	**0.04**
Flow	0.12	**-**	-0.06	-0.02	**-0.08**
Temperature (°C)	***-0.84***	**0.02**	***0.20***	***0.26***	***0.48***
Turbidity	0.19	0.00	0.02	1.44	**0.07**

Bolded values = p<0.05

Bolded italic values = p<0.001

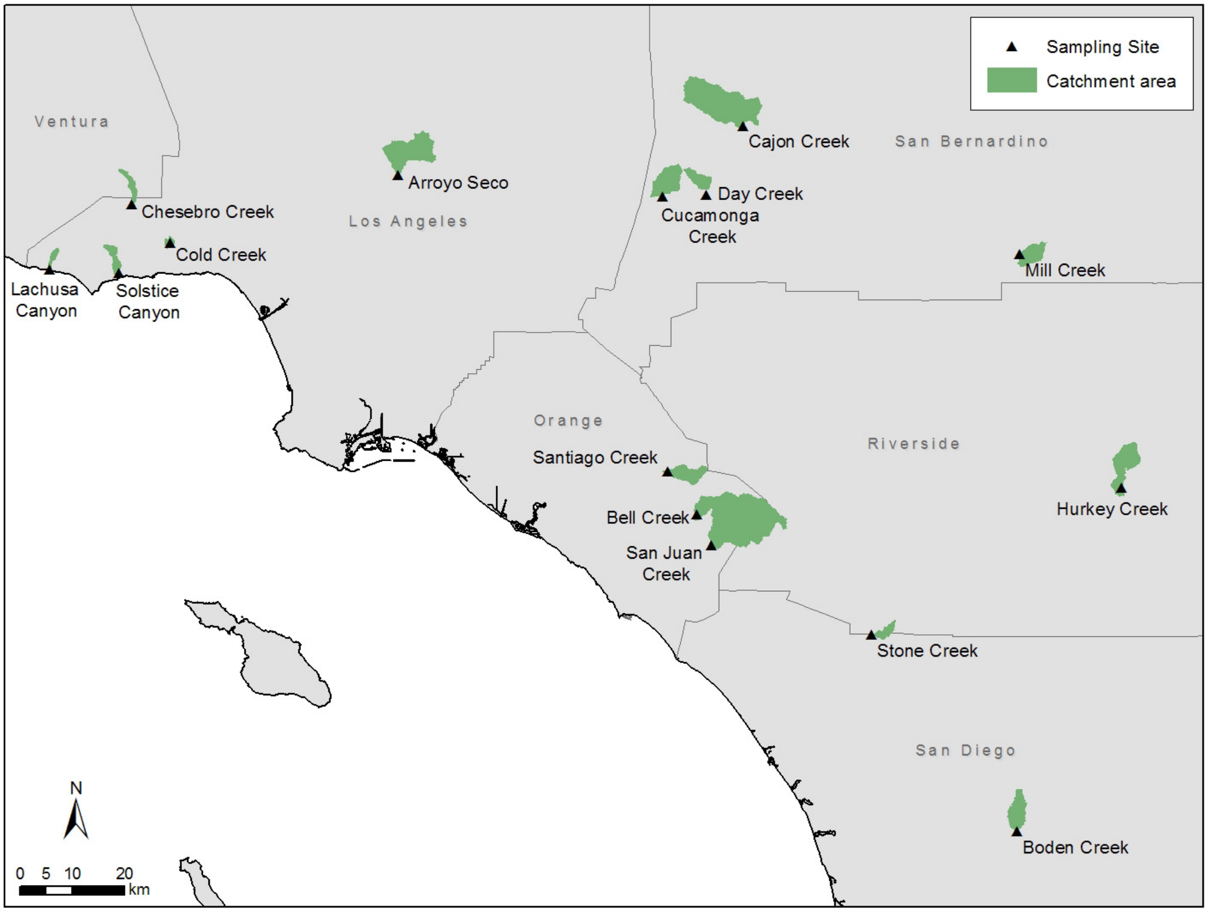

Figure 1. Map of natural stream sampling sites and their respective catchments within southern California.

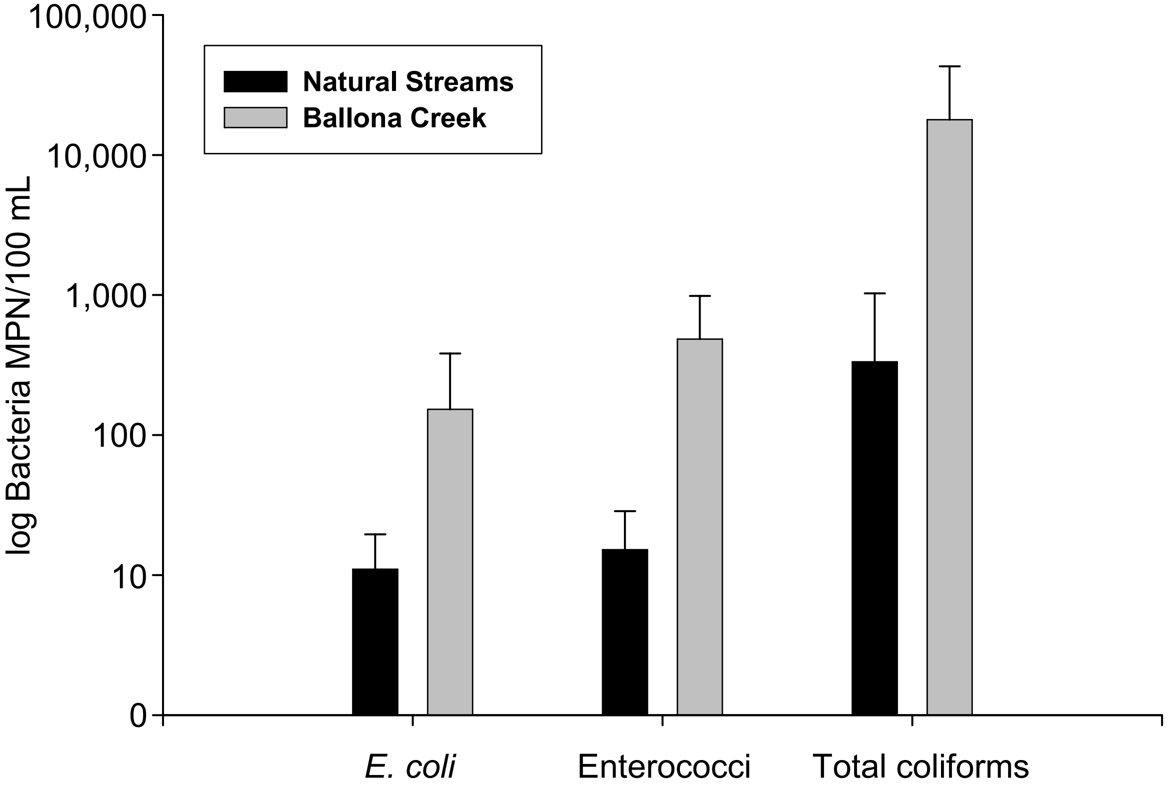

Figure 2. Comparison of dry weather log10 fecal indicator bacteria (FIB) densities (± standard deviations) between natural streams in undeveloped watersheds and developed Ballona creek watershed from May 2006-May 2007 in southern California, USA.

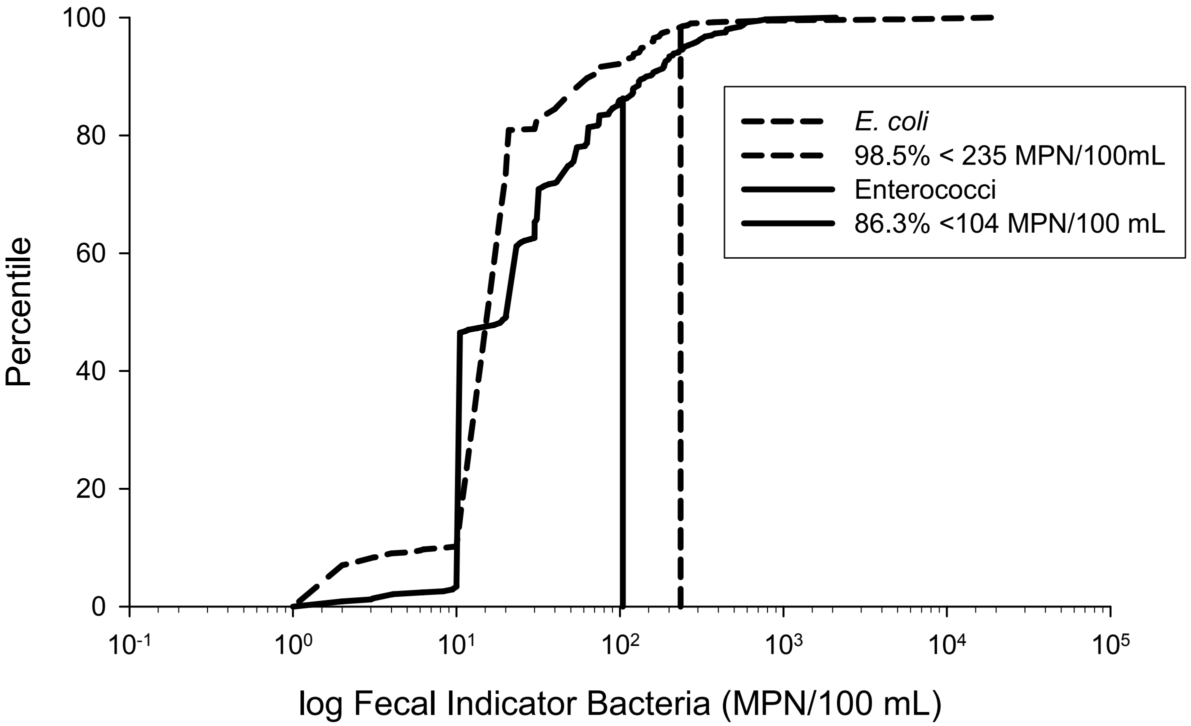

Figure 3. Dry season fecal indicator bacteria cumulative density frequency plots (CDFs) of natural streams relative to freshwater quality standards from May 2006 to May 2007 in southern California, USA.

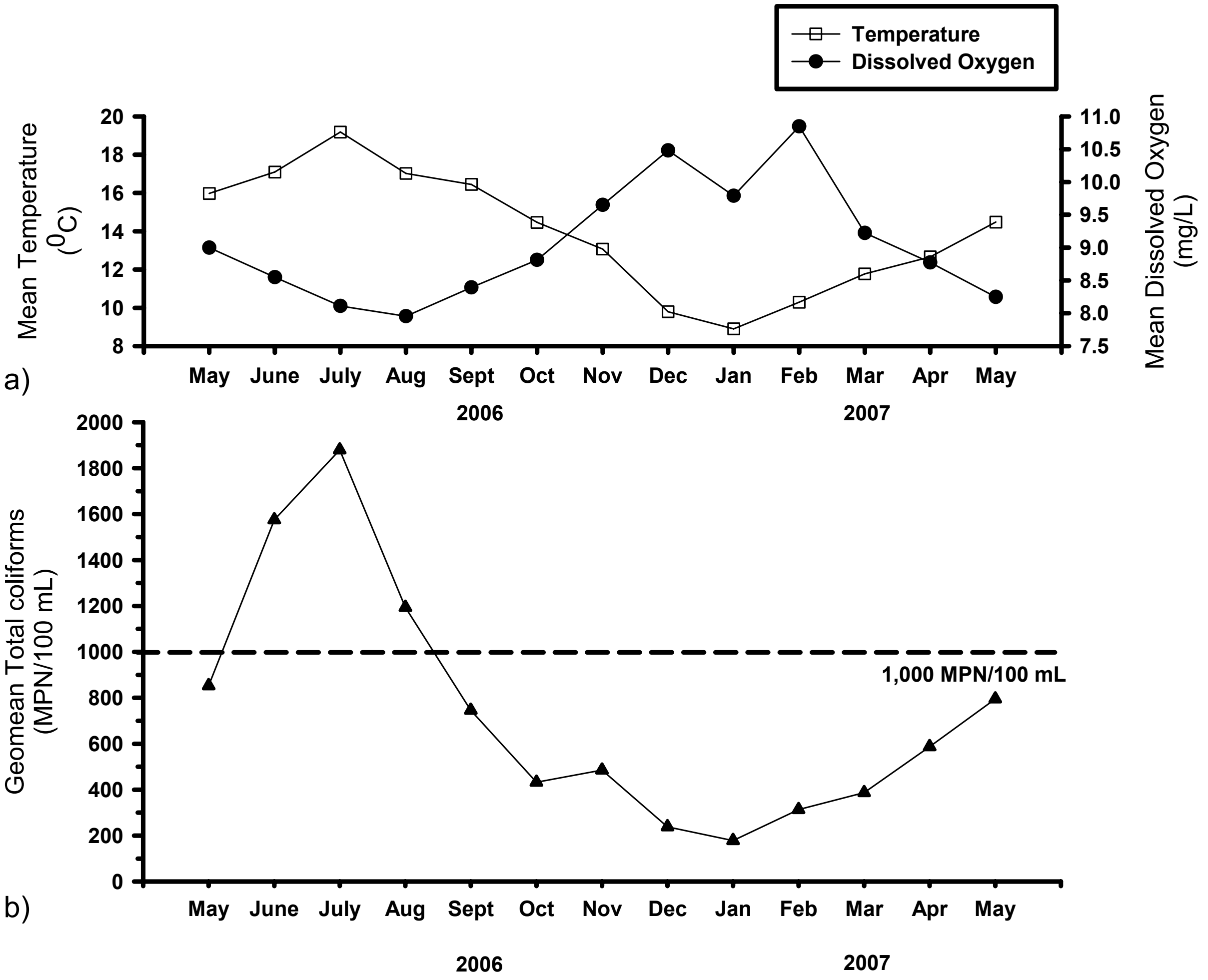

Figure 4. Mean monthly temperature (°C) and dissolved oxygen (mg/L) comparison (a) and geomean total coliform densities in natural streams in southern California (b) between May 2006 and May 2007. Summer months (June-August) were substantially higher than all other seasons ($p < 0.01$). *E. coli* and enterococci exhibited similar results. The dotted line indicates the 30-d geomean for total coliforms equal to 1,000 MPN/100 ml. All points above the line represent bacteria water quality exceedances.

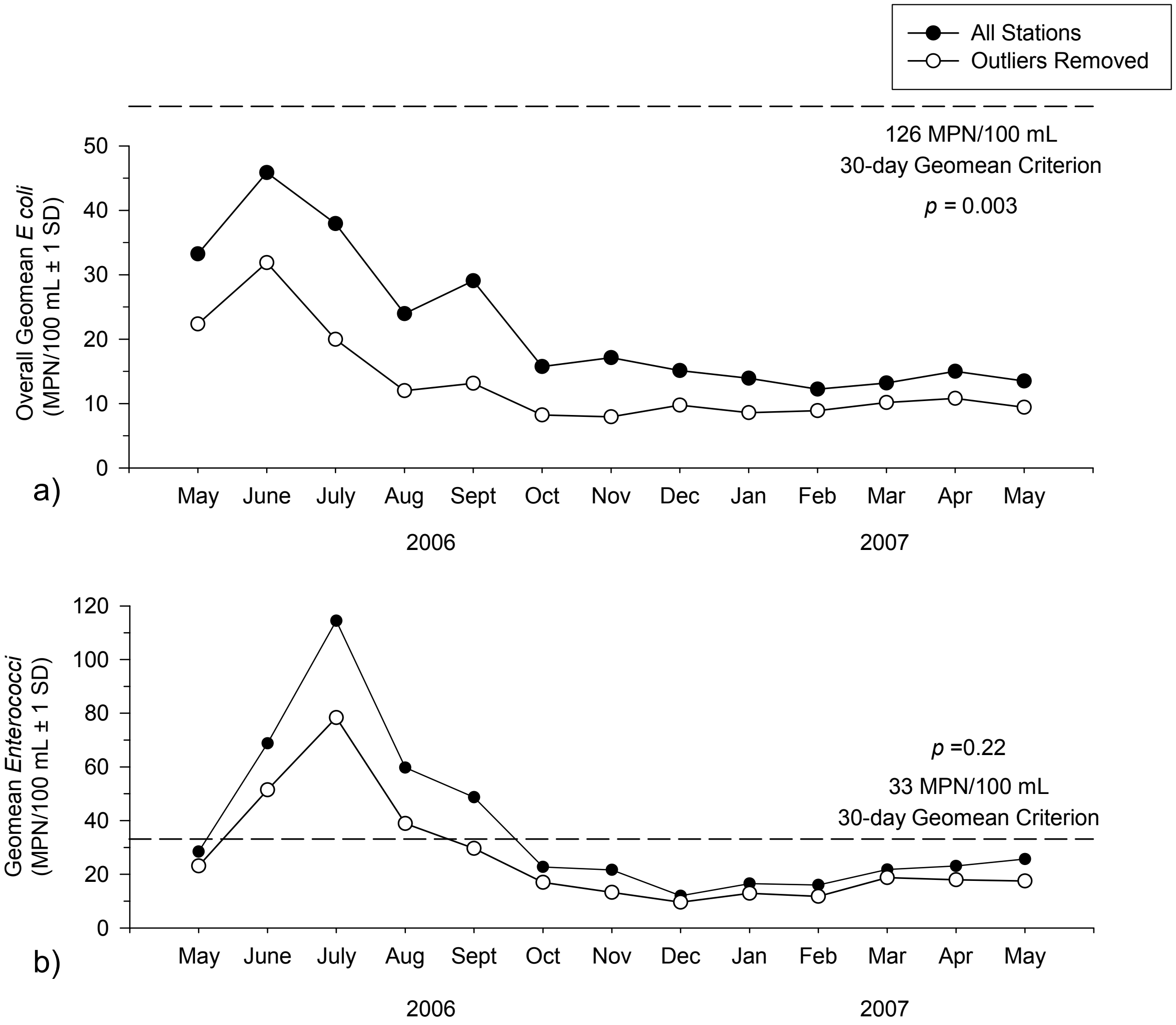

Figure 5. ***E.coli*** **a) and enterococci b) geomean densities in natural streams in southern California between May 2006 and May 2007. Summer months (June-August) were substantially higher than all other seasons. The dashed line indicates the monthly water quality standard equal to 235 MPN/100 ml and 104 MPN/100 ml for *E. coli* and enterococci respectively. All points above the line represent bacteria water quality exceedances.**

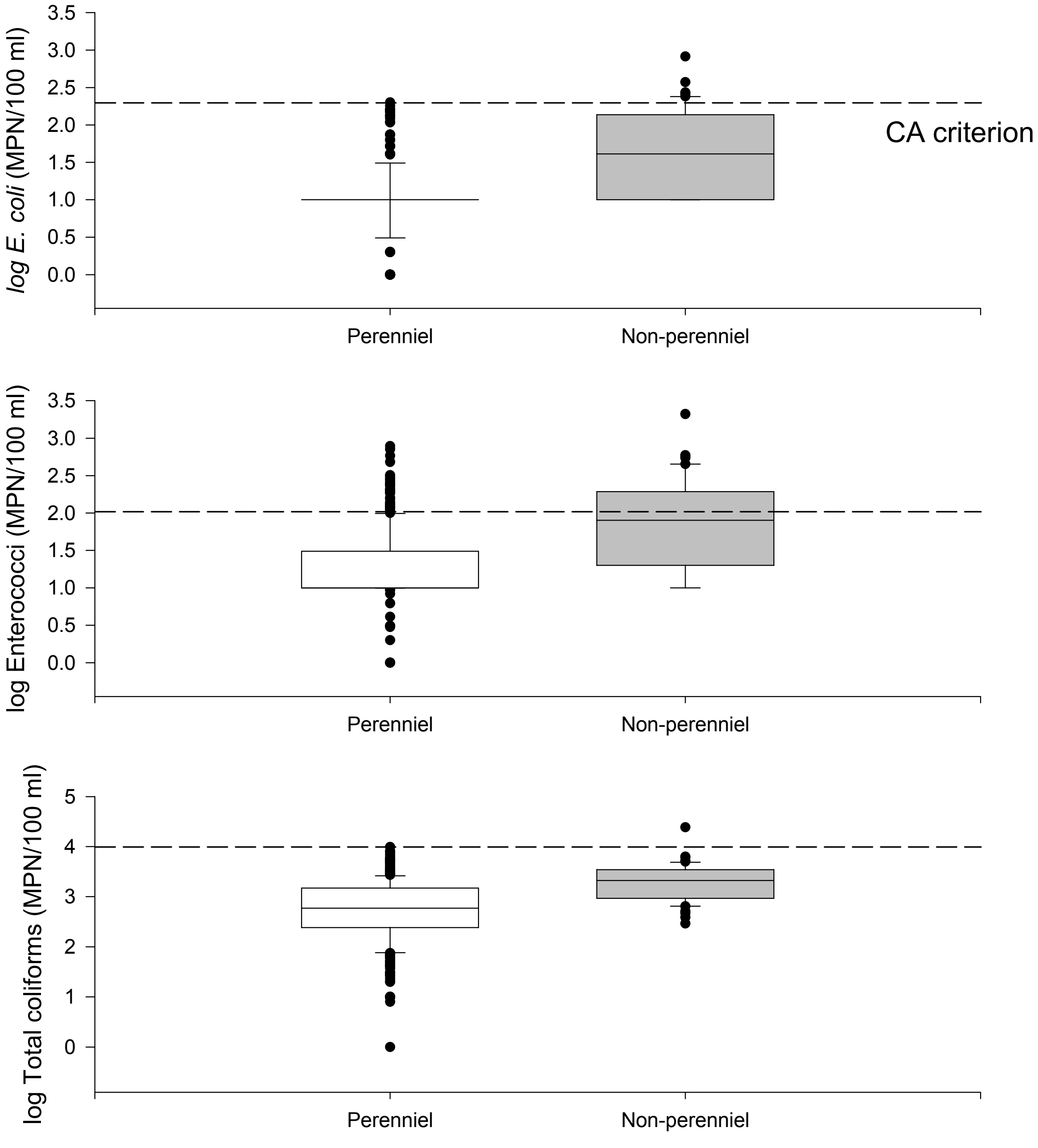

Figure 6. Perennial and non-perennial stream comparison of log10 fecal indicator bacteria densities (MPN/100 ml) in southern California during the present study. The dotted line indicates the State single-sample bacterial water quality criterion. Significant differences in indicator densities existed between streams but ranges generally overlapped ($p < 0.05$). Boxplots show mean, median, 25th and 75th percentiles.

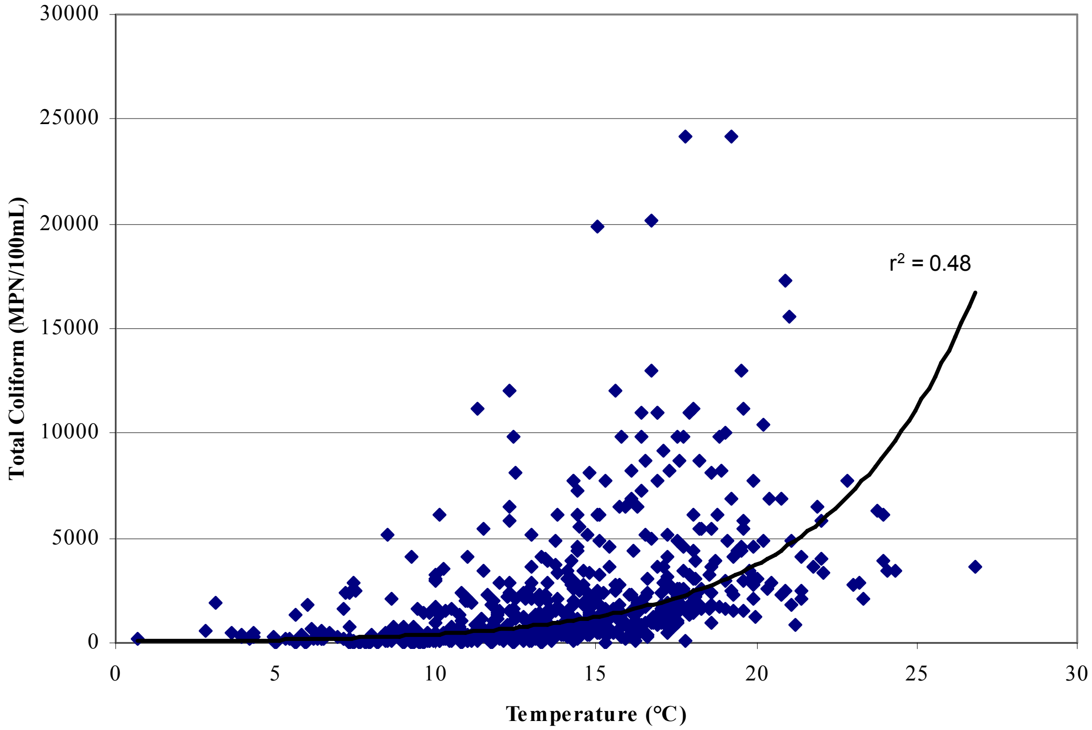

Figure 7. Natural stream temperatures in southern California versus total coliform densities (MPN/100 ml) during dry weather for an entire year. Solid line indicates the exponential trend line (r2 = 0.48).

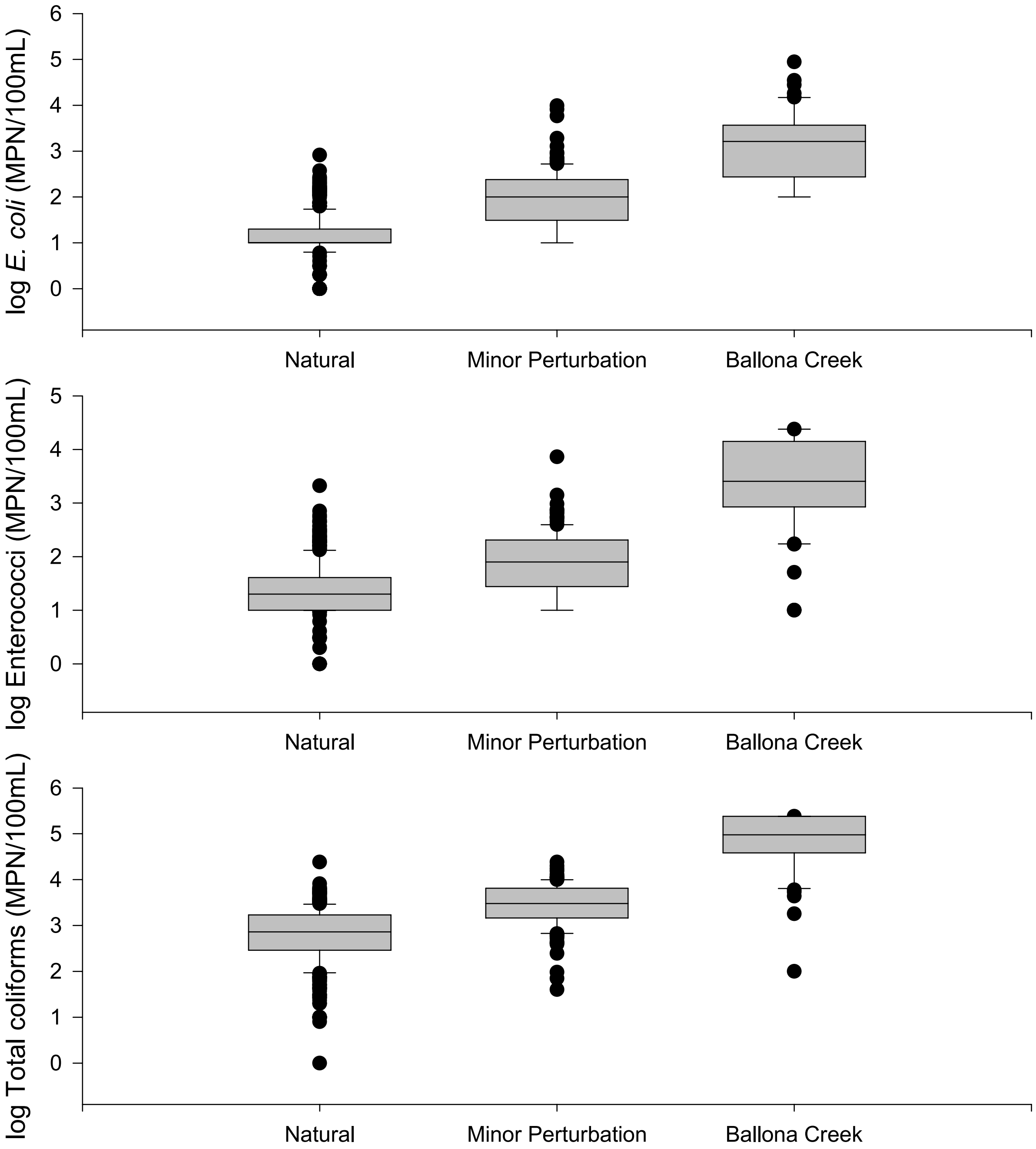

Figure 8. Distribution of log *E. coli* a); enterococci b); and total coliforms c) concentrations in natural streams, streams with minor perturbations, and in developed Ballona Creek watershed in southern California, USA. Natural streams were significantly lower then all other streams (p <0.001). Minor perturbation streams were significantly lower than developed Ballona Creek (p <0.001).

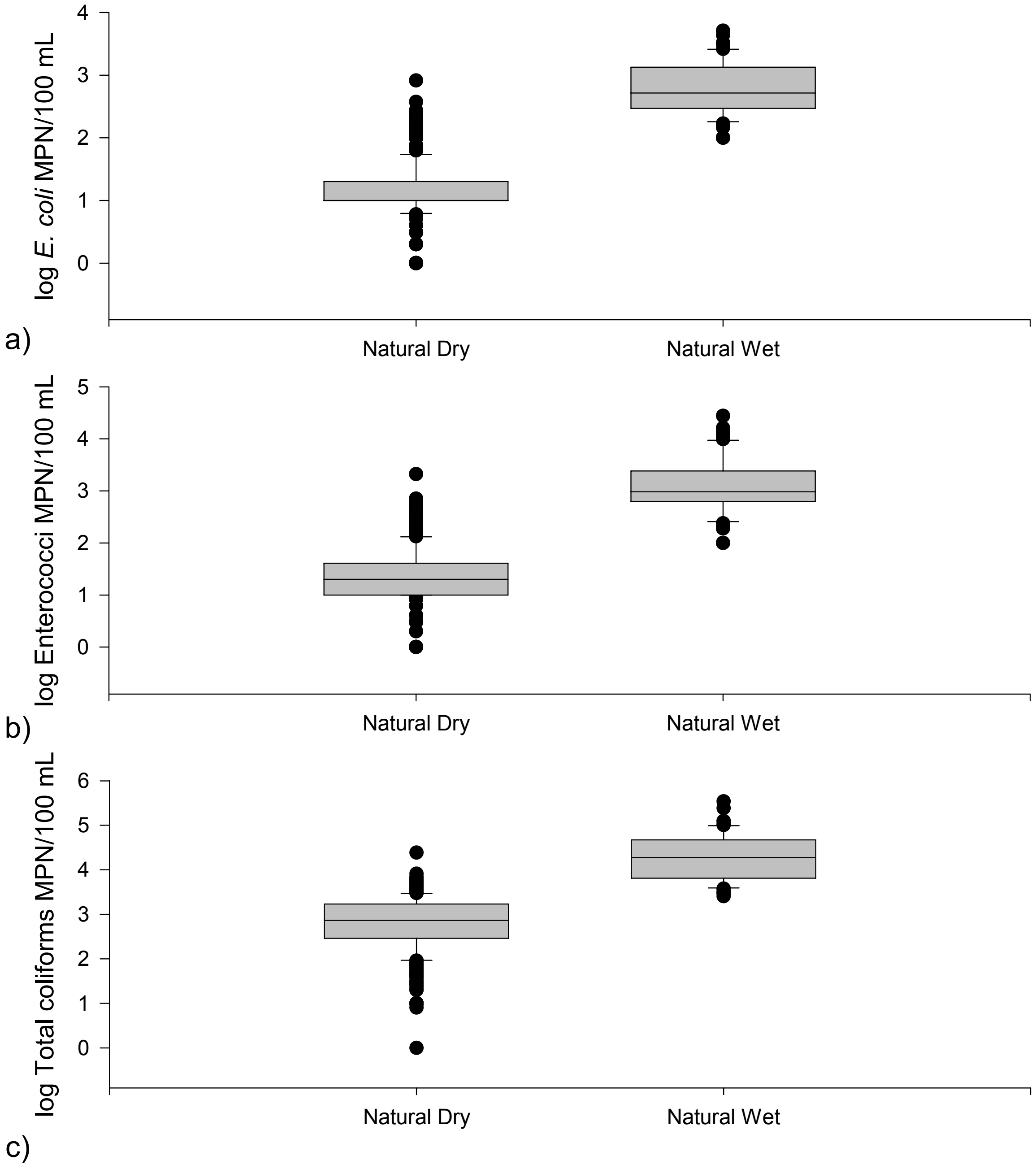

Figure 9. Distribution of log *E. coli* a); enterococci b); and total coliforms c) concentrations in natural streams during dry weather (present study) compared to wet weather (Natural Loadings; 2003-2005 and Los Angeles River watershed; 2001-2005) studies in southern California, USA. Dry weather bacteria concentrations were significantly lower then wet weather concentrations (p <0.001).

APPENDIX A - SUMMARY BACTERIA DATA FOR ALL NATURAL STREAM SITES

Table A1. List of natural stream sampling sites, characteristics and their daily fecal indicator bacteria densities (MPN/100 ml).

		Concentration (MPN/100 ml)								
		E. coli			**Enterococci**			**Total coliforms**		
Sampling site	Watershed	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Arroyo Seco	LA River	10	15.2	148	10	20.5	250	10	1291.9	6867
Cold Creek	Malibu Creek	10	13.6	108	10	15.3	480	10	443.3	6131
Lachusa Canyon	Los Alisos Canyon	10	16.1	161	10	20.6	197	146	1486.5	8164
Solstice Canyon	Solstice Canyon	10	17.0	200	10	20.6	262	10	1109.2	5475
Chesebro Creek	Malibu Creek	10	90.3	9804	10	68.2	7270	96	2940.4	24192
Bell Creek	San Juan	10	80.5	820	10	164.6	2098	292	2008.7	24196
San Juan Creek	San Juan	20	74.7	259	10	25.2	299	1664	2848.2	6294
Santiago Creek	Santa Ana	10	23.0	134	10	34.7	228	469	1869.1	3873
Hurkey Creek	San Jacinto	10	18.9	5500	10	36.9	780	210	688.6	7700
Mill Creek	Santa Ana	1	2.1	20.9	1	12.7	190	1	75.0	435
Cucamonga Creek	Cucamonga	6	11.1	180	10	26.3	580	10	399.6	2000
Day Creek	Santa Ana	4	11.0	160	10	25.2	240	31	545.7	9800
Cajon Creek	Santa Ana	10	55.0	520	20	159.2	960	730	4794.5	13000
Stone Creek	Santa Margarita	10	138.2	5830	10	52.7	1408	40	1728.4	15530
Boden Creek	San Dieguito	10	45.3	18600	10	98.3	563	388	1658.5	20140
Mean		9.40	40.79	2829.66	10.07	52.08	1053.67	273.80	1592.51	10253.13
StDev		2.04	19.84	2662.11	1.82	25.32	911.35	222.68	622.94	3837.71

Table A2. Monthly *E. coli* geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

	***E. coli* Geomeans**												
Sampling site	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	10.0	37.5	56.1	11.5	26.0	12.5	10.0	10.0	10.0	10.0	10.0	16.5	10.0
Lachusa Canyon	82.8	30.7	20.0	12.5	28.5	10.0	10.0	14.1	10.0	10.0	16.0	10.0	25.1
Cold Creek	14.4	42.1	10.0	27.6	10.0	14.2	10.0	10.0	10.0	10.0	10.0	10.0	20.0
Solstice Canyon	32.2	59.6	11.9	15.2	29.9	10.0	10.0	40.0	12.6	10.0	15.2	10.0	20.0
Chesebro Creek	150.3	276.0	444.2	233.5	1336.8	111.3	27.1	58.7	11.9	25.3	65.8	28.9	10.0
Bell Creek	25.9	125.6	104.0	146.0									
San Juan Creek	36.0	121.6	84.2										
Santiago Creek	10.0	22.8	53.6										
Hurkey Creek	5500.0	18.9	14.1						22.6	10.0	10.0	10.0	
Cucamonga Creek	10.0	10.0	10.0	12.4	10.0	10.0	10.0	20.6	10.0	13.2	10.0	10.0	10.0
Mill Creek	10.0	10.0	5.0	2.6	2.8	1.4	1.0	1.0	1.1	2.0	1.0	1.0	1.0
Day Creek	10.0	20.0	10.0	11.0	10.0	13.2	10.0	10.0	10.0	11.9	10.0	10.0	10.0
Cajon Creek	38.3	180.1	146.9	104.1	225.3	96.6	76.1	42.4	35.3	12.6	12.6	10.0	10.0
Stone Creek	65.7	129.2	269.5	134.6		156.1	441.1	57.8	240.1	82.8	20.2	99.4	112.1
Boden Creek	1082.5	26.1									21.5	63.5	30.7

Table A3. Monthly enterococci geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

	Enterococci Geomeans												
Sampling site	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	41.0	63.0	105.7	23.9	54.6	18.1	11.9	10.0	10.0	10.0	10.0	10.0	14.1
Lachusa Canyon	20.2	13.2	15.1	17.4	21.6	20.1	11.5	14.1	14.6	34.0	82.3	24.7	17.6
Cold Creek	12.6	18.8	115.6	16.6	16.5	10.0	10.0	10.0	10.0	10.0	11.5	17.6	10.0
Solstice Canyon	25.1	23.8	39.8	61.0	47.5	16.9	13.2	10.0	12.6	10.0	12.5	10.0	35.1
Chesebro Creek	59.0	200.3	563.1	146.5	252.2	31.1	41.1	24.9	11.9	29.0	51.5	26.8	62.0
Bell Creek	12.6	402.1	467.8	158.0									
San Juan Creek	20.2	47.5	10.0										
Santiago Creek	14.6	59.0	40.8										
Hurkey Creek	380.0	121.6	744.2						18.9	10.0	10.0	19.5	
Cucamonga Creek	33.9	90.2	241.1	85.8	31.2	14.3	10.0	14.1	10.0	11.9	12.6	18.4	10.0
Mill Creek	10.0	10.0	20.2	35.8	16.5	23.2	14.8	4.0	16.1	3.2	22.6	25.8	3.9
Day Creek	21.5	43.3	125.8	92.1	42.4	18.8	24.6	11.9	11.5	21.4	10.0	11.9	14.1
Cajon Creek	87.1	307.1	486.6	367.7	253.0	157.0	217.8	56.6	66.9	100.3	74.1	95.2	200.0
Stone Creek	53.6	163.0	192.3	133.8	79.0	31.8	53.4	12.6	46.1	18.5	11.9	45.9	74.2
Boden Creek	143.4	208.4									69.4	44.9	98.4

Table A4. Monthly total coliforms geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

	Total coliforms Geomeans												
Sampling site	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	708.0	1854.8	4200.8	1859.1	2506.6	1480.0	1155.9	534.0	134.9	588.4	1547.1	1843.3	2926.5
Lachusa Canyon	1611.2	1825.6	2724.7	3350.6	2074.7	998.6	1139.4	1206.9	725.0	1655.0	807.2	1009.0	2176.8
Cold Creek	997.6	1743.3	3567.4	1312.3	1347.5	488.0	250.7	109.2	70.7	78.3	123.9	218.3	277.4
Solstice Canyon	1064.2	1404.8	2278.4	2998.4	1048.4	499.8	550.8	654.2	761.3	1218.5	529.5	1783.9	2549.3
Chesebro Creek	2546.1	4655.0	9044.6	8141.9	8332.1	4770.4	2142.9	1017.2	789.6	1085.4	1515.9	1722.6	2540.4
Bell Creek	518.6	4780.6	2513.8	1483.0									
San Juan Creek	1748.1	3406.8	4139.9										
Santiago Creek	1189.6	1846.4	2985.1										
Hurkey Creek	6500.0	2102.0	5040.8						348.1	224.5	326.7	347.1	
Cucamonga Creek	419.1	688.2	1334.1	650.0	740.5	362.5	364.9	122.4	155.4	253.2	318.7	434.9	720.0
Mill Creek	170.6	224.0	126.4	139.1	35.3	91.9	151.7	27.5	30.8	24.0	48.3	52.0	115.7
Day Creek	311.1	746.5	1146.1	1320.5	668.1	267.3	417.4	374.0	232.4	569.0	450.4	498.0	2674.7
Cajon Creek	5915.7	8730.8	7512.4	3300.6	7335.3	9693.4	2667.5	3993.7	2747.3	2242.1	2946.6	5461.8	8200.0
Stone Creek	347.3	3493.6	4887.8	5727.9	7310.9	2482.6	1959.5	321.2	734.3	617.5	673.7	1610.4	1151.6
Boden Creek	7229.3	3207.2									603.5	1295.2	1302.1

Table A5. Dry season *E. coli* geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

		E. coli Dry Season Geomeans				
Sampling site	**Watershed**	Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	25.0	22.4	13.7	10.0	13.3
Lachusa Canyon	Los Alisos Canyon	45.9	15.9	13.4	12.0	13.7
Cold Creek	Malibu Creek	24.8	16.5	11.2	10.0	11.9
Solstice Canyon	Solstice Canyon	49.7	19.7	12.6	12.6	13.0
Chesebro Creek	Malibu Creek	213.3	531.7	56.0	21.5	32.8
Bell Creek	San Juan	48.3	115.8			
San Juan Creek	San Juan	74.2	75.2			
Santiago Creek	Santa Ana	16.8	31.4			
Hurkey Creek	San Jacinto	119.5	16.9		15.3	10.0
Cucamonga Creek	Cucamonga	10.0	10.8	12.7	11.1	10.0
Mill Creek	Santa Ana	10.0	4.2	1.2	1.3	1.0
Day Creek	Santa Ana	17.4	10.3	10.9	10.6	10.0
Cajon Creek	Santa Ana	84.7	126.2	102.7	20.1	11.2
Stone Creek	Santa Margarita	95.1	181.5	292.4	80.7	82.9
Boden Creek	San Dieguito	148.1	14.1		10.0	43.1

Table A6. Dry season enterococci geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

		Enterococci Dry Season Geomeans				
Sampling site	**Watershed**	Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	54.6	49.4	15.6	10.0	11.0
Lachusa Canyon	Los Alisos Canyon	15.9	17.1	14.2	30.6	35.0
Cold Creek	Malibu Creek	15.2	35.0	10.0	10.0	14.5
Solstice Canyon	Solstice Canyon	22.1	52.9	14.7	12.2	13.7
Chesebro Creek	Malibu Creek	118.8	365.1	33.7	21.1	46.1
Bell Creek	San Juan	60.1	338.0			
San Juan Creek	San Juan	26.8	23.4			
Santiago Creek	Santa Ana	24.2	49.9			
Hurkey Creek	San Jacinto	127.2	386.5		14.2	14.9
Cucamonga Creek	Cucamonga	47.0	138.9	14.1	11.3	16.5
Mill Creek	Santa Ana	10.0	20.6	11.4	12.4	8.0
Day Creek	Santa Ana	28.7	77.4	20.5	13.7	11.9
Cajon Creek	Santa Ana	140.7	383.2	145.4	89.6	96.7
Stone Creek	Santa Margarita	83.1	151.8	40.0	22.0	51.2
Boden Creek	San Dieguito	154.0	305.7		28.6	81.6

Table A7. Dry season total coliforms geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

		Total coliforms Dry Season Geomeans				
Sampling site	**Watershed**	Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	1066.8	2610.9	1230.6	422.2	2163.9
Lachusa Canyon	Los Alisos Canyon	1663.9	2899.9	1092.8	1034.7	1099.7
Cold Creek	Malibu Creek	1069.4	2133.9	295.8	97.2	180.3
Solstice Canyon	Solstice Canyon	1278.2	2165.1	543.3	616.5	1900.3
Chesebro Creek	Malibu Creek	3776.6	8814.0	2535.0	889.0	2281.4
Bell Creek	San Juan	1169.5	2955.9			
San Juan Creek	San Juan	2001.5	4426.6			
Santiago Creek	Santa Ana	1417.1	2465.3			
Hurkey Creek	San Jacinto	2952.6	3345.5		326.8	310.5
Cucamonga Creek	Cucamonga	508.0	958.8	254.1	216.2	500.4
Mill Creek	Santa Ana	185.3	104.5	82.8	31.6	79.5
Day Creek	Santa Ana	425.2	1001.3	374.8	348.5	795.6
Cajon Creek	Santa Ana	6926.3	5634.3	5220.2	2595.1	5267.6
Stone Creek	Santa Margarita	1343.4	5682.0	2193.0	516.7	1361.0
Boden Creek	San Dieguito	5146.1	2216.8		514.8	1163.2

Table A8. Annual dry season fecal indicator bacteria geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

		Annual Dry Season Geomeans		
Sampling site	**Watershed**	*E. coli*	Enterococci	Total Coliforms
Arroyo Seco	LA River	15.2	20.5	1291.9
Lachusa Canyon	Los Alisos Canyon	16.1	20.6	1486.5
Cold Creek	Malibu Creek	13.6	15.3	443.3
Solstice Canyon	Solstice Canyon	17.0	20.6	1109.2
Chesebro Creek	Malibu Creek	90.3	68.2	2940.4
Bell Creek	San Juan	80.5	164.6	2008.7
San Juan Creek	San Juan	74.7	25.2	2848.2
Santiago Creek	Santa Ana	23.0	34.7	1869.1
Hurkey Creek	San Jacinto	18.9	36.9	688.6
Cucamonga Creek	Cucamonga	11.1	26.3	399.6
Mill Creek	Santa Ana	2.1	12.7	75.0
Day Creek	Santa Ana	11.0	25.2	545.7
Cajon Creek	Santa Ana	55.0	159.2	4794.5
Stone Creek	Santa Margarita	138.2	52.7	1728.4
Boden Creek	San Dieguito	45.3	98.3	1658.5

APPENDIX B - SUMMARY OF PHYSICAL PARAMETERS AT ALL NATURAL STREAM SITES

Table B1. Annual dry season averages of measured physical parameters in natural streams during May 2006-May 2007 in southern California, USA.

	Physical Parameter Averages					
Sampling site	Conductivity	DO	Flow Rate	pH	Temperature	Turbidity
	□s	mg/L	m^3/s		°C	
Arroyo Seco	411.9	na	0.038	na	13.8	na
Lachusa Canyon	1431.1	na	0.006	na	16.2	na
Cold Creek	604.0	na	0.005	na	13.8	na
Solstice Canyon	1051.6	na	0.011	na	15.4	na
Chesebro Creek	3089.0	na	0.005	na	11.9	na
Bell Creek	738.8	8.7	0.018	8.0	18.7	1.1
San Juan Creek	518.8	10.4	0.028	8.2	21.1	0.7
Santiago Creek	636.9	9.6	0.017	8.1	22.2	0.5
Hurkey Creek	129.9	na	0.006	7.8	11.6	na
Cucamonga Creek	9.8	9.8	0.138	8.0	12.3	na
Mill Creek	0.7	9.4	0.080	8.0	10.6	12.3
Day Creek	13.7	9.9	0.317	8.0	12.6	1.8
Cajon Creek	37.7	8.7	0.082	7.9	15.7	8.0
Stone Creek	1171.6	7.2	0.002	7.5	16.4	16.1
Boden Creek	1012.0	7.5	0.005	7.8	15.3	6.1

APPENDIX C - INTERLABORATORY CALIBRATION RESULTS

RESULTS

SCCWRP is currently coordinating an investigation of bacteria levels in reference drainages throughout southern California. This is a cooperative study involving multiple jurisdictions that are each contributing to the project through combinations of in-kind services and direct funding. Because numerous analytical labs will be participating in analysis of fecal indicator bacteria, it was necessary to conduct a laboratory intercalibration study to ensure that comparable results could be achieved from all participating laboratories. This memo summarizes the results of this intercalibration study.

Eight laboratories from five counties participated in the calibration exercise, a performance-based approach used to evaluate analytical accuracy, reproducibility of results and to ensure that data from participating laboratories were comparable (Table C1). The calibration exercise occurred on March 22th, 2006 and consisted of each lab receiving six common samples for analysis (Table C2). Necessary dilutions or aliquot volumes were processed to insure that reportable values could be determined. Bacterial results were reported for total coliform, *Escherichia coli* (*E. coli*), and enterococcus as organism type per 100 ml of sample. Precision was examined by assessing repeatability variance (based on intralaboratory data) and reproducibility variance (based on interlaboratory data). All participating labs were required to fall within a +/- 0.5 median log count comparability goal (Noble *et al.* 2000).

All laboratories had low repeatability variability for all three constituents and all results fell within the median log comparability criteria. Based on all results there were not large variations between the laboratories (i.e. neither of the laboratories were consistently higher or lower for any parameters) in a given sample or for all samples. However, one lab (CSD) reported higher values than the rest, but this can be explained by their inadvertent double diluting of the sample. Also, both Truesdail and Weck laboratories tended to report lower values than the rest. These laboratories should be extra cautious and invest extra efforts in data interpretation in order to not bias the results of bacterial concentrations on the low side.

Figures C1-3 are an example of how the laboratories compared with the different analyses and how well the laboratories were able to reproduce results. These plots are representative of all the data and illustrated good comparability between the analytical labs. As a result of this study we conclude that there should be no bias introduced into the dataset due to sample analysis by different laboratories. All the data and plots are available from SCCWRP upon request.

Table C1. List of participating laboratories and counties involved in the reference bacteria/watershed interlaboratory calibration.

Laboratory Name	County
E. S. Babcock, & Sons, Inc.	Riverside
City of San Diego	San Diego
CRG Marine Laboratories, Inc.	Los Angeles
HCA Public Health Laboratory	Orange
Truesdale Laboratories, Inc.	Orange
Weck Laboratories	Los Angeles
Aquatic Bioassay & Consulting Laboratory (ABC)	Ventura
Weston Solutions, Inc.	San Diego

Table C2. List of the six common samples and their representative sewage dilutions in (ml) which each laboratory received for the interlaboratory calibration.

Media	Dilution (ml)
DI Water	-
Santiago Creek	-
Sewage Dilution 1	3
Sewage Dilution 2	1.5
Sewage Dilution 3	1
Sewage Dilution 4	0.5

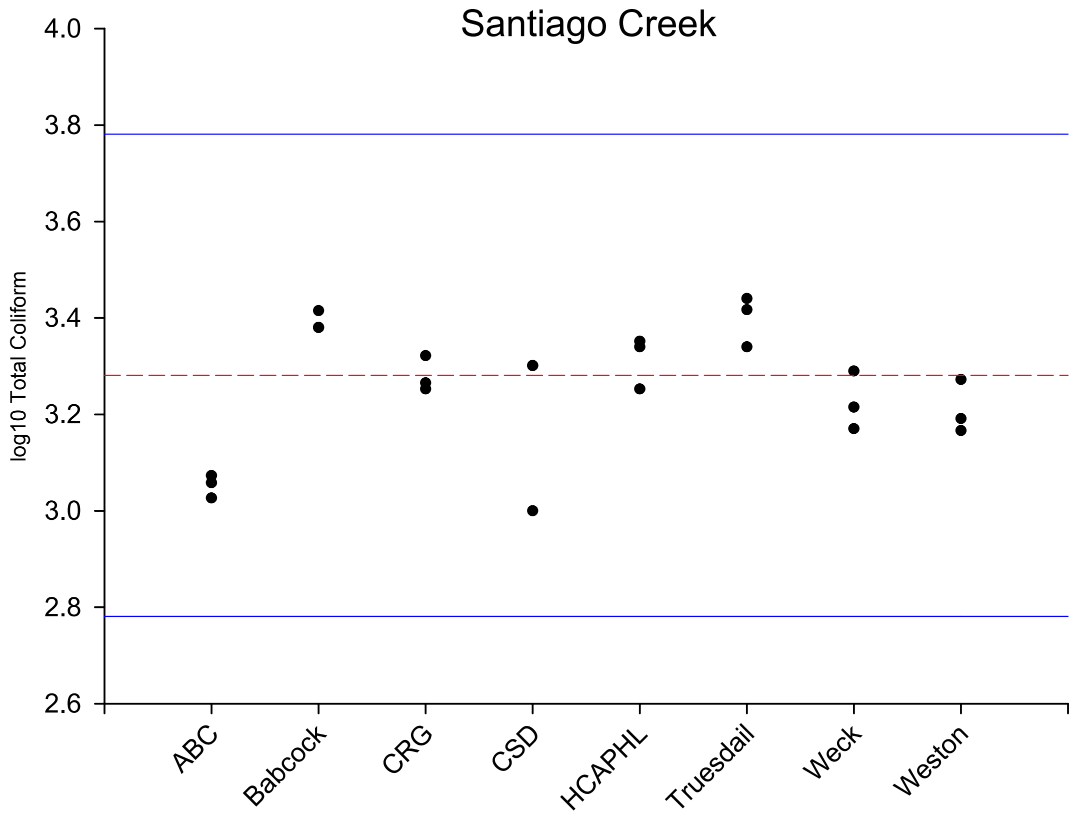

Figure C1. Laboratory comparison results for log transformed total coliform data at Santiago Creek, Orange County. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.

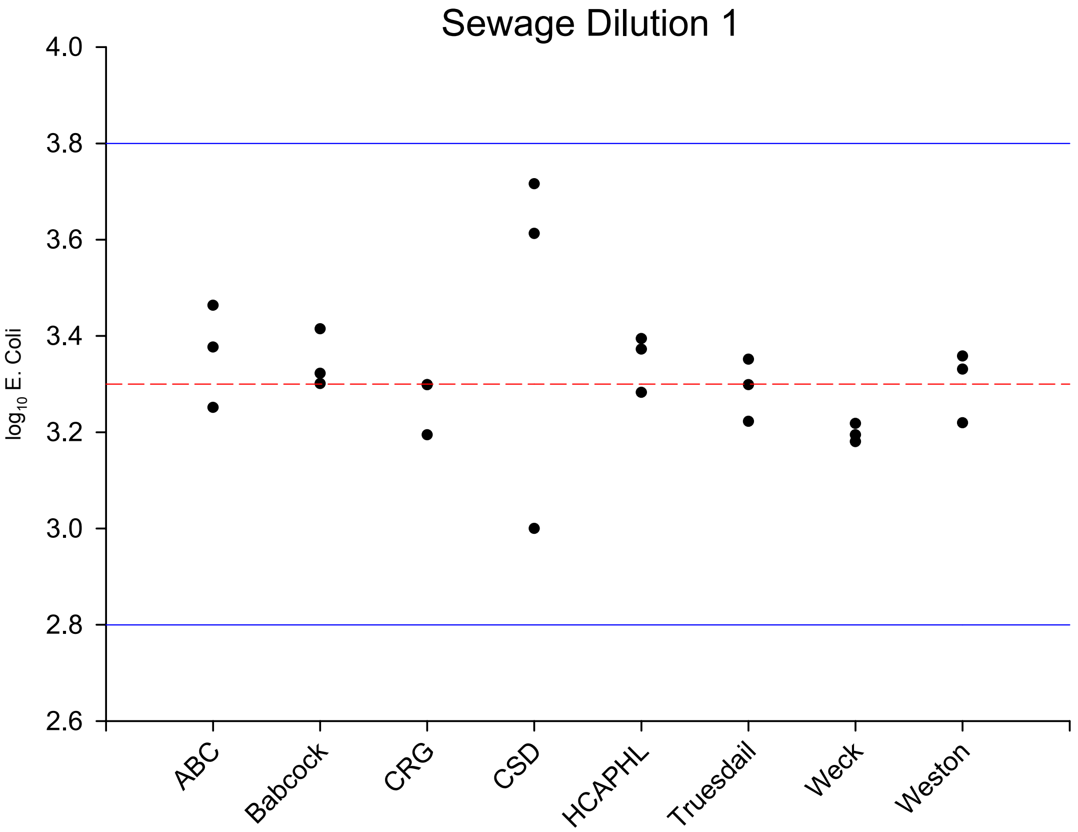

Figure C2. Laboratory comparison results for *E. coli* using a 3 ml sewage dilution. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.

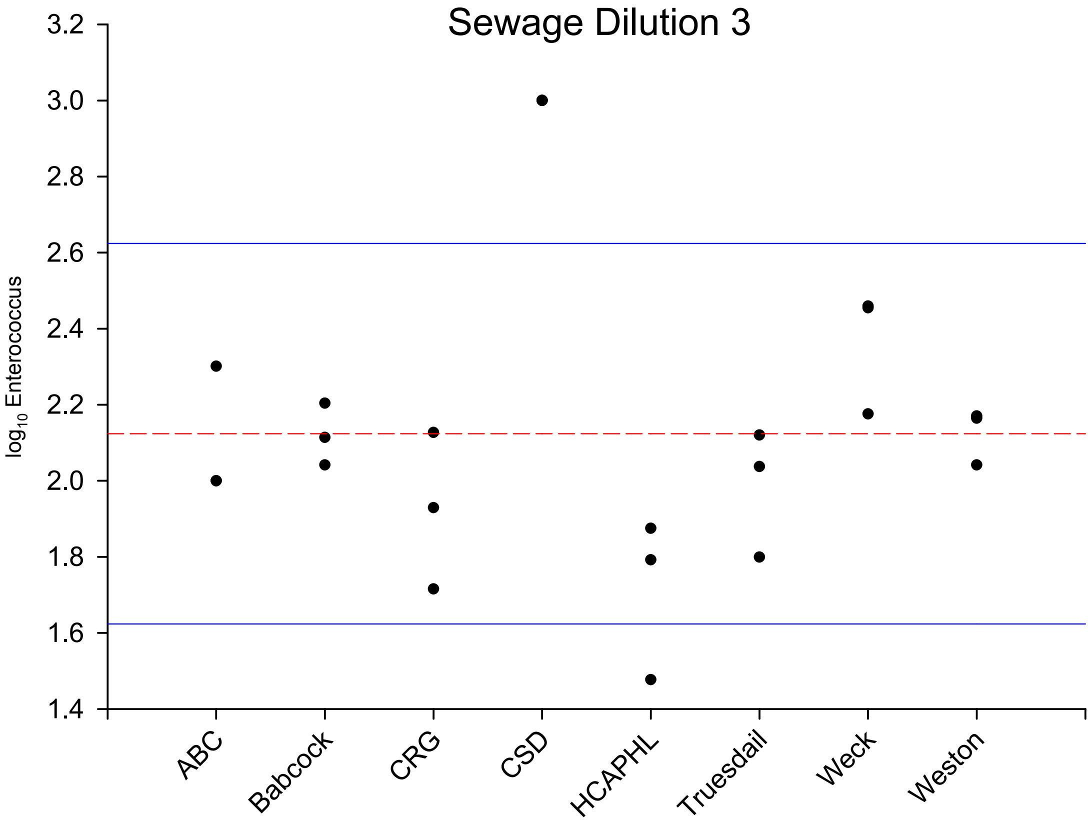

Figure C3. Laboratory comparison results for Enterococcus using a 1 ml sewage dilution. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.