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Fecal indicator bacteria (FIB) levels during dry weather from Southern California reference streams

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Received: 20 March 2008 / Accepted: 25 June 2008 / Published online: 14 August 2008
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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* (*E. coli*), enterococci and total coliforms] were measured from 15 unimpaired streams in 11 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 to 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 the contributions from natural sources.

Keywords Dry weather water quality · Indicator bacteria · Reference condition · Background water quality · TMDL

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 water bodies being listed as impaired under Section 303(d) of the Clean Water Act. Management of impaired water bodies may

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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 and Storrie Ltd. and Proctor and Redfern Ltd. 1981; US EPA Environmental Protection Agency 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 (2008). 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 3 years. Catchment land use was determined by plotting watershed boundaries over land cover maps from the National Oceanographic Administration (NOAA; year 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 (Fig. 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 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

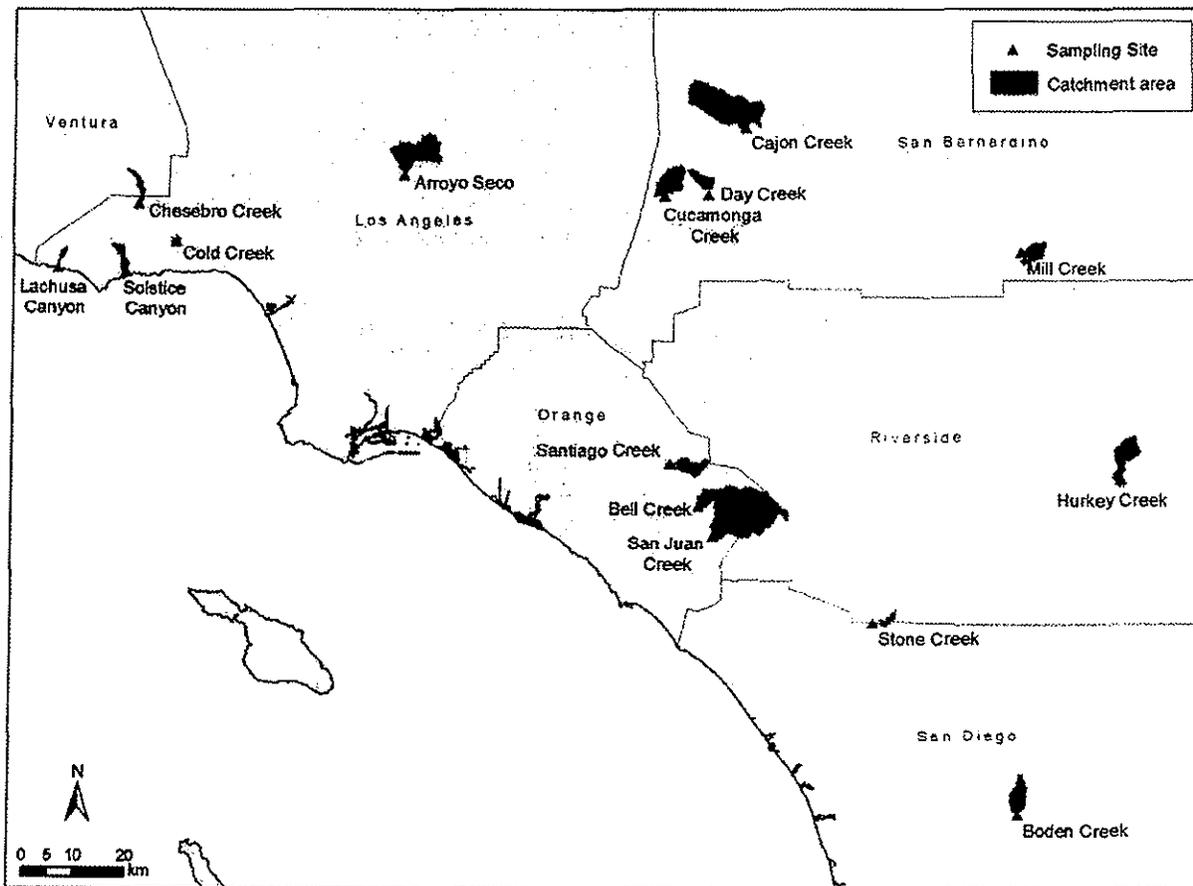


Fig. 1 Map of natural stream sampling sites and their respective catchments within southern California

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

Site name	Watershed	County	Catchment size (km ²)	Number sampling events (weeks/year)	Mean flow (m ³ /s)	Geomean (30-day)					
						<i>E. coli</i>		Enterococci		Total coliforms	
						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

^aIntermittent stream

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, USA). 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 and others 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.). Eight laboratories cooperated on sample analysis. Laboratory intercalibration studies were completed to ensure consistent methodology, data quality, and repeatability between 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 labs indicated that interlab differences should not be a confounding factor in interpreting the results of the study.

Bacteroides 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). 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-day 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-day geomean or mean yearly bacterial concentration (MPN/100 ml) and contributing watershed area (square kilometer) at a specific site. Second, dry weather FIB concentrations were compared with the state of CA 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 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; Yoon and Stein 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

Table 2 State of California marine water quality standards for fecal indicator bacteria as established in Assembly Bill 411

Fecal indicator bacteria	CA maximum allowable density (MPN/100 ml)	
	Single-sample	30-day geometric mean
Enterococci	104	33
<i>E. coli</i>	235	126
Total coliforms	10,000	1,000
Additional indicator <i>Bacteroides thetaiotaomicron</i>	Presence/absence of a human source	

Currently a freshwater quality standard for total coliforms does not exist

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/km², 3 ± 1.7 MPN/100 ml/km², and 106 ± 61.4 MPN/100 ml/km² 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 the developed Ballona Creek are typically in the 10³ range. Densities and fluxes were significantly lower for all indicator bacteria at the natural sites relative to data from developed areas ($p < 0.001$, Fig. 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 (Fig. 3). The average enterococci level of these exceedances was 292 MPN/100 ml, with a maximum of 2,098 MPN/100 ml (Orange County) and a minimum of 160 MPN/100 ml (San Bernardino

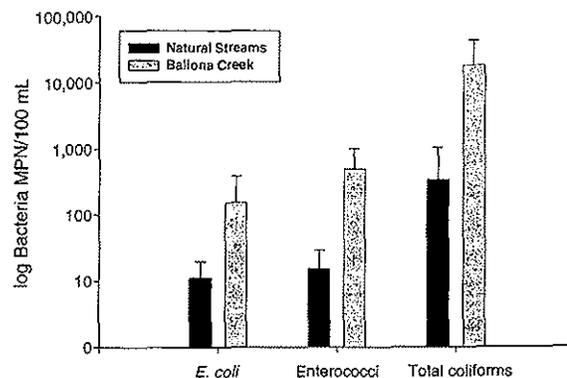


Fig. 2 Comparison of dry weather log₁₀ fecal indicator bacteria (FIB) densities (\pm standard deviations) between natural streams in undeveloped watersheds and developed Ballona creek watershed from May 2006 to May 2007 in southern California, USA

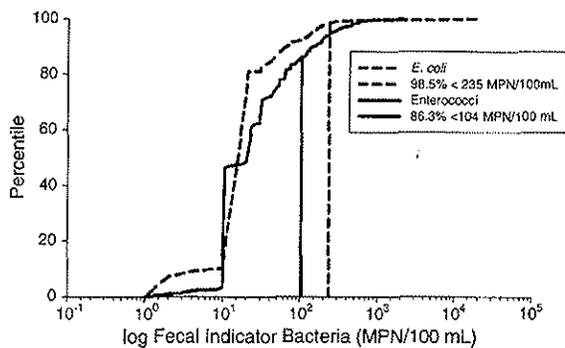


Fig. 3 Dry season fecal indicator bacteria cumulative density frequency plots (CDFs) of natural streams relative to freshwater quality standards from May 2006 to May 2007 in southern California, USA

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 5,500 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-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 and a minimum of 146 and 1 MPN/100 ml, respectively (Orange County). For total coliforms, 45% exceeded the 30-day geomean of 1000 MPN/100 ml with a maximum and a minimum of 5,040 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 to August, Table 3). 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 3).

Temporal and spatial patterns in FIB levels

Bacteria levels for all three indicators were significantly higher during the summer than during

Table 3 Percent single-sample exceedance of fecal indicator bacteria (FIB) levels in natural streams during dry weather from May 2006 to May 2007

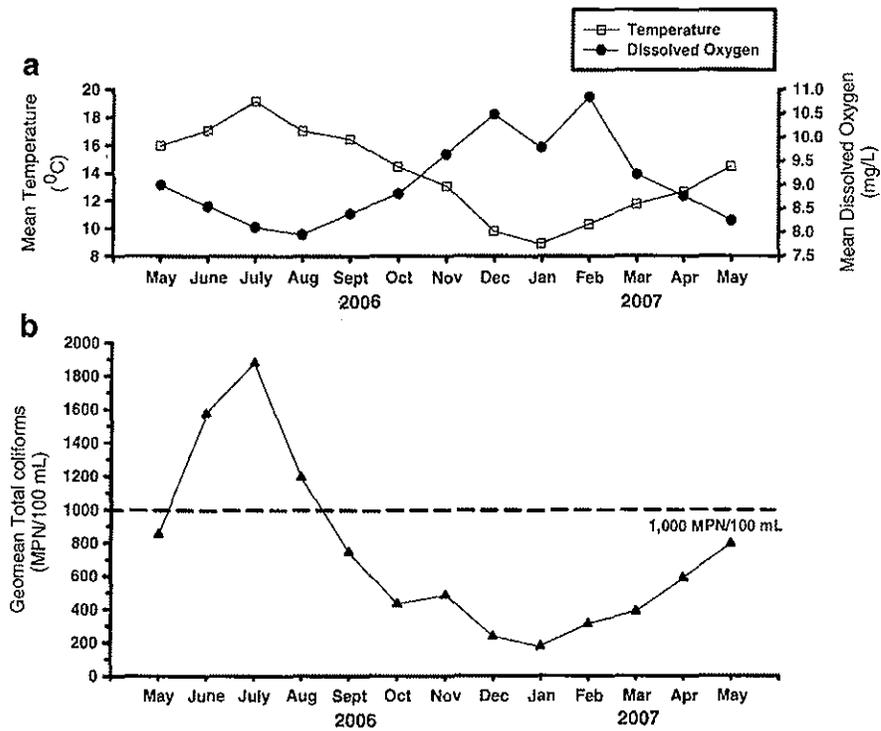
	Exceedance (%)		
	<i>E. coli</i>	Enterococci	Total coliforms
Season			
Spring 06	0.0	41.7	75.0
Summer	12.5	75.0	83.3
Fall	0.0	0.0	28.6
Winter	0.0	0.0	11.1
Spring 07	0.0	22.2	44.4
Month			
May 2006	0.0	27.3	45.5
June 2006	0.0	66.7	75.0
July 2006	0.0	72.7	90.9
August 2006	12.5	62.5	75.0
September 2006	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
February 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

Numbers in bold are significantly different ($p < 0.01$)

all other seasons (Table 3, $p < 0.01$). 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 \pm 3.2 SD, but increased substantially during the summer, exceeding the criterion and peaking in July at 2586 MPN/100 ml \pm 3.1 SD (Fig. 4b). Total coliform geomeans decreased gradually throughout the winter nearing zero in February, 2007 (289 MPN/100 ml \pm 4.2 SD) as stream temperatures fell below 10°C, before gradually returning to baseline geomeans throughout spring, 2007 (Fig. 4a and b). Similar seasonal patterns were observed for *E. coli* and enterococci (Fig. 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 4). For enterococci, approximately 47% of the San Diego County samples exceeded the daily threshold and 100% exceeded the monthly standard (Table 4). However, the Orange County and San Diego County

Fig. 4 Mean monthly temperature ($^{\circ}\text{C}$) 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 to August) were substantially higher than all other seasons ($p < 0.01$). *E. coli* and enterococci exhibited similar results. The dotted line indicates the 30-day geomean for total coliforms equal to 1,000 MPN/100 ml. All points above the line represent bacteria water quality exceedances



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

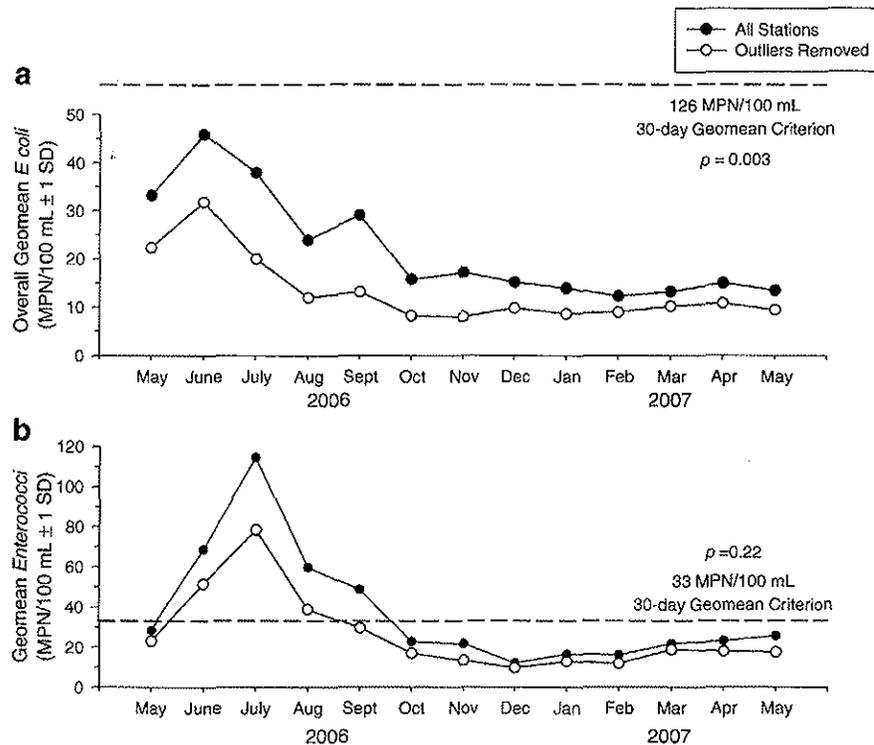
Background bacteria levels differed based on the duration of stream flow (Table 1). *E. coli* and enterococci densities were significantly different in perennial vs. intermittent streams ($p < 0.05$, Fig. 6). Concentrations for *E. coli* and enterococci at perennial streams were 1.0 ± 0.4 and 1.3 ± 0.5 , 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 $^{\circ}\text{C}$ at each of the sites, reaching a maximum of 28 $^{\circ}\text{C}$ 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). For example streams in San Bernardino County ranged from 650 to 1,200 m in elevation and averaged 12.7 $^{\circ}\text{C}$. The highest monthly average water temperatures (20.4 $^{\circ}\text{C}$) were recorded in Orange County where streams were approximately 200 m in altitude. Monthly mean DO concentrations decreased sharply to approximately 8 mg/L at stream temperatures above 15 $^{\circ}\text{C}$, and concentrations increased to approximately 11 mg/L at stream temperatures below 10 $^{\circ}\text{C}$. Few statistically significant relationships existed among the other physical variables. Stream temperature and total coliforms were significantly positively correlated (Table 5, $p < 0.001$, $r^2 = 0.48$) with total coliform densities increasing exponentially at temperatures above 10 $^{\circ}\text{C}$ (Fig. 7, $r^2 = 0.48$). A weaker, but still significant, positive correlation

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



existed between stream temperature and *E. coli* or enterococci ($p < 0.04$, $r^2 = 0.20$ 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 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). Dissolved oxygen concentrations varied inversely with stream temperatures throughout the study (Fig. 4a).

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

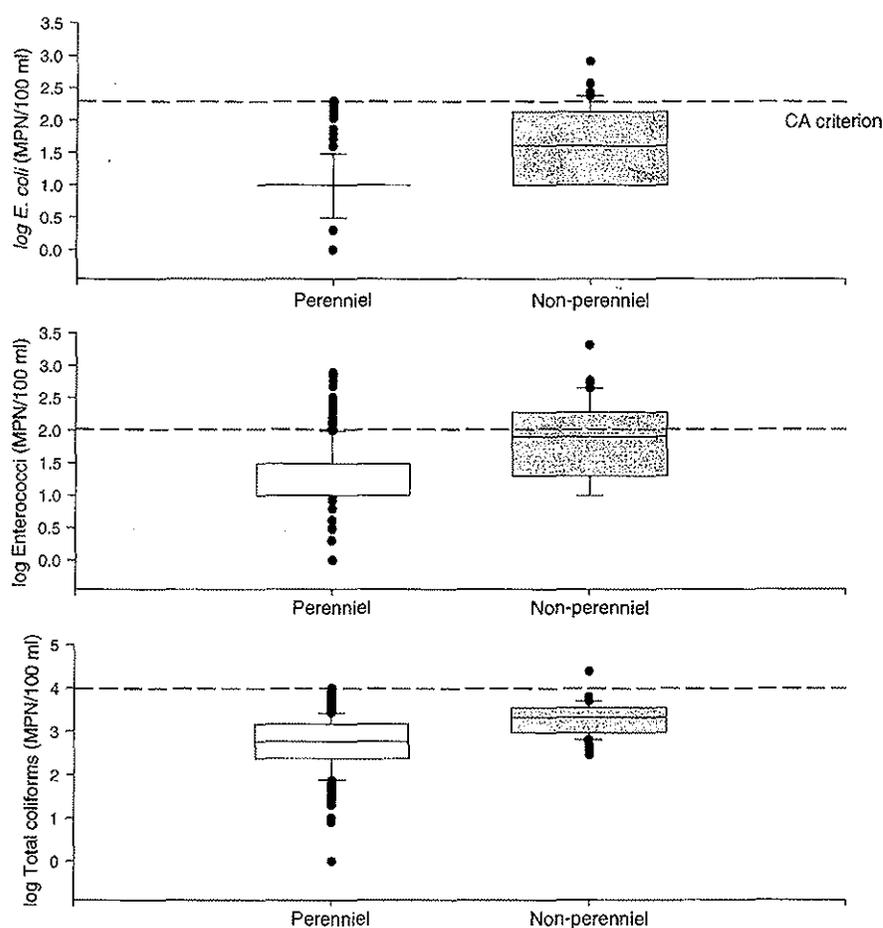
	Exceedance (%)		
	<i>E. coli</i>	Enterococci	Total coliforms
Daily			
Los Angeles County	0.0	6.3	0.0
Orange County ^a	12.9	38.7	3.2
San Bernardino	0.0	13.1	0.0
San Diego ^a	5.3	47.4	0.0
Monthly			
Los Angeles County	0.0	7.7	46.2
Orange County ^a	25.0	75.0	100.0
San Bernardino	0.0	23.1	0.0
San Diego ^a	0.0	100.0	80.0

^aCounties in which samples were collected only during spring and/or summer due to intermittent streams with less stable flow regimes

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 ori-

Fig. 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 ($p < 0.05$). Box plots show mean, median, 25th and 75th percentiles



gin (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

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 to May 2007

Parameter	Pearson r^2 values				
	DO (mg/l)	Flow (m ³ /s)	<i>E. coli</i>	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	0.20	0.26	0.48
Turbidity	0.19	0.00	0.02	1.44	0.07

Bold values, $p < 0.05$; bold italic values, $p < 0.001$

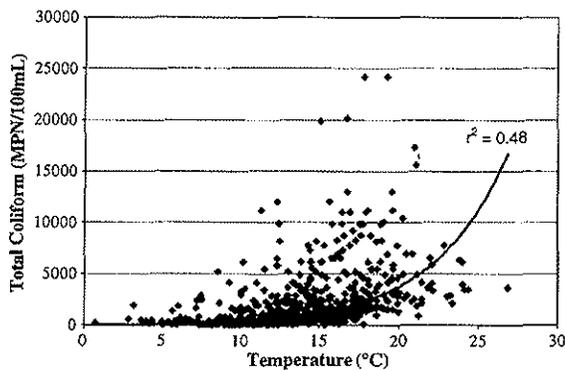


Fig. 7 Natural stream temperatures in southern California versus total coliform densities (MPN/100 ml) during dry weather for an entire year. Solid line indicates the exponential trend line ($r^2 = 0.48$)

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, 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 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 sun-

light. More recently, growth or regrowth of fecal indicator bacteria in tropical and temperate soils during the summer months has also been reported (US EPA United States Environmental Protection Agency 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 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 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 $0.5 \text{ m}^3/\text{s}$ ($2 \text{ ft}^3/\text{s}$). In addition, median annual bacterial densities were higher in intermittent streams than in perennial, with the dif-

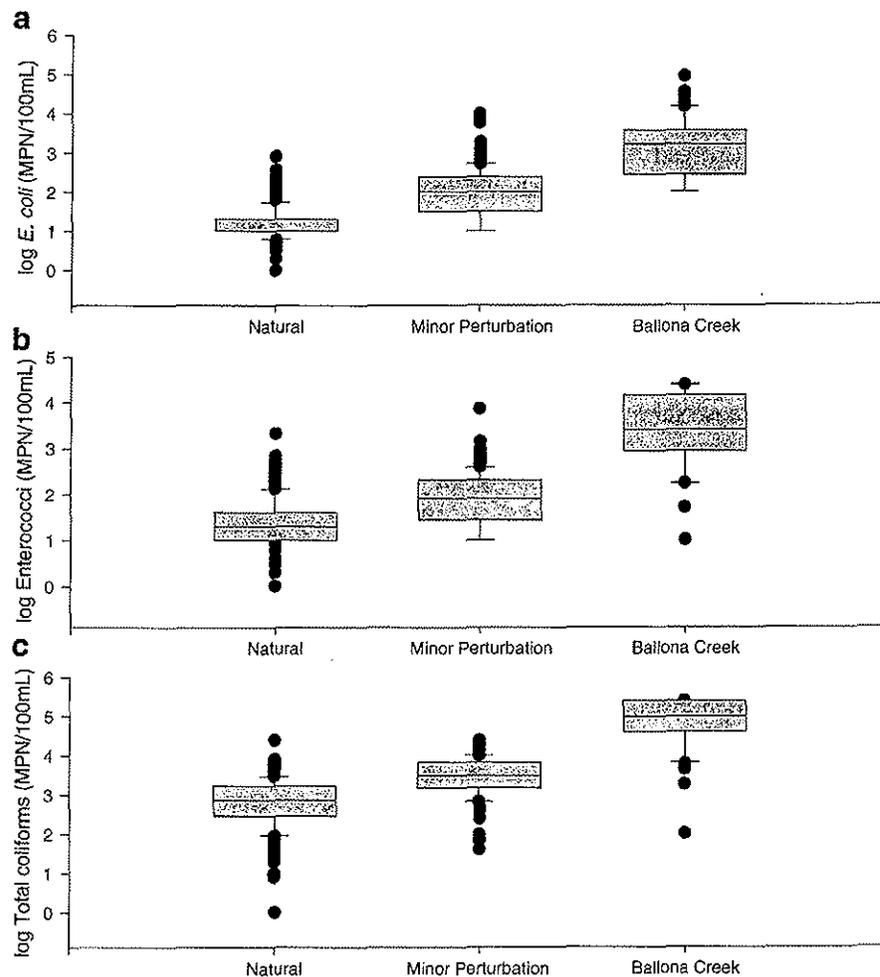
ferences being mainly due to high FIB levels in the period immediately prior to streams drying up. Despite the differences between perennial and intermittent streams, the annual ranges of observed bacteria levels overlapped substantially. Therefore, the combined range of bacteria levels for perennial and intermittent streams observed in this study should reflect expected levels in natural streams throughout southern 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 (Fig. 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

Fig. 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 than all other streams ($p < 0.001$). Minor perturbation streams were significantly lower than developed Ballona Creek ($p < 0.001$)

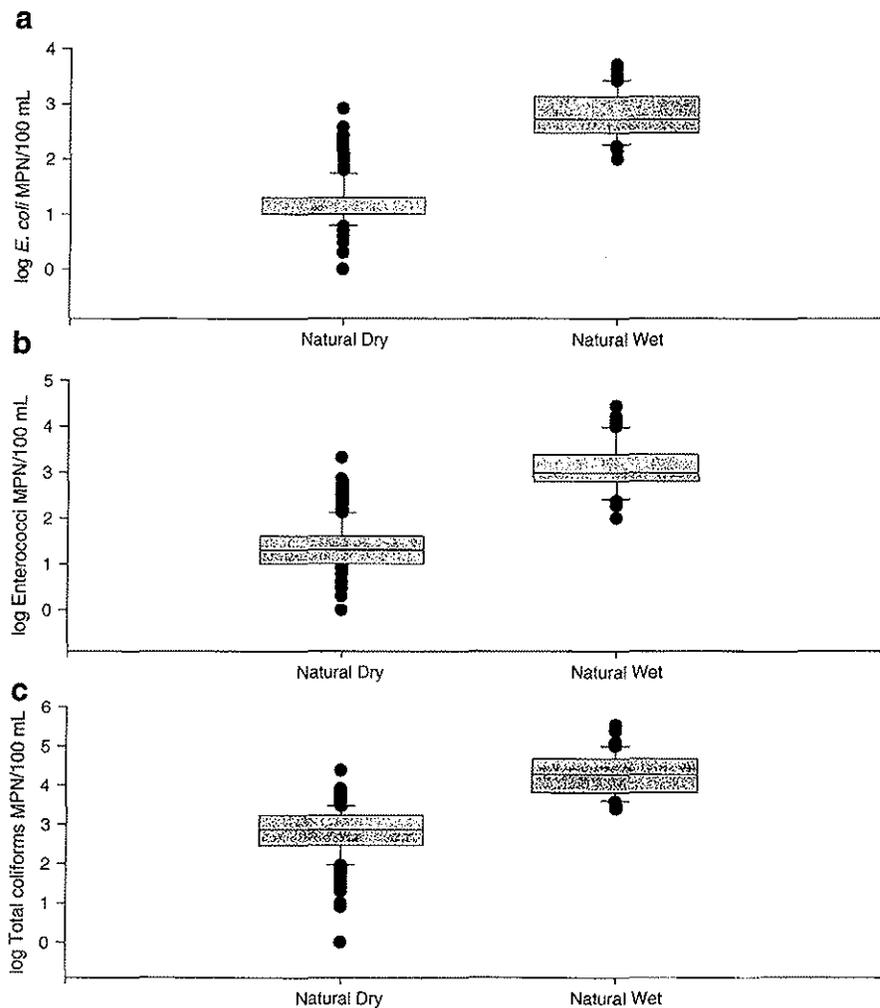


levels in storm water is important because FIB are major constituents of concern in storm water runoff that can result in impairment of receiving waters (Noble et al. 2003; Schiff et al. 2003; Stein and Tiefenthaler 2005). Stein and Yoon (2007) reported storm water geometric mean FIB levels from natural 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 (Fig. 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 3 days of rainfall from beaches at the bottom of natural catchments exceeded water quality thresholds for at least one bacterial indicator. Analogous measurements collected 3 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 freshwa-

Fig. 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 than wet weather concentrations ($p < 0.001$)



ter pose a relative human health risk level of 1.22 (i.e. low level risk). We observed 30-day geometric *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.
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 $0.5 \text{ m}^3/\text{s}$ ($2 \text{ ft}^3/\text{s}$). 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 3 days following storms. Analogous measurements collected 3 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.

Acknowledgements The authors of this study would like to thank Ron Vandergoot, Manny Martinez, Lisa Gonzales, Scott Jakubowski, Tom Clem, David Ortega, Dave Woelfel, Bill Rice, Linda Parady, Steven Di Donna, Nick Miller, Melissa Jarvis and Chris Gliniak for their assistance and dedication in collecting the dry weather bacteria samples. We also thank Kenneth Lidell, Dave Woelfel and Janet Dietzman for database entry and submittal. Next we would like to acknowledge Associated Labs of Orange (Debbie Smith), City of San Diego (Ric Amador), CRG Marine Laboratories, Inc (Rich Gossett), E.S. Babcock and Sons, Inc. (Carol Kase), Orange County Water District (Menu Leddy), Truesdail Laboratories, Inc. (Kenneth Lidell), and Weck Laboratories, Inc. (Marilyn Romero) whom provided the bacterial analyses. We would also like to thank Charlie McGee (Orange County Sanitation District) and John Griffith (SCCWRP) who provided valuable suggestions and guidance with the interlaboratory calibration. Finally we would like to thank Andrew Fields (SCCWRP) for database QA/QC and data analysis. Funding for this study was provided by the Malibu TMDL SAG, City of Laguna Nigel and other Orange County Cities, City of San Diego, County of San Diego, Riverside County Flood Control District, San Bernardino County Flood Control District on behalf of the San Bernardino County Stormwater Program, Santa Ana Regional Water Quality Control Board, and the Southern California Coastal Water Research Project (SCCWRP).

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