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Use of Fecal Steroids To Infer the Sources of Fecal Indicator Bacteria in the Lower Santa Ana River Watershed, California: Sewage Is Unlikely a Significant Source

JAMES A. NOBLET,[†] DIANA L. YOUNG,[‡] EDDY Y. ZENG,*[,‡,§] AND SEMSI ENSARI[||]

Department of Chemistry, California State University, San Bernardino, California 92407, Southern California Coastal Water Research Project, 7171 Fenwick Lane, Westminster, California 92683, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China, and Henry Samueli School of Engineering, University of California, Irvine, California 92697

The Santa Ana River (SAR), CA and adjacent wetlands have been identified as potential sources of fecal indicator bacteria (FIB) to the surf zone at Huntington Beach, CA. A suite of fecal steroids, including coprostanol (COP), epicoprostanol (eCOP), cholesterol (CHOE), cholestanol (CHOA), α-cholestanone (aONE), β-cholestanone (bONE), β-sitosterol (bSIT), stigmasterol (STIG), stigmastanol (STAN), and campesterol (CAM), were used as chemical markers to examine whether sewage was a significant source of FIB within the lower Santa Ana River watershed. A total of 54 water samples were collected from three locations in the intertidal zone near the mouth of the Santa Ana River at different tidal stages. Steroid ratios in SAR samples were different from those found in raw and treated sewage from a local wastewater treatment plant or in nearby effluent plume and did not appear to be influenced by the sampling location, daily tides, and spring/neap tidal cycle. The characteristics of steroid ratios suggested a diagenetic rather than a biogenic source for the COP content of the samples. The log-based concentrations of COP and FIB in the SAR samples were not significantly correlated, inconsistent with sewage being the source of FIB in the study area. In addition, multivariate statistical analysis showed that the concentrations of FIB were better correlated with bird fecal steroids than with the typical sewage sterols. The results implied that sewage was not a significant source of fecal steroids, and therefore perhaps FIB to the study area. Instead, birds may be one possible source of the intermittently high levels of FIB observed in the lower Santa Ana River watershed and the nearby surf zone.

Introduction

Public beaches are an important economic, recreational, and cultural resource for southern California. In an effort to protect beach-goers from waterborne disease, effective from July 1999 a state legislation requires that water quality be routinely monitored at public beaches with 50 000 or more annual visitors (*1*). Beach water quality monitoring programs are required to collect samples and measure three types of FIB—total coliforms (TC), fecal coliforms (a subset of which is *E. coli*, or EC), and the enterococcus group (ENT). The State of California has set uniform contact water quality standards for the three FIB. If the concentration of any FIB in a sample exceeds the respective standard, the local health officer is required to post warnings or close the beach to swimmers if a sewage spill is suspected (*2*).

Huntington State and City Beaches, CA, have been particularly affected by the new regulations. There have been a total of 684 health advisories posted at Huntington State and City Beaches between July 1999 and April 2002, 84% of which were due to ENT exceeding the contact water quality standards (*3*). Huntington Beach even received national attention in the summer of 1999, when a large section of the beach was closed to the public (*4*), due to persistently high levels of all three FIB groups. In response, the Orange County Sanitation District (OCSD; Fountain Valley, CA) that discharges treated wastewater via a sewage outfall to the coastal ocean off Huntington Beach conducted a comprehensive survey and inspection of the local sewage infrastructure, but no significant sewage leaks were identified (*5*). The lack of an obvious sewage source spawned a number of studies to identify possible mechanisms for sewage outfall plume transport to the coastal zone (*6*), gain an understanding of the temporal variability of surfzone FIB concentrations (*4*), and identify other possible sources of fecal contamination (*7*). In the latter study, Grant et al. (*7*) did find compelling evidence that a local constructed wetland, Talbert Marsh, was a net source of ENT to the surfzone. Moreover, the study identified birds and urban runoff as the most likely sources of FIB, particularly ENT, to the marsh, which in turn acted as a reservoir and secondary source of ENT to the local surf zone.

The results of the study by Grant et al. (*7*) prompted a similar but expanded follow-up study on the sources and dynamics of fecal indicators in the lower Santa Ana River watershed (*3*). Some results published recently suggested that FIB in the surf zone off Huntington Beach originated largely from runoff from the Santa Ana River watershed (*8*, *9*). As one component of this larger follow-up study, the objective of the research described herein was to use the selected fecal steroids (Table 1) to examine whether sewage was an important source of FIB in the lower Santa Ana River watershed and thus potentially to nearby areas of Huntington Beach. These compounds (particularly COP) have been recognized and used as chemical markers of sewage contamination for decades (*10*−*16*).

The present study focused on the use of steroid ratios to elucidate source information, as several previous studies have shown the utility of measuring a suite of C_{27}−C_{29} sterols, stanols, and stanones for source tracking of fecal contamination (*17*−*20*). Individual steroids were used in limited cases only. For example, COP was used with FIB to examine whether sewage was the important source of FIB in the study area, because significantly linear correlations between log-based concentrations of COP and EC were found in sewage-polluted aquatic environments (*21*). To facilitate the interpretation of fecal steroid data, total suspended solids, TC, EC, and ENT, were also measured concurrently with the selected chemical markers.

* Corresponding author phone: 86-20-85291421; fax: 86-20-85290706; e-mail: eddyz@sccwrp.org.
[†] California State University, San Bernardino.
[‡] Southern California Coastal Water Research Project.
[§] Guangzhou Institute of Geochemistry.
[||] University of California, Irvine.

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TABLE 1. Nomenclature and Quantitation Ions of the Target Analytes Measured in the Present Study

common name (acronym)	chemical name	quantitation ion
coprostanol (COP)	5β-cholestan-3β-ol	370
epicoprostanol (eCOP)	5β-cholestan-3α-ol	370
cholesterol (CHOE)	5-cholestan-3β-ol	368
chloestanol (CHOA)	5α-cholestan-3β-ol	445
α-chloestanone (aONE)	5α-cholestan-3-one	231
β-chloestanone (bONE)	5β-cholestan-3-one	316
β-sitosterol (bSIT)	24-ethyl-5-cholesten-3β-ol	396
stigmosterol (STIG)	24-ethyl-5,22-cholestadiene-3β-ol	394
stigmostanol (STAN)	24-ethyl-5α-cholesten-3β-ol	473
campesterol (CAM)	24-methyl-5-cholesten-3β-ol	382

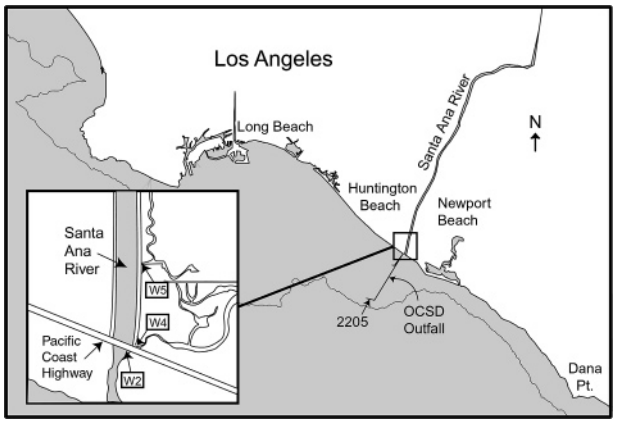

FIGURE 1. Map of field area near the mouth of the Santa Ana River and the outfall of the Orange County Sanitation District (OCSD), indicating the locations of chemical marker sampling stations, W2, W4, W5, and 2205.

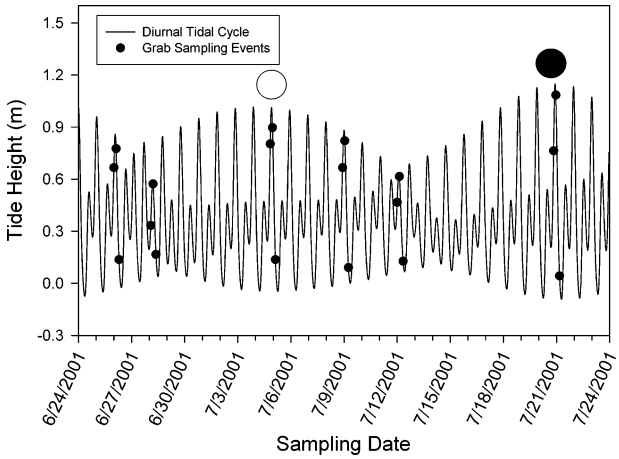

FIGURE 2. Sampling intervals relative to the daily and fortnightly tidal cycles. The dates of the full moon (○) and the new moon (●) are so indicated. Samples were collected from all three stations, W2 (Santa Ana River), W4 (lower slough), and W5 (upper slough) (Figure 1) at each interval.

Methods

Sample Collection. Three locations were chosen for sampling in the Santa Ana River during June and July of 2001. One station was located just below and west of the Pacific Coast Highway bridge (W2), and the two other stations were located at the entrance of two isolated sloughs, a lower slough (W4) and an upper slough (W5) that are subject to tidal fluxes of seawater (Figure 1). A total of six sampling excursions were conducted with the time frame encompassing two spring-neap tidal transitions. During each excursion, three samples were collected from each station at different tidal stages (Figure 2). Overall, 54 water samples (~18 L each) were collected by a field crew using multiple grabs with a stainless steel bucket and poured into precleaned five-gallon glass bottles. One bottle filled with ultrapure water was carried along with the field crew during sampling and treated as a field blank.

Samples of both raw and treated sewage were obtained from the OCSD. In situ plume samples were collected from station 2205 (at the 35-m depth from the sea surface), an OCSD marine monitoring site, near the OCSD outfall (Figure 1) using a CTD-Rosette Sampler (Sea Bird Electronics, Bellevue, WA). The plume samples captured OCSD outfall-discharged wastewater mixed with seawater (~1:300 wastewater:seawater). In addition, samples of fresh (i.e., still wet) bird feces were collected from the field area, combined into a single composite sample, and kept frozen until analyzed. All water samples were stored at 4 °C until filtered.

Sample Extraction. Water samples (the raw sewage was diluted 1:10 with deionized water before filtration) were filtered within 24 h of collection with acid washed and combusted 142-mm diameter (0.7-μm pore size) pure glass filters (Environmental Express, Mt. Pleasant, SC), using a nitrogen (chromatographic grade) pressurized filtration system. Only 4 L of each filtrate sample was retained (in 4-L amber glass bottles), and the remaining filtrate was discarded. Filtrate samples were extracted within 24 h after filtration, whereas filters containing suspended solids were placed in individual glass jars with Teflon-lined closures and stored at −20 °C for up to one year until extracted. For extraction of the composite bird feces sample, a small aliquot (~22 mg) of thawed bird feces was dissolved in 1 L of deionized water.

Each aqueous sample was extracted using 90-mm Empore C_{18} solid-phase extraction disks, following the procedure of Zeng and Khan (*22*). Loaded filters were dried in individual glass desiccators after Capangpangan et al. (*23*), weighed, and extracted using supercritical fluid extraction (SFE). The suspended solids concentration was calculated from the filter mass difference and the sample volume. The SFE extractor consisted of a 10-mL stainless steel extraction cartridge, layered from the bottom with a glass fiber filter, about 2 mm of granular copper (20−30 mesh), and the dried filter containing suspended particles. The extraction was performed using an SFX-220 Supercritical Fluid Extraction System (ISCO, Lincoln, NE) with heated restrictors. Just prior to extraction, samples were spiked with 5 μL of a 1.0-ppm recovery surrogate solution (androstanol or 5α-androstan-3β-ol) and 300 μL of acetone as a polarity modifier. Samples were subject to a 10-min static extraction, followed by a 30-mL dynamic extraction, at 80 °C and 365 atm, with the restrictors at 100 °C, and methylene chloride solvent collection. The extracts were spiked with cholesterol-d_6 (Cambridge Isotope Laboratories, Andover, MA) as an internal standard and taken to dryness under a nitrogen stream. The residue was then derivatized for 10 min at 70 °C in 0.1 mL of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane (Pierce, Rockford, IL).

TABLE 2. Distribution of Selected Steroid Compounds in 54 Santa Ana River Samples Analyzed

	particulate (μg/g-dry wt)[b]			dissolved (ng/L)[b]			total (ng/L)		
common name[a]	mean (min, max)	stdev[c]	detected[d]	mean (min, max)	stdev[c]	detected[d]	mean (min, max)	stdev[c]	detected[d]
coprostanol	0.10 (nd,[e] 0.49)	0.11	40	2 (nd, 21)	4	14	5 (nd, 26)	5	43
epicoprostanol	0.059 (nd, 0.396)	0.10	20	nd (nd, nd)	nd	0	2 (nd, 13)	4	20
cholesterol	14.7 (1.79, 87.1)	14.3	54	326 (186, 608)	101	54	811 (303, 4230)	582	54
cholestanol	2.35 (0.17, 7.18)	1.87	49	19 (nd, 41)	8	50	92 (12, 226)	57	54
α-cholestanone	1.33 (nd, 5.16)	1.29	49	nd (nd, 10)	1	1	28 (nd, 124)	26	46
β-cholestanone	3.99 (nd, 4.77)	0.76	31	14 (nd, 54)	16	31	42 (nd, 145)	38	49
β-sitosterol	3.88 (nd, 17.28)	3.50	53	15 (nd, 44)	11	44	139 (nd, 587)	111	53
stigmosterol	0.64 (nd, 2.45)	0.58	42	27 (13, 55)	10	54	21 (nd, 77)	20	54
stigmostanol	1.01 (nd, 5.82)	1.02	46	88 (24, 272)	50	54	119 (28, 294)	62	54
campesterol	0.94 (nd, 5.69)	0.97	49	na[f] (na, na)	na	0			
$\sum$steroids	25.4 (3.52, 114.2)	20.1	54	492 (262, 945)	150	54	1263 (474, 5517)	785	54

[a] See Table 1 for acronyms and chemical names. [b] Particulate = solid particulate matter with size > 0.7 μm; dissolved = filtrates with size < 0.7 μm. [c] stdev = standard deviation. [d] Number of samples in which the compound was present at detectable levels. [e] nd = not detected at the designated reporting levels. [f] na = not analyzed for in the respective phase.

While filtrate samples were extracted within 24 h after filtration, filters containing suspended solids were placed in individual glass jars with Teflon-lined closures and stored frozen (−20 °C) for up to one year until extracted. Thus, the filtrate samples were analyzed in the order they were received from the field, whereas the filters were pulled from the freezer in no particular order for analysis. This mode of processing randomized the order in which the suspended solid samples were analyzed, thus presumably mitigating any temporal bias in the analytical results.

Instrumental Analysis. The derivatized extracts were analyzed for steroids (CAM measured in particulate phase only) with a Saturn 2000 ion trap GC/MS system (Varian Inc., Walnut Creek, CA). Chromatographic separation was provided by a 60 m × 0.32 mm i.d. (0.25-μm film thickness) DB-XLB column (J&W Scientific, Folsom, CA), temperature-programmed from 60 °C (held for 0.5 min) to 320 °C (held for 15 min) at a rate of 20 °C/min. Ultrahigh purity helium was used as carrier gas at a constant flow of 1.3 mL/min. Autosampler injection was conducted in the split/splitless mode with injector temperature initiated at 60 °C (held for 0.3 min) and ramped to 310 °C (held for 10 min) at 150 °C/min. The mass spectrometer was operated at the electron ionization mode with an emission current of 50 μamps, and spectra were scanned from 50 to 500 m/z at an ionization storage level of 35 m/z and an ionization time factor of 100%. The molecular ions used for quantitation are listed in Table 1.

Analytical Performance. Preliminary experiments were conducted to evaluate the analytical methods. Samples of 4-L ultrapure water were spiked with the analyte standards at 25 ng/L each and processed using the procedure described above. The average recovery for the entire suite of steroids ($n = 6$) was 108 ± 11%. The performance of actual sample analysis was monitored with a recovery surrogate, androstanol. Recoveries of androstanol from the aqueous sample analyses, including field blanks and duplicates ($n =$ 63), ranged from 64.3 to 139% with a mean recovery of 91 ± 13% (*24*).

During the interim period between analyses of the dissolved and particulate phase samples, experiments were conducted to determine the optimum conditions for SFE of the particle-laden filters. SFE performance was optimized using both a spiked inert material, reagent grade Celite 545-AW (Supelco, Bellefonte, PA), and also a reference sediment, IAEA-408, which had certified concentrations for six of the sterol target analytes (IAEA, Vienna, Austria). Again, SFE recoveries in the actual sample analyses were monitored using androstanol as a recovery surrogate. Recoveries for all the SFE filter extractions ($n = 68$) ranged from 44 to 152%, with a mean of 89 ± 24%. Because this study focused primarily on steroid ratios, none of the data presented herein were adjusted for recoveries.

The lowest concentration calibration standard for all analyses was 0.1 mg/L, which corresponds to a minimum quantitation limit of ~2.5 ng/L for the dissolved phase samples. The reporting limit for the particle phase was dependent on the sample mass, which varied significantly. However, for the average suspended solids sample mass of 320 mg, the corresponding minimum quantitation limit would be ~30 ng/g.

Microbiological Analyses. Samples were collected hourly at each sampling station by the University of California-Irvine (UCI) field crews and transported to the UCI laboratory within 6 h. Samples (~20 mL) were immediately analyzed for TC, EC, and ENT using the Colilert and Enterolert defined substrate tests (IDEXX Laboratories, Westbrook, MN). Only a subset of these data was presented herein in cohesion with the chemical marker data.

Data Evaluation. Four types of data analysis were used to evaluate the chemical marker data. First, steroid ratios, such as the percentage of COP (COP/$\sum$steroids) and the ratios of the 5β to 5α epimers for the stanols and stanones, i.e., 5β/(5α +5β) for both COP/(COP+CHOA) and bONE/(aONE+bONE) were calculated. Second, selected individual and grouped chemical marker ratios and concentrations were compared graphically with the contemporaneous FIB data. Third, correlation between the log-based concentrations of COP and FIB was assessed to determine if there was any significant relationship. Finally, multivariate statistical methods were used to quantify the relationships between the chemical marker and FIB data. The statistical analyses included cluster analysis and Pearson product moment correlation analysis performed using MINITAB Release 13.32 (Minitab, State College, PA). Pearson correlation values were calculated for the relationship between two different sums of steroids and the FIB.

Results and Discussion

Distribution of Target Compounds. The target compounds were widely distributed in the SAR samples, with all but eCOP detected in either the particulate or dissolved phase of at least 42 samples or 78% out of 54 samples (Table 2). CHOE was the most ubiquitous and abundant of the steroids, the only analyte detected in all the samples from both the particulate and dissolved phases, and its concentrations were substantially higher than all other analytes. CHOA, bSIT, STIG, and STAN were also detectable in almost all the samples. Conversely, eCOP was detected only in the particulate phase.

TABLE 3. Particle−Water Distribution Coefficients, log(C_p/C_w), of Selected Steroid Compounds in the Santa Ana River Samples[e]

common name[a]	mean log(C_p/C_w)	stdev[b]	95% CI[c]
coprostanol	4.04 ($n = 11$)	0.39	0.23
epicoprostanol	n/a[d]	n/a	n/a
cholesterol	4.52 ($n = 54$)	0.36	0.10
chloestanol	4.91 ($n = 50$)	0.38	0.10
α-chloestanone	4.27 ($n = 1$)	n/a[c]	n/a
β-chloestanone	4.35 ($n = 16$)	0.35	0.17
β-sitosterol	5.30 ($n = 44$)	0.37	0.11
stigmosterol	4.41 ($n = 42$)	0.27	0.08
stigmostanol	4.04 ($n = 46$)	0.42	0.12
campesterol	n/a	n/a	n/a

[a] See Table 1 for acronyms and chemical names. [b] stdev = standard deviation. [c] CI = confidence interval. [d] n/a = not applicable. Epicoprostanol was not detectable in the filtrate samples; campesterol was not measured in the dissolved phase; and caffeine was not measured in the particulate phase. [e] C_p and C_w are the concentrations in the particulate and dissolved phases, respectively.

The distribution data in Table 2 allowed us to calculate the log-transformed ratios of analyte concentrations in the particulate (C_p) to the dissolved phase (C_w) (Table 3) and estimate the equilibrium state of the system. Since the organic carbon content of the particulate phase was not determined, a nominal value of 1% was assumed for this assessment. Takada and Eganhouse (*14*) estimated the log octanol−water partition coefficient (log K_{ow}) for COP to be 6.5 to 7.5 from the water solubility of CHOE. Assuming that the organic carbon-normalized partition coefficient (K_{oc}) is approximated by $K_{oc} = 0.41\ K_{ow}$ (*25*), then the log(C_p/C_w) for the steroids would be about 4.11 to 5.11 at equilibrium. This is in general agreement with the measured average values from this study, which ranged from 4.04 (±0.39) to 5.30 (±0.37) (Table 3), implying that the system appears to be at approximate equilibrium between the dissolved and particulate phases. Because equilibrium partitioning of these hydrophobic chemicals between the particulate and dissolved phases takes time to establish, an equilibrium state means that the suspended solids and the associated fecal steroids may have been more likely derived from tidal scouring and resuspension of local bottom sediments than from an offshore source, such as the OCSD outfall system.

Characteristics of Steroid Ratios. Because COP has been identified as a major component of fecal steroids in sewage contaminated samples, values of COP/∑steroids may be proportional to the extent of sewage contamination. In addition, Grimalt et al. (*18*) found that plotting COP/(COP+CHOA) against bONE/(aONE+bONE) provided a unique method for distinguishing between sewage and nonsewage derived pollution in complex environmental systems. They found that COP/(COP+CHOA) and bONE/(aONE+bONE) ratios greater than 0.7 implied an in vivo production of COP and thus were indicative of sewage pollution.

The average COP/∑steroids values in suspected sources and SAR samples exhibited several interesting features (Figure 3). First, both the particulate and dissolved phase SAR samples had COP/∑steroids ratios that were markedly lower than either raw and treated sewage from the OCSD or plume samples collected near the OCSD outfall (station 2205; Figure 1). Second, the average COP/∑steroids ratio in the particulate samples was comparable to that of seagull feces and slightly lower than that of duck feces reported in the literature (*19*). Finally, all the SAR samples had higher COP/∑steroids ratios than the bird fecal sample collected at the field area. The substantially lower COP/∑steroids ratio with OCSD treated sewage than with OCSD raw sewage was apparently a result of COP reduction due to the treatment process.

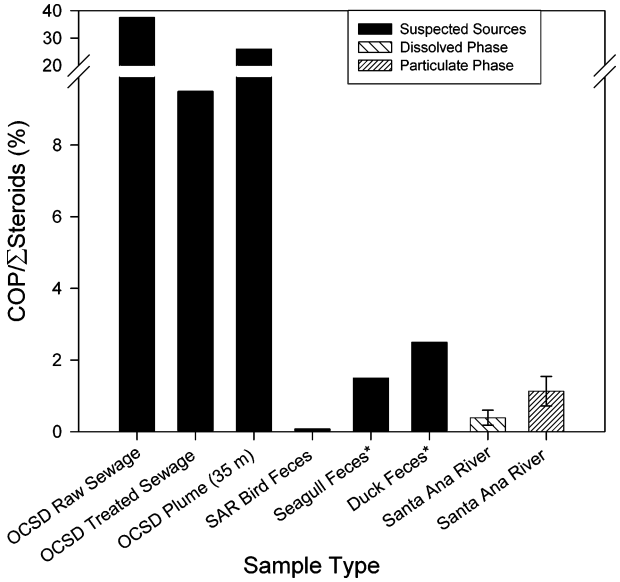

FIGURE 3. Ratios of coprostanol/total steroids (COP/∑steroids) in suspected sources and field samples. Those sources marked with an asterisk (∗) are from the literature. Error bars are the 95% confidence intervals ($n = 54$).

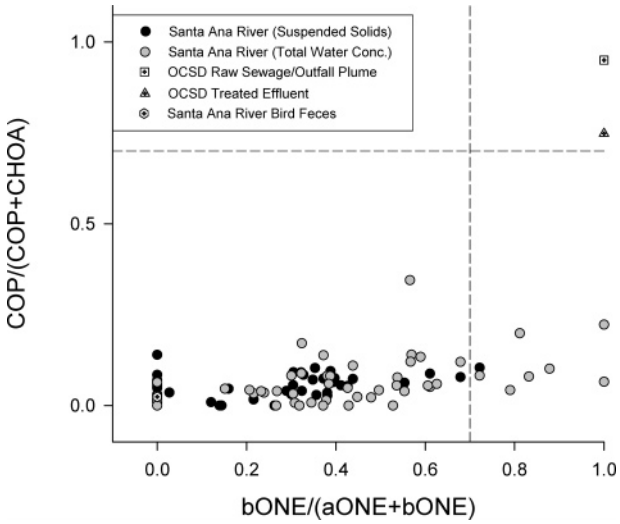

FIGURE 4. Ratios of COP/(COP+CHOA) versus bONE/(aONE+bONE) in samples from the Santa Ana River and selected potential fecal sources, where ratios above 0.7 on one or both axes are typical for sewage sources (dashed lines). The data points for Orange County Sanitation District (OCSD) raw sewage and outfall plume essentially overlap.

The 5β/(5α +5β) ratios for the SAR samples (both the particulate and dissolved phases) and suspected sources were calculated and plotted as stanols and stanones (Figure 4). Suspected sources investigated included OCSD raw and treated sewage, offshore OCSD outfall plume, and SAR bird feces. The 5β/(5α +5β) ratios for samples collected from the offshore OCSD outfall plume were identical to those for raw sewage. All of the sewage related samples were found to plot in the upper right corner of the graph, i.e., > 0.7 for both the COP/(COP+CHOA) and bONE/(aONE+bONE) ratios. This result is in agreement with several other studies that have used these parameters for source identification and found that sewage samples typically are very near unity for both ratios (*18*). In contrast, the SAR samples are spread across the bottom of the graph, covering the full range of possible values from 0 to 1. All SAR samples were below 0.7 for the stanol ratio, and all but eight samples were below 0.7 for the

stanone ratio. The bird fecal sample collected from the SAR field area is plotted very near the origin, and thus the local birds are clearly differentiated from the sewage sources of fecal contamination. A qualitative assessment by expanding the steroid ratios for the particulate SAR samples (Figure 4) indicates that the SAR data do not exhibit any type of pattern related to the sampling location, daily tidal stage, or fortnightly tidal cycle.

Source Assessment for Fecal Steroids. Apparently, both COP/∑steroids and 5β/(5α +5β) data showed that the SAR samples did not have the steroid profile typical of raw sewage, treated effluent or the OCSD outfall plume (Figures 3 and 4). In particular, the 5β/(5α +5β) data can be inferred for further assessment of potential sources for fecal steroids because they provide information about the relative contributions of two different production pathways for COP and CHOA.

COP and CHOA are produced in vivo by transformation of CHOE to a cholestenone and then to 5α or 5β cholestanone, followed by reduction to COP or CHOA (*14*, *18*). In contrast, in situ formation of COP or CHOA in anaerobic sediments occurs by direct reduction of the 5−6 double bond of CHOE. Since CHOA is thermodynamically more stable, its formation is favored by the in situ reduction. In humans and some marine mammals, formation of COP is highly favored (*17*). Hence the ability to distinguish between sources is a function of the ability to determine the relative contributions from in vivo and in situ production of the fecal steroids. However, it has been shown that CHOA can be produced biogenically by a number of aerobic organisms, such as phytoplankton, zooplankton, and macrophytes (*18*, *26*). Therefore, in areas of high productivity, the utility of the COP/(COP+CHOA) ratio may be reduced. Since the equivalent bONE/(aONE+bONE) ratio would not be affected by the biogenic production of CHOA, Grimalt et al. (*18*) suggested that it may be used as a complimentary parameter for distinguishing sewage pollution from other sources of fecal steroids.

The low COP/(COP+CHOA) ratios for the SAR samples (Figure 4) implied an in situ origin for the fecal steroids detected in this study. However, the significant variability in the bONE/(aONE+bONE) ratio may suggest at least some in vivo contribution to the fecal steroid pool. Another possibility is that low levels of diagenetically produced COP could become suspended during vigorous tidal flows and then oxidized under aerobic conditions back to the stanone. Laboratory incubation experiments using radiolabeled compounds have demonstrated that interconversion of stanols and stanones does occur (*18*). In addition, available fate data on COP in natural waters suggest that its half-life is less than 10 days under aerobic conditions (*14*).

Comparison of Fecal Indicator Bacterial and Chemical Marker Data. A comparison of the FIB data with several chemical marker ratios and concentrations derived from the SAR samples was performed (Figure 5). Over the time frame of the study, there was only a single sampling interval, where there was a large spike in all three FIB (Figure 5A). Of all the possible chemical marker parameters, only the sum of the most abundant sterols commonly found in bird feces (not only in the bird feces sample collected in this study), primarily CHOE, showed a comparable spike in the data (Figure 5B). The simultaneous sharp increase in bird fecal steroid and FIB concentrations is consistent with a previously reported hypothesis that birds may be a possible source of FIB to the watershed (*7*). Another study using enterococci antibiotic resistance patterns for source identification indicated that bird feces were the source of surf zone contamination in Huntington Beach on some days, although coastal salt marsh and sewage plume might also have impacted water quality at other times (*27*).

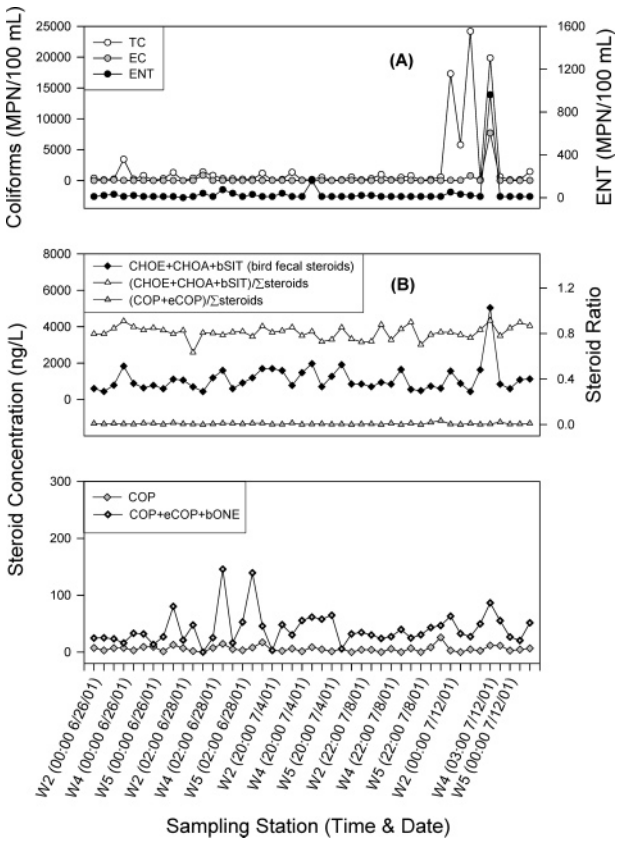

FIGURE 5. Plots of concentrations of total coliform (TC), fecal coliform (EC), and enterococcus (ENT) (A), and selected steroid ratios (B and C). CHOE − cholesterol; CHOA − cholestanol; bSIT − β-sitosterol; COP − coprostanol; eCOP − epicoprostanol; and bONE − β-cholestanone. To simplify the *x*-axis labeling, only one out of three timepoints is displayed at each station on a specific date.

Coefficients of correlation between the log-based concentrations of COP and FIB were 0.096 (TC), 0.007 (EC), and 0.030 (ENT), respectively (Figure 6). The small correlation coefficients indicates that sewage is unlikely an important source of FIB in the study area, as COP was found to significantly relate to EC in sewage-contaminated aquatic systems (*21*). Correlation analyses were also conducted on the log-based concentrations of bird fecal steroids (CHOE+CHOA+bSIT) and FIB, and the correlations were also low (correlation coefficients were 0.022, 0.077, and 0.19, respectively).

It is noteworthy that the concentrations of TC, EC, and ENT were mostly below the single-sample standards, 10 000 (TC), 400 (EC), and 104 (ENT) most probable number (MPN), and 30-day geometric mean (1000, 200, and 35 MPN, respectively) (*7*) over the duration of the study. There was only one instance where all three indicators were above their respective standards simultaneously (station W4, 0300 h, 7/12/01; Figure 5A). Although the correlation between FIB and steroid compounds did not point to sewage as an important source of FIB in the study area, it would be difficult to extrapolate the conclusion to entire Huntington Beach because of the small number of samples that had elevated FIB concentrations.

Similarity Evaluation. A preliminary cluster analysis on the SAR samples was conducted to identify the degree of similarity among the parameters included in the analysis. Based on the results of the cluster analysis and the steroid composition of potential sources, two groupings of steroids were selected as additional parameters. One group of sewage related steroids, COP+eCOP+bONE (sum1), and another

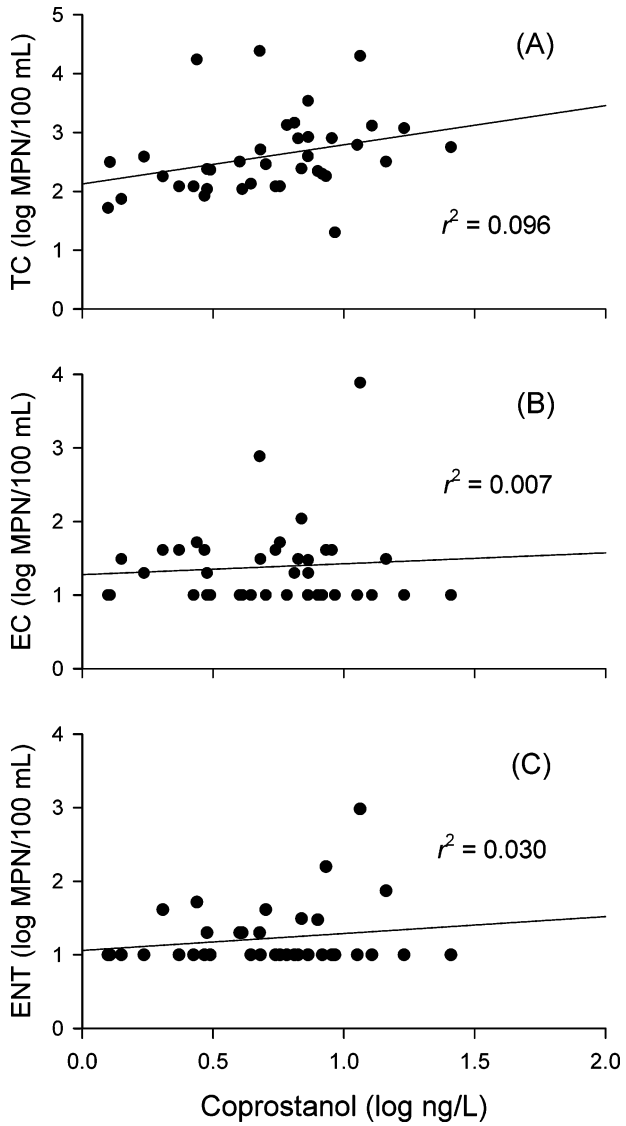

FIGURE 6. Correlation between the concentrations (log-based) of coprostanol and (A) total coliform (TC); (B) *E. coli* (EC); and (C) enterococcus (ENT) in the Santa Ana River samples.

group of bird fecal steroids, CHOE+CHOA+ bSIT (sum2). The bird fecal steroids were the three most abundant steroids commonly found in bird feces as well as in our bird feces sample. These two sums were evaluated relative to the FIB data (log transformed) using Pearson Product Moment Correlation Analysis. There were significantly moderate correlations between log EC ($r = 0.536$, $p < 0.001$), log ENT ($r = 0.637$, with $p < 0.001$), and sum2. A somewhat lower correlation was found between log ENT ($r = 0.398$, $p < 0.01$) and sum1. The strengths of these correlations suggest a strong link between FIB and bird fecal steroids. On the other hand, COP did not generate any significant correlations with the FIB data (Figure 6), consistent with the notion that the FIB are unlikely of sewage origin.

Final Remarks. The hypothesis that the fecal steroids in the study area are primarily of nonsewage origin is further supported by the agreement of the chemical marker data obtained in this study with previous studies in similar environmental settings. For example, Venkatesan and Kaplan (*24*) found that in Santa Monica Bay, California, COP comprised 50−80% of the total steroids near sewage outfalls but dropped to about 3% at stations distant from the source. Also, Phillips et al. (*28*) conducted a study in 1994 which measured fecal steroids in sediments at two locations in the Santa Ana River and one location in Talbert Marsh. That study found sediment COP concentrations of 67 to 380 ng/g, which are comparable to our data for the suspended solids phase ranging from below detection to 490 ng/g (Table 2). These data suggest that the local sediments may be the source of the suspended solids and that the COP content of the sediments has not changed significantly since 1994.

It should be noted that birds are not the only possible source of the FIB based on the fecal steroid data. Interestingly, dogs have fecal steroid composition very similar to that of birds (*29*). To distinguish between these potential FIB sources, Leeming et al. (*29*) used the ratio of fecal coliforms to *Clostridium perfringens*. The feces from birds and humans have virtually no *C. perfringens* spores (<0.01%), whereas domestic pets (dogs and cats) contain nearly equal amounts of both *C. perfringens* and fecal coliforms. Thus, this additional analysis could potentially be used in future studies to distinguish between FIB contributed by birds and domestic pets. A recent study also identified multiple sources of FIB, including bird droppings, contaminated subsurface water, leaking drains, and runoff as well as human sewage, at Avalon Bay, Catalina Island, California (*30*).

In summary, our evaluation of the limited data obtained in this study indicates that raw and/or treated sewage was not an important source of fecal steroids found in the study area. Therefore, it may also be inferred that a local, recent sewage leak or other sewage discharge was not a likely source of the FIB in the lower Santa Ana Watershed.

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