Fecal indicator bacteria levels during dry weather from southern California reference streams

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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. 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. 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. To help establish a regional reference data set, bacteria levels (i.e., *Escherichia coli*, enterococci and total coliforms) were measured from 15 unimpaired streams in 10 southern California watersheds weekly for one full year. Concentrations measured from reference areas were typically between one to two orders of magnitude lower than levels found in developed watersheds. Nearly 82% of the time, samples did not exceed daily and monthly bacterial indicator thresholds. *E. Coli* had the lowest daily percent exceedance (1.5%). A total of 13.7% of enterococci exceeded daily thresholds. 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. Accounting for natural background levels will allow for management targets that are more reflective of bacterial contributions from natural sources.

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. 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 *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 stormwater in southern California. However, most previous studies have focused on either short measurements during or immediately following stormwater runoff or on bacteria in coastal waters. 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 was to establish a "reference" level of bacteria that can be used to set appropriate water quality management targets. More specifically, the following questions were addressed: 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). 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. Catchment land use was determined by plotting watershed boundaries over (year 2003) land cover maps from the National Oceanic and Atmospheric Administration (NOAA) Coastal Change Analysis Program (http://www.csc.noaa.gov/crs/lca/ccap.html). The 15 selected sites are located across 5 counties (Los Angeles, Orange, Riverside, San Bernardino and San Diego) and 10 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).

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 hours and flow was no more than 20% above baseflow. Weekly sampling continued as long as measurable stream flow was present. 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

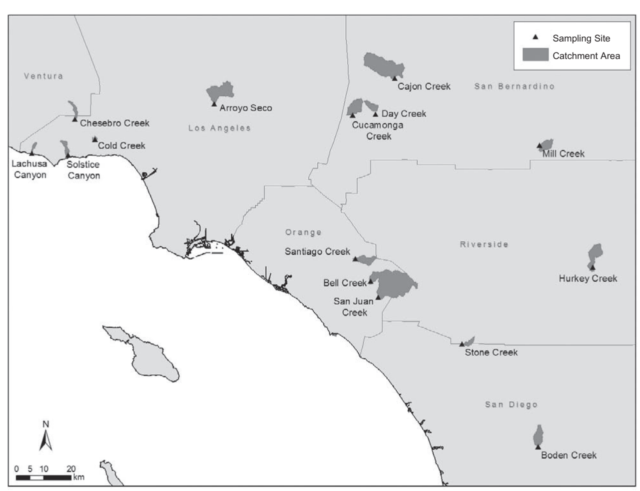

Figure 1. Natural stream sampling sites and their respective catchments within southern California.

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

						Geometric Mean (30-day)					
County	Site Name	Watershed	Catchment Size (km^2)	Number Sampling Weeks/Yr	Mean Flow (m^3/sec)	*E. coli* (MPN/100 ml)	sd	Enterococci (MPN/100 ml)	sd	Total Coliforms (MPN/100 ml)	sd
Los Angeles	Arroyo Seco	LA River	41.5	47	0.04	15.24	2.2	20.48	2.5	1291.90	2.9
	Cold Creek	Malibu Creek	1.4	49	0.00	13.59	1.9	15.33	2.4	443.30	4.3
	Lachusa Canyon	Los Alisos Canyon	3.9	49	0.01	16.08	2.2	20.55	2.3	1486.50	2.1
	Solstice Canyon	Solstice Canyon	8.7	49	0.01	16.97	2.3	20.64	2.4	1109.21	2.7
	Chesebro Creek	Malibu Creek	7.6	49	0.00	90.30	5.5	68.25	4.2	2940.41	2.9
Orange	Bell Creek	San Juan	18.0	12[a]	0.02	80.45	4.3	164.60	5.5	2008.67	3.2
	San Juan Creek	San Juan	99.9	9[a]	0.03	74.66	2.5	25.25	3.3	2848.15	1.7
	Santiago Creek	Santa Ana	17.0	10[a]	0.02	22.99	2.8	34.75	3.1	1869.15	2.0
Riverside	Hurkey Creek	San Jacinto	29.7	29	0.01	18.89	4.4	36.92	4.8	688.57	3.3
San Bernardino	Mill Creek	Santa Ana	15.2	55	0.08	2.06	2.7	12.74	3.3	75.00	3.0
	Cucamonga Creek	Cucamonga	24.1	52	0.14	11.14	1.7	26.35	3.3	399.64	2.4
	Day Creek	Santa Ana	11.7	55	0.32	11.02	1.6	25.18	2.9	545.71	2.4
	Cajon Creek	Santa Ana	82.8	52	0.08	54.98	3.2	159.21	2.5	4794.47	2.0
San Diego	Stone Creek	Santa Margarita	7.0	50	0.00	138.18	3.9	52.72	3.6	1728.44	3.2
	Boden Creek	San Dieguito	19.8	18[a]	0.01	45.33	6.1	98.26	2.9	1658.46	2.5
Mean			25.9	39	0.05	40.79	3.2	52.08	3.3	1592.51	2.7
sd			14.5	9	0.04	19.84	0.7	25.32	0.5	622.94	0.3

[a]Intermittent stream

completion of the initial water sample for approximately 25% of the samples. A field blank sample was also collected at each site once per month. All water samples were collected in pre-sterilized 125-ml high-density polyethylene (HDPE) sample bottles. Collected water samples were immediately placed on ice and transported to laboratories within 6 hours 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 to obtain a total flow value for the transect. Values from three transects were averaged to estimate overall flow at each site (Rantz *et al*. 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 IDEXX® 24-hour for total coliforms. Eight laboratories cooperated on sample analysis. Laboratory intercalibration studies were completed to ensure consistent methodology, data quality, and repeatability among laboratories. All laboratories had good repeatability for all three bacterial indicators, and all results fell within the median log comparability criteria. The low variability between laboratories indicated that inter-lab differences should not be a confounding factor in interpreting the study results.

B. 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 2000). In the present study, 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 California 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).

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

	CA Maximum Allowable Density (MPN/100 ml)	
	Single-sample	Geometric Mean 30-day
Fecal Indictor Bacteria		
Enterococci	104	33
E. coli	235	126
Total Coliforms	10,000	1000
Additional Indicator		
Bacteroides thetaiotaomicron	Presence/absence of a human source	

Data Analysis

Three analyses were used to characterize FIB levels from natural streams. First, the 30-day geometric means, variances, concentration ranges, 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-day geometric mean (geomean) or the 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 California standards for single-sample and 30-day geomean maximum allowable densities (Table 2). Cumulative density frequency plots (CDFs) were produced to compare observed bacterial concentrations to the California 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 and 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 strong associations between FIB concentrations and continuous variables (i.e., temperature and flow existed (Helsel and Hirsch 2002); in this case, the null hypothesis 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; median density values in the developed Ballona Creek are typically in the 10 MPN/100 ml 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 year long 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

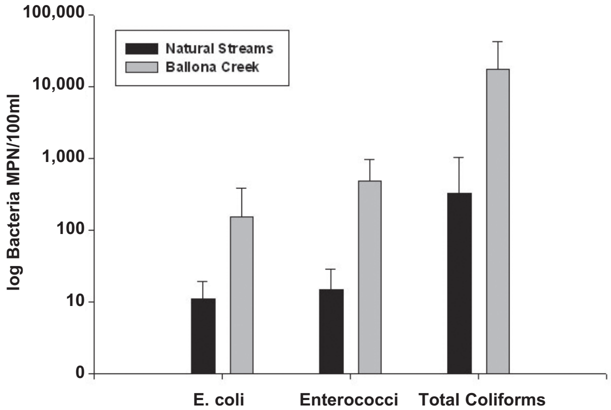

Figure 2. Comparison of dry weather log10 fecal indicator bacteria (FIB) densities (± standard deviation) between natural streams in undeveloped watersheds and developed Ballona creek watershed from May 2006 to May 2007 in southern California.

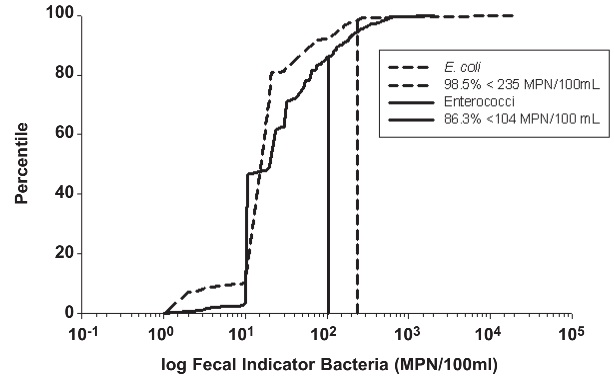

Figure 3. Dry season fecal indicator bacteria (FIB) cumulative density frequency plots (CDFs) of natural streams relative to freshwater quality standards from May 2006 to May 2007 in southern California.

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 of 5500 MPN/100 ml and and a minimum of 241 MPN/100 ml (Orange County). For total coliforms, 3% exceeded the single-sample standard of 10,000 MPN/100 ml. Table 3 presents a comparison of *E. coli*, enterococci, and total coliforms exceedances by county.

A total of 39% of enterococci samples from the natural sites exceeded the 30-day 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-day geomean threshold of 126 MPN/100 ml with a maximum of 146 MPN/100 ml and a minimum of 1 MPN/100 ml (Orange County). For total coliforms, 45% exceeded the 30-day geomean of 1000 MPN/100 ml with a maximum of 5040 MPN/100 ml and a minimum of 23 MPN/100 ml.

Temporal and Spatial Patterns in FIB Levels

Bacteria levels for all three indicators were significantly higher during the summer than during all other seasons (p <0.01; Table 4). Seventy-five percent of enterococci and 83% of total coliforms exceedances occurred during the summer months

Table 3. Table 4. Assessment of percent exceedances between counties in southern California during the present study.

	Exceedance (%)		
	E. coli	Enterococci	Total Coliforms
Daily			
Los Angeles County	0	6.3	0
Orange County[1]	12.9	38.7	3.2
San Bernardino	0	13.1	0
San Diego[1]	5.3	47.4	0
Monthly			
Los Angeles County	0	7.7	46.2
Orange County[1]	25	75	100
San Bernardino	0	23.1	0
San Diego[1]	0	100	80

[1] Counties in which samples were collected only during spring and/or summer due to intermittent streams with less stable flow regimes

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

	Exceedance (%)		
	E. coli	Enterococci	Total Coliforms
Season			
Spring 06	0	41.7	75
Summer	**12.5**	**75**	**83.3**
Fall	0	0	28.6
Winter	0	0	11.1
Spring 07	0	22.2	44.4
Month			
May-06	0	27.3	45.5
Jun-06	0	66.7	75
Jul-06	0	**72.7**	**90.9**
Aug-06	**12.5**	**62.5**	**75**
Sep-06	0	42.9	57.1
Oct-06	0	0	14.3
Nov-06	0	0	28.6
Dec-06	0	0	14.3
Jan-07	0	0	0
Feb-07	0	12.5	25
Mar-07	0	22.2	11.1
Apr-07	0	11.1	44.4
May-07	0	25	62.5
Annual	1	26.4	41.8

(June-August). For example, 30-day geomeans for total coliforms were slightly below the water quality standard in May 2006 with levels approximately 878 MPN/100 ml ±3.2 standard deviance (sd), but increased substantially during the summer, exceeding the criterion and 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 5).

Perennial vs. Non-perennial Streams

Background bacteria levels differed based on the duration of stream flow (Table 1). *E. coli* and enterococci densities were significantly different in perennial vs. intermittent streams, but ranges generally overlapped (p <0.05; Figure 6). Mean 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 log10 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).

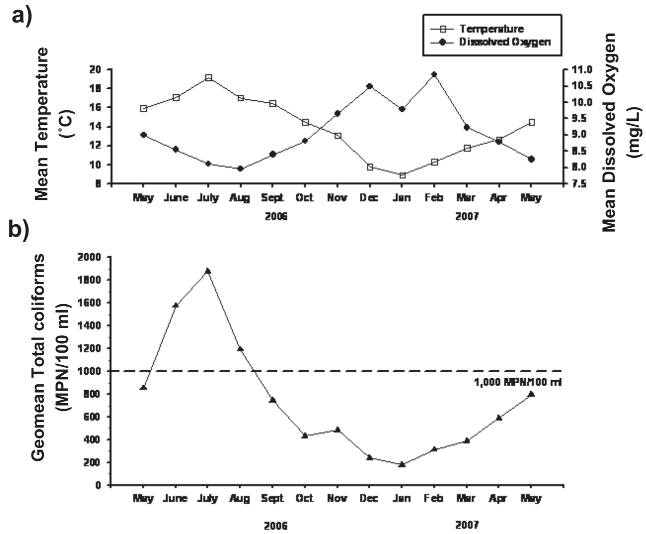

Figure 4. Mean monthly temperature (°C) and dissolved oxygen (mg/L) comparison (a) and geometric mean densities for total coliforms (b) in natural streams in southern California between May 2006 and May 2007. These parameters were substantially higher during the summer (June-August) than all other seasons (p <0.01). Dotted line indicates the 30-day geometric mean for total coliforms; all points above the line represent water quality exceedances for bacteria (b).

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 approximately 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 County) and significantly shaded creeks had the lowest average temperatures (Table 1). 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

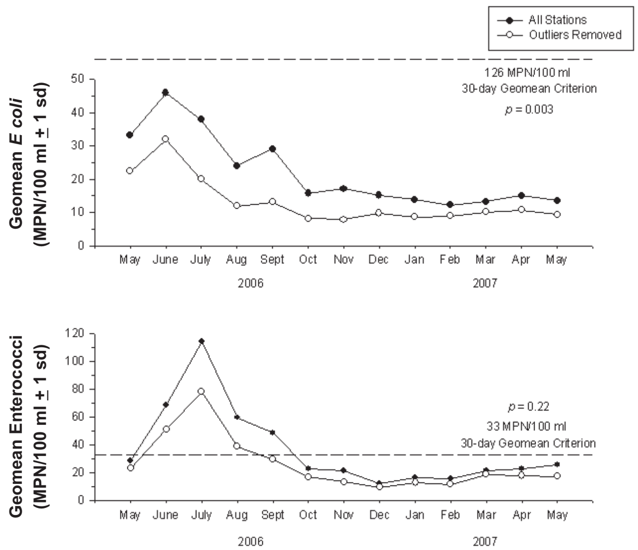

Figure 5. *E.coli* and enterococci geometric mean densities in natural streams in southern California between May 2006 and May 2007. These densities were substantially higher during the summer (June-August) than all other seasons. Dashed lines indicate monthly water quality standards for *E. coli* and enterococci. All points above the line represent water quality exceedances for bacteria.

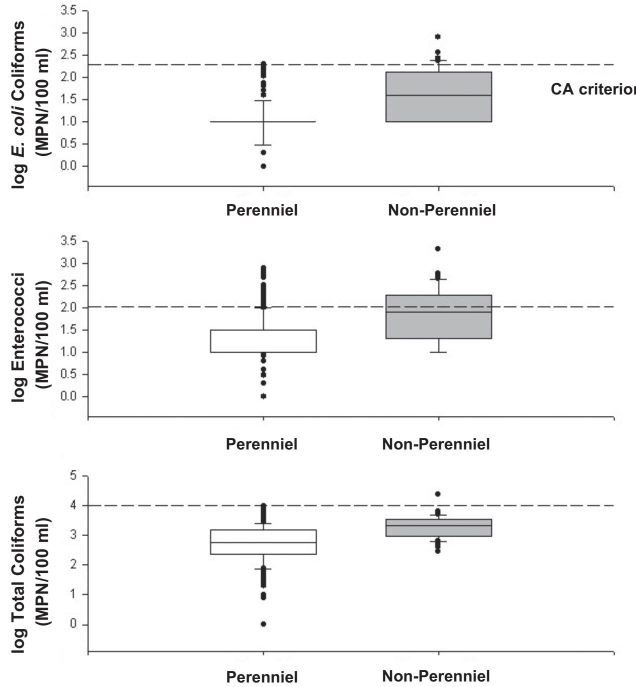

Figure 6. Perennial and non-perennial stream comparison of log10 fecal indicator bacteria (FIB) densities in southern California during the present study. Dotted line indicates the State single-sample bacterial water quality criterion. Boxplots show mean, median, and 25th and 75th percentiles.

200 m in altitude. Stream temperature and total coliforms had a significantly positive correlation ($p < 0.001$, $r^2 = 0.48$; Table 5), with total coliform densities increasing exponentially at temperatures above 10°C ($r^2 = 0.48$; Figure 7). 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 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 DO and both conductivity and stream temperature ($p < 0.05$, $r^2 = -0.5$ and $p < 0.001$, $r^2 = -0.84$, respectively; Table 5). 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; concentrations increased to approximately 11 mg/L at stream temperatures below 10°C. Few statistically significant relationships existed among the other physical variables.

Discussion

Enterococci, *E. coli*, and total coliforms 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 DO

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 and May 2007.

Parameter	Pearson r^2 Values				
	DO (mg/L)	Flow (m^3/s)	*E. coli* (MPN/100 ml)	Enterococci (MPN/100 ml)	Total Coliform (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**	***0.2***	***0.26***	***0.48***
Turbidity	0.19	0	0.02	1.44	**0.07**

Bolded values = p <0.05
Bolded italic values = p <0.001

content of the water. Decreased oxygen solubility associated with higher temperature may combine with lower DO 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 DO. 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).

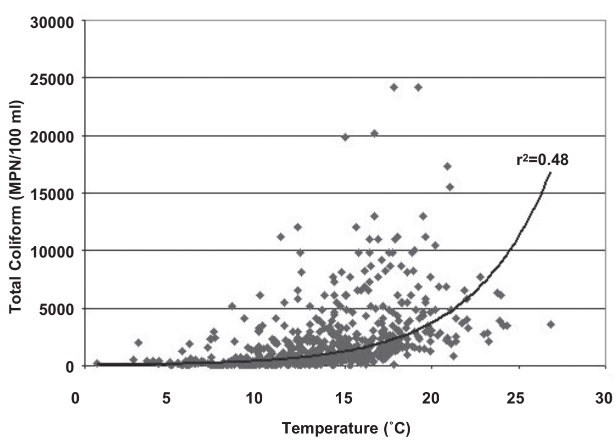

Figure 7. Natural stream temperatures in southern California versus total coliform densities during dry weather for an entire year. Solid line indicates the exponential trend line.

Higher FIB densities and incidence of water quality standard exceedances 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* 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 patterns of use by wildlife and birds. A number of studies have shown that wildlife and other animals can be sources of bacteria in runoff (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 103 - 105 CFU/g from native wildlife in Australian watersheds. Ricca and Cooney (1998) reported that droppings from feral populations of pigeons, geese, and herring gulls in the environment around Boston Harbor, MA, contained up to 108 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 suggest that feces from pets and raccoons are important contributors to FIB levels in streams and storm sewers (Ram *et al*. 2007). Moreover, bacteria levels appeared to increase in the late summer and fall coincident with increased raccoon den mobility following breeding.

Decreased stream flow may also contribute to higher bacteria levels during the summer months. Although the present study did not demonstrate a 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. In addition, median annual bacterial densities were higher in intermittent streams than in perennial, with the differences being primarily due to high FIB 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 California.

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 present study had bacteria levels 2 - 3 log units greater than the natural sites that were retained. Natural streams had significantly lower bacteria concentration levels than all other streams (p <0.001), and minor perturbation streams had significantly lower levels than Ballona Creek (p <0.001; 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 had been affected by a recent fire. These small perturbations in the watershed led to dramatic changes in bacteria levels resulting in increasingly non-reference site conditions. Although these sites were not included in the

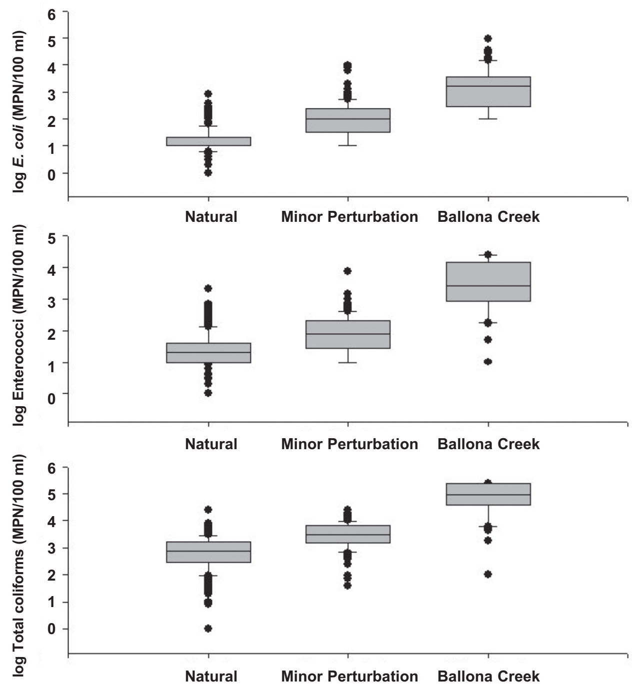

Figure 8. Distribution of log *E. coli*, enterococci, and total coliforms concentrations in natural streams, streams with minor perturbations, and in the Ballona Creek watershed in southern California.

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 stormwater is important because FIB are major constituents of concern in stormwater 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 stormwater geometric mean FIB levels from natural streams 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 (p <0.001; 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

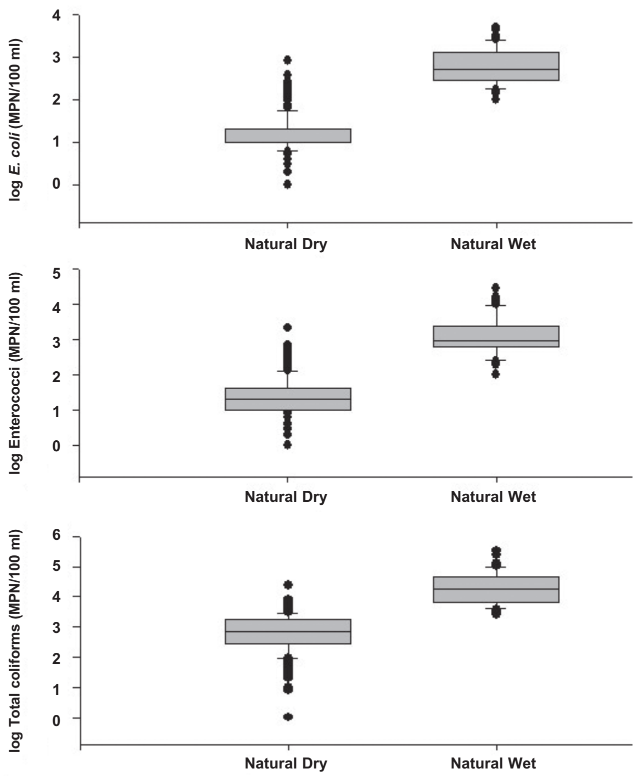

Figure 9. Distribution of log *E. coli*, enterococci, and total coliforms concentrations in natural streams during dry weather (present study) compared to wet weather (Natural Loadings 2003-2005 and Los Angeles River watershed 2001-2005) studies in southern California.

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 indicate that streams in undeveloped watersheds contain low levels of FIB of non-human origin. Whether the levels observed pose a potential health risk is an important management question. 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). The present study 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 inferred that background levels in natural streams have a low likelihood of posing a human health risk. However, this inference should be made with caution based on previous exposure and risk studies conducted in undeveloped areas known to receive wastewater or stormwater discharges containing human fecal sources. Moreover, further study is required to more fully understand the relationship between background FIB levels and the contribution of *E. coli* as an indicator of non-human fecal sources and its implication in management decisions regarding human health risks.

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