Wet and Dry Weather Natural Background Concentrations of Fecal Indicator Bacteria in San Diego, Orange, and Ventura County, California Streams

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EXECUTIVE SUMMARY

Elevated levels of fecal indicator bacteria (FIB) are a common problem in urban surface water and may lead to impairment of beneficial uses, such as swimming or other contact recreation. Once impaired, common regulatory solutions include establishing Total Maximum Daily Loads (TMDLs), incorporating those TMDLs into National Pollutant Discharge Elimination System (NPDES) permits, and other water quality management plans. A reference system approach is a critical element of these TMDLs where natural sources are documented, and a number of exceedance days are allocated based on the frequency at which "reference" sites with natural sources of bacteria exceed established FIB water quality standards. The goal of this study was to characterize the natural background concentrations of bacteria, nutrients, heavy metals and conventional constituents in undeveloped catchments during wet weather (storm), the three days following a storm, and dry weather (non-storm) conditions, and to categorize FIB water quality objective (WQO) exceedance frequency by geomorphologic, hydrologic, biotic and abiotic factors. This report focuses on presenting the findings for FIB, specifically *Escherichia coli* (*E. coli*; EC), enterococcus (ENT), total coliforms (TC) and fecal coliforms (FC).

Specific questions addressed in the study were:

1. How does the WQO exceedance frequency for FIB vary between wet weather, summer dry weather and winter dry weather?
2. How does FIB vary by stream landscape and site-specific factors, including:
    - Catchment size and geology?
    - Wet weather parameters such as size, timing of storm, and number of antecedent dry days? and
    - Dry weather factors such as flow, stream physiochemical parameters (temperature, conductivity, and turbidity), chemical parameters (nutrients, organic carbon, metals, and conventional constituents) and trophic status, as measured by algal abundance?

Flow-weighted sampling of FIB was conducted during a total of eight storm events at 5 sites, for a total of 118 FIB samples over the period of January 2012 to May 2015. In addition, 427 FIB samples were collected during weekly dry weather sampling in 10 intermittent stream sites in 10 watersheds located in San Diego, Orange and Ventura Counties from January 2012 to August 2014. Nutrients, trace metals and other conventional constituents were collected biweekly; while these data will be presented in a separate report, they are used here as supporting data. Sites were selected to meet reference screening criteria and to represent a mix of watershed size (varying from <33 km^2 to > 66 km^2) and sedimentary versus igneous/metamorphic geology. The human-associated fecal microbial source marker HF183 was used to eliminate sites or samples with potential human contamination. FIB concentrations and fluxes from this study were compared with data from previous studies in the region.

The study had five major findings:

1) **FIB exceedances occurred in natural sites and were highest in summer dry weather. (April –August).** Exceedances of single sample WQOs occurred at annual frequencies ranging from 1.4 to 3% for TC and EC, and up to 30% for ENT. No exceedances of FC single sample WQOs were observed. Annual frequencies of 30-day geomean WQO exceedances were 0% for EC and FC, but 48 and 30% for ENT and TC, respectively. Exceedance frequencies were highest in the summer, spiking up to 40% for ENT single sample WQO and 68% for the 30-day geomean WQO. Exceedance frequencies were 15-20% higher then rolling-versus monthly 30-mean is used.

2) **Storm EMC exceedances were low except for ENT.** Based on seven storms, EMC exceedances of single sample WQOs were 0% for EC, FC and TC. For ENT, exceedances on the day of storm was 87% for ENT and 37% for the three “grab” days following the end of the storm. This frequency increased for EC and TC to 29% if the pollutograph maximum is used. The number of storm events captured was not sufficient to investigate the effect of geology or watershed size on storm EMCs.

3) **The HF183 human-associated fecal microbial source marker was successfully used to exclude sites and samples with potential human fecal contamination, ensuring that the documented exceedance rates are attributable to non-human sources.** 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, availability of nutrients and presence of decaying organic matter.

4) **Temperature, and to a lesser extent, nutrients and organic carbon, were the major factors associated with higher summer dry weather FIB concentrations and exceedance frequencies. No significant relationships were found with either watershed size or geology during dry weather.** Water column FIB concentrations could not be attributed directly to instream benthic algal biomass as a measure of stream trophic status, which was low and showed no distinct seasonal variation. In contrast, FIB, temperature, organic carbon and nitrogen spiked at the end of the season, coincident with the end of stream flow. This is a naturally-occurring cycle where organic carbon and nutrients are increasingly recycled from organic matter as flow diminishes and temperature increases, conditions which coincide with increased FIB concentrations.

5) **Storm EMC fluxes were 2-3 times higher than dry weather FIB fluxes documented during this study.** Wet and dry weather fluxes were comparable to those documented in previous southern California regional studies. This comparability of the study data to previous results suggests that data from this study can be used, in addition to other regional datasets, for regulatory applications of reference study results in the greater southern California region.

1. INTRODUCTION

1.1 Background and Context

Enterococci (ENT), *Escherichia coli* (EC), fecal and total coliforms (FC and TC) are commonly used fecal indicator bacteria (FIB) to detect the possible presence of fecal contamination and associated pathogenic (disease-causing) microorganisms in waterbodies. Elevated levels of FIB in surface waters are a major concern for many municipalities, health departments, and regulatory agencies. Persistent or excessive FIB 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. In coastal southern California, where over 280 waterbodies have been listed as impaired for FIB pollution, a regulatory strategy to mitigate these water quality problems is the promulgation of total maximum daily loads (TMDLs), which are typically implemented and enforced through National Pollutant Discharge Eliminations System (NPDES) Permits, Waste Discharge Requirements (WDRs), Conditional Waivers of WDRs, or enforcement orders. 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. However, one of the challenges in developing appropriate targets and assigning responsibility to address the presences of FIB is accounting for biogenic inputs, or the natural contribution from undeveloped catchments.

Natural areas can also be a source of bacteria originating from wildlife, including birds and mammals, pets, and livestock (Griffith *et al.* 2006). Tiefenthaler *et al.* (2008) found that FIB exceeded WQOs for enterococcus in up to 14% of perennial wadeable streams located in undeveloped catchments in southern California. Natural sources can be significant contributors to bacteria levels in urban storm water in southern California, with the most probable sources being birds and other wildlife (Grant *et al.* 2001, Ahn *et al.* 2005, Tiefenthaler *et al.* 2008). Characterizing FIB concentrations and loads during non-storm conditions, hereafter referred to as “dry weather,” is critical because this is the period in which the streams and the coastal waters to which they drain receive the most human use, and thus the risk that humans will be exposed to pathogens is greatest. The Stein and Yoon (2007) and Tiefenthaler *et al.* (2008) studies focused on wet and dry weather, respectively, but the majority of their sites were perennial wadeable streams; more information is needed on FIB concentrations and exceedance frequencies in the intermittent streams that are prevalent in San Diego and southern Orange County. In addition, few studies have examined levels of FIB in conjunction with other environmental factors, such as nutrients, heavy and trace metals, and other conventional contaminants that may help explain the variability observed in previous studies (e.g., Tiefenthaler *et al.* 2008).

In response to this need for additional information, stormwater agencies that are regulated by the San Diego Regional Water Quality Control Board (SDRWQCB), led by San Diego and Orange

Counties and the City of San Diego, funded a study of natural background concentrations of regulated stormwater constituents in intermittent wadeable streams, focused on their region.

1.2 Study Goals, Geographic Scope, and Key Questions

The goal of this study was to characterize natural background concentrations of bacteria, nutrients, heavy metals and conventional constituents in minimally disturbed watersheds during wet weather and dry weather conditions. In addition, the study sought to categorize water quality objective (WQO) exceedance frequency by hydrologic factors, geomorphologic factors, and biotic and abiotic factors. The geographic focus of the study was San Diego and southern Orange County; sites from Ventura County have been substituted where suitable reference sites could not be found further south. This report focuses on results for FIB, specifically: ENT, EC, FC, and TC.

Ultimately, the management application of this study is to quantify FIB WQO exceedance rates in minimally disturbed streams in southern California. In turn, these rates can be used to derive reasonable and accurate targets for regulated pollutants of concerns in streams based on a reference approach. Exceedance frequencies reported herein are not intended to be used directly in a regulatory application, since alternative approaches to exceedance calculations exist, and historic data can also be considered for inclusion. Our assumption is that the underlying data generated in this study are available to support stakeholder discussions with regulatory agencies.

The general approach and specific design elements of the project were driven by a series of monitoring questions. The study questions, developed by a group of stakeholders including the City and County of San Diego and their MS4 Co-permittees, and the County of Orange and its MS4 Co-permittees the SDRWQCB, and San Diego Coast Keeper, are provided in Table 1.1.

Table 1.1. List of questions generated by SDRWQCB stakeholders.

Study Questions for Reference Streams

1. How does the WQO exceedance frequency for FIB vary between wet weather, summer dry weather and winter dry weather?
2. How does the FIB concentration vary by stream landscape and site-specific factors, including:
    a. Size of storm and timing of storm (wet weather only)
    b. Size or dominant geology of catchment (wet and dry weather)
    c. Flow rate (dry weather)
    d. Stream physiochemical (temperature, conductivity, turbidity) and chemical parameters (nutrients, organic carbon, metals, and conventional constituents; wet and dry weather)?
    e. Trophic status, as measured by algal abundance (dry weather only)?

2. METHODS

2.1 Geographic Setting

Coastal southern California is a semi-arid region with a Mediterranean climate. Lower elevations are characterized by chaparral, oak woodlands, and sage scrub. The region is bordered by the Transverse Ranges to the North, the Peninsular Ranges to the East, and the Mexican border to the South. Both Transverse and Peninsular ranges contain peaks that exceed 10,000 feet, and are characterized by pine forests. The mountains receive a large portion of their precipitation as snow, which typically contributes water to streams until mid- to late-summer. Much of the higher elevations are undeveloped and remain protected in national forests and a network of national, state, and county parks. The lower elevations have been pervasively altered by urbanization or conversion to agriculture. Wildfires and drought are frequent in the region. Under average rainfall conditions, there is a natural gradient in annual average rainfall from Santa Barbara County (17 inches) to San Diego and southern Orange Counties (10 inches). However, during this study, a significant drought occurred, causing a 15 to -35% deficit relative to long-term average rainfall (Table 2.1).

Table 2.1. Average rainfall (inches) by hydrological water year and County in coastal southern California.

City	2011-2012	2012-2013	2013-2014	2014-2015	Long-Term Mean	4-Year Deficit[1]
Ventura	11.67	9.42	6.56	8.32	17.76	-35.07
Los Angeles	8.69	5.85	6.08	7.45	14.93	-31.65
Irvine	6.32	4.99	3.64	6.84	13.33	-31.53
San Diego	8.03	6.50	5.06	6.58	10.34	-15.19
Campo	11.29	9.25	6.95	8.38	15.73	-27.05

[1] Deficit is a reduction from the long-term mean rainfall for the region.

In this region, annual cycles of stream flow are driven primarily by the seasonality of California's Mediterranean climate of wet winters and a prolonged dry season. Rugged terrain generates intense precipitation over watersheds draining to stream drainage networks. Watershed slope, geomorphology, and geology are major factors controlling runoff ratios (outflow/precipitation). In undeveloped watersheds, several storms are typically required to maintain stream baseflow during dry weather conditions. Meteorological forcing (air temperature, precipitation) is the dominant mechanism of interannual variability in the timing and magnitude of stream baseflow (Knowles 2002), including periods of drought, such as the record drought of 2013 that occurred as this study was being conducted.

Over the past 50 years, the region has rapidly urbanized. Currently, approximately 66% of southern California Coastal watersheds are undeveloped open space, 30% urban, and 4% agricultural (National Land Cover Database, 2011). Urbanization of coastal watersheds has limited the number of available reference sites, particularly those representing streams in the coastal plain.

2.2 Conceptual Approach and Study Design

Natural concentrations and loads of bacteria, metals, and nutrients during wet and dry weather can be highly variable; therefore, replication in space (many sites) over multiple events is required to adequately characterize median values and estimate variability. Sources of natural variability include physical (geology, catchment size, temperature, etc.), hydrological (rainfall magnitude, frequency, groundwater), and biological (land cover type, composition of flora and fauna, etc.).

The overall approach to this study consisted of characterizing wet and dry weather FIB concentrations and loads at a suite of sites that represent existing minimally disturbed conditions in southern California. The specific study design consisted of an intensive sampling regime with collection of weekly dry weather bacteria samples at onset of sustained baseflow (typically in January) until streams ceased flowing. When bacteria concentrations exceeded single-sample regulatory WQOs for EC or ENT. HF183, a human-associated microbial source tracking (MST) marker (Layton *et al.* 2013), was analyzed on an archived split sample to eliminate samples that could be contaminated with human fecal material. In addition, samples for nutrients, trace metals (total and dissolved), and conventional constituents were collected biweekly. While a separate report is available for these latter constituents (Sutula *et al.* 2015), they are used here to explore explanatory factors associated with patterns in FIB.

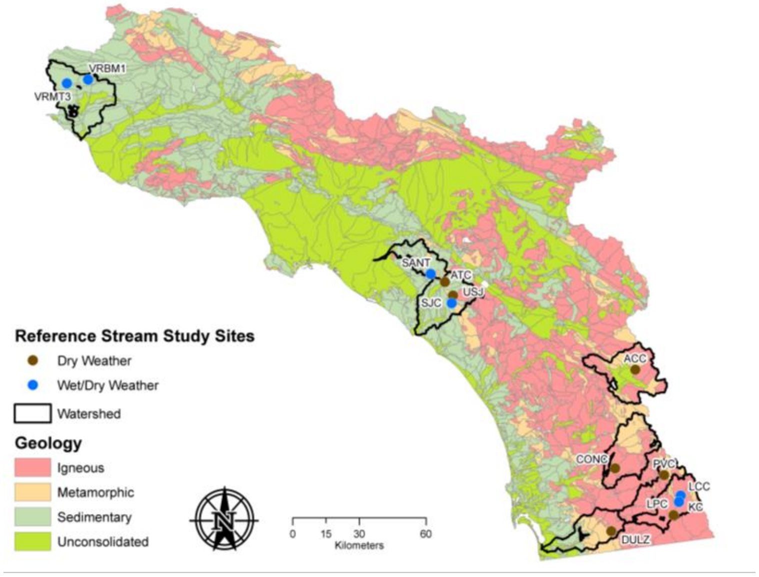

Figure 2.1. Study region with location of sites in Ventura, Orange, and San Diego Counties, in southern California. Colors designated dominant geology where pink = igneous/metamorphic and green = sedimentary geology.

The study was conducted under a rigorous program of quality assurance and quality control (QA/QC). A report of the QA/QC results for the study is given in Appendix A; the QA/QC Project Plan governing this program is available upon request.

2.3 Sampling Design and Criteria for Site Selection

The basic sampling design of the reference stream study was guided by an investigation into the differences in concentrations and loads of natural streams for nutrients, bacteria, and metals by catchment geology (igneous/metamorphic (IM) vs. sedimentary (SED) and catchment size.

2.3.1 Dry and Wet Weather Design Elements

For dry weather, a target design of 10 sites was established, with five sites in each of IM and SED catchment geology. Three catchment size classes (10 -34, >34 and < 60, and >60 km^2) were established based on the range of watershed sizes found in the Surface Water Ambient Monitoring Program (SWAMP) Southern California Regional Stream Bioassessment Program (R. Mazor, personal communication). Catchment areas greater than 10 km^2 were sought to reduce the possibility that sites would have ephemeral dry weather flows and flashy wet weather flows. An effort was made to select sites to achieve an even distribution across watershed size categories. Because of the lack of reference sites in this region, particularly with SED geology, this even distribution was challenging to achieve.

For wet weather, a target design of six sites was established, with three sites in each of IM and SED catchment geology and two sites in each of three catchment sizes, for a targeted total of 18 site events. A target of three storms was set for each site, attempting to capture different storm sizes (small < 0.5 inches; medium > 0.5 and < 1 inches; large >1 inches rainfall) and timing of storm (early season = before January 1; mid-season = January 1–March 1; late season = after March 1).

Because sampling for this study occurred during an extended drought, reference sites did not typically flow during events smaller than 0.5 inches rainfall. In addition, early season storms were difficult to capture as it took several storms to recharge the aquifer of these sites sufficiently for them to flow during wet weather. For this reason, all but one of the storm events captured were mid-late season. Finally, because of the drought itself, only eight storm events were successfully monitored after three years of wet weather deployments. Data from one of these storms were excluded due to positive human fecal MST hits. A decision was made to end field sampling in May 2015 and to utilize existing data from other studies to supplement data from this study for management applications.

2.3.2 Site Selection Criteria

Sites were selected for inclusion in the study based on criteria developed by Stein and Yoon (2007), and 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;
3. Sites should have either year-round or prolonged dry weather flow to allow sampling during at least a portion of the dry season;
4. Sites should not be within watersheds that have burned during the previous 3 years;
5. Sites should not be on the 303(d) list; and
6. Field reconnaissance should reveal no evidence of anthropogenic effects, such as septic tanks, isolated residences, and/or excessive wildlife or human use.

A review of the existing data on water quality in natural areas of southern California was conducted using data from USEPA's Environmental Monitoring and Assessment Program (EMAP), the State of California's Surface Water Ambient Monitoring Program (SWAMP), the U.S. Geological Survey's (USGS) Hydrologic Benchmark Network, and the USGS's National Water Quality Assessment. Geologic type (IM or SED) for the coastal watersheds in southern California was determined by plotting catchment boundaries over a digitized geology map. The rest of catchment characteristics (i.e., watershed size) were assessed with imagery from NOAA C-CAP land use/cover (NOAA 2001), CalWater watershed boundaries, and USGS topography (digital elevation model), using ArcView GIS 10.3a (ESRI, Redlands, CA).

After sampling was initiated, the human-associated HF183 MST marker (Layton *et al.* 2013) was used to confirm that the sites had no detectable human fecal contamination. Sites were also eliminated if water level records suggested upstream impoundments or water withdrawals (Table 2.2). Insufficient sites were available in San Diego and southern Orange Counties. For this reason, additional sites from the Ventura River Watershed were used to supplement the study. From an original list of 14 sites, a final suite of 10 sites in 10 watersheds was used for data analyses (Figure 2.2; Table 2.2, Table 2.5), because of indications of anthropogenic disturbance or other issues with site-suitability (e.g. ephemeral flows).

Table 2.2. Stream Reference Sampling Sites. S = Small, M = Medium, L = Large, IM = Igneous/Metamorphic, SED = Sedimentary; W = Wet Weather, D = Dry Weather.

Station Code	Stream Name	HUC_12_Watershed	Latitude	Longitude	Area (km^2)	Size	Geology	County	Designation
USJ	Upper San Juan Creek	Upper San Juan Creek	33.6185	-117.5096	19.3	S	IM	Orange	D
LCC	Long Canyon Creek	Tributary to Kitchen Creek	32.7784	-116.4429	23.3	S	IM	San Diego	W/D
KC	Kitchen Creek	Kitchen Creek-Cottonwood Creek	32.7528	-116.4519	39.9	M	IM	San Diego	W/D
PVC	Pine Valley Creek	Upper Pine Valley Creek	32.864	-116.5185	43.1	M	IM	San Diego	D
ACC	Agua Caliente Creek	San Luis Rey River	33.2956	-116.6386	46.1	M	IM	San Diego	D
SJC	San Juan Creek	Upper San Juan Creek	33.5879	-117.5165	96.9	L	IM	Orange	W/D
CONC	Conejos Creek	San Diego River	32.8983	-116.7525	116	L	IM	San Diego	D
SANT	Santiago Creek	Santiago Canyon Ck	33.7085	-117.6151	17	S	SED	Orange	W/D
VRBM1	North Fork Matilija	North Fork Matilija	34.5186	-119.2708	40	M	SED	Ventura	W/D
VRMT3	Matilija Creek	Matilija Creek	34.5037	-119.37458	116	L	SED	Ventura	W/D
Sites Removed Due to Positive MST Hits or Anthropogenic Influence									
LPC	La Posta Creek	La Posta Creek	32.7002	-116.4801	115	L	IM	San Diego	D
ATC	Arroyo Trabuco	Arroyo Trabuco	33.6745	-117.5469	31.0	S	SED	San Diego	D
DULZ	Dulzura Creek	Dulzura Creek	32.6442	116.78141	105	L	IM	San Diego	W/D
CCCP	Cristianitos Creek	Lower San Mateo Ck	33.4273	-117.5698	80.0	L	SED	Orange	W/D

2.4 Field Sampling

2.4.1 Dry Weather

Weekly dry-season bacteria sampling, along with biweekly nutrients, trace metals, and conventional constituent sampling, was conducted from April 4, 2012 through August 31, 2014. Bacteria samples were collected with the goal of 5 samples within each 30-day period. Benthic chlorophyll-a and ash-free dry mass were collected 1 to 4 times per season per site, depending on duration of flow, using the methodology of Fetscher *et al.* (2014). A site was eligible for sampling if it had not received measurable rainfall for at least 72 hours and if flow was no more than 20% above base flow. Weekly and biweekly 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 4 to 42 weeks (Table 2.3).

At each sampling location and during each round of sample collection, water quality readings [i.e., temperature (°C), dissolved oxygen (DO; mg/L), pH, salinity (ppt), and conductivity (µS/cm)] were measured using handheld field probes (i.e., Orion 125 and YSI Pro Plus). 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 subsection was computed and summed to compute a total flow for the transect. Values from three transects were averaged to estimate overall flow at each site (Rantz & others 1982). Hobo® water level data loggers were also deployed at each site to record continuous real-time flow and temperature measurements.

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 10% of the samples. A field blank sample was collected at each site once a month. All water samples were collected in pre-sterilized, 1 L, high-density polyethylene (HDPE) sample bottles. Collected water samples were immediately placed on ice and transported to the laboratories within 6 hours of sample collection for subsequent analyses.

Table 2.3. Summary of dry weather samples collected at each site over the period of April 4, 2012 to August 31, 2014.

County	Site	Total Weeks Completed 2012 through 2014	Sample Count by Constituent			
			Bacteria	Nutrients	Metals	Conventionals
San Diego	ACC	22	35	18	18	18
	CONC	4	5	2	2	2
	KC	35	50	22	22	22
	LCC	42	65	23	23	23
	PVC	33	54	18	18	18
Orange	SANT	32	52	16	16	16
	SJC	35	53	20	20	20
	USJ	29	45	21	21	21
Ventura	VRBM1	29	34	20	20	20
	VRMT3	29	34	20	20	20
Total			427	180	180	180

Site IDs: ACC=Agua Caliente Creek, CONC=Conejos Creek, KC=Kitchen Creek, LCC=Long Canyon Creek, PVC=Pine Valley Creek, SANT=Santiago Canyon Creek, SJC=San Juan Creek, USJ=Upper San Juan Creek, VRBM1=North Fork Matilija Creek, VRMT3=Matilija Creek

2.4.2 Wet Weather Field Sampling

Flow-weighted sampling of concentrations and continuous discharge monitoring were conducted over the course of the storm to estimate pollutant loading (Table 2.4). These data are depicted graphically by graphing time (on x-axis) versus flow and concentration (y-axes), otherwise known as a "pollutograph" (Appendix D, Figure D2). Ten to sixteen grab samples, including QA samples, were collected over the duration of the storm event, then once per day on the following three days of the storm. *In-situ* field measurements for conductivity, pH, temperature, and turbidity were recorded at each site to coincide with each pollutograph grab sample. Occasionally, this proscribed sequence of sampling was interrupted, either by the advent of another storm or because of logistical issues (e.g. rock slides); these events were noted when they occurred.

Flow and precipitation were measured throughout the duration of the storm event at each reference site, when feasible. Flow was monitored using a Sigma 950 bubbler flow meter to measures the stage of the river as a function of pressure. Channel dimensions were recorded at the time of installation and used in tandem with Manning’s Equation to calculate flow. Total discharge and total rainfall during the event were also recorded.

Table 2.4. Summary of wet weather samples collected at each site over the period of January 1, 2012-August 31, 2014.

County	Site[a]	Total FIB Samples	Total Metals, Nutrients, Conventional Samples	Sampling Period (Date)	Storm Events		
					Size	Rainfall (cm)	Season
San Diego	CCCP	13	13	3/17-18/2012	Medium	2.06	Mid
	KC	17	17	1/25-29/2013	Large	4.04	Mid
	LCC	14	14	4/26-29/2012	Medium	1.17	Late
		14	14	1/25-29/2013	Large	4.39	Mid
Orange	SANT	15	15	12/13-17/2012	Large	3.71	Early
		16	16	12/19-21/2013	Medium	1.47	Mid
	SJC	14	14	3/25-29/2012	Large	2.67	Mid
Ventura	VRBM1	15	15	2/26/2014	Large	5.54	Mid
	VRMT3	2[b]	8	2/26/2014	Large	5.54	Mid
Total		118	126				

[a] Site IDs: CCCP-Cristianitos Creek, KC-Kitchen Creek, LCC-Long Canyon Creek, SANT- Santiago Canyon Creek, SJC - San Juan Creek, VRBM1 - North Fork Matilija Creek, VRMT3 - Matilija Creek

2.5 Laboratory Analyses

2.5.1 Fecal Indicator Bacteria (FIB) and MST

Water quality samples were analyzed for four types of FIB at SCCWRP laboratories: EC, ENT, FC and TC. EC and TC were measured using Colilert®-18 (IDEXX, Westbrook, ME). Enterococcus was measured using Enterolert® (IDEXX, Westbrook, ME). Both IDEXX methods were performed following the manufacturer's instructions using the Quanti-tray system. Fecal coliforms were measured by Standard Methods for Examination of Water and Wastewater (SM) 9222 D. For each sample, an additional 100 ml of water was vacuum filtered through 47-mm diameter, 0.4-µm pore-size polycarbonate filters and stored at -80° C for later analysis for the human-associated fecal marker HF183. Samples were analyzed for the human-associated marker only if EC or ENT exceeded the single-sample WQO (i.e., EC > 235 MPN/100 ml or ENT > 61 MPN/100 ml) typically used in California (and contained in the San Diego Basin Plan).

On those samples that exceeded FIB WQOs, the HF183 Taqman assay (Layton *et al.* 2013) was conducted by SCCWRP laboratories to determine whether a human-associated fecal signal was present. Each 25 µl qPCR reaction contained 900 nmol l^{-1} of each primer, 250 nmol l^{-1} of the probe, and 2 µl of sample DNA, plus 1x Taqman Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA) and 0.2 mg ml^{-1} bovine serum albumin (Sigma, St. Louis, MO). The reaction was conducted on a CFX96 (Bio-Rad, default ramping speed of 3.3°C second^{-1}) using the following thermal conditions: 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C.

2.5.2 Conventional Constituents

Conventional constituents, consisting of alkalinity (Alk), chloride (Cl), total hardness as calcium carbonate, sulfate (SO4), total dissolved solids (TDS), and total suspended solids (TSS) were analyzed by Physis Laboratory. SO4 and Cl were measured by ion chromatography by EPA Method 300.0. Alkalinity was measured by titration using Standard Method (SM) 2320B by pH meter. Total hardness was measured by EDTA titration via SM 2340B. TDS and TSS were analyzed using the gravimetric technique (SM 2540-C and SM2540-D, respectively) described by Banse *et al.* (1963). They were measured with a flow injection analyzer (Lachat Instruments model Quik Chem 8000).

2.5.3 Nutrients

The University of Maryland's Chesapeake Bay laboratory conducted the nutrient and organic carbon analyses. Water samples were analyzed for ammonia, dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), nitrate+nitrite (N+N), particulate nitrogen (PN), particulate organic carbon (POC), particulate phosphate (PP), ortho-phosphate, total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP). Analyses of nutrients by automated colorimetry are described by the SM (1998) and the US Environmental Protection Agency (EPA) methods. Nitrate + nitrite was analyzed using EPA method 353.2 and ammonia was analyzed using the EPA 350.1. TDN was analyzed using USGS I-2650-03. DOC was determined by high temperature catalytic combustion using a Shimadzu 5000 total organic carbon (TOC) Analyzer (EPA 415.1). Orthophosphate was analyzed using EPA method 365.3. TP was analyzed using EPA365.3. PN and POC was analyzed using EPA method 440. PP was analyzed using Aspila *et al.* 1976 and EPA method 365.5. TN and TP were calculated from the sum of the total dissolved nutrients and the particulate nutrients. Dissolved organic phosphorus (DOP) and nitrogen (DON) were calculated by subtracting the dissolved inorganic fraction from the TDP and TDN, respectively.

2.5.4 Heavy and Trace Metals

Heavy and trace metals, analyzed by Physis Laboratories, were prepared by digestion, followed by analysis using EPA method 200.8. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to analyze total recoverable and dissolved concentrations of cadmium, chromium, copper, lead, iron, manganese, nickel, selenium, and zinc.

2.5.6 Benthic Chlorophyll-a and AFDM

Benthic chlorophyll-a and AFDM were analyzed by SCCWRP laboratories. Chlorophyll a was extracted with 90% acetone for 48 hours, then measured on a Turner 10-AU Field Fluorometer according to the EPA rapid bioassessment protocol for streams and wadeable rivers (USEPA

1992). To determine ash-free dry mass, filters were dried at 60°C for 24 hours and weighed; all organic material was combusted in a muffle furnace at 500° C for two hours, then reweighed.

2.5.7 Quality Assurance Quality Control (QA/QC)

QA/QC samples were submitted blind to the laboratory. These included field duplicates and blanks on approximately 10% of samples, with additional volume sufficient to run laboratory blanks. Every laboratory batch analysis included QA/QC checkup with certified reference materials, duplicate analyses, matrix spike/matrix spike duplicates, calibration standards traceable to the National Institute of Standards, and method blanks. Appendix A, Table A1-1 shows the list of analytes, along with minimum detection limits (MDLs) and applicable units for each analyte. QA/QC on FIB samples was excellent, with 100% of QA/QC samples meeting objectives including: positive control samples within 80-120% recovery, no growth on filters, lab replicate RDP <25%, and precision on sample duplicates within ±25%.

The HF183 MST marker was analyzed on samples exceeding EC or ENT single sample criteria. Of those sites with positive results for HF183, ATC and DULZ were removed from further dry weather sampling because of the high percentage of positives (Table 2.5). Likewise, CCCP was removed as a wet weather site because of consistent positive HF183 marker hits for all samples in the pollutograph. For other sites, data for samples that had positive MST results were removed from analyses (Table 2.5).

Table 2.5. Summary of HF183 MST analyses. ATC, LPC, DULZ, and CCCP were eliminated from further sampling and analyses because of the high percentage of positive reactions and/or evidence of anthropogenic impacts.

Site	N	% Positive Reactions	% Sample Both Replicates Positive	Mean Log Gene Copies per 100 ml
ACC	12	0	0	-
ATC	9	44	44	3.035456
CCCP	16	13	98	3.745296
CONC	3	0	0	-
DULZ	5	50	40	2.342621
KC	15	0	0	-
LCC	30	8	3	2.21717
LPC	23	2	0	2.361501
PVC	9	0	0	-
SANT	18	0	0	-
SJC	23	0	0	-
USJC	12	0	0	-
VRBM1	19	0	0	-
VRMT3	18	11	0	2.589069

2.6 Data Analyses

Data analyses were conducted to answer the three principle questions:
1) What are the concentrations, and fluxes of FIB?
2) What is the exceedance frequency of FIB concentration in streams, and how does this vary by wet weather, winter and summer dry weather?
3) What factors explain variability in FIB concentrations?

Four types of analyses were used to answer these questions, explained in greater detail below:

- Calculation of baseline bacteria levels;
- Exceedance frequency calculations;
- Exploration of factors associated with FIB variability; and
- Comparison of flow weighted mean concentrations and FIB fluxes with previous studies.

Baseline bacteria levels. First, the storm event mean concentrations (EMCs), dry weather single sample or 30-day geomean, variances, and ranges of concentrations, and fluxes were calculated to provide an estimate of baseline bacterial levels. Calculations were summarized by wet weather, winter dry (October– March), summer dry (April – August), and annual (January – December, though stream flow ceased by August). Note that sampling of winter dry weather typically began in January, because several storm events were required to induce stream flow in these reference watersheds.

Using only those samples for a single storm, the event EMC was calculated according to Equation 1:

$$\text{EMC} = \frac{\sum_{i=1}^{n} C_i * F_i}{\sum_{i=1}^{n} F_i} \qquad \textbf{Eq. 1}$$

where EMC = flow-weighted mean (FWM) for a particular storm, C_i = individual runoff sample concentration of the sample, F_i = instantaneous flow at the time of the sample, and n = number of samples per event.

FIB concentrations were log-transformed prior to calculations to improve normality. In all cases, non-detectable results were assigned a value of one-half the minimum detection limit, based on the inability to log transform a value of zero. Mass loading was calculated as the product of the EMC and the storm volume. Flux estimates facilitated loading comparisons among watersheds of varying sizes. Flux was calculated as the ratio of the mass loading per storm and watershed area.

Dry weather 30-day geomean concentrations were calculated with 5 samples within a 30-day period, consistent with calculation procedures outlined in the San Diego Basin Plan for fecal coliform. For this study, 30-day geomean concentrations were calculated by calendar month. When the number of samples was four (e.g., sampling postponed due to wet weather events), then all field duplicates from that month were regarded as individual samples to ensure at least 5 samples within the calendar month were available for the analyses. Months for which there were less than four samples were dropped from geomean calculations; the sensitivity of the result to this method of calculation is explored in Appendix B.

Exceedance Frequency Calculations. Second, wet and dry weather FIB concentrations were compared with the WQO for single-sample and 30-day geomean maximum allowable densities (Table 2.6). Cumulative frequency distributions (CFDs) were produced to compare observed bacterial concentrations to the WQOs and to calculate accumulated relative exceedance percentages. The sensitivity of 30-day geomean concentrations and exceedance frequencies to geomean calculation method (calendar month vs. rolling geomean, number of samples in geomean) is explored in Appendix B. For wet weather, exceedance of WQO can be applied to either the storm EMC or maximum of the pollutograph. Here, we present both, but nominally emphasize the storm EMC.

Table 2.6. State of California freshwater FIB quality standard. FC units are in CFU/100 ml; all others are in MPN/100 ml.

FIB Analyte	Maximum Allowable Density		*Applicable to Region's Freshwater Waterbodies?*		
	Single Sample	30-day Geomean	San Diego	Orange	Ventura
Enterococcus	61	33	Yes	No	No
E. Coli	235	126	Yes	Yes	Yes
Fecal Coliform	400	200	Yes	No	No [1]
Total Coliform	10,000	1,000	No	No	No

[1] In the Los Angeles Region Basin Plan, Fecal Coliform WQOs only apply to waterbodies designated as REC-2 and are a geomean of 2000 MPN/100mL and no more than 10% of samples can exceed 4000 MPN/100mL

Investigation of Factors Associated with FIB Variability. Factors associated with the variability in FIB concentrations were investigated. Differences in concentration by winter and summer dry weather, geology, and watershed size were tested using a one-way analysis of variance (ANOVA), with a significance level $p < 0.05$ (Sokal and Rohlf 1995). For wet weather, because an insufficient number of storms were available to test watershed size, only significant differences in geology were tested using a one-way ANOVA. Relationships of FIB EMC were explored qualitatively using total rainfall (storm size), Julian day of hydrological year (from October 1; storm timing), and number of antecedent dry days as continuous variables.

For dry weather, relationships with continuous factors such as flow, physiochemical parameters (conductivity, pH, DO, temperature, turbidity), and chemical water quality (conventional constituents, nutrients, and heavy and trace metals) were investigated first using Spearman's correlation to determine if there were strong associations between each one of these factors and FIB concentrations. Once promising factors were identified, stepwise regression was used to consider multiple factors and the covariance among them simultaneously to generate the final relationships for wet and dry weather separately.

Comparison of FIB Loads and Fluxes with Previous Studies. In order to make region-wide comparisons among watersheds of varying sizes, storm EMC and dry weather flow-weighted mean concentrations and flux estimates were computed. For dry weather, flow-weighted mean concentrations were calculated by multiplying each single event concentration by the daily discharge, then dividing the total dry weather load for the season by the sum of the daily discharge for each site. Flux was calculated as the ratio of either the storm EMC or the dry weather flow- weighted mean concentration (MPN/100 ml) and contributing watershed area (km^2) at a specific site. Water quality statistics from this study were compared to previous FIB reference studies to assess differences between perennial vs. intermittent streams and to determine whether San Diego and southern Orange County were significantly different from Los Angeles County. Differences in concentration or flux were tested using a one-way 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.

All statistical analyses were conducted in SAS (V.9.4, 2014) and in R Studio (R Core Development Team, 2014).

3 RESULTS

3.1 Annual and Seasonal FIB Flow- Weighted Mean Concentrations and Fluxes from Reference Sites

Annual mean dry weather FIB fluxes from reference sites were are shown in Table 3.1. The wet weather fluxes of FIB from the seven storm events were generally 2 to -3 times that of annual dry weather (p-value << 0.05), with the exception of FC, which showed no significant seasonal difference (Figure 3.1). EC and ENT dry weather flow-weighted mean concentrations and fluxes at the natural sites (based on single-sample measurements) were generally 2 to -8 times higher in the summer vs. during winter dry weather. These differences were statistically different for p-value < 0.05 for EC and ENT (Figure 3.1, Table 3.1). Appendix C, Tables C1 - C5-5 provide detailed dry weather flow-weighted mean concentrations, and fluxes, detailed by site, season, and year.

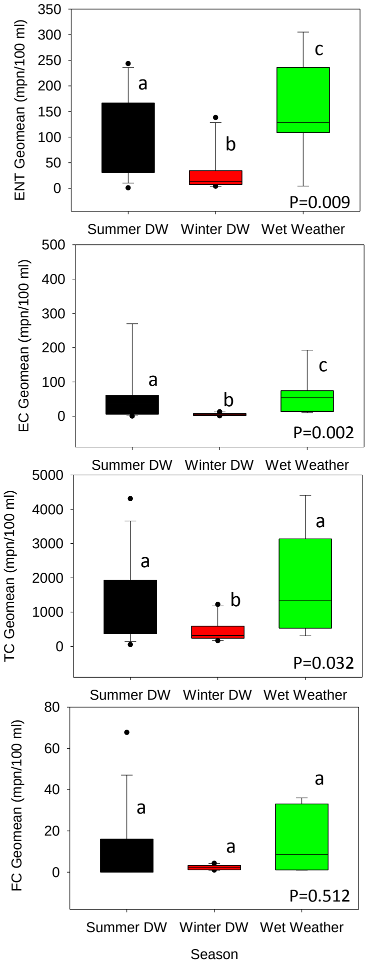

Figure 3.1. Box and whiskers plots of differences in mean FIB concentrations by season (summer dry and winter dry weather (DW). P-value at $\alpha = -0.05$ of one-way ANOVA is shown on the plot. Different lower case letters indicate significant statistical difference in ANOVA among season.

Table 3.1. Summary of wet and dry weather mean FIB concentrations and fluxes from all reference sites. Wet weather fluxes are day of storm only.

FIB Analyte	Wet Weather		Dry Weather Flow-Weighted Mean Concentration (MPN/100 ml)			Fluxes (MPN/100 ml km^{-2})		
	EMC (MPN/100 ml)	Flux (MPN/100 ml km^{-2})	Summer Dry	Winter Dry	Annual Dry	Summer Dry	Winter Dry	Annual Dry
EC	62.3	2.0 ±3.1	40.7	5.8	32.0	1.6 ±3.2	0.1 ±0.07	1.0 ±2.6
ENT	160.6	6.7 ±9.4	98.0	35.0	91.0	2.1 ±1.8	0.75 ±0.72	1.6 ±1.6
TC	1864.0	69.0 ±124.1	1,026.0	546.3	1,036.0	35.4 ±29.9	12.0 ±7.9	26.7 ±26.8
FC	15.4	0.7 ±1.5	13.9	2.4	8.2	0.59 ±1.05	0.07 ±0.05	0.32 ±0.77

3.2 Frequency of Exceedance of FIB Standards at Reference Sites

3.2.1 Dry Weather Exceedances

Among reference sites in this study, low annual single sample exceedance rates were found for EC, FC and TC (0 - 3.4%), but high rates were found for ENT (29.7%; Table 3.2). Significant differences in concentrations by season (Figure 3.1) were also reflected in a roughly 25% higher exceedance frequency in summer dry vs. winter dry weather. Sites varied considerably in their individual exceedance rates (Figure 3.2).

In contrast to single sample values, geomean exceedances were 0% for EC and FC, but 48.3% and 30.4% for ENT and TC, respectively. Most of these exceedances occurred in the summertime. The 30-day geomean exceedance frequency was relatively insensitive to the number of samples included in the geomean (i.e., n = 1 - 5; Appendix B). Exceedance frequencies were 15-20% higher then rolling-versus monthly 30-mean is used.

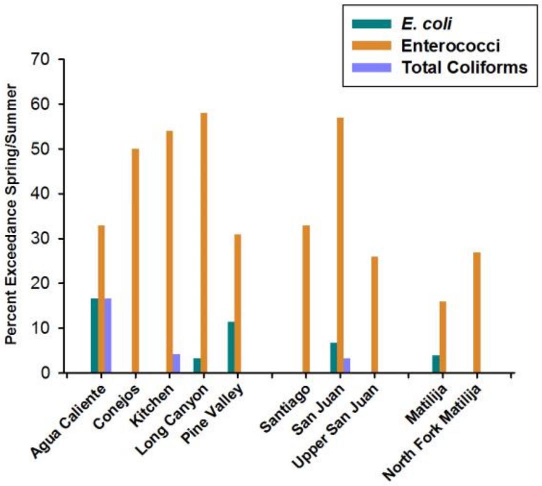

Figure 3.2. Variability in dry weather exceedance frequency by reference site for EC, ENT, and TC.

Table 3.2. Summary of FIB median, ranges, and exceedance frequencies for single sample and 30-day geomean water quality objectives. Annual dry weather gives a comparison of 30-day rolling geomean versus monthly geomean exceedance frequencies.

Parameter	**Sample Size, Means and 30-day Geomeans (MPN/100 ml) and Exceedance Rates**			
	EC	**ENT**	**FC**	**TC**
Annual Dry Weather				
Overall Annual (N Size)	297	300	216	298
Single Sample Median (Range)	9 (1-1986)	30 (1-649)	2 (1-247)	488 (34-22,865)
Single Sample Exceedance Rate (%)	3.4	29.7	0.0	1.7
Number of Months included in Monthly Geomean	55	58	40	56
30-day Monthly Geomean % Exceedance	0	48.3	0	30.4
Number of Months included in Rolling 30-day Geomean	97	97	60	97
30-day Rolling Geomean % Exceedance	0	67.0	0	44.3
Winter Dry Weather				
Winter Dry (N Size)	85	87	86	85
Single Sample Median (Range)	4 (1-630)	6 (1-351)	1 (1-29)	260 (20-18,596)
Single Sample Exceedance Rate (%)	1.2	6.9	0.0	1.2
Number of Months included in Geomean	15	17	16	15
30-day Geomean % Exceedance	0	5.9	0	6.7
Summer Dry Weather				
Summer Dry (N Size)	212	213	130	213
Single Sample Median (Range)	10 (1-1986)	45 (1-649)	2 (1-247)	816 (34 -22,865)
Single Sample Exceedance Rate (%)	4.2	39.0	0.0	1.9
Number of Months included in Monthly Geomean	40	41	24	41
30-day Monthly Geomean % Exceedance	0	65.9	0	39.0

3.2.2 Wet Weather Exceedances

Wet weather exceedances of the single sample maximum WQO were highest for ENT: 86% of the seven events had EMCs that exceeded WQOs, while 37% of grab days exceeded enterococcus. There were no exceedances of WQOs for EC, FC, and TC for either storm events or grab days for all qualifying storm events.

Table 3.3 Summary of wet weather EMCs (day of storm) and Grab Days 1 to 3 and exceedance frequencies for the seven storm events.

	EC		ENT		TC		FC	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Mean EMC- Day of Storm	62.3	134.8	160.6	243.6	1,864.1	4,755.4	15.4	44.3
Mean of Grab Days	25.2	32.0	84.2	134.0	694.6	559.9	5.4	16.4
Exceedance Frequencies	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Pollutograph EMC	0/7	0%	6/7	86%	0/7	0.0	0/5	0%
Pollutograph Max	2/7	29%	6/7	85%	2/7	29%	0/5	0%
Grab Day	0/19	0%	7/19	37%	0/19	0.0	0/13	0%

Day-of-storm exceedance frequencies of WQO increased to 29% for EC and TC if using the pollutograph maximum.

3.3 Factors Associated with Variability in FIB Concentration at Dry Weather Reference Sites

3.3.1. Watershed Size and Geology

Neither watershed size nor geology (IM vs. SED) were significantly correlated to FIB concentration (p-value >0.05). However, box and whiskers plots of both TC and ENT show a fairly constrained range of concentrations for sites of SED geology, while the range in concentration was much larger for that of IM sites (Figure 3.3).

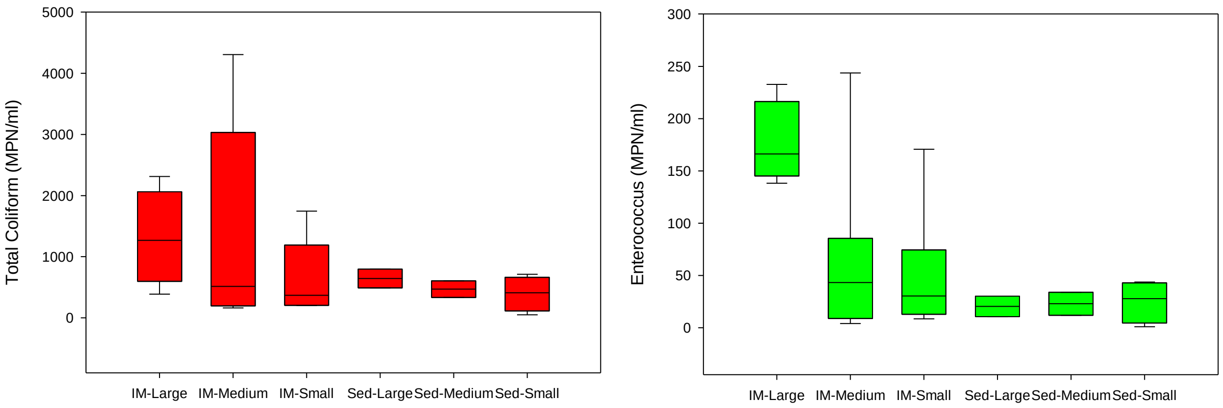

Figure 3.3 Box and whiskers plots of TC and ENT concentrations in sites grouped by geology (IM, SED) vs. watershed size.

3.3.2 Association of FIB Concentration with Other Stream Factors

Given that ENT and EC concentration were significantly different by season (p-value <0.01) and moderately different for TC (p-value = 0.06), we investigated the factors associated with this pattern, as temperature, flow (and ratio of runoff to baseflow), physiochemical parameters, and chemical water quality are all likely to vary as a function of season (e.g., Figure 3.4).

Overall, water temperature, nutrients and organic carbon were among the factors that had the strongest relationship with ENT, EC, and TC concentrations in reference streams. Temperature showed highly significant spearman correlation with ENT, EC, and TC (Table 3.4), and in stepwise regression models, its coefficient was highly significant at p-value <0.01 for all three FIB analytes. No parameters were found to be significantly correlated with FC.

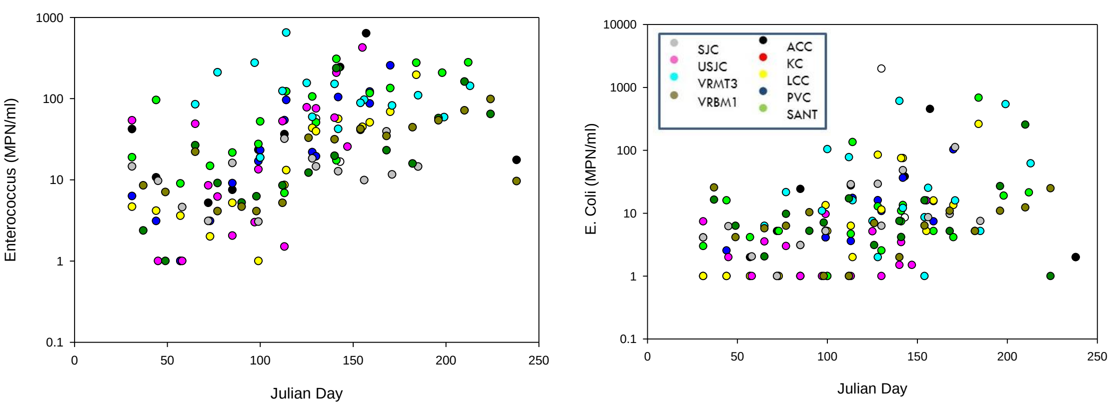

Figure 3.4. Plot of ENT concentrations (left panel) and EC concentrations (right panel) as a function of Julian Day, showing a general increase in concentration from January through August.

Table 3.4 Summary of explanatory factors that had correlations >±0.3. All shown were highly significant (p-value <0.0001) with FIB concentrations.

Category	Constituent	EC	Ent	TC
Conventional	TSS		0.37	0.39
Metal	Fe-Tot	0.30	0.57	0.47
	Mn-Tot		0.52	0.37
	Pb-Tot		0.48	0.39
	Se-Tot		-0.33	
	Cd-Tot			
Nutrient	PN	0.39	0.36	0.51
	POC	0.30	0.38	0.52
	TN			0.44
	TP			0.35
Physical	Flow			-0.30
	Water Temp.		0.50	0.53

While flow generally was not significantly correlated (p-value >0.05), it does appear that concentrations spike up in the lower ranges of flow with all FIB analytes to some degree (Figure 3.5). For TC, FC and EC, all exceedances occurred in the lower 25th or 33th percentile of flows. For ENT, this relationship was noisy, but still present.

TSS, nutrients and organic carbon were also consistently positively correlated with ENT, EC, and TC (Table 3.3) and consistently among top variables explaining variability in concentration. The specific form of nutrient (dissolved inorganic, dissolved organic, or particulate N or P) depended on the FIB analyte. For example, PN was highly correlated to both EC and TC (p-value <0.001), while DOC, DOP, TP, and NO3 were significantly correlated with ENT (p-value <0.05), especially DOC (p-value <0.001). Particulate and dissolved organic carbon and nitrogen showed similar patterns as FIB spiked up with declining flows (Figure 3.5). POC:PN ratios at low flow showed a mix of both algal carbon sources (benthic C:N of 8 - 13) and terrestrial carbon sources (C:N >25).

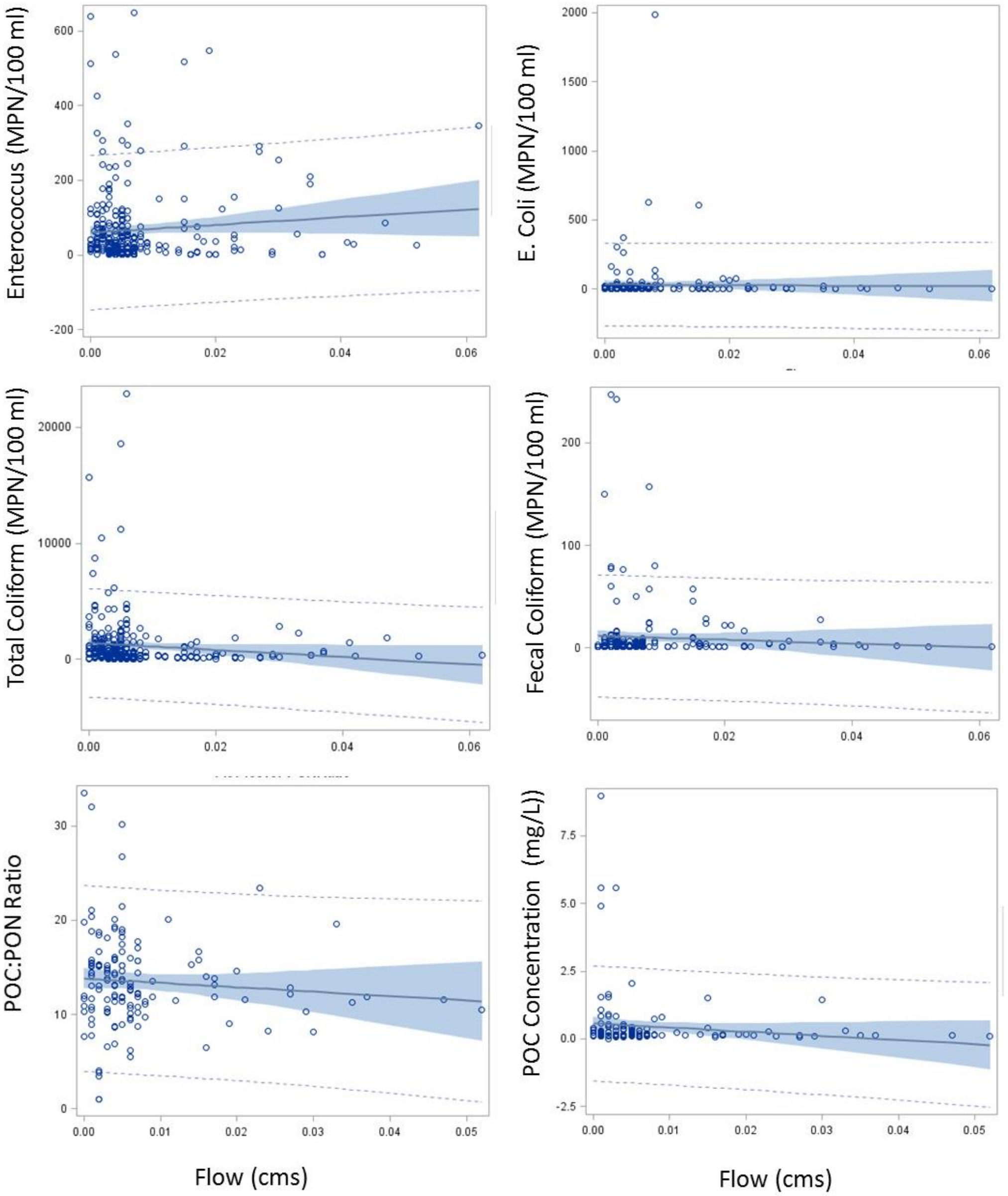

Figure 3.5. Relationship between flow, FIB concentration, POC concentration, and POC:PN ratio of TSS and DOC, grouping across years and sites. Regression lines are indicated by solid line; shaded blue is 95th percentile prediction interval in least squares regression, all of which were not significant.

While correlated to water column nutrients, it was less obvious that a direct tie between FIB, benthic algal biomass (benthic chlorophyll-a) and AFDM could be demonstrated. Biomass was low compared to the median of Stormwater Monitoring Coalition reference sites, and had no discernable increase with Julian day (Spearman Tau P-value >0.05; Figure 3.6). No significant relationship was found between FIB concentration and maximum benthic chlorophyll-a or AFDM.

Other chemical constituents such as total heavy and trace metals were also correlated, though it is less clear what the mechanism may be (Table 3.3). For example, total Fe was a consistently correlated with EC, ENT, and TC (p-value < 0.01). In some cases, total Pb, total Mn, total Cd, and TSS were positively correlated, but not significant in stepwise models.

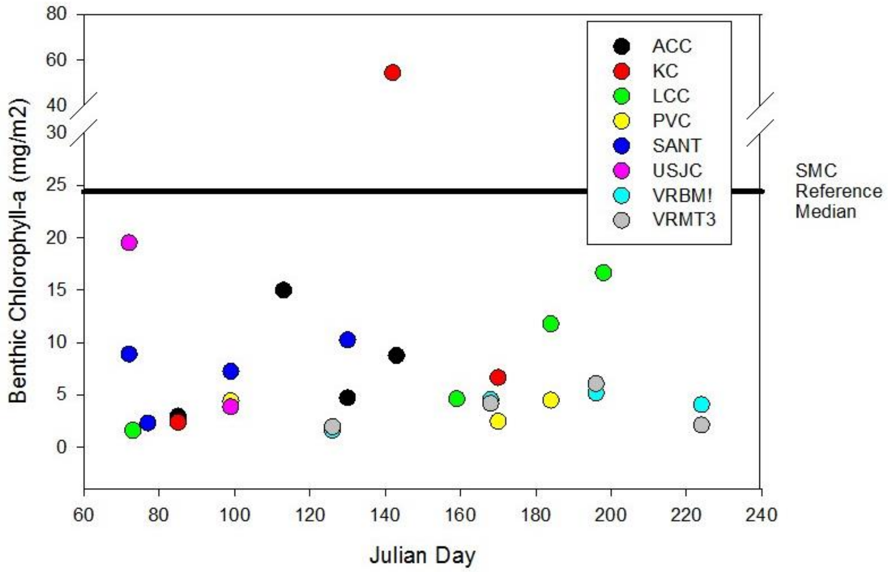

Figure 3.6. Trends in benthic chlorophyll-a as a function of Julian day. Color designates the site as shown in inset legend. The line references the median of SMC Bioassessment Program reference sites.

3.4 Factors Associated with Variability in Concentrations in Wet Weather Samples

The number of storm events captured was not sufficient to investigate the effect of geology or watershed size on storm EMCs. Overall, storm size, as measured by total rainfall on the day of the storm, exhibited a positive correlation with EC, ENT, TC, and FC, but the relationship was heavily driven by one point and exhibited a lot of scatter (Figure 3.7). Similar patterns were observed with storm timing, with an equal amount of scatter. Storms with fewer number of antecedent days in which last storm had occurred in general exhibited a higher range of storm FIB EMCs than those which had a protracted dry period (Figure 3.8).

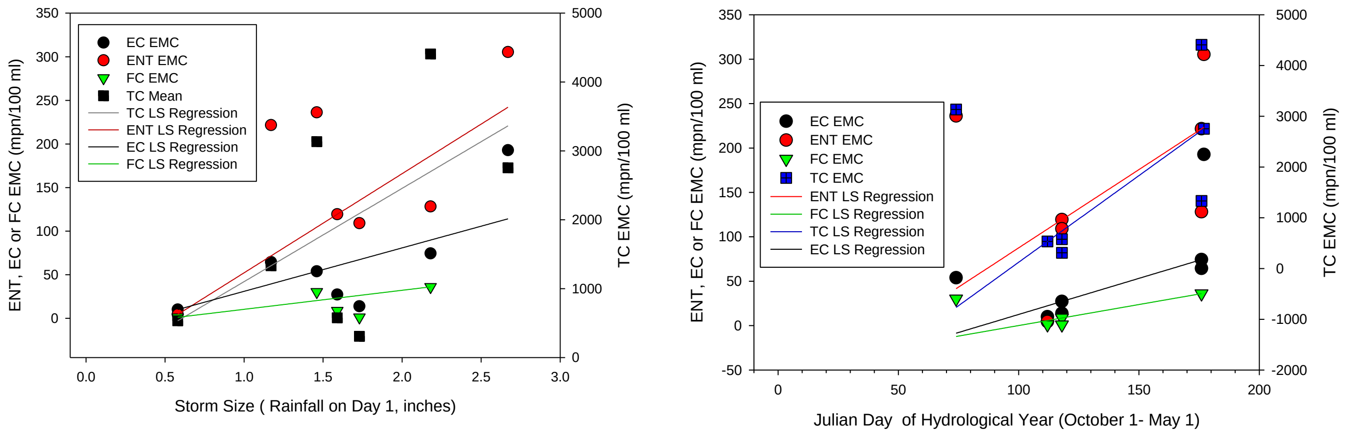

Figure 3.7. Relationship between storm size measured as rainfall on Day 1 (left panel) and storm timing (right panel) versus concentrations of ENT, EC, FC, and TC. Lines show least squares (LS) regression. Right panel x-axis shows storm timing, measured as the Julian Day of the hydrological year, beginning October 1. Day 75 is December 14, and Day 176 is April 26.

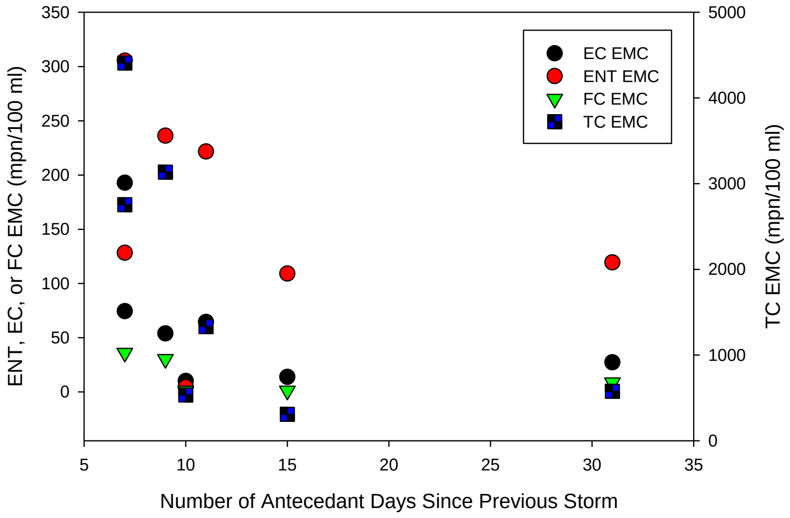

Figure 3.8. Relationship between number of antecedent days since previous storm versus EMC concentrations of ENT, EC, FC, and TC.

4. DISCUSSION

4.1 Exceedance Frequencies

FIB are found in natural streams, with populations present throughout the year and typically increasing during warm summer months (Stein and Yoon 2007, Tiefenthaler *et al.* 2008). FIB densities observed in the reference streams in this study were generally well below WQOs for EC, FC and TC (Geldreich 1978, Toranzos 2007), except for ENT. The annual rate of single sample exceedance of FIB WQOs for ENT in this study was double that previously documented in southern California Coastal streams during dry weather (14%, Tiefenthaler *et al.* 2008), spiking up to 40% during summer dry weather. However, the rates between the two studies are likely to be comparable when factoring in those of Tiefenthaler *et al.* (2008), which incorporated fall sampling, during which exceedances were very low. Dry weather single sample exceedances for other FIB indicators were comparable between the two studies (1.4 - 3%), as were annual 30-day geomean exceedances for all TC and ENT (39 and 45%, respectively, Tiefenthaler *et al.* 2008). Use of rolling 30-day geomean rather than a monthly mean to calculate exceedance frequency increased exceedance frequencies 15-20% for ENT and TC.

With the exception of ENT, wet weather EMC WQO exceedance frequencies in this study were lower than previously documented for “open space” in the L.A. River watershed by Stein et al. (2007), which ranged from 66 to 100% exceedance. Exceedance frequencies for wet weather “grabs” on Grab Days 1 to 3 following the storm event were comparable to that documented for EC and TC, but three times that documented by Tiefenthaler *et al.* (2008) for ENT (11%).

Our use of the HF183 human-associated MST marker (Layton *et al.* 2013) to eliminate entire sites as well as eliminate single samples with positive human marker hits provides confidence that the FIB exceedances in reference streams were likely of non-human origin (Carson *et al.* 2005). Non-human sources of FIB may be due to direct inputs from waterfowl and animals, soil erosion, or growth and colonization associated with decomposition of organic matter (Ricca and Cooney 1998, Byappanahalli *et al.* 2003, Toranzos 2007).

4.2 Dry and Wet Weather Relationship with Factors

We found that dry weather FIB concentrations peaked during the summertime and were significantly correlated with temperature, nutrients and organic carbon. No significant difference was found by geology or watershed size during dry weather. Increased presence and activity of wildlife during warmer months may be contributing (Baxter-Potter and Gilliland 1988, Bagshaw 2002, Stein *et al.* 2007), but previous authors have suggested that higher bacteria levels observed during summer months are largely attributable to bacterial growth in streams (Tiefenthaler *et al.* 2008). The positive relationship between temperature and bacteria levels suggests that heat-induced growth may be a contributing factor to seasonally high FIB levels, as optimum temperature ranges of FIB are those that mimic human body temperatures. These conditions can also spur growth of algal biomass and accelerate death and decomposition of organic matter in the stream, further enhancing *in situ* bacterial growth. Biofilm associated with increases in organic decomposition have also been shown to increase survival and growth of all bacteria, including enteric species (Novotny and Olem 1994). In this study, FIB exceedances were not directly linked to *in situ* algal biomass, which was quite low (Fetscher 2014), and had no discernable seasonal trend. In contrast, FIB, POC, PON, and DOC all spiked at the end of the season, coincident with the cessation of stream flow. This indicates that as the stream dries down, organic carbon and nutrients are being increasingly recycled from live and dead organic matter and/or concentrated in diminished flows. POC:PN ratio of particles suggested that the WQO exceedances were associated with a mix of both algal and terrestrial carbon sources.

Relationships were also found between dry weather FIB and total Pb, Fe, and Mn. Interestingly, these were the three metals in which the dominant fraction was particulate, rather than dissolved. Since bacteria are measured as particulate matter, these correlations may point to the association of FIB and metals with TSS overall (Stein and Yoon 2007) and have less to do with a mechanistic, stimulatory effect of metals on FIB per se.

Analyses of factors associated with wet weather EMCs was limited by the low sample size, though the general trend among sites was higher EMCs with increased storm size, fewest number of antecedent storm days, and storms later in the year. Notably, our study did not capture "first flush," during which the FIB loading may have been greater (Sansalone and Buchenberger 1997, Bertrand-Krajewski *et al.* 1998). It may be that higher storm EMC for events occurring later in the year may be capturing the effect of higher temperatures in late spring on FIB concentrations (Tiefenthaler *et al.* 2008).

4.3 Comparison of Storm EMCs, Dry Weather Concentrations and Fluxes with Previous Studies

Dry weather FIB fluxes documented for streams in this study were very comparable to those found previously by Tiefenthaler *et al.* (2 ± 1.4, 3 ± 1.7, and 106 ± 61.4 MPN/100 ml km^{-2} for EC, ENT, and TC, respectively, 2008). Furthermore, when FIB concentrations of streams of variable flow duration were flow-weighted, there were no significant differences between perennial and intermittent streams documented in southern California Coastal stream reference studies (Stein and Yoon 2007, Tiefenthaler *et al.* 2008). Similarly, wet weather EMCs were within the same order of magnitude of Stein and Yoon (2007) estimates for EC, ENT and TC (125, 140, and 4460 MPN/100 ml, respectively). These results suggest that data from this study can be used, in additional to other regional datasets, for regulatory applications of reference study results in the greater southern California region.

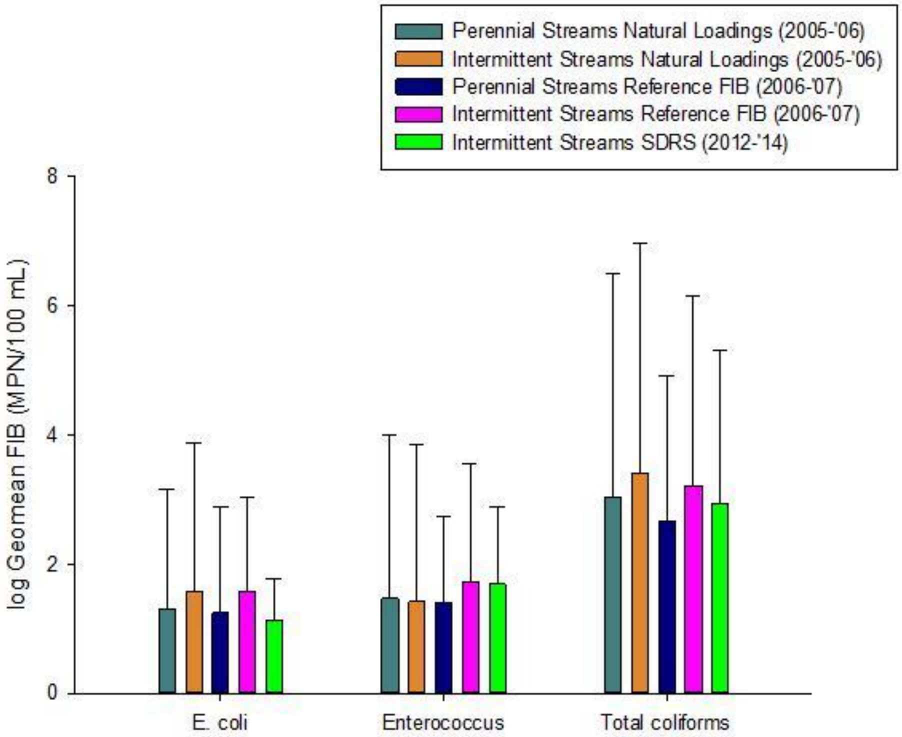

Figure 4.1. Comparison of the geomean of FIB concentrations among perennial and intermittent stream sites in the current study vs. Tiefenthaler *et al.* (2008) and Stein and Yoon (2007).

5. SUMMARY OF FINDINGS

A study of the natural background concentrations of FIB was conducted in wadeable streams during wet and dry weather, focused on San Diego and southern Orange Counties. This study yielded five major findings:

1. **FIB exceedances occurred in natural sites and were highest in summer dry weather. (April-August).** Exceedances of single sample WQOs occurred at annual frequencies ranging from 1.4 to 3% for TC and EC, and up to 30% for ENT. No exceedances of FC single sample WQOs were observed. Annual frequencies of 30-day geomean WQO exceedances were 0% for EC and FC, but 48 and 30% for ENT and TC, respectively. Exceedance frequencies were highest in the summer, spiking up to 40% for ENT single sample WQO and 68% for the 30-day geomean WQO.

2. **Storm EMC exceedances were low except for ENT.** Based on seven storms, EMC exceedances of single sample WQOs were 0% for EC, FC and TC, but 87% for ENT and 37% for the three "grab" days following. Exceedance frequencies for EC and TC increased to 29% if using the pollutograph maximum. The number of storm events captured was not sufficient to investigate the effect of geology or watershed size on storm EMCs.

3. **The HF183 human-associated fecal microbial source marker was successfully used to exclude sites and samples with potential human fecal contamination, providing improved confidence that the documented rates are attributable to non-human sources.** 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, availability of nutrients and presence of decaying organic matter.

4. **Temperature, and to a lesser extent, nutrients and organic carbon, were the major factors associated with higher summer dry weather FIB concentrations and exceedance frequencies. No significant relationships were found with either watershed size or geology during dry weather.** Water column FIB concentrations could not be attributed directly to instream benthic algal biomass as a measure of stream trophic status, which was low and showed no distinct seasonal variation. In contrast, FIB, temperature, POC, PON, and DOC all spiked at the end of the season, coincident with the end of stream flow. This is a naturally-occurring cycle where organic carbon and nutrients are increasingly recycled from live and dead organic matter as flow diminishes and water temperatures increase, conditions which coincide with increased FIB.

5. **Storm EMC fluxes were 2-3 times higher than dry weather FIB fluxes documented during this study. Wet and dry weather fluxes were comparable to those documented in previous southern California regional studies.** This suggests that data

from this study can be used, in additional to other regional datasets for regulatory applications of reference study in the greater southern California region.

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APPENDIX A. QUALITY ASSURANCE RESULTS

http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/862_StreamFIBs_AppendixA.pdf

APPENDIX B. EXCEEDANCE FREQUENCY CALCULATION SENSITIVITY ANALYSIS

http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/862_StreamFIBs_AppendixB.pdf

APPENDIX C. DRY WEATHER FIB EXCEEDANCE FREQUENCIES, CONCENTRATIONS, AND FLUXES BY SITE

http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/862_StreamFIBs_AppendixC.pdf

APPENDIX D. WET WEATHER POLLUTOGRAPHS, FIB EXCEEDANCE FREQUENCIES, CONCENTRATIONS, AND FLUXES BY SITE

http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/862_StreamFIBs_AppendixD.pdf