

Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River at Imperial Highway, Southern California, 1999–2002

By John A. Izbicki, M. Isabel Pimentel, Menu Leddy¹, and Brian Bergamaschi

In cooperation with the
Orange County Water District

¹Orange County Water District

Scientific Investigations Report 2004-5116

**U.S. Department of the Interior
U.S. Geological Survey**

U.S. Department of the Interior
Gale A. Norton, Secretary

U.S. Geological Survey
Charles G. Groat, Director

U.S. Geological Survey, Reston, Virginia: 2004

For sale by U.S. Geological Survey, Information Services
Box 25286, Denver Federal Center
Denver, CO 80225-0286

For more information about the USGS and its products:

Telephone: 1-888-ASK-USGS

World Wide Web: <http://www.usgs.gov/>

Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Although this report is in the public domain, permission must be secured from the individual copyright owners to reproduce any copyrighted materials contained within this report.

Suggested citation:

Izbicki, J.A., Pimentel, M.I., Leddy, M., and Bergamaschi, B., 2004, Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River at Imperial Highway, Southern California, 1999–2002: U.S. Geological Survey Scientific Investigations Report 2004–5116, 71 p.

Contents

Abstract	1
Introduction	2
Purpose and Scope	2
Previous Studies	2
Description of the Santa Ana River Basin	4
Acknowledgments	4
Approach	6
Sampled Stormflows	6
Analytical Methods	9
Total Coliforms and Fecal Indicator Bacteria	10
Historical Data	10
Total Coliforms and Fecal Indicator Bacteria Concentrations during Stormflow	13
Phospholipid Fatty Acids	16
Phospholipid Fatty Acid Concentrations and Compositions during Stormflow	20
Comparison with Phospholipid Fatty Acids from Other Sources	23
16S rRNA Gene	28
Amplicon Size and Diversity during Stormflow	29
Comparison with Amplicons from Other Sources	31
Dissolved Organic Carbon	31
Dissolved Organic Carbon Concentrations during Stormflow	31
Dissolved Organic Carbon Compositions during Stormflow	34
Ultraviolet Absorbance	34
Excitation/Emission Spectroscopy	36
Dissolved Organic Carbon Fractionation	40
Total Coliform Bacteria, Fecal Coliform Bacteria, and Dissolved Organic Carbon Concentrations in Shallow Ground Water during Stormflow	41
Conclusions	43
References Cited	44
Appendixes	49

Figures

Figure 1. Map showing Santa Ana River Basin, southern California, and location of selected stream gages and sample-collection sites	3
Figure 2. Map showing land use in the Santa Ana River Basin, southern California, 1990.	5
Figure 3. Graphs showing sampled stormflows, precipitation, and stream discharge for the Santa Ana River at Imperial Highway, southern California, during the 1999–2000, 2000–01, and 2001–02 rainy seasons.	7
Figure 4. Graph showing monthly median total and fecal coliform bacteria concentrations in the Santa Ana River at Imperial Highway, southern California, January 1986 to March 1994.	12
Figure 5. Graphs showing total coliform and fecal indicator bacteria (<i>Escherichia coli</i> and enterococci) concentrations in the Santa Ana River at Imperial Highway, southern California, during the first stormflow of the 1999–2000, 2000–01, and 2001–02 rainy seasons.	14
Figure 6. Graphs showing total coliform and fecal indicator bacteria (<i>Escherichia coli</i> and enterococci) concentrations in Mill Creek at Chino-Corona Road, and in the Santa Ana River at Imperial Highway, southern California, November 12, 2001	15
Figure 7. Graphs showing total coliforms, fecal coliforms, <i>Escherichia coli</i> , and enterococci concentrations as a function of suspended-sediment concentration in stormflow in the Santa Ana River at Imperial Highway, southern California, 2000–01 and 2001–02 rainy seasons.	17
Figure 8. Graph showing suspended-sediment concentrations as a function of streamflow during stormflow conditions in the Santa Ana River at Imperial Highway, southern California, 2000–01 and 2001–02 rainy seasons.	18
Figure 9. Graphs showing phospholipid fatty acid concentrations as a function of streamflow, and of total coliform bacteria, and <i>Escherichia coli</i> concentrations in stormflow in the Santa Ana River at Imperial Highway, 1999–2000 and 2001–02 rainy seasons.	21
Figure 10. Graphs showing selected phospholipid fatty acid (PLFA) structural groups as a function of total phospholipid fatty acid concentration in stormflow in the Santa Ana River, 1999–2000 and 2001–02 rainy seasons.	22
Figure 11. Graphs showing total coliform bacteria and total phospholipid fatty acid (PLFA) concentrations as a function of streamflow, and changes in diversity of PLFA and the 16S rRNA gene during the November 12–14, 2001, stormflow, Santa Ana River (SAR) at Imperial Highway, southern California	24
Figure 12. Graph showing the first principal component as a function of the second principal component for the 10 most common phospholipid fatty acids in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons.	26
Figure 13. Graphs showing amplicons from Terminal-Restriction Fragment Length Polymorphism analysis of the 16S rRNA gene with polymerase chain reaction amplification using universal eubacterial primers and PCR amplification using eubacterial primers and digestion with <i>DdeI</i> restriction endonuclease for selected stormflow samples from the Santa Ana River at Imperial Highway, southern California, November 12–14, 2001.	30

Figure 14. Graphs showing amplicons from Terminal-Restriction Fragment Length Polymorphism analysis of the 16S rRNA gene with polymerase chain reaction amplification using universal eubacterial primers and PCR amplification using eubacterial primers and digestion with <i>DdeI</i> restriction endonuclease for selected samples from the Santa Ana River Basin, southern California.	32
Figure 15. Graphs showing changes in dissolved organic carbon concentration and ultraviolet absorbance at 254 nanometers during the first stormflows of the 1999–2000, 2000–01, and 2001–02 rainy seasons, Santa Ana River at Imperial Highway, southern California.	33
Figure 16. Graphs showing selected full-spectrum ultraviolet absorbance scans, Santa Ana River at Imperial Highway, 1999–2000, 2000–01, and 2001–02 rainy seasons.	35
Figure 17. Graphs showing dissolved organic carbon as a function of UV_{254} , and UV_{285} as a function of UV_{254} , in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons	37

Tables

Table 1. Summary of stormflow sample collection in the Santa Ana River at Imperial Highway, southern California, 1999–2000, 2000–01, 2001–02 rainy seasons	8
Table 2. Summary of historical bacteria concentrations and bacteria concentrations measured during pre-stormflow and stormflow conditions in the Santa Ana River at Imperial Highway, southern California	11
Table 3. Description of selected phospholipid fatty acid structural groups	19
Table 4. Eigenvectors composing the first and second principal components of phospholipid fatty acid structural groups and the 10 most common fatty acids in stormflow in the Santa Ana River at Imperial Highway, 1999–2000, and 2001–02 rainy seasons	25
Table 5. Total phospholipid fatty acid concentrations and percent of total PLFA for the 10 most commonly detected fatty acids in selected samples from the Santa Ana River basin, southern California	27
Table 6. Equations describing the relation between dissolved organic carbon and ultraviolet absorbance in early-season and late-season stormflows in the Santa Ana River, California	38
Table 7. Dissolved organic carbon concentrations and hydrophobic-acid, hydrophobic-neutral, hydrophilic-acid, and transhydrophilic-acid fractions of dissolved organic carbon in stormflow samples collected from the Santa Ana River at Imperial Highway and from Mill Creek at Chino-Corona Road, southern California, 2001–02 rainy season	42

Appendixes

Appendix A. Total coliforms, fecal coliforms, <i>Escherichia coli</i> , enterococci, and suspended-sediment concentrations for stormflow in the Santa Ana River at Imperial Highway, in Mill Creek, and in shallow ground water from the Santa Ana River Basin, southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons	50
Appendix B1. Total phospholipid fatty acid concentrations and selected fatty acid structural groups in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons	53
Appendix B2. Normal saturated phospholipid fatty acids in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons	54
Appendix B3. Mid-chain branched saturated phospholipid fatty acids in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons	55

Appendix B4. Terminally branched saturated phospholipid fatty acids) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons	57
Appendix B5. Monoenoic phospholipid fatty acids in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons	58
Appendix B6. Branched monoenoic phospholipid fatty acids in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons	60
Appendix B7. Polyenoic phospholipid fatty acids in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons	61
Appendix C. Dissolved organic carbon and ultraviolet absorption data for stormflow in the Santa Ana River at the diversion downstream from Imperial Highway and at the subsurface collection and recharge system, southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons	63
Appendix D. Data for field parameters, major ions, and nutrients in the Santa Ana River at the diversion downstream from Imperial Highway, southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons	68

Conversion Factors, Datum, and Abbreviations

CONVERSION FACTORS

Multiply	By	To obtain
acre-foot (acre-ft)	1,233	cubic meter
cubic feet per second (ft ³ /s)	0.02832	cubic meter per second
foot (ft)	0.3048	meter
inch (in.)	2.54	centimeter
inch (in.)	25.4	millimeter
mile (mi)	1.609	kilometer
square mile (mi ²)	12.590	square kilometer

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$^{\circ}\text{F}=1.8\ ^{\circ}\text{C}+32.$$

Specific conductance is given microsiemens per centimeter at 25 degrees Celsius ($\mu\text{S}/\text{cm}$ at 25°C).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter ($\mu\text{g}/\text{L}$).

DATUM

Sea level: In this report, “sea level” refers to the National Geodetic Vertical Datum of 1929 (NGVD of 1929)—a geodetic datum *derived* from a general adjustment of the first-order level nets of both the United States and Canada, formerly called Sea Level Datum of 1929.

ABBREVIATIONS

ABI	Applied Biosystems
bp	base pairs
CaCO ₃	calcium carbonate
CFU/100mL	colony forming units per 100 milliliters
CH ₄	methane
CM-1	absorbance per centimeter length
CO ₂	carbon dioxide
DDMMSS	degrees, minutes, seconds
DBP	disinfection by product
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
EEM	excitation/emission spectroscopy
GIS	geographic information system
HAA	haloacetic acids
HCL	hydrochloric acid

μL	microliter
μM	micromole
μS/cm	microsiemen per centimeter at 25°C
mL	milliliters
MPN	most probable number
MTBE	methyl-tert-butyl ether
MTF	multi-tube fermentation
2N	normality of two
N ₂	nitrogen gas
Na ₂ HPO ₄	sodium phosphate
NaOH	sodium hydroxide
ng	nanogram
nm	nanometer
NPOC	nonpurgeable organic carbon
OCWD	Orange County Water District
PCR	polymerase chain reaction
PLFA	phospholipid fatty acid
pmole/g	picomole per gram
pmole/L	picomole per liter
PO ₄	phosphate
rpm	revolutions per minute
SAR	Santa Ana River
SARWQH	Santa Ana River Water Quality and Health (study)
SCARS	subsurface collection and recharge system
16S rRNA	a bacterial ribosomal ribonucleic acid
SO ₄	sulfate
SUVA	specific ultraviolet absorbance
THM	trihalomethane
T-RFLP	Terminal-Restriction Fragment Length Polymorphism
UVA	ultraviolet absorption
UV ₂₅₄	ultraviolet absorption at 254 nanometers
UV ₂₈₅	ultraviolet absorption at 285 nanometers
QSU	quinine sulfate unit

Organizations

OCWD	Orange County Water District
SCAG	Southern California Association of Governments
USGS	U.S. Geological Survey

Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River at Imperial Highway, Southern California, 1999–2002

By John A. Izbicki, M. Isabel Pimentel, Menu Leddy, and Brian Bergamaschi

Abstract

The Santa Ana River drains about 2,670 square miles of densely populated coastal southern California, near Los Angeles. Almost all the flow in the river, more than 200,000 acre-feet annually, is diverted to ponds where it infiltrates and recharges underlying aquifers pumped to supply water for more than 2 million people. Base flow in the river is almost entirely treated municipal wastewater discharged from upstream treatment plants and, in the past, stormflow was considered a source of high-quality water suitable for use as a source of ground-water recharge that would dilute poorer quality water recharged during base flow.

Stormflow in the Santa Ana River at the Imperial Highway diversion contains total coliform bacteria concentrations as high as 3,400,000 colonies per 100 mL (milliliters). Fecal indicator bacteria concentrations, including fecal coliforms, *Escherichia coli*, and enterococci, were as high as 310,000, 84,000, and 102,000 colonies per 100 mL, respectively. Although concentrations were high owing to urban runoff during the first stormflow of the rainy season, the highest concentrations occurred during the recessional flows of the first stormflow of the rainy season after streamflow returned to pre-storm conditions. Molecular indicators of microbiological organisms in stormflow, including phospholipid fatty acid (PLFA) and genetic data, show that the diversity of the total microbial population decreases during stormflow while fecal indicator bacteria concentrations increase. This suggests that the source of the bacteria must be poorly diverse and dominated by only a few types of bacteria. Although direct runoff of fecal indicator bacteria from urban areas occurs, this process cannot explain the very high concentrations of fecal indicator bacteria in runoff from upstream parts of the basin characterized by urban, agricultural (including more than 300,000 head of dairy cattle), and other land uses. Although other explanations are possible, fecal indicator bacteria concentrations and molecular microbiological data indicate accumulation and extended

survival of bacteria in streambed sediments, and subsequent mobilization of those sediments and associated bacteria during stormflow. Both PLFA and genetic data indicate that water from dairy-waste storage ponds was not present during sampled stormflows. This is consistent with the relatively dry conditions and the absence of large stormflows during the study.

Dissolved organic carbon (DOC) concentrations in stormflow ranged from 3 to 15.3 mg/L. In general, concentrations increased during stormflow and were distributed across the stormflow hydrograph in a manner similar to that of fecal indicator bacteria. DOC concentrations typically remained high for several days after flow returned to pre-storm conditions. Ultraviolet absorbance, excitation emission spectroscopy, and sequential fractionation of DOC using XAD-8 and XAD-4 resins showed that the composition of DOC changed rapidly during stormflow. Hydrophobic and hydrophilic acids were the largest fraction of DOC composing between 27 and 45 percent and between 24 and 37 percent of the DOC, respectively.

The fraction of DOC composed of hydrophobic acids decreased due to urban runoff and increased during the recession of the first stormflow of the rainy season; the hydrophilic-acid fraction generally decreased throughout the stormflow hydrograph; the transhydrophilic-acid fraction did not vary greatly during stormflow; and the hydrophobic-neutral fraction increased from low values in base flow to almost 30 percent of the DOC after more soluble and more mobile hydrophobic and hydrophilic acids were washed from urban areas. Comparison of ultraviolet absorbance data with data collected during previous studies shows that the optical properties and, presumably, the composition of the DOC were different in this study than DOC collected during wetter periods.

Samples of shallow ground water collected during stormflow from beneath recharge facilities along the Santa Ana River show that most fecal indicator bacteria and DOC were removed as water infiltrated into the streambed.

2 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Introduction

The Santa Ana River drains about 2,670 square miles (mi²) of the densely populated coastal area of southern California south of Los Angeles (fig. 1). Base flow in the river is maintained largely by the discharge of treated municipal wastewater from upstream wastewater-treatment plants (Burton and others, 1998). Almost all the flow in the Santa Ana River, more than 200,000 acre-feet (acre-ft) of water annually, is diverted to ponds where it infiltrates and recharges aquifers underlying Orange County. Pumpage from these aquifers is the primary source of water supply for about 2 million people. Although wastewater discharged to the river is highly treated, there may be public health issues associated with the long-term use of water from the Santa Ana River for ground-water recharge to aquifers pumped for public supply.

Stormflow has long been considered a source of high-quality water, suitable for dilution of water recharged from the river during base flow. Recent work (Izbicki and others, 2000) has shown that stormflow in the Santa Ana River may contain increased concentrations of pesticides, trace elements, and other contaminants—although these contaminants do not typically exceed respective public health goals. Stormflow in the Santa Ana River also may include runoff from urban and agricultural areas that may contain high concentrations of microbiological organisms that can degrade water quality.

Purpose and Scope

The purpose of this study was to measure changes in the concentration of total coliforms and fecal indicator bacteria in the Santa Ana River during stormflow. Measured changes were related to changes in selected molecular indicators of microbiological organisms, such as phospholipid fatty acid (PLFA) and the profile obtained from analysis of deoxyribonucleic acid (DNA), to determine if sources having greatly different microbial populations were contributing to stormflow during different parts of the hydrograph. In addition, changes in the concentration and composition of dissolved organic carbon (DOC) were measured due to (1) the possible association of organic carbon with fecal material, (2) health concerns associated with carbon of wastewater origin in water used to recharge aquifers pumped for public supply, and (3) concerns about the reactivity of the DOC and potential for disinfection by-product formation when recharged water is extracted and chlorinated for public supply.

This study was done by the U. S. Geological Survey (USGS) in cooperation with the Orange County Water District

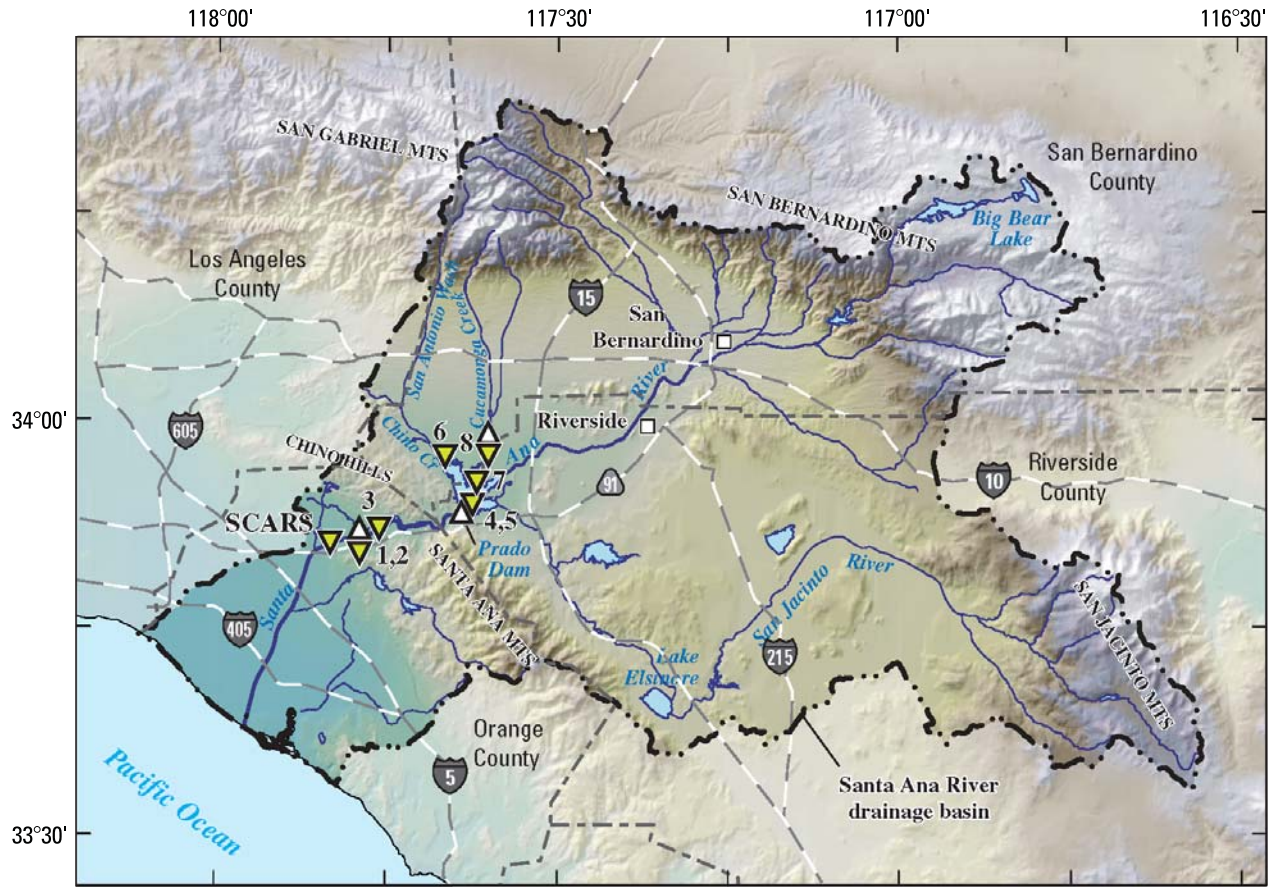
(OCWD) as part of the Santa Ana River Water Quality and Health (SARWQH) study. The purpose of the SARWQH study was to evaluate the use of the Santa Ana River as the primary source of recharge for aquifers underlying Orange County, California.

Previous Studies

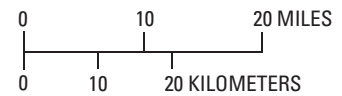
Contamination of surface water and ground water by fecal bacteria is a public health concern due to the potential for transmission of disease through contact or ingestion. Urban areas are especially sensitive to microbial contamination because, during stormflow, water and fecal material may run directly into streams from city streets and sewers without retention and filtration by soils. Concern over microbial contamination of surface water is especially high in southern California where runoff from large urban areas drains directly to the ocean (Schiff and Stevenson, 1996; Nobel and others, 2000; Leecaster and Weisberg, 2001; Schiff and Kinney, 2001; Schiff and others, 2001). Recent closure of recreational beaches due to bacterial contamination has resulted in economic losses and concern about the quality of runoff from urban areas.

The chemical and microbiological quality of surface water in southern California may be of greater concern in areas where surface water is used directly for public supply or to recharge aquifers pumped for public supply. These issues have been addressed as part of the Santa Ana River Water Quality and Health study (SARWQH study) by Orange County Water District. Historical changes in water quality in the Santa Ana River were studied by Burton and others (1998). The chemical quality of stormflow, with respect to pesticides, selected trace elements, and dissolved organic carbon, was studied by Izbicki and others (2000). Although microbial data have been collected from the Santa Ana River by the Orange County Health Department from 1986 to 1994 (Orange County Health Department, written commun., 1999), the rapid, short-term changes in microbial quality resulting from stormflow runoff in the Santa Ana River previously have not been studied.

To address potential public health effects, the chemical and microbiological quality of water recharged from the Santa Ana River to aquifers underlying Orange County that are used for public supply also has been studied as part of the SARWQH study. Similar studies have been done in parts of Los Angeles County (Leenheer and others, 2001; Schroeder and Anders, 2002) and other areas where treated wastewater is used to recharge aquifers pumped for public supply.



Base from U.S. Geological Survey digital elevation data, 1999
Albers Equal-Area Conic Projection



EXPLANATION

— · — Basin boundary

- - - County line

△ Stream gage

▽ Sample-collection site—

Number corresponds to sample location in text

- 1 Santa Ana River at the diversion downstream from Imperial Highway (11075620)
- 2 Stormdrain tributary to Santa Ana River at Imperial Highway and Santa Ana River at Imperial Highway (11075600)
- 3 Santa Ana River streambed upstream from Imperial Highway
- 4 Santa Ana River upstream from Prado Dam
- 5 Prado wetland soil
- 6 Dairy waste pond near Pine Avenue
- 7 Mill Creek at Chino-Corona Road
- 8 Cucamonga Creek near Mira Loma

SCARS Subsurface collection and recharge system



Figure 1. Santa Ana River Basin, southern California, and location of selected stream gages and sample-collection sites.

4 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Public health issues related to the use of water from the Santa Ana River to recharge aquifers pumped for public supply are complex. Part of the SARWQH study focused on travel time from recharge to wells (Davisson and others, 1996, Gamlin and others, 2001; Herndon and others, 2003) and the percentage of wastewater in water extracted by the wells. Other parts of the SARWQH study focused on the concentration and composition of carbon of wastewater origin (Reinhard and others, 1996; Ding and others, 1999) in water extracted by public supply wells. The concentration and composition of DOC in water used to recharge aquifers are of additional concern in the Santa Ana River Basin and in other areas because of the potential for DOC to form disinfection by-products, such as trihalomethanes (THMs) or haloacetic acids (HAA), during chlorination (Reckhow and others, 1990; California Department of Water Resources, 1994, Krazner and others, 1996; Fujii and others, 1998; Rostad, 2002, Rostad and others, 2000).

In addition to the SARWQH study, the Santa Ana River is one of the USGS National Water-Quality Assessment (NAWQA) study units, and ground-water and surface-water quality within the basin are studied as part of that ongoing program (Belitz, 1999).

Description of the Santa Ana River Basin

Streams of the Santa Ana River drainage basin, which are part of the Santa Ana River hydrologic unit, discharge to the west into the Pacific Ocean. The Santa Ana River hydrologic unit is approximately 2,670 mi² and encompasses parts of San Bernardino, Riverside, Los Angeles, and Orange Counties (fig. 1). The study area topography ranges from steep, rugged mountains with peaks as high as 11,500 ft above sea level, to broad alluvial valleys and a coastal plain. Warm, dry summers and cool, moist winters characterize the climate of the study area, and average annual precipitation ranges from about 12 inches (in.) near the coast to about 18 in. in the inland valleys. Annual precipitation totals can exceed 40 in. in some of the higher mountains. Most precipitation occurs during the “winter” rainy season between November and March.

The Santa Ana River Basin has undergone major development in recent years, and has experienced a rapid increase in population. In 1990, the population was greater than 4.5 million (Santa Ana Regional Water Quality Control Board, 1995). Land use in the area ranges from dense urban development in the coastal plain and inland valleys to undeveloped wilderness in the high mountains (fig. 2). In 1995, approximately 340 animal-confinement facilities having over 340,000 animals, mostly dairy cows, operated within the Santa Ana River Basin (Santa Ana Regional Water Quality Control Board, 1995). Most of these are in the area just north of Prado Dam that is drained by Chino and Cucamonga creeks (fig. 1).

The Santa Ana River Basin can be divided into an upper and a lower basin at Prado Dam (fig. 1). Prado Dam is operated according to a complex set of procedures intended to minimize flood damage in the lower part of the basin and to maximize the availability of surface water for ground-water recharge (U.S. Army Corps of Engineers, 1994). To minimize adverse effects on endangered species in the riparian habitat upstream from Prado Dam, water is not stored behind the dam during much of the dry season (U.S. Army Corps of Engineers, 1994). Storage of water behind Prado Dam during the wet season typically includes temporary flooding of the riparian habitat upstream from the dam after stormflows until water can be released downstream for ground-water recharge. In wet years, the upstream area may be flooded throughout most of the rainy season and into the dry season as late as June. In dry years, water may be stored only briefly behind the dam after stormflow. When water is stored behind the dam, releases may be increased prior to large stormflows to increase available storage for flood control. Releases are frequently decreased while runoff is occurring downstream from the dam to minimize damage to urban areas from flooding and to maximize storage of stormflow for subsequent release for ground-water recharge.

Local water is extensively managed for public supply, and additional water is imported into the study area from northern California and the Colorado River. Most of the water used for public supply upstream from Prado Dam is discharged to the Santa Ana River, its tributaries, or adjacent shallow ground water as treated municipal wastewater. Nearly half of the base flow of the Santa Ana River is treated to remove nitrate by a series of artificial wetlands upstream from Prado Dam, and nearly all of the flow (including stormflow) in the river, more than 200,000 acre-ft annually, is diverted near Imperial Highway by the Orange County Water District to recharge underlying aquifers. Only water from larger stormflows that exceed the capacity of the diversion facility is not diverted for recharge.

Acknowledgments

This study, by the U.S. Geological Survey, was funded by the Orange County Water District, Fountain Valley, Calif., as part of their Santa Ana River Water Quality and Health study (SARWQH). The authors thank the U.S. Army Corps of Engineers for precipitation and stage data for Prado Dam; John Vandenberg of the Orange County Water District for data on surface flow at the diversion downstream from Imperial Highway; Minoo Ghajar, of the Orange County Public Health Department, for the analysis of total coliforms and fecal indicator bacteria. The authors also thank Katherine Patel, Mike Weihner, Gregory Woodside, and Nira Yamachika of the Orange County Water District, and the scientific review panel of the SARWQH study for sharing their expertise and for their input throughout this study.

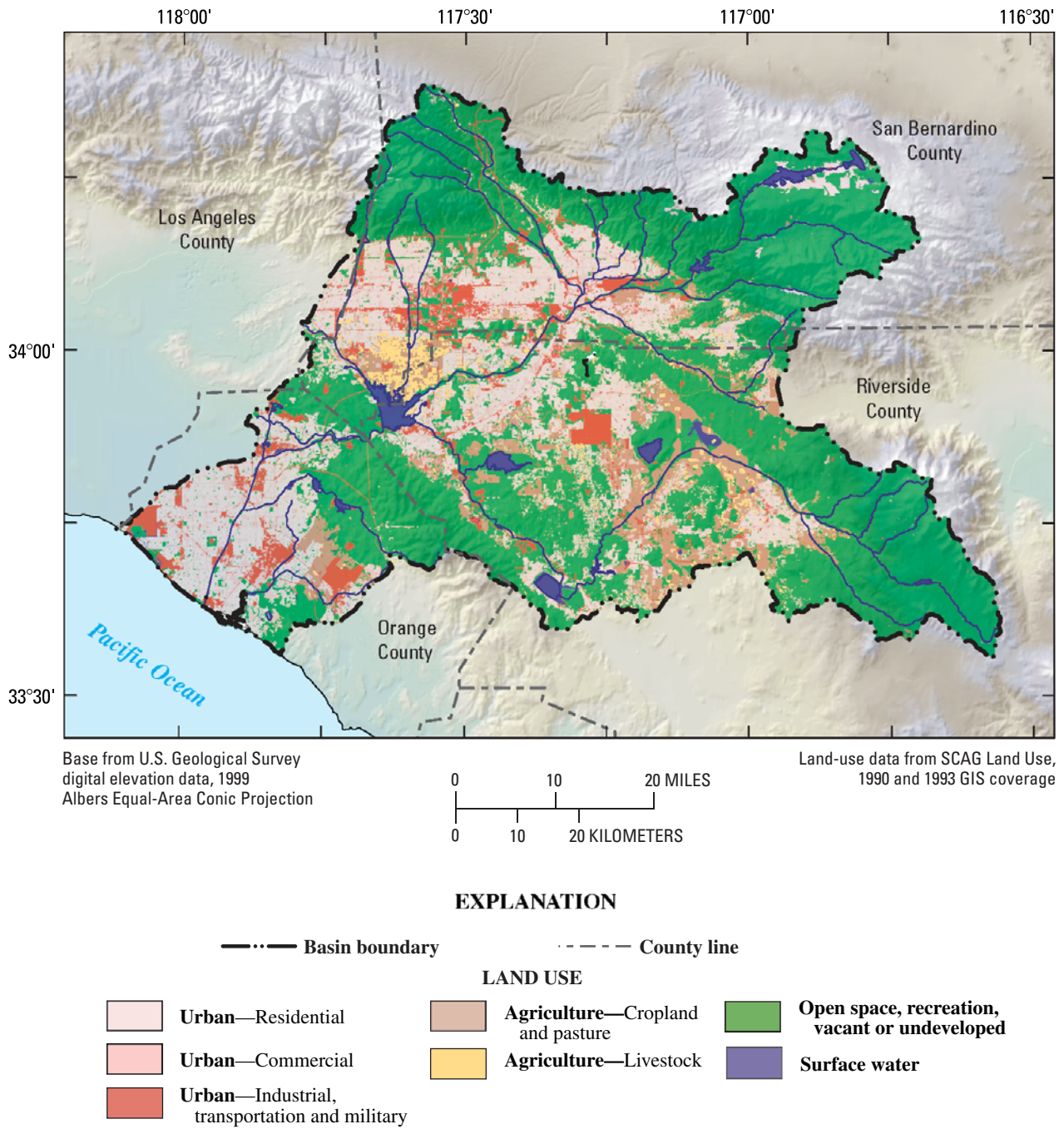


Figure 2. Land use in the Santa Ana River Basin, southern California, 1990.

Approach

Eight stormflows were sampled on the Santa Ana River between January 25, 2000, and March 8, 2002. Discrete samples were collected to characterize conditions in the river prior to stormflow, on the rising limb of the stormflow hydrograph, near the peak flow, and during the recession of the stormflow hydrograph. Bacteria samples were collected from the bridge at Imperial Highway using depth-integrated samplers. Equipment used for the collection and handling of bacteria samples was sterilized in an autoclave prior to use and sterile procedures were used to ensure that samples intended for microbial analysis were not contaminated during collection and handling. Additional samples were collected more frequently from automated samplers at the diversion point about one-quarter of a mile downstream from the bridge at Imperial Highway. The automated samplers were not used for microbiological samples because sterile collection and handling of samples was not possible using these samplers; however, samples from the automated samplers were analyzed for nonmicrobial and less expensive parameters, such as field parameters, some chemical constituents, or optical property data such as ultraviolet absorbance. Data collected from the automated samplers helped verify that large changes in the chemical composition of stormflow did not occur between sample-collection intervals at the bridge farther upstream. All samples were processed in the field at the time of collection and shipped to laboratories for analysis within 24 hours of sample collection.

Concentrations of total coliforms and fecal indicator bacteria—including fecal coliforms, *Escherichia coli* (*E. coli*), and enterococci—were evaluated across the stormflow hydrograph. Phospholipid fatty acid (PLFA) and genetic data (deoxyribonucleic acid, DNA) were used to determine if sources having greatly different microbial populations were contributing to stormflow runoff during different parts of the hydrograph. PLFA and genetic data were not used to identify specific organisms that may, or may not, have been present in a sample; instead, bacterial concentrations were compared with changes in PLFA, genetic profile, and dissolved organic carbon (DOC) concentrations to determine if there was an association between bacteria and DOC. Changes in DOC composition during stormflow were evaluated on the basis of optical properties, such as ultraviolet absorption and excitation-emission spectroscopy, and selected operationally-defined fractions. Changes in DOC composition also are important owing to concern about carbon of wastewater origin in water recharged to aquifers and the potential of the DOC to form disinfection by-products if chlorinated prior to distribution for public supply.

Sample pH, specific conductance, and alkalinity were measured in the field. Samples for total coliforms and fecal

indicator bacteria were placed in coolers and chilled and delivered to the Orange County Public Health Laboratory within 24 hours for analysis. Samples for DOC, and optical property analysis were field filtered through baked glass-fiber filters, placed in coolers, chilled, and shipped overnight delivery to a USGS Laboratory in Sacramento within 24 hours of collection for analysis. Samples for PLFA analysis were placed in coolers, chilled, and shipped overnight delivery to a commercial laboratory within 24 hours of collection for analysis. Samples for Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis were placed in coolers, chilled, and delivered to Orange County Water District laboratories within 24 hours of collection for analysis.

Sampled Stormflows

Eight stormflows were sampled at Imperial Highway during the 1999–2000, 2000–01, and 2001–02 rainy seasons ([fig. 3](#)). The data-collection period was drier than average and the 2001–02 rainy season was the driest rainy season on record (Los Angeles Times, 2002). The small amount of precipitation that did occur was typically of short duration, and not widely distributed across the basin. As a result, stormflows were generally smaller in magnitude and shorter in duration than were stormflows sampled between 1995 and 1998 by Izbicki and others (2000). Precipitation and flow statistics associated with sampled stormflows are summarized in [table 1](#).

Sample collection included the first stormflow of each rainy season. The first stormflow in the 1999–2000 and 2000–01 rainy seasons was later than usual and occurred in January. Sample collection also included late-season stormflows that occurred under a wide range of antecedent conditions—such as the February 11–15, 2001, stormflow that was preceded by relatively dry conditions that allowed highly mobile or soluble material to accumulate in the basin; or the March 5–7, 2001, stormflow that was preceded by a series of larger storms that washed highly mobile or soluble material from the basin. Although detention ponds intended to control runoff from dairies in the basin were filled to near capacity as a result of that series of storms, widespread failure of detention ponds was not reported during the 2000–01 rainy season.

During this study, only small amounts of water were stored behind Prado Dam after stormflow for later release to the river and the diversion at Imperial Highway farther downstream. As a result, with the exception of the March 5–7, 2001, stormflow, most stormflows sampled as part of this study were discrete events and water was not greatly altered by mixing with water stored from previous stormflows or by leaching of organic material from the flooded riparian habitat behind the dam.

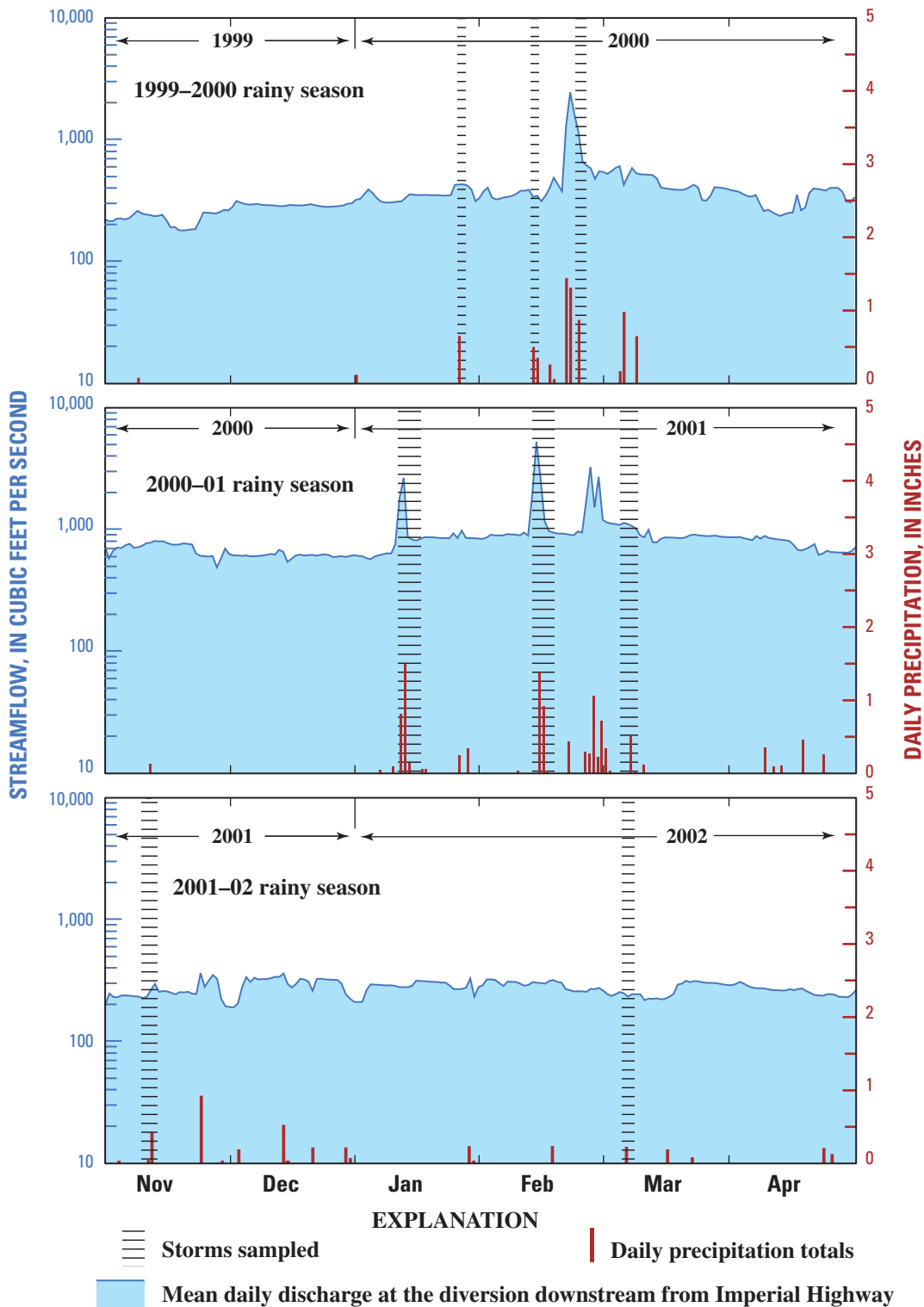


Figure 3. Sampled stormflows, precipitation, and stream discharge for the Santa Ana River at Imperial Highway, southern California, during the 1999–2000, 2000–01, and 2001–02 rainy seasons.

8 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Table 1. Summary of stormflow sample collection in the Santa Ana River at Imperial Highway, southern California, 1999–2000, 2000–01, 2001–02 rainy seasons

[Data provided by Orange County Public Health Department; in., inches; ft³/s, cubic feet per second; >, greater than]

Sample collection dates	Rainfall at Prado Dam (in.)	Peak flow at Imperial Highway (ft ³ /s)	Peak flow below Prado Dam (ft ³ /s)	Comments
01/25/00 to 01/26/00	0.62	569	401	First stormflow of rainy season
02/12/00 to 02/13/00	.79	644	307	
02/22/00 to 02/27/00	2.04	>900 ¹	3,900	Preceded by a large storm
01/10/01 to 01/14/01	2.38	4,111	4,100	First stormflow of rainy season
02/11/01 to 02/15/01	2.27	5,400	5,800	Preceded by dry conditions
03/05/01 to 03/07/01	.5	618	505	Preceded by a series of larger storms
11/12/01 to 11/14/01	.43	399	372	First stormflow of rainy season
03/06/02 to 03/08/02	.25	256	295	

¹Accurate measurement of flow was not possible because of mechanical problems at the diversion at Imperial Highway

Analytical Methods

Total coliforms, fecal coliforms, *Escherichia coli* (*E. coli*), and enterococci were analyzed within 24 hours of collection by the Orange County Public Health Laboratory, Anaheim, California. During the 1999–2000 and 2000–01 rainy seasons, samples were analyzed at dilutions ranging from 1:10 to 1:10,000 using a combination of three procedures: (1) multi-tube fermentation (MTF) for total coliforms, (2) Colilert-18 with Quanti-tray 2000 (IDEXX Laboratories Inc., Westbrook, Maine) for fecal coliforms and *E. coli*, and (3) Enterolert (IDEXX Laboratories Inc., Westbrook, Maine) for enterococci. Results are Most Probable Number per 100 milliliters (MPN/100ml). Because *E. coli* numbers were greater than fecal coliform bacteria numbers, the analytical methods were changed after the 2000–01 rainy season. During the 2001–02 rainy season, samples were analyzed at similar dilutions using m-Endo LES agar for total coliforms (American Public Health Association, 1998 9222 B), M-FC agar for fecal coliforms (American Public Health Association, 1998), modified m-TEC agar for *E. coli* (U.S. Environmental Protection Agency, 2000), and m-EI agar for enterococci (American Public Health Association, 1998). Results for the 2001–02 rainy season are reported as colonies per 100 mL. Field blanks showed no evidence of contamination from sample collection equipment.

Phospholipid fatty acid analyses were done by Microbial Insights, Rockford, Tenn. Analysis for fatty acid methyl esters was done using standard protocol (Microbial ID Inc., 1992; Cavigelli and others, 1995). Lipids were saponified using 3.25N NaOH in methanol, heated, and then methylated using 3.25N HCl in methanol and extracted in a mixture of methyl-tert-butyl ether (MTBE) and hexane. After extraction and separation the organic phase was washed using dilute NaOH and the methylated fatty acids were analyzed by gas chromatography (Microbial ID Inc., 1992; Cavigelli and others, 1995). Blank samples collected from the automated samplers during the 1999–2000 rainy season showed small amounts of contamination from the automated sampling equipment. PLFA samples collected during the 2001–02 rainy season were collected from the bridge upstream of Imperial Highway using depth-dependent sample collection equipment. No evidence of contamination was observed in these samples.

Genetic profiles of the microbial communities from stormflow samples were done using a combination of PCR (polymerase chain reaction) and Terminal Restriction Fragment Length Polymorphism (T-RFLP) by Orange County Water District. Bacterial cells suspended in a 3-liter water

sample were separated from particulate material by adding a 1 percent solution of Na₂HPO₄ and shaking the sample for 15 minutes. Debris was allowed to settle for about 30 minutes, following which 500 ml of the sample was removed and bacterial cells were concentrated by centrifugation at 8,000 rpm for 60 minutes. Total deoxyribonucleic acid (DNA) from the resulting bacterial pellet was extracted using the UltraClean Soil DNA isolation kit (MoBio Laboratories Inc., Solana Beach, California), as specified by the manufacturer, and was stored at –20°C for further analysis. To obtain a genetic profile from as many different species as possible, PCR was used to amplify a portion of the 16S ribosomal ribonucleic (rRNA) gene. “Universal” PCR primers (Avaniss-Aghajani, and others 1996; Brunk and others, 1996) were used to amplify the hypervariable region of the 16S rRNA that results in amplicons (amplified PCR products) that range from 480 to 500 base pairs (bp) in size. The forward “Universal” primer was synthesized with fluorescent 5-hexachlorofluorescein (Hex) labeled at the 5' end (IDT, Coraville, Iowa). Amplification was done using a GeneAmp PCR system 9600 (Applied Biosystems, ABI, Foster City, California) with the following concentration of PCR components in a final volume of 100 µL: 1X PCR buffer with 1.5 µM MgCl₂ (ABI), 200 µM each deoxynucleotide triphosphate (dNTP, ABI), 0.2 µM of each primer, 2.5 units of AmpliTaq Polymerase (ABI) and approximately 2–4 ng of total DNA. The cycling protocol used 95°C for 11 minutes for initial denaturation, 35 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 3 minutes, and a final extension of 72°C for 10 minutes. Approximately 10–30 ng of PCR amplicons derived from each sample were digested using 10Units of *DdeI* restriction endonuclease (New England Biolabs, Beverly, Massachusetts) in a total volume of 20 µL for 1 hour at 37°C to obtain a T-RFLP pattern of the different species present in the sample. The size of the amplicons was determined by capillary electrophoresis using a 310 Genetic Analyzer (ABI) as follows: 2 µL of the restricted amplicons were mixed with 12.5 µL of a mixture of deionized formamide and Tamara 500 internal size standard (ABI). The mixture was then denatured at 94°C for 5 minutes and immediately chilled on ice prior to electrophoresis using the Genescan mode (ABI). The size of the restricted amplicons were determined by comparison with Tamara internal size standard using the Local Southern method in the Genescan software, version 2.1 (ABI). Only the 5' end of the restricted amplicons are fluorescently labeled: therefore, digestion of the amplicons with restriction enzymes results in only one band even if multiple cut sites exist. The sensitivity of this method is limited to about one base pair.

10 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Dissolved organic carbon and optical-property measurements were done by the USGS, Sacramento, California (Bird and others, 2003). Dissolved organic carbon was measured on filtered samples using a Shimadzu TOC-5000A organic carbon analyzer calibrated using potassium hydrogen phthalate standards prepared in organic-free water, with standard concentrations bracketing the concentration of the samples. Aliquots of sample (4.5 mL) were acidified using 30 μ L of 2N HCL and sparged using N₂ for 3 minutes to remove inorganic carbon as CO₂. The non-purgeable organic carbon (NPOC) was analyzed by direct injection of liquid sample into a high-temperature (680°C) combustion tube packed with a platinum catalyst, and the CO₂ produced was detected using a nondispersive infrared photometric cell. Each analysis represents the mean of three injections, standard deviation of the three injections ranged from 0.002 to 0.145 mg/L. Precision of the technique is reported to be between 0.3 and 2.9 percent of the total concentration (Bird and others, 2003).

Optical measurements made as part of this study included UV Absorbance and excitation/emission spectroscopy (EEM). Samples were gravity filtered to reduce DOC contamination by lysing algae or bacterial cells during mechanical filtration. UV measurements were done from 190 to 310 nm using a PE Lambda 3B UV/VIS spectrophotometer having a 1-centimeter path-length cell. EEM measurements were done by irradiating the sample across 255 to 600 nm range of wavelengths and simultaneously recording the emitted fluorescence across 250 to 700 nm range of wavelengths. EEM measurements were done using a SPEX Fluorimax-3 excitation/emission fluorometer.

Separation of dissolved organic carbon (DOC) into operationally defined fractions and measurements on those fractions was done by George Aiken, USGS, Boulder, Colorado. Operationally defined fractions of DOC were defined by their ability to pass-through or sorb on a column of XAD-8 or XAD-4 resins under different pHs (Aiken and others, 1992). The fractions measured include hydrophobic acids, hydrophilic acids, transhydrophilic acids, and the hydrophobic-neutral fractions of the DOC. Hydrophobic acids are defined as that portion of DOC that sorbs on a column of XAD-8 resin at pH 2 and is eluted at pH 13. Hydrophilic acids are defined as that portion of DOC that passes-through columns of XAD-8 and XAD-4 resins at pH 2. Transhydrophilic acids are defined as that portion of DOC that does not sorb on XAD-8 resin at pH 2, sorbs on XAD-4 resin at pH 2, and is eluted from the XAD-4 resin using NaOH at pH 13. The hydrophobic-neutral fraction is defined as the fraction of DOC that sorbs and cannot be eluted from XAD-8 and XAD-4 resin at pH 13. Once separated, the DOC and optical properties of the hydrophobic, hydrophilic, and transhydrophobic acid fractions were measured using analytical techniques similar to those described in the previous paragraph. Because the hydrophobic neutral fraction cannot be eluted, its concentration was calculated as the difference

between the total DOC concentration and the DOC concentrations of the eluted fractions at pH 2 (hydrophobic acids) and pH 11 (hydrophilic acids plus transhydrophilic acids). Because small amounts of DOC may be contributed from the XAD-4 resins during the fractionation procedure, at low DOC concentrations the four fractions do not necessarily sum to 100 percent.

Total Coliforms and Fecal Indicator Bacteria

Historical Data

Total and fecal coliform bacteria concentrations at Imperial Highway measured on a weekly to monthly basis by Orange County Public Health Department from January 1986 to March 1994 provide a baseline to evaluate stormflow data collected as part of this study. During that period, total coliform bacteria concentrations ranged from 130 colonies per 100 mL to greater than the maximum quantification level of 16,000 colonies per 100 mL. Fecal coliform bacteria concentrations ranged from 20 colonies per 100 mL to greater than the maximum quantification level of 16,000 colonies per 100 mL (table 2). High concentrations of total and fecal coliform bacteria were measured in both the dry season and the rainy season, and concentrations in excess of the maximum quantification level occurred in all months of the year and were not well correlated with streamflow.

Despite variability in individual samples, monthly historical median total and fecal coliform bacteria concentrations were higher toward the end of the dry season and decreased during the rainy season (fig. 4). The reason for high median values during the dry season is not clear. Flow in the Santa Ana River during the dry season is maintained by discharge of highly treated municipal wastewater having low bacteria concentrations. There is scant surface runoff during the dry season and few discharges from other sources. It may be possible that total coliform and fecal coliform bacteria survive and grow in the shallow warm water or bed of the Santa Ana River and its tributary streams during this time. Growth of fecal coliform bacteria has been observed in environmental settings (Francy and others, 1996) and may be a special concern in shallow, concrete-lined channels tributary to the Santa Ana River. Some of these channels have year-round flow and are highly productive, supporting dense algal growth during the latter part of the dry season. Improperly treated wastewater, small amounts of urban runoff associated with lawn watering and other uses, windblown material from agricultural areas, or fecal material from other sources could inoculate these channels with fecal bacteria and produce elevated concentrations of fecal bacteria in the Santa Ana River farther downstream.

Table 2. Summary of historical bacteria concentrations and bacteria concentrations measured during pre-stormflow and stormflow conditions in the Santa Ana River at Imperial Highway, southern California

[Historical data collected and analyzed by Orange County Public Health Laboratory; data from this study collected by U.S. Geological Survey and analyzed by Orange County Public Health Laboratory; shallow ground water collected from Orange County Water District subsurface-collection and recharge system (SCARS) about 4 feet below the bed of the off-channel recharge facility along the Santa Ana River about 1 mile downstream from Imperial Highway; bacteria results reported as colonies per 100 mL; ft³/s, cubic feet per second; <, less than; >, greater than; —, no data]

		Historical data			Data from this study		
		January 1986 to March 1994			January 2000 to March 2002		
		Dry season (April–September)	Rainy season (October–March)		Pre- stormflow	Stormflow, including recessional flow	Shallow ground water
<200 ft ³ /s	<200 ft ³ /s	>200 ft ³ /s					
Flow	Minimum	44	52	200	231	224	—
	Median	169	173	280	357	491	—
	Maximum	446	199	4,500	537	5,400	—
Total Coliforms	Minimum	170	300	130	1,100	200	<10
	Median	9,000	9,000	5,000	2,650	25,000	50
	Maximum	>16000	>16000	>16000	8,000	3,400,000	130
Fecal Coliforms	Minimum	<20	80	<20	<10	25	<10
	Median	600	700	500	25	4,000	<10
	Maximum	>16000	>16000	>16000	1,700	310,000	10
<i>Escherichia coli</i>	Minimum	—	—	—	100	40	<10
	Median	—	—	—	210	3,080	<10
	Maximum	—	—	—	700	84,000	<10
Enterococci	Minimum	—	—	—	120	<10	<10
	Median	—	—	—	120	5,800	10
	Maximum	—	—	—	940	100,000	20

12 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

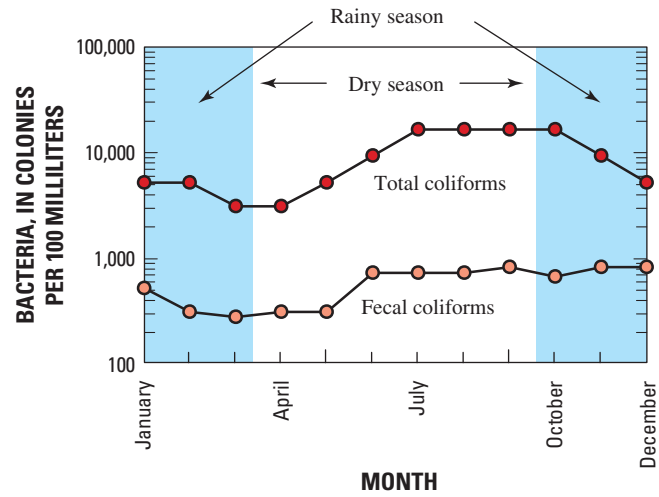


Figure 4. Monthly median total and fecal coliform bacteria concentrations in the Santa Ana River at Imperial Highway, southern California, January 1986 to March 1994. (Data provided by the Orange County Public Health Department.)

Historical data collected across a wide range of flows suggest that total and fecal coliform bacteria concentrations were lower during the rainy season. During the rainy season the median total and fecal coliform bacteria concentrations during low flows (less than 200 ft³/s) were slightly higher than concentrations measured during higher flows (table 2). However, available historical data had an upper quantification limit; as a result, concentrations greater than 16,000 colonies per 100 mL were not evaluated and maximum concentrations are not known. In addition, samples were collected at weekly or greater time intervals that do not allow for the evaluation of short-term changes in water quality that occur during stormflow. Short-term changes in microbial concentrations during stormflow were addressed as part of this study.

Total Coliforms and Fecal Indicator Bacteria Concentrations during Stormflow

Total and fecal coliform bacteria concentrations in stormflow from the Santa Ana River at Imperial Highway were as high as 3,400,000 and 310,000 colonies per 100 mL, respectively. *E. coli* and enterococci concentrations were as high as 84,000 and 100,000 colonies per 100 mL, respectively (table 2, Appendix A). In general, total coliform and fecal indicator bacteria concentrations increased during stormflow, although decreases in total coliform and fecal indicator bacteria concentrations were recorded during the March 6–8, 2002, stormflow. Precipitation and subsequent runoff were small in magnitude during that stormflow, and streamflow actually decreased at Imperial Highway as a result of regulation at Prado Dam. In most stormflows, total coliforms, fecal coliforms, *E. coli*, and enterococci concentrations were well correlated. During the 2000–01 rainy season, *E. coli* concentrations exceeded fecal coliform bacteria concentrations in some samples—especially at higher concentrations. This should not occur because *E. coli* is one of many fecal coliform bacteria: as discussed previously, analytical methods were changed during the 2001–02 rainy season to correct this problem.

Total coliform and fecal indicator bacteria concentrations during the first stormflows of the winter rainy season were higher than concentrations during stormflows later in the season. Changes in bacterial concentrations during the first stormflows of the rainy season followed a similar pattern regardless of the magnitude of the stormflow (fig. 5). In general, total coliform and fecal indicator bacterial concentrations increased rapidly as runoff from urban areas downstream from Prado Dam contributed to stormflow, and

concentrations then declined slightly after bacteria were washed from urban streets and drains. After a period of several hours, bacterial concentrations increased to their highest values as parts of the basin upstream from Prado Dam contributed to stormflow. The basin is extensively urbanized upstream from Prado Dam, but it is unlikely that runoff from urban areas upstream from the dam can account for total coliform and fecal indicator bacteria concentrations that are as much as an order of magnitude greater than concentrations measured during runoff from urban areas downstream from the dam (fig. 5). Other sources of bacterial contamination also must be present. The basin also contains numerous dairies in the Chino and Cucamonga Creek areas, as well as other land uses, that may contribute to increased bacteria concentrations during stormflow. Data collected as part of this study also show that total coliform and fecal indicator bacteria concentrations in the Santa Ana River at Imperial Highway remained high even after flow in the river had returned to pre-storm levels.

The highest bacteria concentrations were measured November 12–14, 2001, during the first stormflow of the 2001–02 rainy season; although precipitation was only about 0.5 in., it was very intense and most of the water fell during a brief interval. Orange County Water District personnel sampling downstream from dairy operations—on Mill Creek at Chino-Corona Road, a tributary to the Santa Ana River about 11 mi upstream from Imperial Highway—recorded total coliform bacteria concentrations as high as 7,300,000 colonies per 100 mL during this stormflow (fig. 6). Fecal coliform bacteria, *E. coli*, and enterococci concentrations were similarly elevated, having concentrations of 530,000, 90,000, and 1,210,000 colonies per 100 mL, respectively. The bacteria concentrations at Imperial Highway were highest about 14 hours after the highest concentrations at the Mill Creek site. Samples were not collected along the main stem of the Santa Ana River during this stormflow, and contributions from urban areas along the main stem of the Santa Ana River during this stormflow are not known.

Total coliforms and fecal indicator bacteria concentrations in stormflows that occurred later in the rainy season were variable and did not follow a consistent pattern during stormflow. For example, bacteria concentrations and their distribution across the stormflow hydrograph during large stormflows preceded by extended dry periods, such as the February 11–15, 2001, stormflow, were similar to those measured during the first stormflow of the rainy season. In contrast, bacteria concentrations actually decreased during small late-season stormflows that were preceded by other stormflows that may have washed bacteria from the basin.

14 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

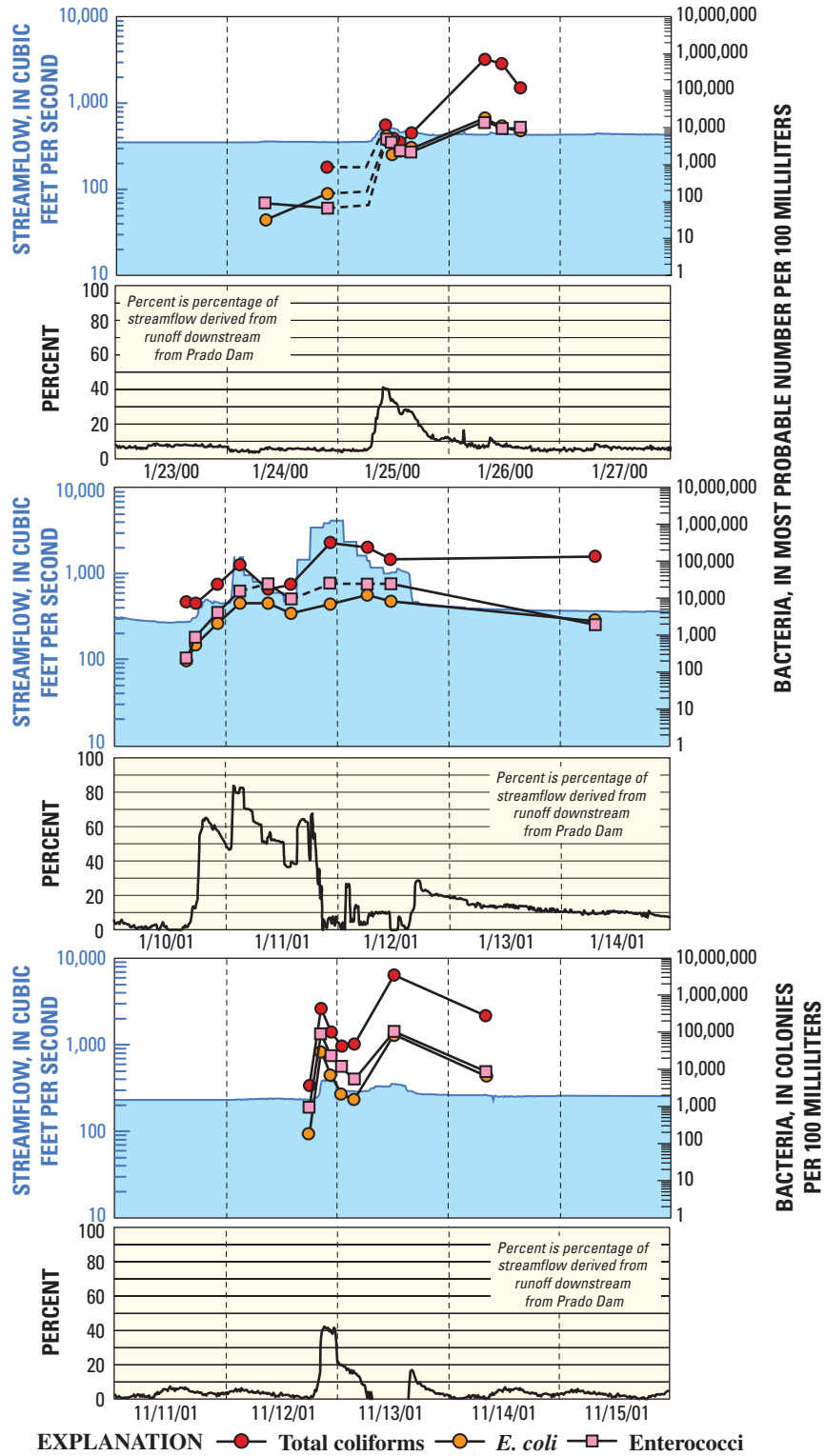


Figure 5. Total coliform and fecal indicator bacteria (*Escherichia coli* and enterococci) concentrations in the Santa Ana River at Imperial Highway, southern California, during the first stormflow of the 1999–2000, 2000–01, and 2001–02 rainy seasons.

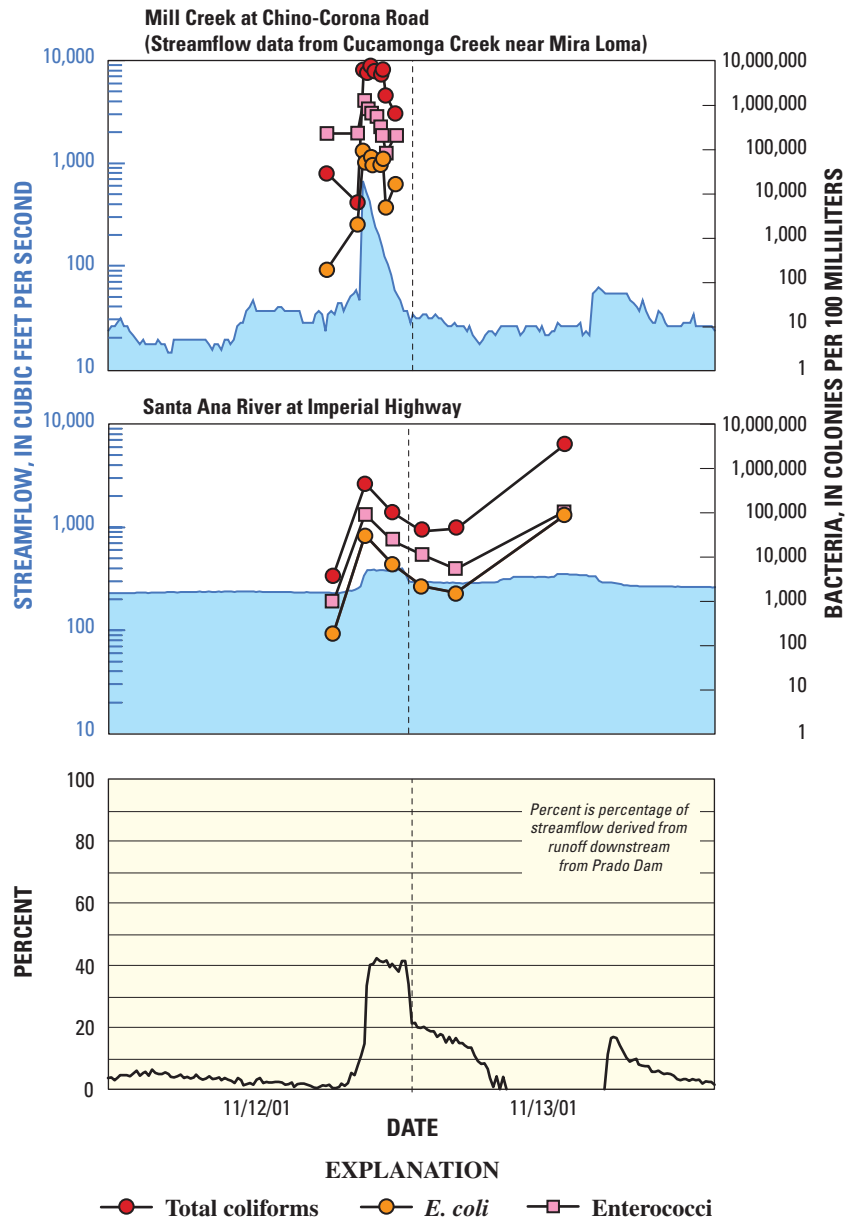


Figure 6. Total coliform and fecal indicator bacteria (*Escherichia coli* and enterococci) concentrations in Mill Creek at Chino-Corona Road, and in the Santa Ana River at Imperial Highway, southern California, November 12, 2001. (Samples collected by Orange County Water District; bacteria data from Orange County Health Department.)

16 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Several researchers have shown that fecal indicator bacteria may be present at large concentrations in streambed sediments (Von Donsel and Geldreich, 1971; Myers and others, 1998) and these bacteria may survive for extended periods in those sediments (Burton and others, 1987). Matson and others (1978) theorized a conceptual model of bacteria mobilization that includes storage of fecal bacteria in streambed sediments and scouring of sediment and associated bacteria as velocities increase during stormflow. Total coliforms and fecal indicator bacteria concentrations in the Santa Ana River at Imperial Highway increased with suspended-sediment concentrations, consistent with the model of Matson and others (1978), until suspended-sediment concentrations exceeded about 500 mg/L (fig. 7). The higher suspended-sediment concentrations occurred during the larger stormflows, and suspended sediment mobilized during these stormflows eventually exceeded the available bacteria. Total coliforms and fecal indicator bacteria concentrations were lower, per unit of suspended sediment, during the first stormflow of the rainy season (fig. 7) because suspended-sediment concentrations were higher during the first stormflow of the rainy season (fig. 8). The model of Matson and others (1978) is consistent with low bacteria concentrations measured during smaller stormflows when streamflow velocities are not large enough to re-suspend streambed sediments. The model is not consistent with high total coliforms and fecal indicator bacteria concentrations that persist in the Santa Ana River for several days after streamflow returned to pre-storm conditions. The data show that sediment disturbed and re-deposited after stormflow continues to contribute these bacteria to the river until a new equilibrium is established between the streambed and overlying water. This results suggests that the model initially proposed by Matson and others (1978) could be modified to include a period after the cessation of stormflow when recently disturbed sediment equilibrate with the overlying water. Because accumulation and survival of bacteria in streambed sediments was not measured as part of this study, data are not available to test the suitability of the modified model.

Phospholipid Fatty Acids

Fatty acids are components of all living cells. At the cellular level, they may be used for energy storage or they may be part of cellular organelles and structures where they participate in metabolic activity (Tunlid and White, 1992). Fatty acids that contain phosphorous, known as phospholipid

fatty acid (PLFA), are commonly found in cell walls and in the membranes of cellular organelles where they participate in activities such as nutrient uptake and waste removal. Specific PLFAs are associated with specific metabolic activities and may be indicators of the types of organisms present (Edlund and others, 1985; Dowling and others, 1986; White and others, 1996; Paul and Clark, 1996). Because PLFAs contain phosphorous, they are rapidly degraded in the environment and are typically associated with living (or recently living) organisms (White and others, 1979; White, 1994). Because PLFAs can be measured at very low concentrations (in the picomole range) they can be used to identify changes in microbial populations in environmental settings (Vestal and White, 1989). Total PLFA concentrations also are an indicator of total microbial biomass. However, concentrations do not necessarily relate directly to other measures of cellular populations, such as most probable number or colonies per 100 mL, used to quantify fecal indicator bacteria because microorganisms differ greatly in size and large organisms may contain larger quantities of PLFAs than smaller microorganisms.

Fatty acids have a wide range of compositions, and a shorthand notation has been developed to identify specific fatty acids (Paul and Clark, 1996). This notation follows the form

$$X:Y\omega Z,$$

where

- X is the number of carbon atoms,
- Y is the number of carbon-carbon double bonds,
- ω is the end of the molecule, and
- Z is the number of carbon atoms between the terminal double bond and the methyl end of the molecule.

For unsaturated fatty acids, the stereochemistry of the carbon chain is described by the suffixes *c*, cis (this is the most common form, and the suffix is usually omitted from the shorthand notation), or *t*, trans. For fatty acids having branched carbon chains, *i* describes isomethyl branching (second C from the methyl end), *a* describes anteisomethyl branching (third from the methyl end); and *Me*, follows position of the methyl branching. The prefix *cy*, identifies a cyclopropane ring within the carbon chain. Fatty acids can be categorized into common structural groups (table 3). The groups are useful to categorize the wide diversity of fatty acids and describe changes that are occurring within the fatty acid assemblages.

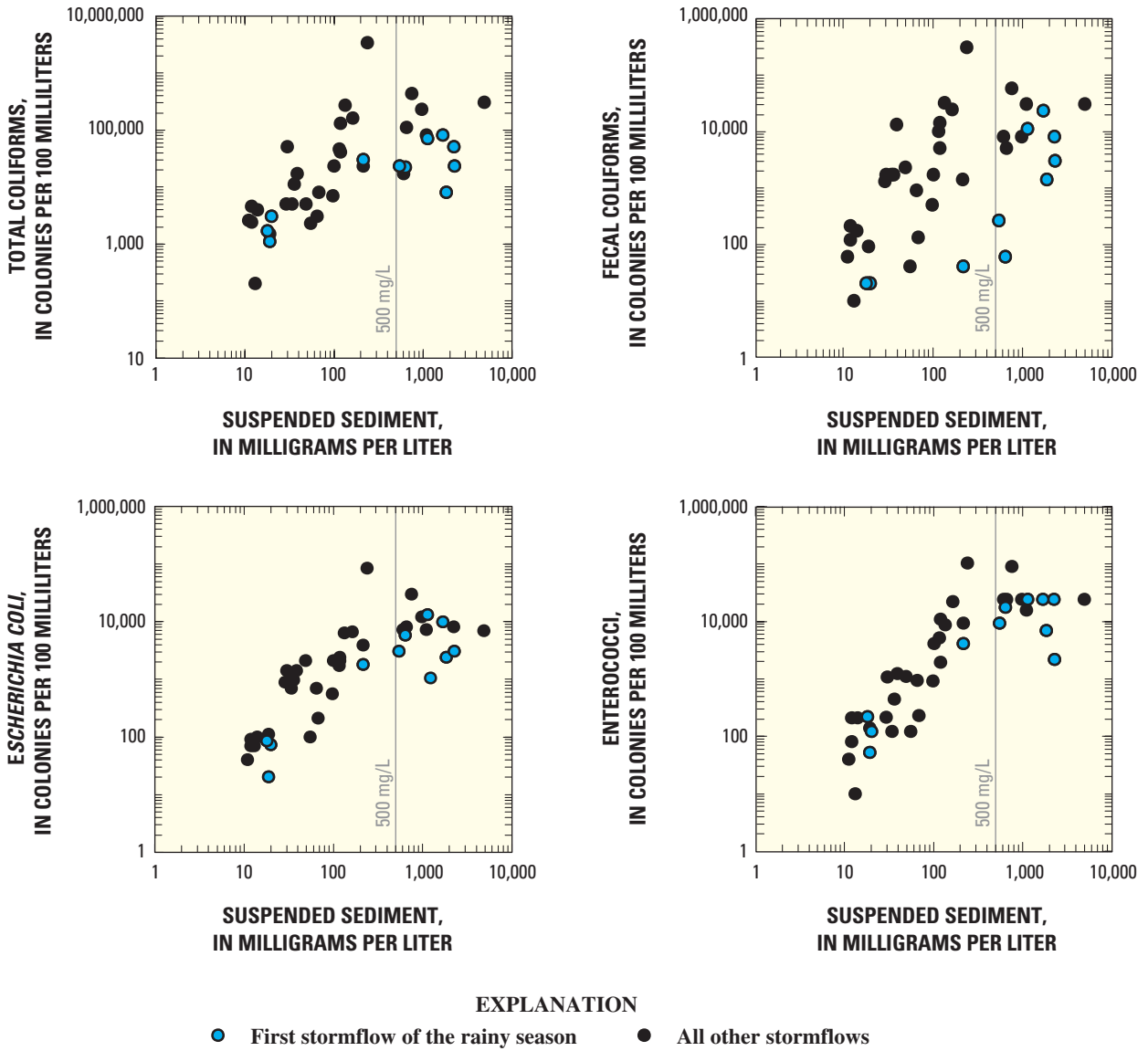


Figure 7. Total coliforms, fecal coliforms, *Escherichia coli*, and enterococci concentrations as a function of suspended-sediment concentration in stormflow in the Santa Ana River at Imperial Highway, southern California, 2000–01 and 2001–02 rainy seasons.

18 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

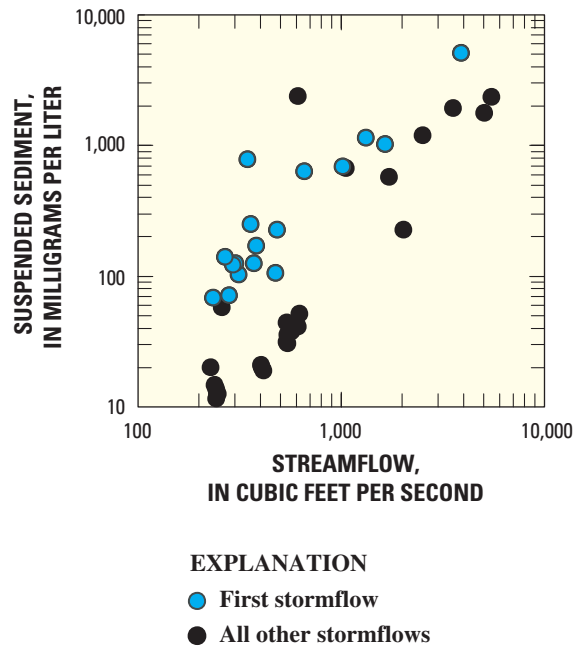


Figure 8. Suspended-sediment concentrations as a function of streamflow during stormflow conditions in the Santa Ana River at Imperial Highway, southern California, 2000–01 and 2001–02 rainy seasons.

Table 3. Description of selected phospholipid fatty acid (PLFA) structural groups

[Standard nomenclature used for fatty-acid names follows the form $X:Y\omega Z$, where X is the number of carbon atoms, Y is the number of carbon-carbon double bonds, ω is the end of the molecule, and Z is the number of carbon atoms between the terminal double bond and the methyl end of the molecule, i , iso methyl branching, and Me , methyl branching]

Structural group	Representative fatty acid	Shorthand notation
Monoenoic	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	16:1 ω 7
Branched monoenoic	$\text{CH}_3\text{CH}(\text{CH}_2)_4\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ CH_3	<i>i</i> 17:1 ω 7
Polyenoic	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	18:2 ω 7
Normal saturated	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	16:0
Terminally branched saturated	$\text{CH}_3\text{CH}(\text{CH}_2)_{12}\text{COOH}$ CH_3	<i>i</i> 16:0
Mid-chain branched saturated	$\text{CH}_3(\text{CH}_2)_2\text{CH}(\text{CH}_2)_{10}\text{COOH}$ CH_3	10 <i>Me</i> 16:0

20 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Although researchers in previous studies have identified marker fatty acids and associated these compounds with specific organisms or groups of organisms (Edlund and others, 1985; Dowling and others, 1986; Paul and Clark, 1996; White and others, 1996), even a brief review of the available literature shows a number of inconsistencies in the approach. Recent work by Haack and others (1994) showed that even in simplified model microbial communities, many fatty acids are not specific to individual organisms but rather are found in a wide range of different organisms having similar metabolic processes. In this study, changes in the relative abundance of specific fatty acids or fatty acid structural groups were used to show changes in the types and diversity of microorganisms present during different parts of the stormflow hydrograph and throughout the rainy season without inferring the presence of specific organisms.

Phospholipid Fatty Acid Concentrations and Compositions during Stormflow

Phospholipid fatty acid concentrations and composition were measured during five stormflows during the 1999–2000 and 2001–02 rainy seasons (Appendix B). Additional samples for comparison with stormflow samples were collected from other sources such as storm drains, bed material from the Santa Ana River, and a dairy-waste storage pond (table 5, later in this report).

Total PLFA concentrations in samples from the Santa Ana River at Imperial Highway ranged from 18,200 to 725,000 picomoles per liter (pmole/L). In general, total PLFA concentrations were lower in samples collected prior to the beginning of stormflow. However, pre-storm concentrations were dependent on antecedent conditions, and samples collected prior to the onset of stormflow, when antecedent conditions were relatively wet, had higher PLFA concentrations than did samples collected after relatively dry periods. Total PLFA concentrations increased with increases in streamflow and total coliform and fecal indicator bacteria concentrations (fig. 9). Concentrations were distributed through the stormflow hydrograph in a similar manner to bacterial concentrations. In the same manner as total coliforms and fecal indicator bacteria (*E. coli*), PLFA concentrations commonly remained high during the recessional flows of the first stormflow of the rainy season—even after flow had returned to pre-storm conditions.

More than 80 PLFAs were identified in samples collected as part of this study (Appendix B). Monoenoic (monounsaturated) fatty acids, normal saturated fatty acids,

and polyenoic (polyunsaturated) fatty acids composed most of the total fatty acids measured. Structurally more complex fatty acids having branched or ring structures composed, on average, less than 12 percent of the total fatty acids measured. Changes in fatty acid groups and individual fatty acids within those groups reflect the complex changes in the total microbial population (not just total coliform and fecal indicator bacteria measured as part of this study) present within the Santa Ana River that occur as water from different sources having different microbial populations contributes to stormflow.

As total PLFA concentrations increased, the monoenoic fatty acid fraction increased (fig. 10), and in some samples the two most common monoenoic fatty acids, 16:1 ω 7 and 18:1 ω 7, composed more than half of the total PLFAs. For this to occur the source of bacteria contributing these fatty acids must be poorly diverse and the total microbial population must be dominated by only a few types of bacteria. As total fatty acid concentrations increased, normal saturated and polyenoic fatty acid concentrations generally decreased. However, there was more variability in the relation between the polyenoic PLFA fraction, total PLFA concentrations, and stormflow than in the monoenoic or normal fatty acid fractions (fig. 10). For example, during the first stormflows of the winter rainy season, the polyenoic fatty acid fraction initially increased as runoff from urban areas downstream from Prado Dam contributed to stormflow, and in some stormflows the polyenoic fraction composed as much as 40 percent of the total PLFA during that time. The polyenoic fatty acid fraction subsequently decreased to less than 20 percent of the total PLFA during the recessional flows when runoff from areas upstream from Prado Dam was present and the total PLFA was composed largely of a few monoenoic fatty acids. Even though much of the basin upstream from Prado Dam is urbanized, the fatty acids (and consequently the total bacterial population) present in the Santa Ana River during the recession of the early season stormflows are different from the fatty acids present in urban runoff contributed by areas downstream from Prado Dam.

Changes in PLFA composition during stormflow were expressed as changes in the diversity of the 10 most common fatty acids using the Simpson reciprocal and Shannon-Wiener indexes (Goodman, 1975). The indexes are calculated as follows:

$$\text{Simpson reciprocal index (D)} = 1/\sum(P_i^2),$$

$$\text{Shannon-Wiener index (H)} = -\sum(P_i \ln[P_i]),$$

where

P is the number of organisms (or, in this case, the fatty acid fraction) of each type *i* observed.

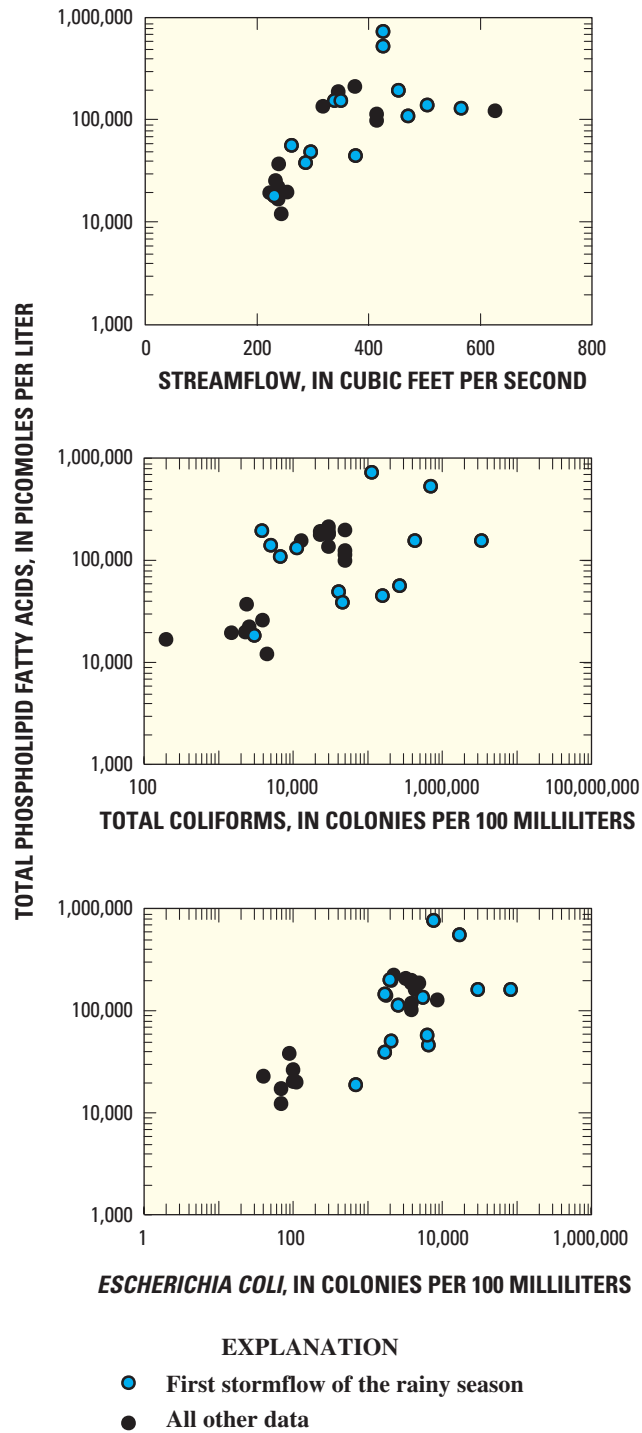


Figure 9. Phospholipid fatty acid concentrations as a function of streamflow, and of total coliform bacteria, and *Escherichia coli* concentrations in stormflow in the Santa Ana River at Imperial Highway, 1999–2000 and 2001–02 rainy seasons.

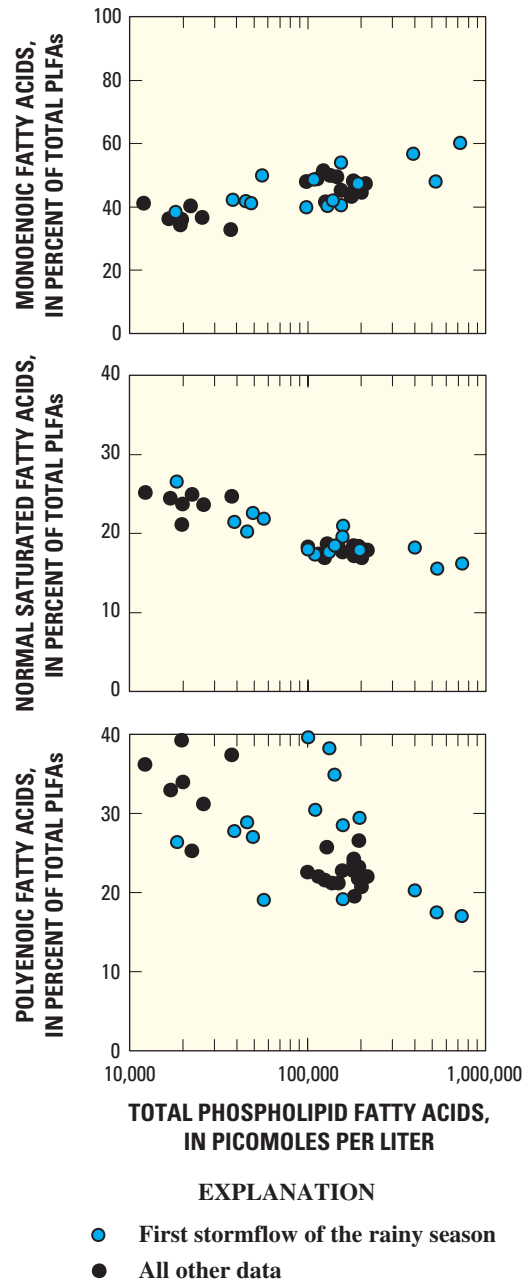


Figure 10. Selected phospholipid fatty acid (PLFA) structural groups as a function of total phospholipid fatty acid concentration in stormflow in the Santa Ana River, 1999–2000 and 2001–02 rainy seasons.

Both the Simpson reciprocal and Shannon-Weiner indices increase with increasing diversity and decrease with decreasing diversity. The indexes provide a convenient way to compare changes in fatty acid composition representative of changes in the total bacterial population during stormflow, with changes in total coliform and fecal indicator bacteria measured during stormflow. For example, as total PLFA concentrations and total coliform concentrations increased during the recession of the November 12–14, 2001, stormflow, PLFA diversity decreased (fig. 11). The decrease in diversity presumably results from a source present within the Santa Ana River largely dominated by only a few bacteria species that contains high concentration of total coliforms.

Comparison with Phospholipid Fatty Acids from Other Sources

Principal component analysis was used to compare stormflow data with samples collected from other sources. Principal component analysis is a multivariate statistical technique that transforms a set of inter-correlated variables into a new coordinate system (Kshirsagar, 1972; Morrison, 1976; Gnanadesikan, 1977). The transformed variables are uncorrelated linear combinations of the original variables known as principal components. Principal components have a mean of zero and the same variance as the original data set. The values of the principal components are known as scores, and the scores are calculated on the basis of the contribution of each variable to the total variance (Preisendorfer and others, 1981). The magnitude and direction (plus or minus) of each variable to the principal component score is described by an eigenvector.

The eigenvectors describing the first and second principal components for the 10 most common fatty acids in stormflow are listed in table 4. The first principal component explained almost 40 percent of the total variance and contained large positive eigenvectors for a monoenoic (18:1 ω 9), two saturated (18:0, 16:0), and three polyenoic fatty acids (20:5 ω 3, 18:2 ω 6, and 18:3 ω 3); and large negative eigenvectors for two monoenoic (16:1 ω 7, and 16:1 ω 5) and a terminally branched saturated fatty acid (*i*15:0). The second principal component explained about 20 percent of the total variance and contained large positive eigenvectors for a terminally branched (*i*15:0), a polyenoic (18:2 ω 6), and a monoenoic (16:1 ω 5) fatty acid; and large negative eigenvectors for a polyenoic (20:5 ω 3) and a monoenoic (16:1 ω 7) fatty acids.

A plot of the first principal component as a function of the second principal component (fig. 12) shows that samples from the recession of the first stormflows of the rainy season had negative scores for both the first and second principal components. These scores are consistent with decreases in fatty acids having large positive eigenvectors for both the first and second principal components (such as the monoenoic fatty acid 18:1 ω 9 and the polyenoic fatty acids 18:2 ω 6 and 18:3 ω 3), and increases in fatty acids having large negative eigenvectors for the first and second principal component (such as the monoenoic fatty acid 16:1 ω 7) (table 4). In contrast, the first principal component was positive and the second principal component was negative for samples collected from the Santa Ana River when urban runoff predominated. These scores are consistent with decreases in fatty acids having large negative eigenvectors for the first principal component and large positive eigenvectors for the second principal component (such as the normal saturated fatty acid *i*15:0), and increases in fatty acids having large positive eigenvectors for the first principal component and large negative eigenvectors for the second principal component (such as the polyenoic fatty acid 20:5 ω 3). The results of the principal component analysis reflect changes in the composition of the total PLFA and, presumably, changes in the total microbial population they represent during stormflow.

Samples from selected sources in the Santa Ana River Basin show a wide range of fatty acid concentrations and compositions (table 5). Some of the samples have distinctive fatty acid composition that could be useful in the identification of bacteria from these sources.

The highest fatty acid concentrations in water, about 100,000 pmole/L, were from Cucamonga Creek, downstream from dairy operations, and from a dairy-waste storage pond (table 5). The two most common fatty acids in water from Cucamonga Creek were the monoenoic fatty acids 18:1 ω 7 and 16:1 ω 7. The sample from the dairy-waste pond contained high concentrations of terminally branched saturated fatty acids *i*14:0, *i*15:0, and *a*15:0—which, with the exception of *i*15:0 (shown in table 5), were not among the 10 most commonly detected fatty acids in stormflow.

The lowest concentrations, between about 5,000 and 24,000 pmole/L, were from an urban stormdrain tributary to the Santa Ana River near the Imperial Highway diversion. Although the fatty acid composition of water from the stormdrain varied widely throughout the rainy season, several samples contained high concentrations of the monoenoic fatty acid 18:1 ω 7 and the polyenoic fatty acid 20:5 ω 3.

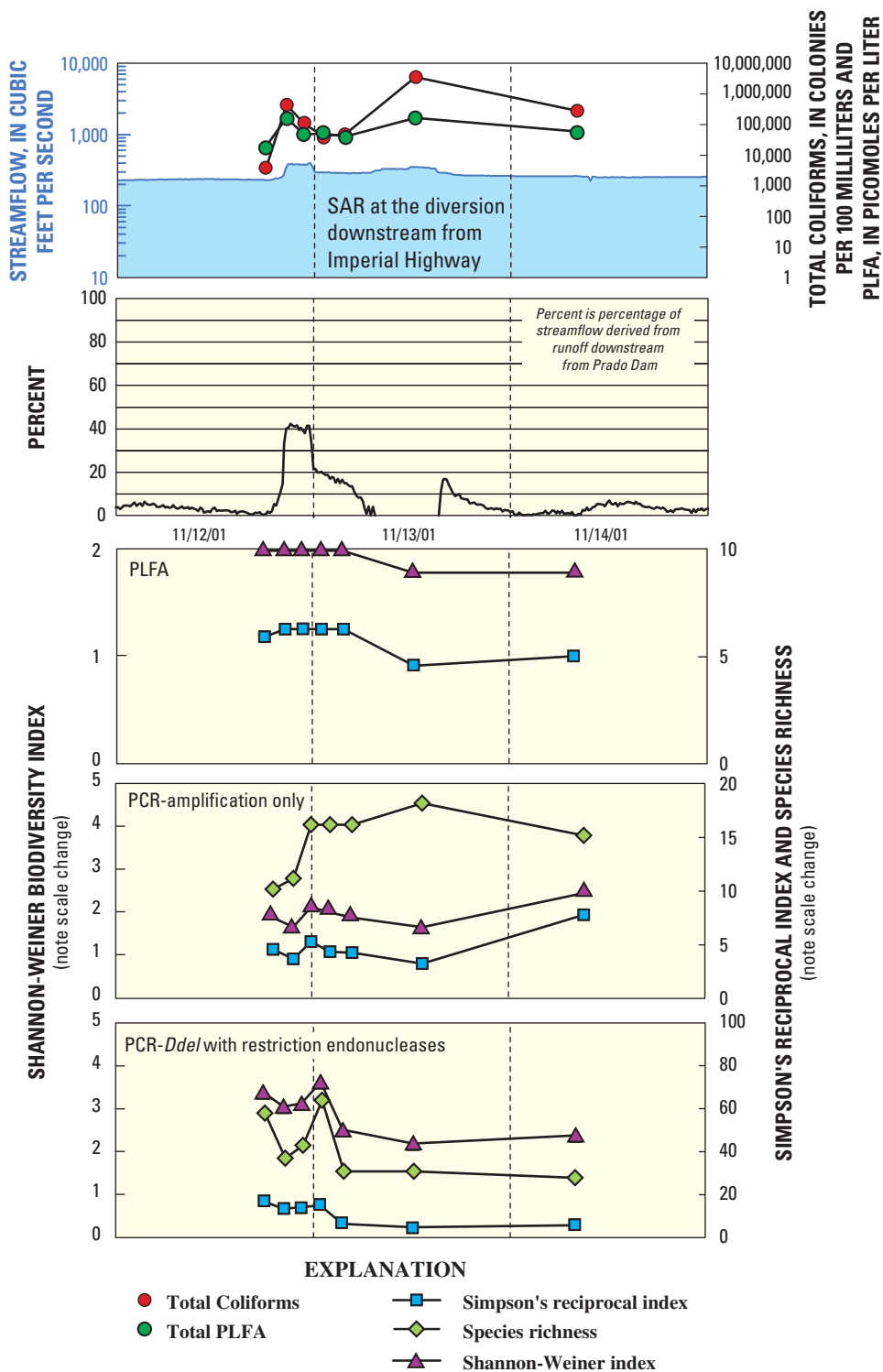


Figure 11. Total coliform bacteria and total phospholipid fatty acid (PLFA) concentrations as a function of streamflow, and changes in diversity of PLFA and the 16S rRNA gene during the November 12–14, 2001, stormflow, Santa Ana River (SAR) at Imperial Highway, southern California. (PCR, polymerase chain reaction; 16S rRNA, a bacterial ribosomal ribonucleic gene.)

Table 4. Eigenvectors composing the first and second principal components of phospholipid fatty acid structural groups and the 10 most common fatty acids in stormflow in the Santa Ana River at Imperial Highway, 1999–2000, and 2001–02 rainy seasons

[Fatty acids listed in order of average abundance; ↑, expected increase in fatty acid fraction; ↓, expected decrease in fatty acid fraction; —, no data]

Phospholipid fatty acid	First principal component	Second principal component	Recessional flow, first stormflow of rainy season	Urban runoff, first stormflow of rainy season
16:1 ω 7	–0.37	–0.40	↑	—
18:1 ω 7	–.08	–.05	—	—
<i>i</i> 16:0	.28	–.16	—	—
18:1 ω 9	.43	.22	↓	—
20:5 ω 3	.30	–.43	—	↑
18:2 ω 6	.30	.42	↓	—
18:3 ω 3	.30	.16	↓	—
<i>i</i> 15:0	–.31	.52	—	↓
16:1 ω 5	–.34	.31	—	—
18:0	.32	.08	—	—

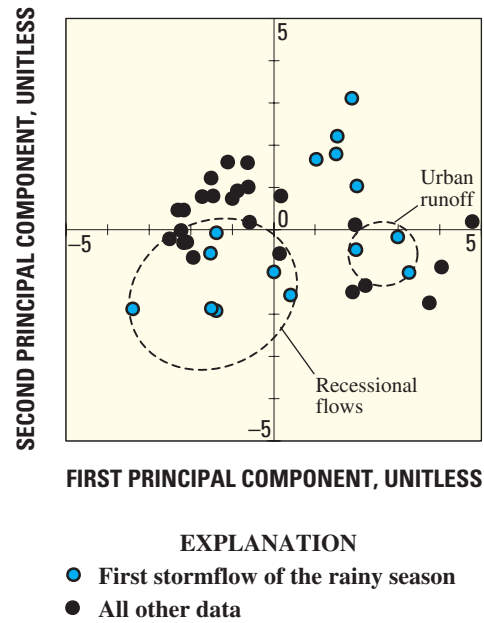


Figure 12. The first principal component as a function of the second principal component for the 10 most common phospholipid fatty acids in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons.

Table 5. Total phospholipid fatty acid (PLFA) concentrations and percent of total PLFA for the 10 most commonly detected fatty acids in selected samples from the Santa Ana River basin, southern California

[Data collected by U.S. Geological Survey and analyzed by Microbial Insights, Inc.; DDMSS, degrees, minutes, seconds; PLFA structural-group concentrations in percent of total PLFA concentration; pmole/L, picomoles per liter; pmole/g, picomoles per gram; SAR baseflow, Santa Ana River baseflow sample; urban drain, stormdrain tributary to the Santa Ana River at Imperial Highway; SAR streambed, Santa Ana River streambed upstream from Imperial Highway; Prado wetland soil, wetland soil sample at Prado Dam; dairy waste pond, dairy waste pond near Pine Avenue; Cucamonga Creek baseflow, Cucamonga Creek near MiraLoma; Cucamonga Creek algae, Cucamonga Creek near Mira Loma algae sample; NA, not applicable; —, no data]

Sample	Site No.	Latitude (DDMMSS)	Longitude (DDMMSS)	Sample date	Total PLFA (pmole/g)	Total PLFA (pmole/L)	Terminally branched saturated (percent of total PLFA)	Monoenoic (percent of total PLFA)	Branched monoenoic (percent of total PLFA)	Mid-chain branched saturated (percent of total PLFA)	Normal saturated (percent of total PLFA)	Polyenoic (percent of total PLFA)
SAR baseflow	1	335123	1174723	08/29/01	NA	59,600	7.7	33	1.0	1.3	28	29
Urban drain	2	335122	1174718	12/09/99	NA	5,150	3.2	40	1.5	1.2	18	36
Urban drain	2	335122	1174718	03/02/00	NA	23,900	2.3	26	.6	3.3	22	47
Urban drain	2	335122	1174718	08/29/01	NA	17,200	4.5	40	1.6	3.6	25	25
SAR streambed	3	335223	1174448	12/09/99	3,690	NA	13.1	34	3.7	7.2	21	20
SAR streambed	3	335223	1174448	03/02/00	13,200	NA	9.3	29	2.8	4.8	22	33
SAR streambed	3	335223	1174448	08/29/01	107,000	NA	4.2	28	.3	.5	45	22
Prado wetland soil	5	335323	1173817	12/09/99	6,700	NA	16.0	36	5.4	5.0	22	17
Prado wetland soil	5	335323	1173817	03/02/00	3,080	NA	13.7	37	5.2	11	16	18
Prado wetland soil	5	335323	1173817	08/29/01	25,000	NA	10.9	33	2.0	5.5	22	26
Dairy waste pond	6	335645	1173922	08/29/01	NA	97,000	21.7	23	1.1	1.8	32	20
Cucamonga Creek baseflow	8	335858	1173555	12/09/99	NA	101,000	6.6	36	1.4	2.7	23	30
Cucamonga Creek algae	8	335858	1173555	08/29/01	342,000	NA	9.3	17	.7	.3	27	45

[Percent of total PLFA for the 10 most commonly detected fatty acids; standard nomenclature used for fatty-acid names follows the form $X:Y\omega Z$, where X is the number of carbon atoms, Y is the number of carbon-carbon double bonds, ω is the end of the molecule, and Z is the number of carbon atoms between the terminal double bond and the methyl end of the molecule, i , isomethyl branching.]

Sample	Site No.	Date	16:1 ω 7	18:1 ω 7	16:0	18:1 ω 9	20:5 ω 3	18:3 ω 3	16:1 ω 5	18:0	18:2 ω 6	15:0
SAR baseflow	1	08/29/01	19.4	9.7	3.7	6.7	5.6	—	2.5	2.2	4.6	1.5
Urban drain	2	12/09/99	11.2	25.7	.5	12.8	7.7	2.5	.0	3.8	4.5	.5
Urban drain	2	03/02/00	12.8	8.0	.5	5.0	20.9	6.5	.8	3.5	8.1	.6
Urban drain	2	08/29/01	11.1	22.7	1.0	8.3	4.2	—	1.8	4.8	4.6	.6
SAR streambed	3	12/09/99	11.4	12.0	2.1	6.6	1.4	1.3	2.8	3.0	2.3	5.4
SAR streambed	3	03/02/00	23.9	2.4	2.2	2.8	2.0	—	1.2	.9	3.4	.7
SAR streambed	3	08/29/01	10.1	12.2	1.7	6.2	1.4	1.9	3.3	3.1	4.0	3.6
Prado wetland soil	5	12/09/99	7.0	9.0	1.0	5.9	.3	2.9	3.5	2.0	18.2	3.0
Prado wetland soil	5	03/02/00	9.1	12.1	2.2	6.5	.7	.7	4.2	2.5	4.3	4.2
Prado wetland soil	5	08/29/01	16.6	8.4	1.5	14.3	1.6	—	4.9	1.7	4.3	3.5
Dairy waste pond	6	08/29/01	11.2	8.3	2.4	10.1	.3	—	2.1	4.6	5.5	4.5
Cucamonga Creek baseflow	8	12/09/99	14.5	18.3	1.1	6.1	2.3	11.7	1.2	1.6	4.9	2.9
Cucamonga Creek algae	8	08/29/01	4.6	7.5	6.3	29.6	.2	—	1.4	1.2	9.3	1.6

28 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

The highest fatty acid concentration extracted from solid material, slightly greater than 340,000 picomoles per gram (pmole/g), was from algae sampled from the concrete-lined channel of Cucamonga Creek (table 5). This sample contained high concentrations of the monoenoic fatty acid 18:1 ω 9 but low concentrations of other commonly detected monoenoic fatty acids such as 16:1 ω 7 and 18:1 ω 7. The algae sample was very low in the polyenoic fatty acid 20:5 ω 3. Fatty acids in streambed material varied widely in concentration and composition. Concentrations were higher, about 100,000 pmole/g, in streambed material collected prior to the beginning of the rainy season, and were as low as 3,000 pmole/g in streambed material collected later in the rainy season. Fatty acid composition in streambed material was variable, but the sample collected prior to the beginning of the rainy season contained large amounts of the monoenoic fatty acid 16:1 ω 7.

Comparison of the principal component scores in table 4 with the fatty acid composition from selected sources (table 5) showed that the fatty acid composition of the first stormflow of the rainy season, when urban runoff from the area downstream from Prado Dam predominated, was similar to the fatty acid composition of samples collected from urban stormdrains. Some of the fatty acids present during the recessional flow of the first stormflow of the rainy season also could be explained by contributions from urban stormdrains. However, a combination of fatty acids from urban stormdrains (urban drain baseflow) and material mobilized from the bed of the Santa Ana River (SAR streambed) would be required to account for the large amounts of the monoenoic fatty acid 16:1 ω 7 present during these recessional flows. Fatty acids associated with streambed sediments during the latter part of the sampled stormflows may reflect recently disturbed streambed sediments equilibrating with the overlying water consistent with the proposed modifications to the model of Matson and others (1978).

The algae sample had a high concentration of fatty acid 18:1 ω 9. This fatty acid actually decreased during the recession of the first stormflow of the rainy season, suggesting that large amounts of algal material was not present in the Santa Ana River at that time—even though this material should be readily scoured from the concrete-lined channel of Cucamonga Creek during stormflow.

The sample from the dairy-waste pond had high concentrations of terminally branched saturated fatty acids such as *i*14:0, *i*15:0, and *a*15:0—which, only *i*15:0, is among the 10 most commonly detected fatty acids in the sampled stormflows. Concentrations of these fatty acids did not show a consistent increase or decrease during different parts of the stormflow hydrographs from this study, suggesting that direct release of material from dairy waste ponds did not occur during the sampled stormflows. This is consistent with field observations that showed that the sampled stormflows were not large and did not exceed the design criteria of the waste-storage ponds.

Data collected as part of this study show changes in the fatty acid composition of stormflow, presumably resulting from changing microbiological assemblages, as runoff was contributed from different sources within the basin. Additional data collection covering a greater range of stormflow conditions, which would include additional characterization of the fatty acid composition from different sources in the basin and characterization of seasonal changes in fatty acid composition, would be required to draw definitive conclusions about the sources of the fatty acids and the total microbial population they represent in the Santa Ana River during stormflow.

16S rRNA Gene

Genetic-based methods increasingly have been used to determine sources of fecal contamination in water (Bernstein and others, 2002; Hartel and others, 2002; Kern and others, 2002; Wheeler and others, 2002; Bernhard and Field, 2000). The genes that compose the 16S rRNA gene has been characterized (Paul and Clark, 1996) and widely used for genetic studies of bacteria in water (Samadpour, 2002) and soils (Bruce, 1997; Liu and others, 1997; Dunbar and others, 2000). The 16S rRNA gene can be isolated and specific regions of the gene, such as the hypervariable region, can be amplified using PCR (polymerase chain reaction). The base pair sequence in the hypervariable region is different in different bacterial species and, in a mixture of bacteria, species can be identified on the basis of their unique base pair sequence in that region.

One of the more common approaches for the interpretation of this type of data is to compare the pattern of the 16S rRNA gene with known patterns from organisms in a database developed from known sources (Samadpour, 2002). The development of such a database is often expensive and time consuming and was beyond the scope of this study. Databases developed from other geographic areas were not used because the spatial and temporal range over which these databases can be successfully used for the identification of bacteria is unknown (Hartel and others, 2002). In this study, fragment length patterns from the 16S rRNA gene isolated from different samples of stormflow using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) are representative of the genetic material from the entire microbial population. These data were not compared to libraries of genetic data from different organisms to identify specific organisms from specific sources, but rather these data were used to determine if the microbial community changed during the stormflow hydrograph and if total coliform and fecal indicator bacteria measured during different times within the hydrograph were associated with a similar or dissimilar bacteria populations—and were presumably from a similar or dissimilar source.

Amplicon Size and Diversity during Stormflow

Two methods were used to create the genetic profiles analyzed in this study. In the first method, a segment of the hypervariable region of the 16S rRNA gene, consisting of about 500 base-pairs, was amplified using universal eubacterial primers (Avaniss-Aghajani and others, 1996; Brunk and others, 1996). The forward primer was labeled using a fluorescent dye and the total DNA was amplified using Polymerase Chain Reaction (PCR). In the second method, the labeled amplicons (PCR products) were digested into smaller fragments using *DdeI* restriction endonuclease according to a procedure known as Terminal Restriction Fragment Length Polymorphism (T-RFLP). Digestion of the amplicons produced fragments of different size due to differences in the sequence of base pairs that compose the DNA. The genetic material is more finely subdivided during the digestion step and each fragment is believed to represent a different bacterial species. As a result, the T-RFLP analysis produces a genetic profile of the microbial community present in the water sample.

The undigested PCR products for three selected samples collected during the November 12–14, 2001, stormflow are shown in [figure 13A](#). The samples represent base flow (streamflow prior to the onset of stormflow), urban runoff (from that part of the basin downstream from Prado Dam), and the recessional part of the stormflow hydrograph (streamflow at this time consists largely of runoff from areas upstream from Prado Dam and increasing amounts of base flow as flows decrease), and are representative of the changes in the microbial community across the stormflow hydrograph.

The undigested PCR products consisted of DNA fragments ranging in length from 422 to 483 base pairs. Comparison of the fragments in the three samples from the November 12–14, 2001, stormflow shows that of the 29 different fragments detected only 2, having lengths of 427 and 429 base pairs, were present in all three samples. The fragment having 429 base pairs was commonly detected in other stormflows sampled as part of this study, but the fragment having 427 base pairs was not commonly present in the late-season stormflows sampled as part of this study. Six of the twenty-nine fragments detected were unique to base flow, 6 were unique to urban runoff, and 10 were unique to the recession flow of the hydrograph and presumably represent organisms found only during those parts of the stormflow hydrograph. The data show large changes in the genetic profile, and the microbial community it represents, during stormflow.

More fragments, that were smaller in length, ranging from 28 to 493 base pairs, were detected from the digested samples ([fig. 13B](#)) using T-RFLP. Fragments in the digested samples should not be larger than fragments present in the undigested samples. The absence of the longer fragments in the undigested samples may result from reduced sensitivity of the capillary electrophoresis technique when fewer fragments having greater individual peak height are present in the sample. Endonuclease digestion provided a finer discrimination of the

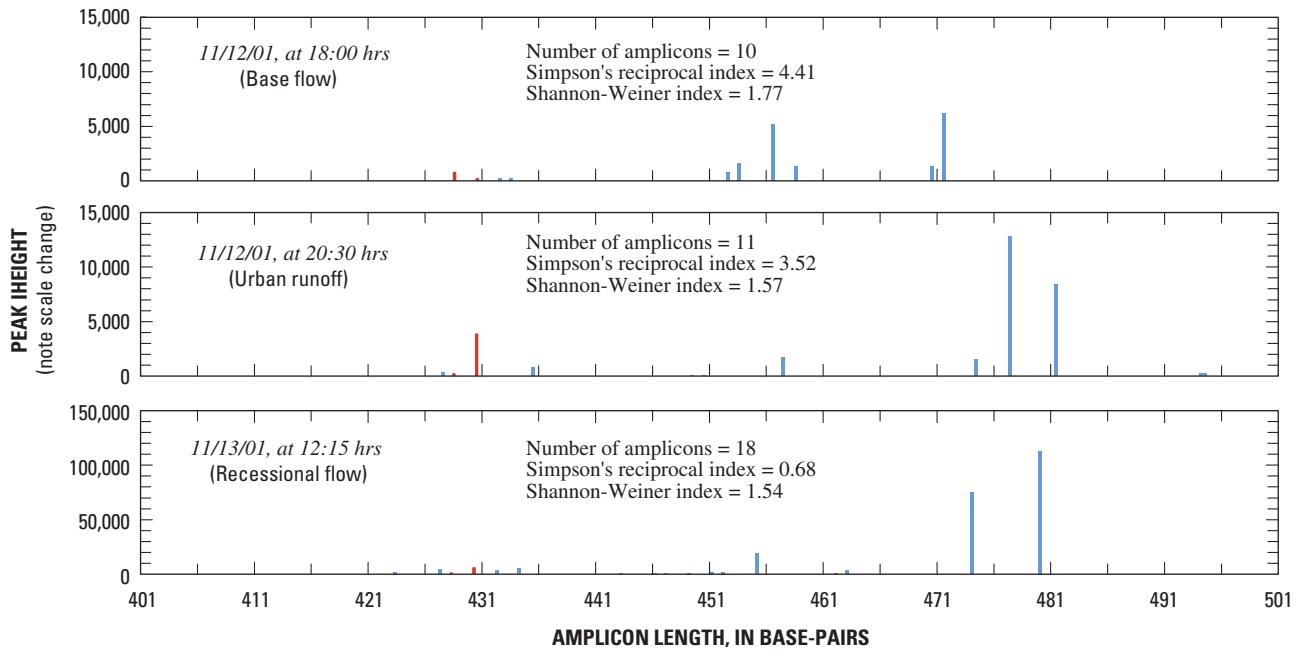
genetic material from different bacteria—although it was more labor intensive. Additional digestion using another restriction enzyme may have yielded even more fragments and provided even finer discrimination of the genetic material. Of the ninety-four different fragments detected only 4, having lengths of 240, 241, 244, and 268 base pairs, were present in all three samples. These fragments were commonly detected in samples collected throughout the November 12–14, 2001, stormflow, but all were unexplainably absent in the last sample collected at 08:00 from the recession of this stormflow on November 14, 2001. Forty of the ninety-four fragments detected were unique to base flow, 20 were unique to urban runoff, and 20 were unique to the recessional flow. The base-flow sample and the urban-runoff sample had the largest number of fragments in common, and the sample from the recession of the stormflow hydrograph had the fewest fragments in common with the other samples shown in red in [figure 13](#). Even though streamflow returned to pre-storm conditions by the end of the sample collection period, the genetic material sampled had not returned to pre-storm conditions. In the same manner demonstrated by the undigested PCR products, these data suggest large changes in the genetic material, and in the microbial community it represents, during stormflow.

PCR products, with and without digestion, showed increasing dominance of a few fragments during the recession of the November 12–14, 2001, stormflow ([fig. 13](#)). The effect is large and during recessional flow several fragments were present at concentrations, as indicated by peak height of the capillary electrophoresis, that were an order of magnitude larger than measured during base flow or during urban runoff. In the same manner shown by the PLFA data, the genetic profile data are consistent with bacterial contributions from sources that have only a limited diversity of bacteria.

Quantitative measures of fragment diversity, including species richness (calculated as the number of fragments present), Simpsons's reciprocal index, and the Shannon-Weiner index, were calculated from the number of fragments present and the peak height in a manner similar to that used for calculating phospholipid fatty acid diversity. These measures of diversity are shown for the entire November 12–14, 2001, stormflow in [figure 11](#). For T-RFLP-with endonuclease digestion-species richness, Simpson's reciprocal index, and the Shannon-Weiner index decrease, indicating decreased diversity during stormflow. The lowest diversity was present during the recessional flows. Results were similar for PLFA and T-RFLP, with endonuclease digestion suggesting that similar conclusions can be drawn about changes in bacterial diversity and sources during stormflow, using either type of data. Indexes of diversity calculated on the basis of fragments obtained without endonuclease digestion increase during stormflow, and conclusions about the changes in the microbial community would be different using these data. It is likely that the genetic profile data without endonuclease digestion are too imprecise to adequately characterize changes in the microbial community during stormflow.

30 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

A



B

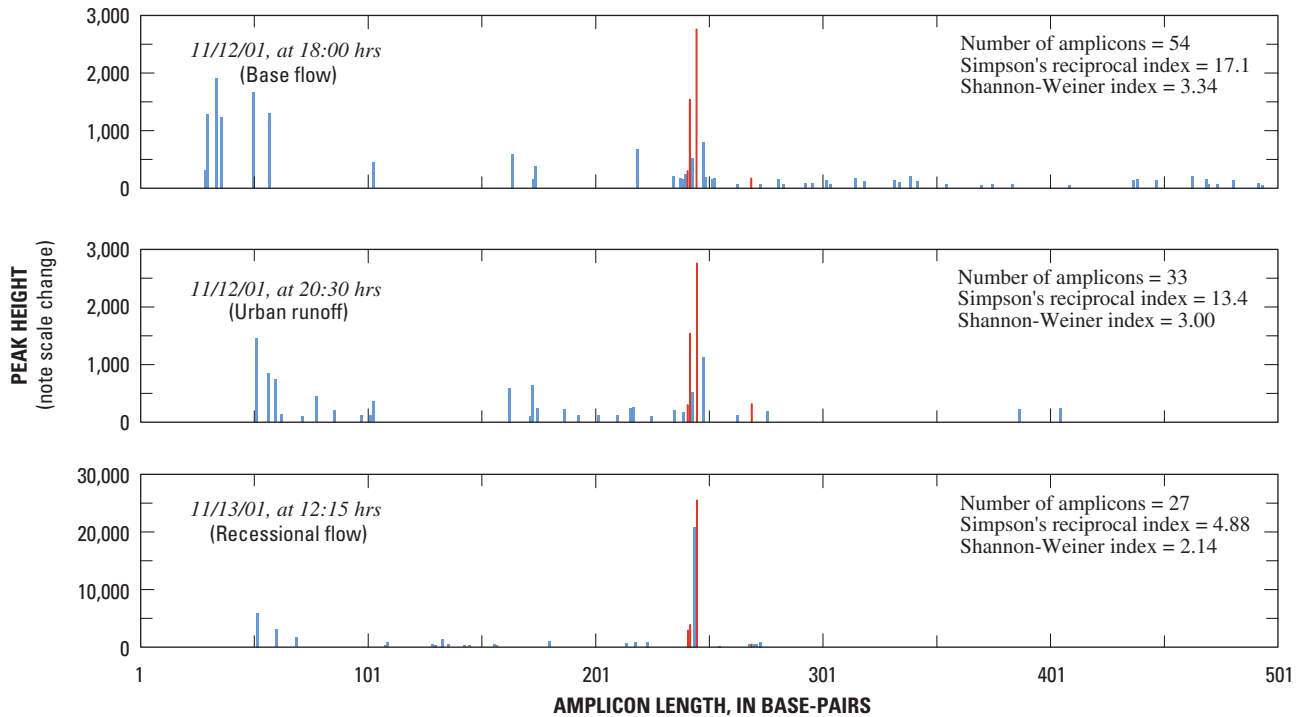


Figure 13. Amplicons from Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis of the 16S rRNA gene with (A) polymerase chain reaction (PCR) amplification using universal eubacterial primers and (B) PCR amplification using eubacterial primers and digestion with *DdeI* restriction endonuclease for selected stormflow samples from the Santa Ana River at Imperial Highway, southern California, November 12–14, 2001.

Comparison with Amplicons from Other Sources

Genetic profiles from stormflow were compared with profiles obtained with and without endonuclease digestion from a small number of samples from different sources. These sources included samples of dairy-pond wastes, algae from the Cucamonga Creek stormflow channel, and base flow discharge from an urban storm drain ([fig 14](#)). These samples are not intended to be inclusive of all possible sources in the Santa Ana River Basin but are representative of several possible sources of bacterial contamination.

Fragments from none of the three sources matched the very large peaks present in urban runoff and during the recession of the November 12–14, 2001 stormflow. However, a combination of urban runoff and algae could produce the very large peaks present in the recession stormflow obtained from both PCR amplification without digestion and PCR amplification with digestion (T-RFLP). The PLFA data suggest that algae did not contribute to stormflow fatty acid composition, and T-RFLP analysis of streambed material were not available to determine if the resuspension of streambed sediments and associated bacteria could contribute to the material sampled during stormflow.

The large peaks present in stormflow are notably absent in the sample from the dairy-waste pond. These data support the suggestion that a direct release of dairy waste did not occur during the November 12–14, 2001 stormflow, which is consistent with field observations that suggest that dairy-waste ponds did not contribute to runoff during this period.

Dissolved Organic Carbon

Some dissolved organic carbon (DOC) within stormflow may be derived from the same sources as are fecal bacteria and, therefore, an understanding of the sources and changes in composition of DOC may help delineate the sources of fecal bacteria during stormflow. In addition, knowledge of the source and composition of DOC is important because there are other health concerns associated with DOC in stormflow. For example, it may be desirable to minimize the amount of DOC owing to the potential for disinfection by-product (DBP) formation during the chlorination of drinking water and to minimize the DOC of wastewater origin in water recharged from the Santa Ana River due to health concerns associated with this material.

Dissolved Organic Carbon Concentrations during Stormflow

The DOC concentrations in samples collected prior to the beginning of stormflow ranged from 4.2 to 7.6 mg/L, with a median concentration of 5.3 mg/L (Appendix C). Concentrations in samples collected prior to the beginning of stormflow were highest later in the rainy season. These values are similar to DOC concentrations in base flow in the Santa Ana River measured by Izbicki and others (2000). DOC concentrations of 3 to 5 mg/L are typical for surface water in arid regions (Thurman, 1986); however, because base flow in the Santa Ana River is primarily treated wastewater, the DOC concentration is largely controlled by the concentration of the wastewater discharges. Some increase in DOC concentrations in the Santa Ana River may occur as water flows through artificial wetlands used to remove nitrate upstream from Prado Dam. Gray and others (1996) showed that nitrate removal within these wetlands changed the composition of DOC in the Santa Ana River.

During this study, DOC concentrations in stormflow ranged from 3.0 to 15.0 mg/L, with a median concentration of 6.7 mg/L. These values are similar to historical (1977–86) DOC concentrations in the Santa Ana River during the rainy season (Burton and others, 1998) and slightly lower than DOC concentrations in stormflow measured by Izbicki and others (2000). DOC concentrations increased during most stormflows and concentrations were higher during early season stormflows. The highest concentrations were measured during the recession of the first stormflows of the rainy season when stormflow was dominated by runoff from the area upstream from Prado Dam ([fig. 15](#)), similar to the distribution of total and fecal coliform bacteria concentrations in stormflow. The elevated DOC concentrations also persisted in the Santa Ana River for several days—frequently remaining high even after streamflow returned to pre-storm conditions. Similar results were obtained for stormflow samples collected from the Santa Ana River during 1995–98 (Izbicki and others, 2000). In general, DOC concentrations in late-season stormflows were lower than concentrations in the first stormflow of the rainy season. However, if antecedent conditions were dry, DOC concentrations in late-season stormflows were higher and approached concentrations measured during the first stormflow of the rainy season.

32 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

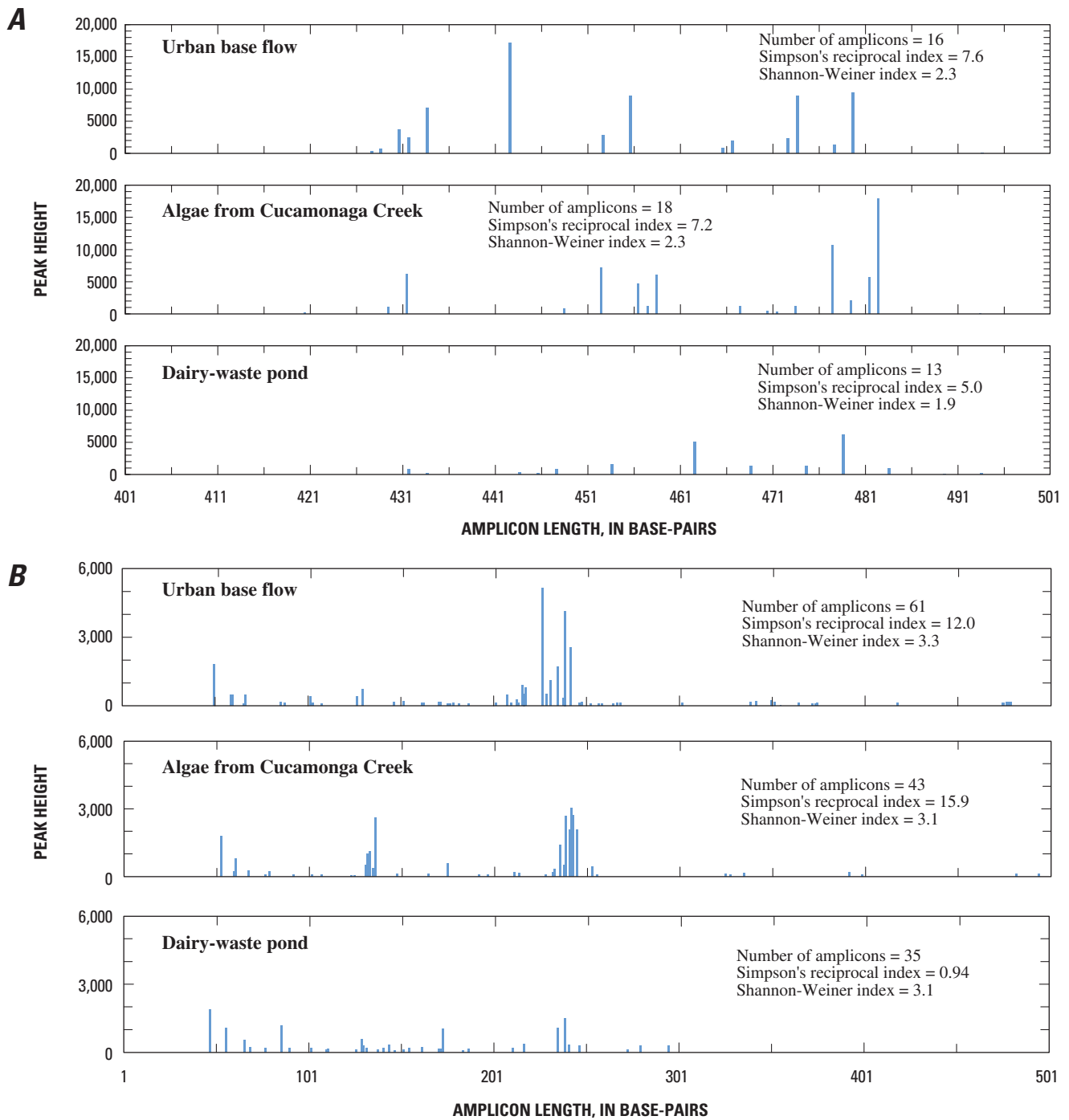


Figure 14. Amplicons from Terminal-Restriction Fragment Length Polymorphism (T-RLFP) analysis of the 16S rRNA gene with (A) polymerase chain reaction (PCR) amplification using universal eubacterial primers and (B) PCR amplification using eubacterial primers and digestion with *DdeI* restriction endonuclease for selected samples from the Santa Ana River Basin, southern California.

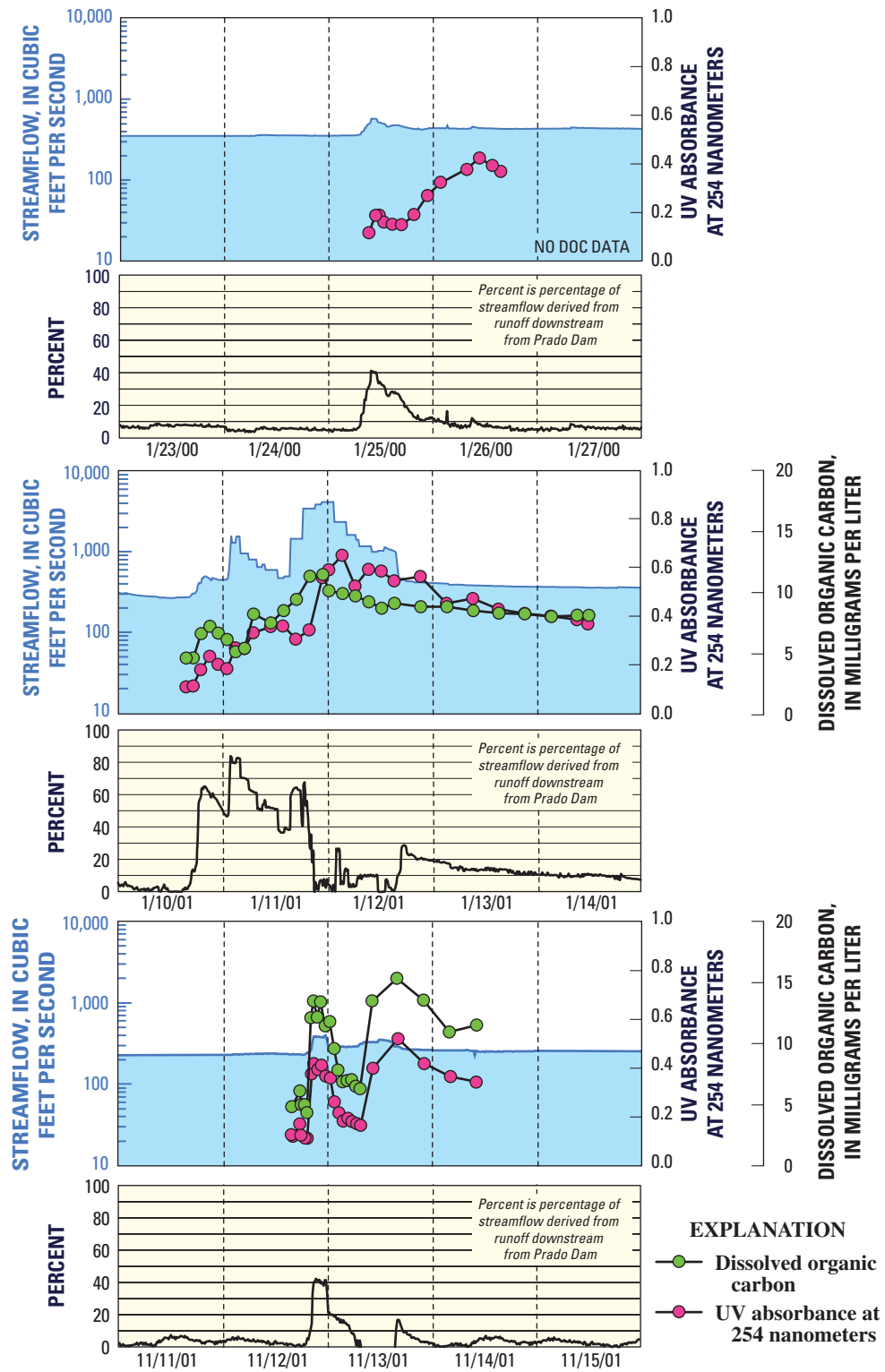


Figure 15. Changes in dissolved organic carbon (DOC) concentration and ultraviolet (UV) absorbance at 254 nanometers during the first stormflows of the 1999– 2000, 2000–01, and 2001–02 rainy seasons, Santa Ana River at Imperial Highway, southern California.

Dissolved Organic Carbon Compositions during Stormflow

Characterization of the composition of DOC may help identify its origin and allow predictions about its fate in the environment. Thorough characterization of DOC is a complex task requiring a hierarchical analytical approach that may determine, among other things, (1) operationally defined organic fractions, (2) functional-group characteristics, (3) molecular weight, and (4) the concentrations of specific organic compounds (Leenheer and Huffman, 1976; Barber, 1992). This hierarchical approach is time consuming, labor intensive, and expensive. Given the number of samples collected and the rapid changes in DOC during stormflow, such a rigorous approach was beyond the scope of this study.

As part of this study, a simplified approach that utilizes changes in the optical properties of water was used to characterize DOC for most samples collected in this study. Exposure to specific wavelengths of light can cause changes in DOC compounds at the atomic level. These changes include the transition of electrons to higher energy levels, rotational or vibrational changes within bonds, or changes in the spin of atomic nuclei (Hart and Schuetz, 1972). Such changes result in the absorbance and fluorescence of energy that is related to the presence of specific functional groups within complex carbon molecules. Optical properties of DOC are especially useful for identification of aromatic (benzene-like ring-structures) and aliphatic (carbon compounds that do not contain benzene-like ring structures). Interpretation of optical data is complex and not quantitative. Simple molecules such as methane (CH₄) have several absorption bands, and more complex molecules may absorb and fluoresce light energy at many wavelengths. Most natural DOC is complex and some material is more aromatic, containing many benzene-like ring structures, while other material is more aliphatic (less aromatic), containing fewer benzene-like ring structures.

To assist in the characterization of DOC, optical data were supplemented with measurements of specific fractions of DOC during the 2001–02 rainy seasons. Ultraviolet absorbance spectra were measured on the DOC fractions, but individual compounds within each fraction were not determined.

Ultraviolet Absorbance

Dissolved organic carbon absorbance within the UV range [220–800 nanometers (nm)] is usually associated with unsaturated molecules such as those containing carbon-carbon double bonds, carbon-oxygen double bonds, or carbon-carbon double bonds in complex aromatic (benzene-like) ring structures (Gutsche and Pasto, 1975). Carbon-carbon double

bonds within aromatic rings generally absorb near 254 nm and carbon-oxygen double bonds generally absorb near 285 nm (Gutsche and Pasto, 1975). However, the exact absorbance wavelength changes with the length and complexity of the associated carbon molecules and other structures. As a result, ultraviolet absorbance spectra do not show pronounced peaks at wavelengths corresponding to specific functional groups but instead are smooth curves. The shape of these curves can vary significantly in individual samples (fig. 16).

UV absorbance spectra can be used to quantify the concentrations of individual compounds in simple mixtures; however, absorbance data are not quantitative for solutions containing complex mixtures of natural and anthropogenic compounds. Nitrate and iron also absorb within the UV range, and some interference due to these constituents has been observed in stormflow samples from the Santa Ana River (Izbicki and others, 2000). For example, comparison of the UV spectra for early season base flow samples collected on January 25, 2000, at 0915 and January 10, 2001, at 1540 shows a large difference in UV absorbance in the range of 190 to about 230 nm (fig. 16). Much of this difference can be attributed to differences in nitrate concentrations, which were 3.7 and 6.6 mg/L as nitrogen, respectively (Appendix D). Because nitrate concentrations in the Santa Ana River decrease during stormflow, and because nitrate does not greatly affect UV absorbance at higher wavelengths (such as 254 and 285 nm), interference from nitrate is small for most stormflow samples. UV absorbance data have been used in previous studies as a surrogate for DOC concentrations (California Department of Water Resources, 1994) and, despite their lack of specificity, UV absorbance data have been used as an indicator of DOC composition (Krazner and others, 1996; Izbicki and others, 2000).

Ultraviolet absorbance was measured between 190 and 310 nm as part of this study (fig. 16); however, for convenience, most analysis was done on data collected at 254 and 285 nm. As previously discussed, these wavelengths correspond to the peak absorbance of carbon-carbon double bonds and carbon-oxygen double bonds, respectively. Ultraviolet absorbance at 254 nm (UV₂₅₄) in pre-stormflow conditions from the Santa Ana River at Imperial Highway ranged from 0.092 to 0.240, with a median absorbance of 0.130. Ultraviolet absorbance at 254 nm in stormflow ranged from 0.102 to 0.649, with a median absorbance of 0.237. UV₂₅₄ was generally well correlated with DOC and increased in a similar manner as urban runoff and other sources contributed to stormflow (fig. 15). Ultraviolet absorbance at 285 nm (UV₂₈₅) was smaller in magnitude but generally well correlated with UV₂₅₄, having a Spearman rank correlation coefficient of $\rho = 0.99$.

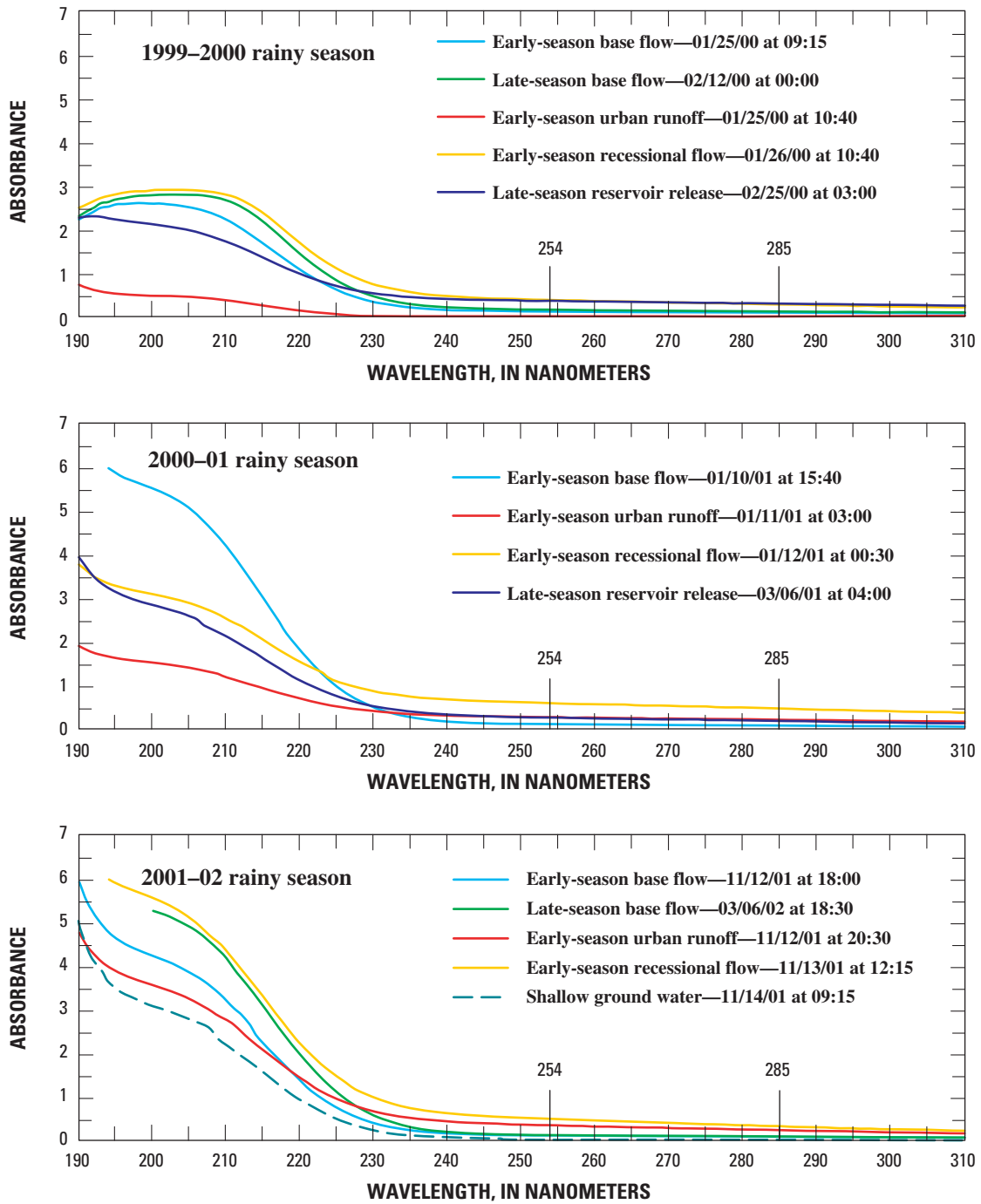


Figure 16. Selected full-spectrum ultraviolet (UV) absorbance scans, Santa Ana River at Imperial Highway, 1999–2000, 2000–01, and 2001–02 rainy seasons.

UV₂₅₄ absorbance data are compared with DOC concentrations in [figure 17](#). The slope of the lines shown in [figure 17](#) have units of DOC in mg/L per absorbance at 254 nm, and the inverse of the slope is conceptually similar to specific UV absorbance (SUVA) discussed in some studies. The data show that there is less UV₂₅₄ absorbance per unit carbon in early season stormflows than in other stormflows. This is consistent with less aromaticity (fewer benzene-like ring structures) in DOC from this stormflow. The equation of the line through the data was not significantly different from the equation used to describe the relation between DOC and UV₂₅₄ absorbance in early season stormflows sampled during 1995–98 in the Santa Ana River at Imperial Highway by Izbicki and others (2000) ([table 6](#)). This result suggests that the DOC in early season stormflows is similar to DOC sampled previously in similar stormflows.

Despite occurring more than 2 months after the usual start of the rainy season, the January 10–14, 2001, stormflow was the first stormflow of that winter's rainy season. The composition of DOC during this stormflow was different from that of the November 12–14, 2001, stormflow and early-season stormflows sampled by Izbicki and others (2000), and was more similar to the composition of DOC in late-season stormflows. Organic carbon that accumulated in the basin during the previous dry season had not been washed off during previous stormflows, but had apparently changed in composition and become more aromatic, resembling the DOC of other late-season stormflows sampled as part of this study. This could occur as a result of the decomposition of simpler, straight-chain, aliphatic carbon compounds such as sugars, starches, and waxes common in plant material.

The equations in [table 6](#) describe the slope of DOC in late-season stormflows, which is significantly less than the slope estimated for similar late-season stormflow data collected by Izbicki and others (2000), and reflect more carbon-carbon double bonds per unit carbon than do previously collected data. Data collected as part of this study were collected under dryer conditions than data collected previously by Izbicki and others (2000). Under the wetter conditions that prevailed during 1995–98, the reservoir behind Prado Dam contained water during the later part of the rainy season. Soils and vegetation within the flooded area contributed DOC to water held behind the dam and downstream reaches of the Santa Ana River. Under the dryer conditions that prevailed during this study, the reservoir behind Prado Dam did not contain water and did not contribute DOC to late-season stormflows.

Comparison of UV₂₅₄ and UV₂₈₅ data ([fig. 17](#)) shows that DOC in the January 10–14, 2001, stormflow has optical properties, and presumably composition, that is more similar to late-season stormflows rather than the first stormflow of the 2000–01 rainy season. This result is consistent with alteration

of the organic carbon prior to the beginning of the rainy season. The slopes of the early-season equations are not significantly different from the slope estimated from similar data collected by Izbicki and others (2000). The optical-property data suggest that the composition of DOC of the early season stormflows sampled as part of this study are similar to the composition of early season stormflows sampled from the Santa Ana River at Imperial Highway between 1995 and 1998 by Izbicki and others (2000).

Excitation/Emission Spectroscopy

In addition to absorbance at selected wavelengths, dissolved organic matter also fluoresces when irradiated at different wavelengths. The technique used in this study to measure this property is known as excitation/emission (EEM) spectroscopy, and the resulting spectra is a three-dimensional map of fluorescence intensity as a function of excitation wavelength and emission wavelength. Like UV absorbance, EEM spectra of natural DOC is a continuum that does not show peaks at specific wavelengths that correspond to specific functional groups or compounds. However, various researchers have identified fluorescence at specific wavelengths that may be associated with proteins (Traganza, 1969; Coble and others, 1990) and humic substances (Coble and others, 1990; Mopper and Shultz, 1993). Other researchers have used EEM spectra for tracking aromatic structures, and unsaturated structures containing carbonyl groups that exhibit strong EEM signals (Kresovitskii and Bolotin, 1988; Skoog and others, 1998).

The intensity of the fluorescence at different excitation and emission wavelengths changed in patterns that were similar from stormflow to stormflow. Numerical approaches that interpret changes in EEM spectra as mixtures from selected source waters (Marhaba and others, 2000) have been used in the Santa Ana River Basin (Brian Bergamaschi, USGS, written commun., 2003). However, quantitative, numerical interpretation of EEM spectra was beyond the scope of this study and no attempt was made to identify specific compounds or functional groups within the dissolved organic matter that contributed to the spectra.

More than 160 excitation/emission spectra were measured on samples collected from eight stormflows during the 1999–2000, 2000–01, 2001–02 rainy seasons. Stormflow samples show a distinct series of changes in fluorescence intensity at different excitation and emission wavelengths as runoff from different sources enters the Santa Ana River during different parts of the stormflow hydrograph. This sequence is illustrated by selected EEM spectra from three sampled stormflows ([fig. 18](#)). The stormflows are discussed from the smallest to the largest stormflow.

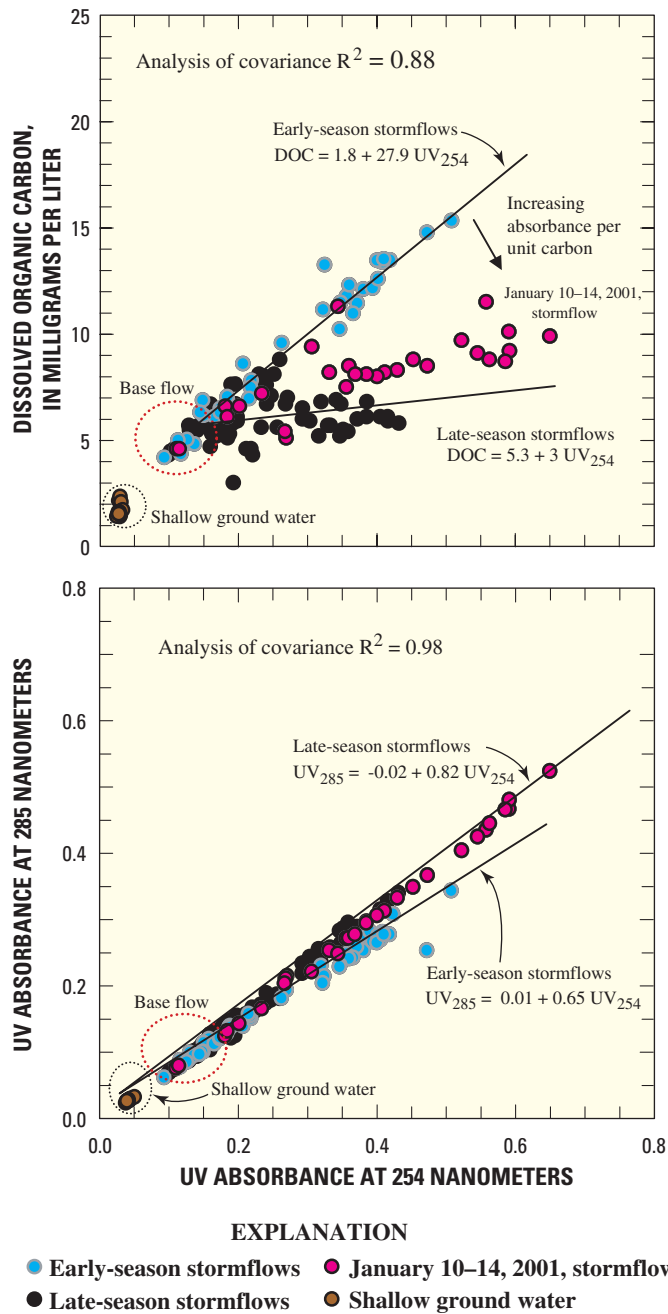


Figure 17. Dissolved organic carbon (DOC) as a function of UV_{254} , and UV_{285} as a function of UV_{254} , in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons. UV, ultraviolet.

38 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Table 6. Equations describing the relation between dissolved organic carbon (DOC) and ultraviolet (UV) absorbance in early-season and late-season stormflows in the Santa Ana River, California

[1995–98 data modified from Izbicki and others, 2000; DOC, dissolved organic carbon; UV₂₅₄, ultraviolet absorbance at 254 nanometers; UV₂₈₅, ultraviolet absorbance at 285 nanometers]

Data-collection period	Early-season stormflows	Late-season stormflows
1995–98	$\text{DOC} = 1.9 + 28.1 \text{ UV}_{254}$	$\text{DOC} = 1.9 + 23.2 \text{ UV}_{254}$
2000–02	$\text{DOC} = 1.8 + 27.9 \text{ UV}_{254}$	$\text{DOC} = 5.3 + 3 \text{ UV}_{254}$
1995–98	$\text{UV}_{285} = 0.78 \text{ UV}_{254} - 0.01$	$\text{UV}_{285} = 0.69 \text{ UV}_{254}$
2000–02	$\text{UV}_{285} = 0.82 \text{ UV}_{254} - 0.02$	$\text{UV}_{285} = 0.01 + 0.65 \text{ UV}_{254}$

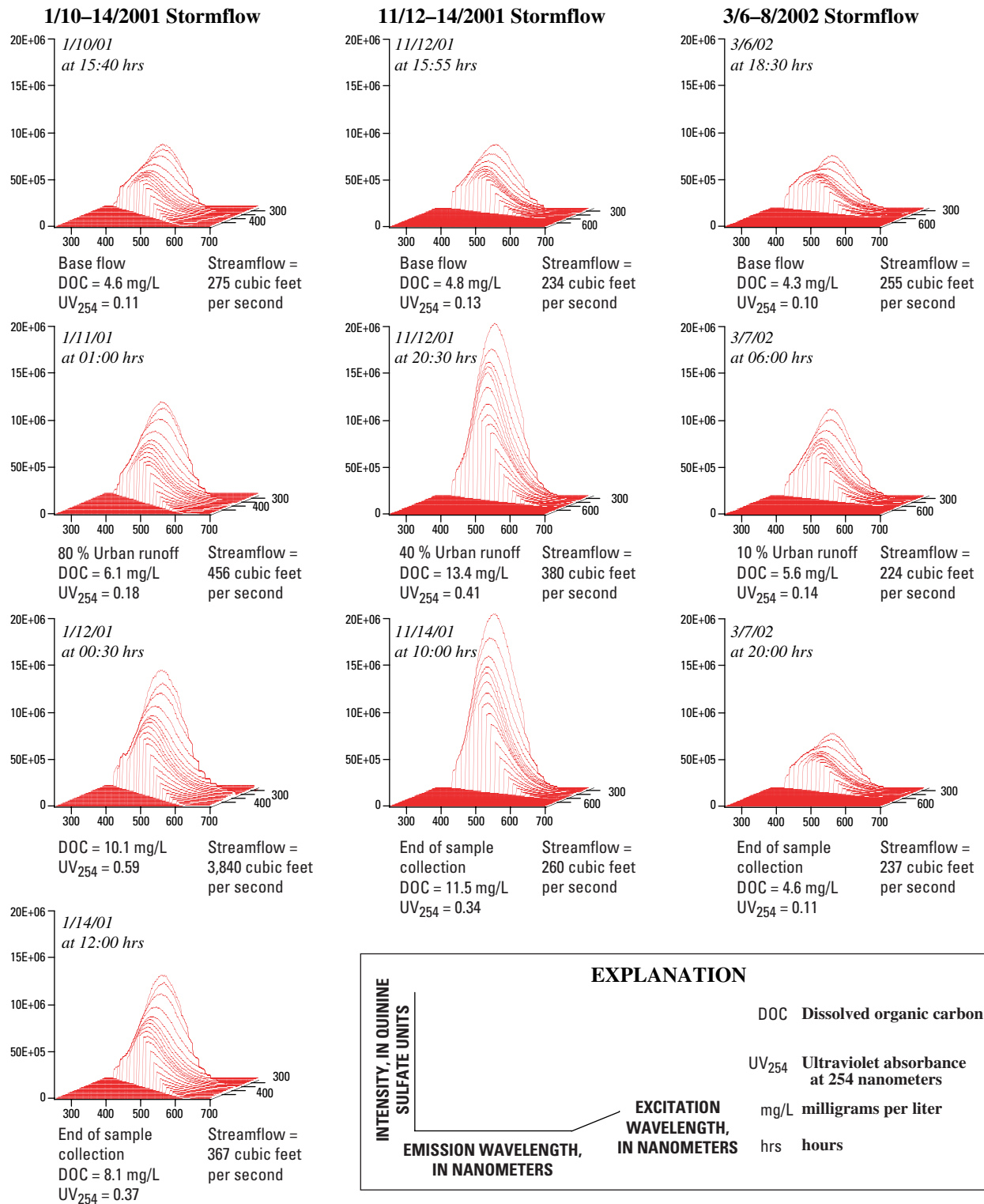


Figure 18. Selected excitation/emission spectra for three stormflows in the Santa Ana River at Imperial Highway, southern California.

40 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Data collected during the March 6–8, 2002, stormflow (fig. 18) show that EEM spectra are sensitive to even small changes in DOC concentrations and composition. This late-season stormflow was one of the smallest stormflows sampled during this study and only 0.25 in. of precipitation was measured at Prado Dam. DOC concentrations ranged from 4.3 to 7.6 mg/L (Appendix C), and changes in total and fecal coliform bacteria concentrations during this stormflow were small (Appendix A). DOC and bacteria concentrations were difficult to relate to stormflow, because most of the precipitation fell near the coast with only small, scattered showers farther inland that produced almost no runoff from upstream parts of the basin, and because flow in the Santa Ana River decreased as a result of regulation at Prado Dam. Despite the small amount of precipitation and subsequent runoff, and only small changes in the DOC concentrations, there are measurable changes in the intensity and shape of the EEM spectra as runoff from urban areas downstream from Prado Dam entered the Santa Ana River on March 7 at 0600 (fig. 18). For example, samples collected prior to the onset of stormflow on March 6 at 1830 have relatively high intensity at excitation wavelengths near 285 nm and emission wavelengths near 375 nm that give the spectra a pronounced “shoulder” in that region (fig. 18). This shoulder is not present in the EEM spectra from samples collected on March 7 at 0600 when runoff from urban areas was present. By 2000 on March 7, the EEM spectra from DOC in the river had returned to pre-storm conditions (fig. 18).

Data collected during the November 12–14, 2001, stormflow show a pattern similar to that observed during the March 6–8, 2002, stormflow. This was the first stormflow of the 2001–02 rainy season and, although only 0.5 in. of precipitation was measured at Prado Dam, most precipitation fell during a short period at the beginning of the storm and precipitation occurred throughout the basin. DOC concentrations ranged from 4.8 to 13.5 mg/L (Appendix C). The changes in total and fecal coliform bacteria concentrations were large during this stormflow and during the recession of this stormflow. Total coliform bacteria concentrations were as high as 3,400,000 colonies per 100 mL and fecal coliform bacteria concentrations were as high as 310,000 colonies per 100 mL (Appendix A). These were the highest concentrations measured in the Santa Ana River at Imperial Highway during this study. The EEM spectra from samples collected prior to the storm on November 12 at 1555 (fig. 18) were similar in magnitude to the samples collected prior to the March 6–8, 2002, stormflow and to most other samples collected prior to the beginning of stormflow; the spectra for the November 12, at 1555, samples had the distinctive “shoulder” near an excitation wavelength of 280 nm and an emission wavelength near 350 nm. The intensity and shape of

the EEM spectra changed by November 12, at 2030, when runoff from urban areas downstream from Prado Dam and associated DOC were present. Unlike the March 6–8, 2002, stormflow, the EEM spectra did not return to pre-storm conditions at any time during the sampling period. This is because DOC from runoff from urban areas upstream from Prado Dam continued to dominate the EEM spectra even though streamflow returned to pre-storm conditions. Total and fecal coliform bacteria concentrations also remained high throughout the stormflow sample-collection period.

Changes in EEM spectra for samples collected during the January 10–14, 2001, stormflow are similar to changes measured on samples collected during the November 12–14, 2001, stormflow (fig. 18)—but the EEM spectra are lower in intensity, presumably owing to the lower DOC concentrations. Despite the lower DOC concentrations and lower EEM intensities, the sample collected January 11 at 0100 had the highest percentage of runoff from urban areas downstream from Prado Dam (slightly more than 80 percent) of all samples collected during this study. These samples illustrate that there is not a 1 to 1 correspondence between runoff from selected sources and the DOC concentration of water from that source. However, the EEM spectra suggest that the composition of DOC in stormflow may remain relatively constant despite changing antecedent conditions that may change the concentration. The intensity and shape of the EEM spectra for samples collected during the January 10–14, 2001, stormflow did not return to pre-storm conditions during the sample-collection period; similarly total and fecal coliform bacteria concentrations remained high during the sample-collection period in a pattern similar to that of the November 12–14, 2001, stormflow.

Dissolved Organic Carbon Fractionation

Operational fractions of DOC, defined by their ability to pass-through or sorb on XAD8 or XAD4 resins under different pHs (Aiken and others, 1992), were measured for two stormflows sampled during the 2001–02 rainy season. The fractions include hydrophobic acids, hydrophilic acids, transhydrophilic acids, and the hydrophobic-neutral fractions of the DOC.

The hydrophobic-acid fraction typically consists of aliphatic carboxylic acids having five to nine carbons, one- and two-ring aromatic carboxylic acids, one- and two-ring phenols, and aquatic humic and fulvic substances (Aiken and others, 1992). Hydrophobic acids typically account for about 30 percent of the DOC in natural water but may account for as much as 50 to 90 percent of the DOC in highly colored water (Thurman, 1986).

The hydrophilic-acid fraction typically consists of more readily soluble compounds and may contain sugar acids, alcohols, polyfunctional organic acids, and aliphatic acids with five or fewer carbon atoms (Thurman, 1986; Aiken and others, 1992). This fraction contains fewer aromatic compounds; therefore, it should contain relatively fewer THM and DBP precursors in comparison with the hydrophobic-acid fraction (Fujii and others, 1998).

The hydrophobic acids were the largest fraction within the DOC during both base flow and stormflow, composing about 33 percent (median value) of the DOC during the two sampled stormflows (table 7). The DOC concentration and the percent hydrophobic acid increased during both stormflows and composed as much as 45 percent of the DOC during the recessional flow of the November 12–14, 2001, stormflow. The highest concentrations were present during the recessional flow. Hydrophilic acids were the next largest fraction within the DOC. The concentrations and percent of total DOC were similar for hydrophobic and hydrophilic acids for pre-storm samples; however, the hydrophilic-acid fraction decreased during stormflow to slightly more than 20 percent of the total DOC—although concentrations of hydrophilic acids subsequently increased during the recessional flows of both sampled stormflows. These results are consistent with increased aromaticity in DOC composition in the recession of stormflows in Santa Ana River inferred from UV absorbance measurements by Izbicki and others (2000) and with UV absorbance data presented in this report (fig. 17). The transhydrophilic-acid fraction remained relatively constant, between 21 and 13 percent of the DOC, during stormflow, although concentrations increased with increasing DOC concentrations (table 7). The hydrophobic-neutral fraction varied widely during stormflow, ranging between 2 and 29 percent of the total DOC. The highest hydrophobic-neutral fractions were present in samples collected during the transition from stormflow dominated by urban runoff from the area downstream from Prado Dam to the recessional flows dominated by runoff from the area upstream from Prado Dam. Motor oils and many other relatively insoluble contaminants associated with runoff from city streets may be within this fraction. These compounds composed a large fraction of the

DOC on the rising limb of the hydrograph after hydrophobic acids from urban areas downstream from Prado Dam were washed from the basin.

Total Coliform Bacteria, Fecal Coliform Bacteria, and Dissolved Organic Carbon Concentrations in Shallow Ground Water during Stormflow

Samples of shallow ground water were collected during stormflow in the 2001–02 rainy season from the subsurface-collection and recharge system (SCARS) operated by OCWD and analyzed for fecal indicator bacteria, DOC concentrations and optical properties. The SCARS system collects water by gravity drainage through three, 1,000-foot-long, 6-inch-diameter perforated pipes buried about 4 ft below the bed of the OCWD off-channel recharge facility along side of the Santa Ana River about 1 mi. downstream from Imperial Highway (Gregory Woodside, Orange County Water District, unpub. data, 2001).

Total coliform bacteria concentrations in shallow ground water collected from the SCARS during two stormflows during the 2001–02 rainy season were low and ranged from less than the detection limit of 10 to 130 colonies per 100 mL (Appendix A). Smaller changes in concentrations were measured for fecal coliforms, *E. coli*, and enterococci bacteria, which rarely exceeded the detection limit of 10 colonies per 100 mL (Appendix A). Similarly, DOC concentrations measured in shallow ground water collected from the SCARS during two stormflows during the 2001–02 rainy season were low and ranged from 1.4 to 2.4 mg/L (Appendix C). DOC concentrations showed only small increases during stormflows. UV absorbance values also were low. These results suggest that most bacteria and much of the DOC are removed as water infiltrates through the bed of the Santa Ana River.

42 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Table 7. Dissolved organic carbon (DOC) concentrations and hydrophobic-acid, hydrophobic-neutral, hydrophilic-acid, and transhydrophilic-acid fractions of dissolved organic carbon in stormflow samples collected from the Santa Ana River at Imperial Highway and from Mill Creek at Chino-Corona Road, southern California, 2001–02 rainy season

[Samples from the Santa Ana River at Imperial Highway collected by the U.S. Geological Survey, samples from Mill Creek at Chino–Corona Road collected by Orange County Water District; all samples analyzed by the U.S. Geological Survey laboratory in Boulder, Colorado; results for dissolved organic carbon may differ slightly from results in Appendix C; mg/L, milligrams per liter; percent, percent of dissolved organic carbon; —, no data]

Sample date	Time	Dissolved organic carbon	Hydrophobic-acid-fraction		Hydrophobic-neutral-fraction		Hydrophilic-acid-fraction		Transhydrophilic-acid-fraction	
		(mg/L)	(mg/L)	(percent)	(mg/L)	(percent)	(mg/L)	(percent)	(mg/L)	(percent)
Santa Ana River at Imperial Highway										
11/12/01	1800	5.9	2.2	37	0.1	2	2.2	37	1.2	21
11/12/01	2030	10.6	4.1	39	.3	3	2.7	25	1.7	16
11/12/01	2240	7.5	2.2	30	1.8	24	1.9	25	1.1	15
11/13/01	0100	6.9	1.9	27	2.0	29	1.8	26	1.0	14
11/13/01	0340	5.9	1.9	33	.8	13	1.8	30	1.1	18
11/13/01	1215	18.5	7.9	43	2.0	11	4.3	23	3.2	17
11/14/01	0800	12.4	5.6	45	.9	7	3.1	25	2.1	17
03/06/02	1830	4.5	1.5	33	.8	17	1.4	32	.8	17
03/07/02	0650	4.9	1.7	34	.8	17	1.5	30	.8	17
03/07/02	1030	5.2	1.8	34	.9	18	1.5	29	.8	15
03/07/02	1330	5.3	1.5	29	1.2	22	1.5	28	.9	17
03/07/02	1630	5.0	1.5	30	1.1	21	1.4	28	.8	15
03/07/02	2300	5.1	1.6	32	.9	18	1.5	29	.8	15
03/08/02	0700	8.6	3.2	37	2.0	23	2.1	24	1.1	13
Mill Creek at Chino-Corona Road										
11/12/01	1720	5.2	1.4	26	.2	4	2.1	41	.9	17
11/12/01	1945	5.8	1.6	28	.5	8	2.2	38	.9	16
11/12/01	2015	34.4	15.5	45	3.4	10	7.6	22	5.2	15
11/12/01	2030	35.0	15.4	44	3.9	11	7.7	22	5.3	15
11/12/01	2045	43.5	20.4	47	4.4	10	9.1	21	6.5	15
11/12/01	2100	28.7	12.9	45	3.2	11	6.3	22	4.3	15
11/12/01	2115	29.8	—	—	—	—	—	—	—	—
11/12/01	2130	27.6	—	—	—	—	—	—	—	—
11/12/01	2145	25.9	11.9	46	1.3	5	8.3	32	4.1	16
11/12/01	2200	23.3	10.5	45	1.9	8	6.5	28	3.7	16
11/12/01	2245	28.0	12.9	46	3.4	12	6.2	22	4.2	15

Conclusions

Total and fecal indicator bacteria concentrations increased in the Santa Ana River at Imperial Highway during stormflow. Total coliform bacteria concentrations in stormflow were as high as 3,400,000 colonies per 100 mL. Fecal coliforms, *Escherichia coli*, and enterococci concentrations were as high as 310,000, 84,000, and 100,000 colonies per 100 mL, respectively. The highest concentrations were measured during the first stormflow of the winter rainy season. Although runoff from urban areas downstream from Prado Dam contributed high concentrations of fecal indicator bacteria, the concentrations measured in the recession flows of the first stormflow of the rainy season—when runoff was derived from areas upstream from Prado Dam—were as much as an order of magnitude higher. High concentrations of fecal indicator bacteria persisted even after the river returned to pre-stormflows and were not likely to have resulted solely from urban runoff.

The highest total and fecal coliform bacteria concentrations (7,300,000 and 530,000 colonies per 100 mL, respectively) measured by OCWD personnel as part of this study were from Mill Creek, downstream from dairy operations and upstream from Prado Dam. Although bacteria concentrations were not measured in the main stem of the Santa Ana River upstream from Prado Dam or other tributaries, runoff from this area directly contributes to high bacteria concentrations measured farther downstream.

Direct runoff of fecal bacteria occurs during stormflow, especially from urban areas, but the occurrence and distribution of the highest total and fecal coliform indicator bacteria concentrations in stormflow also may fit a conceptual model proposed by Matson and others (1978). In this model, bacteria accumulate and survive for extended periods in streambed sediments and are mobilized as those sediments are scoured when flow velocities increase during stormflow. However, data collected as part of this study show that high bacteria concentrations persist after flow returns to pre-storm conditions. To explain the persistence of bacteria, the model initially proposed by Matson and others (1978) could be modified to include a period after the cessation of stormflow when recently disturbed sediment equilibrates with the overlying water.

Because accumulation and survival of bacteria in streambed sediments was not measured as part of this study, data are not available to test the suitability of the model directly. However, molecular microbiological data, including phospholipid fatty acid (PLFA) and genetic profile data, collected as part of this study provide information on changes in the microbial population that occur during stormflow and the

nature of the source responsible for these changes. These data are consistent with the proposed modifications to Matson and others (1978) model.

More than 80 phospholipid fatty acids, reflecting specific metabolic processes by different bacteria that comprise the total microbial population, were detected in stormflow. PLFA concentrations ranged from 18,200 to 725,000 picomoles per liter and increased with bacteria concentrations. Fatty acid concentrations were distributed in a similar manner to bacteria during stormflow and concentrations often remained high after flow returned to pre-storm conditions. In general, fatty acids decreased in diversity and were dominated by a few monoenoic fatty acids as concentrations increased—especially during the recession flow of the first stormflow of the rainy season. The source of bacteria contributing fatty acids to the total bacterial population sampled during stormflow must be poorly diverse and dominated by only a few types of bacteria. Samples from specific sources within the Santa Ana River Basin suggest that changes in fatty-acid concentrations measured during stormflow could be explained by fatty-acid concentrations in runoff from urban storm drains and streambed material. Fatty acids associated with dairy-waste storage ponds were not commonly measured in stormflows sampled during this study.

Genetic profiles from the hypervariable region within the 16S rRNA gene, amplified using polymerase chain reaction (PCR) analyzed without and with endonuclease digestion (T-RFLP) showed decreases in the diversity of the total microbial population with increasing total and fecal coliform bacteria concentrations during stormflow. Results of quantitative analysis of the genetic profiles obtained with endonuclease digestion (T-RFLP) were similar to the results obtained on the basis of the PLFA data. However, the genetic profile data are more specific than PLFA data and indicate a wide range of bacterial organisms that are unique to different parts of the stormflow hydrograph and, presumably, to runoff from different sources in the basin. Quantitative analysis of the genetic profile data obtained without endonuclease digestion produced different results from either the analysis of PLFA data or the genetic profiles obtained using T-RFLP. This result suggests that the digestion of the larger fragments is necessary to obtain an accurate genetic profile of the changes in microbial community that occur during stormflows containing runoff and bacteria from different sources. Development of a library of the genetic make-up and base-pair sequences of bacteria from different sources and comparison of stormflow data to this library, a technique commonly known as microbial “source tracking” was beyond the scope of this study, but comparison of genetic profiles from dairy-waste storage ponds showed that organisms from the waste pond were not present in stormflow.

44 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

The DOC concentration and composition changed rapidly during stormflow and did not return to pre-storm conditions during the sample-collection period but typically remained high for several days after the storm ceased. Comparison of ultraviolet absorbance data with similar data collected between 1995 and 1998 suggests that the composition of DOC from many stormflows sampled as part of this study was different from the composition of DOC in stormflows sampled previously from the Santa Ana River. This may limit interpretations from this study to similar dry periods, and additional data collection may be required before interpretations can be extended to wetter periods.

Total coliform bacteria, fecal indicator bacteria, and DOC concentrations in shallow ground water during the 2001–02 rainy season were low and showed only small changes during stormflows. These data suggest that most bacteria and much of the DOC are removed as water infiltrates through the bed of the Santa Ana River.

References Cited

- Aiken, G.R., McKnight, D.M., Thorn, K.A., and Thurman, E.M., 1992, Isolation of hydrophilic organic acids from water using nonionic macroporous resins: *Organic Geochemistry*, v. 18, no. 4, p. 567–573.
- American Public Health Association, 1998, *Standard methods for the analysis of water and wastewater* (20th ed.): Washington, D.C., variously paged.
- Avaniss-Aghajani, E., Jones, K., Holtzman, A., Aronson, T., Glover, N., Boian, M., Froman, S. and Brunk, C.F., 1996, Molecular technique for rapid identification of *mycobacteria*: *Journal of Clinical Microbiology*, v. 34, p. 98–102.
- Barber, L.H., II, 1992, Hierarchical analytical approach to evaluating the transport and biogeochemical fate of organic compounds in sewage-contaminated ground water, Cape Cod, Massachusetts, in Lesage, S., and Jackson, R.E., eds, *Groundwater contamination and analysis at hazardous waste sites*: New York, Marcel Dekker, Inc., p. 753–770.
- Bird, S.M., Fram, M.S., and Crepeau, K.L., 2003, *Method of Analysis by the U.S. Geological Survey California District Sacramento Laboratory—Determination of Dissolved Organic Carbon in Water by High Temperature Catalytic Oxidation, Method Validation, and Quality-Control Practices*, U.S. Geological Survey Open-File Report 03-366, <http://pubs.water.usgs.gov/ofr03-366/>
- Belitz, Kenneth, 1999, Santa Ana Basin: National Water Quality Assessment Program, U.S. Geological Survey Fact Sheet 054-99.
- Bernhard, A.E., and Field, K.G., 2000, Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S Ribosomal DNA genetic markers from fecal anaerobes: *Applied and Environmental Microbiology*, v. 66, no. 4, p. 1587–1594.
- Bernstein, B.B., Griffith, J.F., and Weisberg, S.B. (eds.), 2002, *Microbiological source tracking workshop: summary of proceedings*: U.S. Environmental Protection Agency Workshop on Microbial Source Tracking, February 5, 2002, Irvine, Calif., 78 p.
- Bruce, K.D., 1997, Analysis of *mer* gene subclasses within bacterial communities in soils and sediments resolved by fluorescent-PCR-Restriction Fragment Length Polymorphism profiling: *Applied and Environmental Microbiology*, v. 63, no. 12, p. 4914–4919.
- Brunk, C.F., Avaniss-Aghajani, E., and Brunk, C.A., 1996, A computer analysis of primer and probe hybridization potential with bacterial small-subunit rRNA sequences: *Applied and Environmental Microbiology*, v. 62, p. 872–879.
- Burton, G.A., Gunnison, Douglas, and Lanza, G.R., 1987, Survival of pathogenic bacteria in various freshwater sediments: *Applied and Environmental Microbiology*, v. 53, no. 4, p. 633–638.
- Burton, C.A., Izbicki, J.A., and Paybins, K.S., 1998, Water-quality trends in the Santa Ana River at MWD Crossing and below Prado Dam, Riverside County, California: U.S. Geological Survey Water-Resources Investigations Report 97-4173, 36 p.
- California Department of Water Resources, 1994, *Five-year report of the municipal water quality investigations program*: variously paged.
- Cavigelli, M.A., Robertson, G.P., and Klug, M.J., 1995, Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure, *Plant and Soil*, v. 170, p. 99–113.
- Coble, P.G., Green, S.A., Blough, N.V., and Gagosian, R.B., 1990, Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy: *Nature*, v. 348, p. 432–435.
- Davisson, M.L., Hudson, G.B., Herndon, R.L., Niemeyer, S., and Beiriger, J., 1996, Report on the feasibility of using isotopes to source and age-date groundwater in Orange County Water Districts Forebay Region, Orange County California. Lawrence Livermore National Laboratory Isotope Sciences Division, May 1996 (UCRL-ID-123953).
- Ding, W.H., Wu, J., Semadeni, M., Reinhard, J., 1999, Occurrence of wastewater indicators in the Santa Ana River and impacted into groundwater: *Chemosphere*, v. 39, p. 1781–1794.
- Dowling, N.J., Widdel, F., and White, D.C., 1986, Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulfate reducers and other sulfide forming bacteria: *Journal of General Microbiology*, v. 132, p. 1815–1825.
- Dunbar, John, Ticknor, L.O., Kuske, C.R., 2000, Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene Terminal-Restriction Fragment Analysis: *Applied and Environmental Microbiology*, v. 66, no. 7, p. 2943–2950.

- Edlund, A., Nichols, P.D., Roffey, R., and White, D.C., 1985, Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species, *Journal of Lipid Research*, v. 26, p. 982–988.
- Francy, D.S., Hart, T.L., and Virostek, C.M., 1996, Effects of receiving-water quality and wastewater treatment on injury, survival, and regrowth of fecal indicator bacteria and implications for assessment of recreational water quality: U.S. Geological Survey Water-Resources-Investigations Report WRIR 96-4199, <http://oh.water.usgs.gov/reports/111/rpt96.4199.html>
- Fujii, Roger, Ranalli, A.J., Aiken, G.R., and Bergamaschi, B.A., 1998, Dissolved organic carbon concentrations and compositions, and trihalomethane formation potentials in waters from agricultural peat soils, Sacramento–San Joaquin Delta, California: Implications for drinking-water quality: U.S. Geological Survey Water-Resources Investigations Report 98-4147, 75 p. <http://ca.water.usgs.gov/archive/reports/wrir984147/>
- Gamlin, J.D., Clark, J.F., Woodside, G., and Herndon, R., 2001, Large-scale tracing of ground water with sulfur hexafluoride: *J. Environmental Engineering*, February, p. 171–174.
- Gnanadesikan, R., 1977, *Methods for statistical data analysis of multivariate observations*: New York, John Wiley and Sons, 311 p.
- Goodman, D., 1975, The theory of diversity-stability relationships in ecology. *Quarterly Review of Biology*, v. 50, p. 237–266.
- Gray, K.A., McAuliffe, K.S., Bornick, Robert, and Simpson, Allen, 1996, Evaluation of organic quality in Prado wetlands and Santa Ana River by pyrolysis-GC-MS: Department of Civil Engineering, Northwestern University, 100 p.
- Gutsche, C.D., and Pasto, D.J., 1975, *Fundamentals of organic chemistry*: Englewood Cliffs, New Jersey, Prentice-Hall Inc., 1,249 p.
- Haack, S.K., Garchow, Helen, Odelson, D.A., Forney, L.J., and Klug, M.J., 1994, Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities: *Applied and Environmental Microbiology*, v. 60, no. 7, p. 2483–2493.
- Hart, H., and Schuetz, R.D., 1972, *Organic chemistry: A short course (4th ed.)*: Boston, Houghton Mifflin Company, 499 p.
- Hartel, P.G., Summer, J.D., Hill, J.L., Collins, Victoria, Entry, J.A., and Segars, W.I., 2002, Geographic variability of *Escherichia coli* ribotypes from animals in Idaho and Georgia: *Journal of Environmental Quality* v. 31, p. 1273–1278.
- Herndon, R.L., Woodside, G.D., Davisson, M.L., Hudson, G.B., 2003, Use of isotopes to estimate groundwater age and flow path: *Southwest Hydrology*, v. 2, No. 1, p. 24–25 and 36.
- Izbicki, J.A., Mendez, G.O., and Burton, C.A., 2000, Stormflow chemistry in the Santa Ana River below Prado Dam and at the diversion downstream from Imperial Highway, southern California, 1995–98. U.S. Geological Survey Water-Resources Investigations Report 00-4127, 92 p.
- Kern, J., Petrauskas, B., McClellan, P., Shanholtz, V.O., and Hagedorn, C., 2002, Bacterial source tracking: a tool for total maximum daily load development. In: Younos, T. (ed.), *Advances in water monitoring research: Water-Resources Publications, LLC*, p.125–167
- Krazner, S.W., Croue, J.P., Buffle, Jacques, Perdue, E.M., 1996, Three approaches for characterizing NOM: *Journal of American Water Works Association*, v. 88, p. 66–79.
- Kresovitskii, B. M., and Bolotin, B. M., 1988, *Organic Luminescent Materials*: Weinheim, Verlagsgesellschaft, 340 p.
- Kshirsagar, A.M., 1972, *Multivariate analysis*: New York, Marcel Dekker, 534 p.
- Leecaster, M.K., and Weisberg, S.B., 2001, Effect of sampling frequency on shoreline microbiology assessments: *Marine Pollution Bulletin*, v. 42, no. 11, p. 1150–1154.
- Leenheer, J.A., and Huffman, E.W.D., Jr., 1976, Classification of organic solutes in water by using macroreticular resins: *Journal of Research of the U.S. Geological Survey*, v. 4, no. 6, p. 737–751.
- Leenheer, J.A., Rostad, C.E., Barber, L.B., Schroeder, R.A., Anders, R., and Davisson, M.L., 2001, Nature and chlorine reactivity of organic constituents from reclaimed water in groundwater, Los Angeles County, California: *Environmental Science and Technology*, v. 35, no. 19, p. 3869–3876.
- Liu, Wen-Tso, Marsh, T.L., Cheng, Hans, and Forney, L.J., 1997, Characterization of microbial diversity by determining Terminal-Restriction Fragment Length Polymorphism of genes encoding 16S rRNA. *Applied and Environmental Microbiology*, v. 63, no. 11, p. 4516–4522.
- Los Angeles Times, 2002, L.A. suffers from driest rain season, *Los Angeles Times*, Sunday, June 30, 2002.
- Marhaba, T.F., Lippincott, R.L., and Van, Doanh, 2000, Characterizing natural organic carbon fractions using spectral fluorescent signatures and post processing by principal component analysis: *Forsenius Journal of Analytical Chemistry*, v. 366, no. 1, p. 22–25.
- Matson, E.A., Horner, S.G., and Buck, J.D., 1978, Pollution indicators and other microorganisms in river sediments: *Journal of Water Pollution Control Federation*, v. 50, p. 13–19.
- Microbial ID, Inc., 1992, *Microbial Identification System Operating Manual, Version 4*, Newark DE, USA.
- Mopper, K., and Schultz, C.A., 1993, Fluorescence as a possible tool for studying the nature and water column distribution of DOC components: *Marine Chemistry*, v. 41, p. 229–238.

46 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

- Morrison, D.F., 1976, *Multivariate statistical methods* (2nd ed.): New York, McGraw-Hill, 415 p.
- Myers, D.N., Koltun, G.F., and Francy, D.S., 1998, *Effects of Hydrologic, Biological, and Environmental Processes on Sources and Concentrations of Fecal Bacteria in the Cuyahoga River, with Implications for Management of Recreational Waters in Summit and Cuyahoga Counties, Ohio*, U.S. Geological Survey Water-Resources Investigations Report 98-4089, <http://oh.water.usgs.gov/reports/Abstracts/wrir.98-4089.html>.
- Nobel, R.T., Dorsey, J.H., Leecaster, M.K., Orozco-Borbon, Victoria, Reid, Daniel, Schiff, K.C., and Weisberg, S.B., 2000, A regional survey of the microbiological water quality along the shoreline of the Southern California Bight, *Environmental Monitoring and Assessment*, v. 64, p. 435–447.
- Paul, E.A., and Clark, F.E., 1996, *Soil microbiology and biochemistry*: New York, Academic Press, 340 p.
- Preisendorfer, R.W., Zwiers, F.W., and Barnett, T.P., 1981, *Foundations of principal component selection rules: SIO Reference Series 81-4*: La Jolla, California, Scripps Institute of Oceanography, 191 p.
- Reckhow, D.A., Singer, P.C., and Malcolm, R.L., 1990, Chlorination of humic materials: byproduct formation and chemical interpretations, *Environmental Science and Technology*, v. 24, p. 1655–1664.
- Reinhard, M., Ding, W.H., Fujita, Y., Semadeni, M., and Wu, J., 1996, Behavior and fate of organic contaminants during groundwater recharge with reclaimed wastewater and Santa Ana River Water—A field and laboratory investigation. Technical Report No. 322, Department of Civil Engineering, Stanford University, variously paged.
- Rostad, Colleen, 2002, Fate of disinfection by-products in the subsurface. In: Aiken, G.A., and Kuniandy, E.L., (eds.), *U.S. Geological Survey Artificial Recharge Workshop Proceedings*, Sacramento, California, April 2–4, 2002. U.S. Geological Survey Open-File Report 02-86, p. 27–30. <http://water.usgs.gov/ogw/pubs/ofr0289/index.htm>
- Rostad, C.E., Leenheer, J.A., Katz, B., Martin, B.S., and Noyes, T.I., 2000, Characterization and disinfection by-product formation potential of natural organic matter in surface and ground waters from northern Florida, Chapter 11 in *Natural Organic Matter and Disinfection By-Products* (S.E. Barrett, S.W. Krasner, and G. Amy, Eds.), ACS Symposium Series 761, American Chemical Society, Wash. D.C., p. 154–172.
- Samadpour, Mansour, 2002, Microbial source tracking: principles and practice, in: Bernstein, B.B., Griffith, J.F., and Weisberg, S.B. (eds.), *Microbiological source tracking workshop: summary of proceedings U.S. Environmental Protection Agency Workshop on Microbial Source Tracking*, February 5, 2002, Irvine, California, p. 5–9.
- Santa Ana Regional Water Quality Control Board, 1995, *Water Quality Control Plan: Santa Ana Region VIII*, Riverside, California [variously paged].
- Schiff, K.C., and Kinney, Patrick, 2001, Tracking sources of bacterial contamination in stormwater discharges to Mission Bay, California: *Water Environment Research*, v. 73, no. 5, p. 534–542.
- Schiff, K.C., and Stevenson, Marty, 1996, San Diego regional storm water monitoring program: contaminant inputs to coastal wetlands and bays, *Bulletin of Southern California Academy of Sciences*, v. 95, p. 7–16.
- Schiff, K.C., Weisberg, S.B., and Dorsey, J.H., 2001, Environmental auditing: microbiological monitoring of marine recreational waters in southern California: *Environmental Management*, v. 27, no. 1, p. 149–157.
- Schroeder, R.A., and Anders, Robert, 2002, Transport and fate of water-quality indicators after 40 years of artificial recharge with treated municipal wastewater to the central ground-water basin in Los Angeles County. In: Aiken, G.A., and Kuniandy, E.L. (eds.), *U.S. Geological Survey Artificial Recharge Workshop Proceedings*, Sacramento, California, April 2–4, 2002. U.S. Geological Survey Open-File Report 02-89, p. 42–46. <http://water.usgs.gov/ogw/pubs/ofr0289/index.htm>
- Skoog, D. A., Holler, F. J., and Nieman, T. A., 1998, *Principals of instrumental analysis*: Orlando, Harcourt Brace and Company, 849 p.
- Thurman, E.M., 1986, *Organic geochemistry of natural waters*: Boston, Nijhoff and Junk, 497 p.
- Traganza, E.D., 1969, Fluorescence excitation and emission spectra of dissolved organic matter in seawater: *Bulletin of Marine Science*, v. 19, p. 897–904.
- Tunlid, A., and White, D.C., 1992, Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soils, in: Stotzky, G., and Bollang, J.M. (eds.), *Soil Biochemistry*, New York, Dekker, p. 229–262.
- U.S. Army Corps of Engineers, 1994, *Water control manual, Prado Dam and Reservoir, Santa Ana River, California*: 83 p.
- U.S. Environmental Protection Agency, 2000, *Improved enumeration methods for the recreational water quality indicators: enterococci and Escherichia Coli*, EPA/821/R-97/004, Office of Science and Technology, Washington, D.C. 53 p.
- Vestal, J.R., and White, D.C., 1989, *Lipid analysis in microbial ecology*, Biosciences, v. 39, p. 535–541.
- Von Donsel, D.J., and Geldreich, 1971, Relationships of Salmonellae to fecal coliforms in bottom sediments: *Water Research* v. 5, p. 1079–1087.
- Wheeler, A.L., Hartel, P.G., Godfrey, D.G., Hill, J.L., Segars, W.I., 2002, Potential of Enterococcus faecalis as a human fecal indicator for microbial source tracking: *Journal of Environmental Quality* v. 31 p. 1286–1293.

- White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobie, R.J., 1979, Determination of the sedimentary microbial biomass of extractable liquid phosphate: *Oecologia*, v. 40, p. 51–62.
- White, D.C., 1994, Is there anything else you need to understand about the microbiota that cannot be derived from analysis of nucleic acids. *Microbial Ecology*, v. 28, p. 163–166.
- White, D.C., Stair, J.O., and Ringelberg, D.B., 1996, Quantitative comparisons of insitu microbial biodiversity by signature biomarker analysis: *Journal of Industrial Microbiology*, v. 17, p. 185–196.

Appendixes

50 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix A. Total coliforms, fecal coliforms, *Escherichia coli*, enterococci, and suspended-sediment concentrations for stormflow in the Santa Ana River at Imperial Highway, in Mill Creek, and in shallow ground water from the Santa Ana River Basin, southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons

[Indicator bacteria measured as most probable number per 100 milliliters (MPN/100 mL), except for storms on 11/2001 and 3/2002, which are in Colony Forming Units per 100 milliliters (CFU/100 mL); 11075600, Santa Ana River at Imperial Highway; Mill Creek, Mill Creek at Chino-Corona Road; SCARS, subsurface collection and recharge system; samples collected by U.S. Geological Survey and analyzed by Orange County Public Health Laboratory; streamflow data collected by the Orange County Water District; ft³/s, cubic feet per second; mg/L, milligrams per liter; <, less than; >, greater than; ≥, greater than or equal to; —, no data]

Station No.	Date	Time	Stream-flow (ft ³ /s)	Total coliforms (MPN/100 mL)	Fecal coliforms (MPN/100 mL)	<i>Escherichia coli</i> (MPN/100 mL)	Enterococci (MPN/100 mL)	Suspended sediment (in mg/L)
11075600	11/18/1999	0830	—	16,000	—	31	86	—
11075600	12/07/1999	0935	—	800	—	160	63	—
11075600	01/25/2000	1035	566	17,000	—	5,500	4,600	—
11075600	01/25/2000	1200	506	5,000	—	1,700	3,900	—
11075600	01/25/2000	1330	454	3,000	—	2,000	1,900	—
11075600	01/25/2000	1600	471	9,000	—	2,600	2,100	—
11075600	01/26/2000	0750	426	900,000	—	17,000	14,000	—
11075600	01/26/2000	1130	435	500,000	—	9,800	9,200	—
11075600	01/26/2000	1530	426	170,000	—	7,700	9,200	—
11075600	02/12/2000	0240	319	30,000	—	1,800	7,700	—
11075600	02/12/2000	0440	627	50,000	—	8,700	17,000	—
11075600	02/12/2000	0710	521	23,000	—	5,200	9,800	—
11075600	02/12/2000	0930	415	50,000	—	3,900	6,100	—
11075600	02/12/2000	1230	376	30,000	—	2,200	5,800	—
11075600	02/12/2000	1630	346	30,000	—	2,100	2,600	—
11075600	02/13/2000	0900	363	30,000	—	1,200	3,400	—
11075600	02/22/2000	2300	—	50,000	—	3,300	8,200	—
11075600	02/23/2000	0800	—	24,000	—	3,900	6,100	—
11075600	02/23/2000	0940	—	50,000	—	4,400	8,700	—
11075600	02/23/2000	1140	—	23,000	—	3,900	4,900	—
11075600	02/23/2000	1300	—	30,000	—	4,900	6,900	—
11075600	02/23/2000	1520	—	30,000	—	4,400	9,800	—
11075600	02/23/2000	1740	—	13,000	—	4,400	9,800	—
11075600	02/24/2000	0830	—	23,000	—	3,900	7,300	—
11075600	02/25/2000	1130	632	14,000	—	3,100	4,600	—
11075600	01/10/2001	1600	276	8,000	130	210	230	68
11075600	01/10/2001	1800	307	7,000	500	550	910	98
11075600	01/10/2001	2300	466	23,000	1,700	2,100	4,100	101
11075600	01/11/2001	0400	1,300	80,000	30,000	7,300	15,000	1100
11075600	01/11/2001	1020	650	17,000	8,000	7,300	>24,000	607
11075600	01/11/2001	1530	475	23,000	1,400	3,900	9,200	215
11075600	01/12/2001	0020	3,840	300,000	30,000	6,900	>24,000	4,900
11075600	01/12/2001	0840	1,620	230,000	8,000	12,000	>24,000	977
11075600	01/12/2001	1400	1,000	110,000	5,000	8,200	>24,000	657
11075600	01/14/2001	1200	367	130,000	5,000	2,400	1,900	119
11075600	02/11/2001	1900	401	1,100	<20	20	52	19
11075600	02/12/2001	0130	396	3,000	<20	73	120	20
11075600	02/12/2001	0430	409	1,700	<20	85	220	18

Appendix A. Total coliforms, fecal coliforms, *Escherichia coli*, enterococci, and suspended-sediment concentrations for stormflow in the Santa Ana River at Imperial Highway, in Mill Creek, and in shallow ground water from the Santa Ana River Basin, southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—Continued

Station No.	Date	Time	Stream-flow (ft ³ /s)	Total coliforms (MPN/100 mL)	Fecal coliforms (MPN/100 mL)	<i>Escherichia coli</i> (MPN/100 mL)	Enterococci (MPN/100 mL)	Suspended sediment (in mg/L)
11075600	02/12/2001	0720	2,000	30,000	40	1,800	4,100	216
11075600	02/12/2001	1100	1,030	22,000	60	5,800	17,000	640
11075600	02/12/2001	1430	1,690	23,000	260	3,100	9,200	546
11075600	02/12/2001	2130	1,890	17,000	330	2,900	4,400	—
11075600	02/13/2001	0430	3,490	8,000	1,400	2,400	6,900	1,850
11075600	02/13/2001	1200	5,400	50,000	8,000	8,200	>24,000	2,240
11075600	02/13/2001	1700	4,970	80,000	23,000	9,800	>24,000	1,690
11075600	02/14/2001	1000	2,480	70,000	11,000	13,000	>24,000	1,140
11075600	02/15/2001	1000	604	23,000	3,000	3,100	2,200	2,280
11075600	03/05/2001	1100	537	5,000	1,700	690	120	34
11075600	03/06/2001	0100	529	50,000	1,700	1,400	1,100	30
11075600	03/06/2001	0300	602	17,000	13,000	1,400	1,200	39
11075600	03/06/2001	0600	614	5,000	2,300	2,100	1,100	49
11075600	03/06/2001	1230	530	—	—	—	—	42
11075600	03/06/2001	1700	536	5,000	1,300	880	210	29
11075600	03/07/2001	0900	562	11,000	1,700	960	440	36
11075600	11/12/2001	1800	231	≥3,600	≥330	≥180	940	65
11075600	11/12/2001	2030	339	430,000	57,000	30,000	89,000	749
11075600	11/12/2001	2240	377	≥100,000	24,000	6,600	22,000	163
11075600	11/13/2001	0100	297	41,000	≥8,800	2,100	11,000	119
11075600	11/13/2001	0340	288	46,000	≥4,600	1,500	5,100	116
11075600	11/13/2001	1215	351	3,400,000	310,000	84,000	100,000	238
11075600	11/14/2001	0800	263	270,000	32,000	6,500	8,600	134
11075600	03/06/2002	1830	255	2,300	40	90	110	55
11075600	03/07/2002	0650	224	2,300	90	110	140	19
11075600	03/07/2002	1030	234	3,900	170	100	210	14
11075600	03/07/2002	1330	240	2,400	210	80	80	12
11075600	03/07/2002	1630	238	2,600	50	40	40	11
11075600	03/07/2002	2300	238	200	10	70	<10	13
11075600	03/08/2002	0700	244	4,500	120	70	210	12
Mill Creek	11/12/2001	1720	—	≥31,000	≥680	≥230	230,000	—
Mill Creek	11/12/2001	1945	—	≥6,900	6,000	2,300	230,000	—
Mill Creek	11/12/2001	2015	—	5,700,000	530,000	90,000	1,210,000	—
Mill Creek	11/12/2001	2030	—	5,100,000	520,000	50,000	780,000	—
Mill Creek	11/12/2001	2045	—	7,300,000	500,000	67,000	680,000	—
Mill Creek	11/12/2001	2100	—	5,600,000	200,000	44,000	640,000	—
Mill Creek	11/12/2001	2115	—	5,200,000	250,000	44,000	530,000	—
Mill Creek	11/12/2001	2130	—	4,700,000	230,000	42,000	320,000	—
Mill Creek	11/12/2001	2145	—	5,900,000	200,000	65,000	220,000	—
Mill Creek	11/12/2001	2200	—	1,600,000	43,000	5,300	82,000	—
Mill Creek	11/12/2001	2245	—	630,000	170,000	18,000	210,000	—
SCARS	11/12/2001	1950	—	<10	<10	<10	<10	—

52 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix A. Total coliforms, fecal coliforms, *Escherichia coli*, enterococci, and suspended-sediment concentrations for stormflow in the Santa Ana River at Imperial Highway, in Mill Creek, and in shallow ground water from the Santa Ana River Basin, southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—Continued

Station No.	Date	Time	Stream-flow (ft ³ /s)	Total coliforms (MPN/100 mL)	Fecal coliforms (MPN/100 mL)	<i>Escherichia coli</i> (MPN/100 mL)	Enterococci (MPN/100 mL)	Suspended sediment (in mg/L)
SCARS	11/12/2001	2100	—	60	<10	<10	<10	—
SCARS	11/12/2001	2250	—	130	10	<10	20	—
SCARS	11/13/2001	0140	—	130	<10	<10	10	—
SCARS	11/13/2001	0410	—	50	10	<10	20	—
SCARS	11/13/2001	0905	—	40	<10	<10	10	—
SCARS	11/13/2001	1240	—	40	<10	<10	<10	—
SCARS	11/13/2001	1620	—	20	<10	<10	10	—
SCARS	11/14/2001	0915	—	90	<10	<10	<10	—
SCARS	03/06/2002	1830	—	10	<10	<10	<10	—
SCARS	03/07/2002	0715	—	10	<10	<10	<10	—
SCARS	03/07/2002	1100	—	<10	<10	<10	<10	—
SCARS	03/07/2002	1330	—	20	<10	<10	<10	—
SCARS	03/07/2002	1700	—	<10	<10	<10	<10	—
SCARS	03/07/2002	2330	—	10	<10	<10	<10	—
SCARS	03/08/2002	0700	—	<10	<10	<10	<10	—

Appendix B1. Total phospholipid fatty acid (PLFA) concentrations and selected fatty acid structural groups in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons

[Data collected by U.S. Geological Survey and analyzed by Microbial Insights, Inc.; PLFA structural-group concentrations in percent of total PLFA concentration; pmole/L, picomoles per liter; 11075620, Santa Ana River at the diversion downstream from Imperial Highway; 11075600, Santa Ana River at Imperial Highway]

Station No.	Date	Time	Total PLFA (pmole/L)	Community structure (percent of total PLFA)					
				Terminally branched saturates	Monoenoic	Branched monoenoic	Mid-chain branched saturates	Normal saturated	Polyenoic
11075620	01/25/00	0915	99,100	3.8	40	1.1	1.0	17	37
11075620	01/25/00	1040	130,000	4.8	40	1.2	1.1	16	36
11075620	01/25/00	1240	140,000	5.4	42	1.4	1.1	17	33
11075620	01/25/00	1440	193,000	6.1	47	1.6	1.0	17	27
11075620	01/25/00	1640	109,000	4.6	48	1.2	1.1	16	28
11075620	01/25/00	2240	393,000	5.7	57	1.6	.9	17	18
11075620	01/26/00	0740	525,000	4.8	48	1.3	.8	14	15
11075620	01/26/00	1530	725,000	7.0	60	2.0	1.1	15	15
11075620	02/12/00	0130	135,000	8.9	50	2.9	2.1	17	19
11075620	02/12/00	0300	146,000	9.4	49	3.0	2.0	17	19
11075620	02/12/00	0430	192,000	8.5	46	2.5	2.5	17	24
11075620	02/12/00	0440	123,000	8.6	51	2.8	2.2	16	19
11075620	02/12/00	0930	98,600	9.2	48	3.2	2.2	17	20
11075620	02/12/00	0930	113,000	9.6	49	3.3	2.3	16	20
11075620	02/12/00	1400	213,000	10.5	47	3.1	2.7	17	20
11075620	02/12/00	2130	189,000	10.4	47	3.2	2.4	17	20
11075620	02/22/00	2200	197,000	10.9	47	3.6	4.5	16	19
11075620	02/23/00	0730	181,000	10.7	48	3.4	4.4	16	17
11075620	02/23/00	1200	190,000	10.2	45	3.2	4.2	17	21
11075620	02/23/00	1430	178,000	10.0	43	3.3	4.4	17	22
11075620	02/23/00	1600	179,000	10.4	44	3.3	4.4	16	22
11075620	02/23/00	2200	155,000	10.0	45	3.1	4.8	17	21
11075620	02/24/00	0100	201,000	11.1	45	3.4	4.7	17	19
11075620	02/24/00	0700	177,000	10.8	43	2.9	5.3	17	21
11075620	02/27/00	0300	126,000	10.7	41	2.9	4.0	18	23
11075600	11/12/01	1800	18,200	9.2	38	1.2	1.8	25	24
11075600	11/12/01	2030	155,000	9.9	40	1.7	1.8	20	26
11075600	11/12/01	2240	44,900	9.3	42	1.6	1.7	19	27
11075600	11/13/01	0100	48,500	10.2	41	1.2	1.4	21	25
11075600	11/13/01	0340	38,100	8.8	42	1.3	2.1	20	26
11075600	11/13/01	1215	155,000	8.8	54	1.0	1.0	18	17
11075600	11/14/01	0800	55,900	9.8	50	1.1	1.7	21	17
11075600	03/06/02	1830	19,600	5.0	36	.7	4.2	23	32
11075600	03/07/02	0650	19,300	3.6	34	1.3	4.0	20	37
11075600	03/07/02	1030	25,600	6.4	36	.9	4.7	23	29
11075600	03/07/02	1330	37,000	4.1	33	.7	3.8	24	35
11075600	03/07/02	1630	22,100	7.2	40	1.0	4.7	24	23
11075600	03/07/02	2300	16,700	4.6	36	1.0	4.2	23	31
11075600	03/08/02	0700	12,100	.0	41	.0	1.0	24	34

54 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix B2. Normal saturated phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons

[Data collected by the U.S. Geological Survey and analyzed by Microbial Insights, Inc.; individual PLFA concentrations in percent of total PLFA; standard nomenclature used for fatty-acid names where the numbers before and after the colon indicate the numbers of carbon atoms and carbon-carbon double bonds, respectively; pmole/L, picomoles per liter; 11075620, Santa Ana River at the diversion downstream from Imperial Highway; 11075600, Santa Ana River at Imperial Highway]

Station No.	Date	Time	Total PLFA pmole/L	14:0	15:0	16:0	17:0	18:0
11075620	01/25/00	0915	99,100	0.2	0.5	14	0.5	2.1
11075620	01/25/00	1040	130,000	.3	.6	13	.6	2.2
11075620	01/25/00	1240	140,000	.6	.6	14	.5	1.9
11075620	01/25/00	1440	193,000	1.0	.7	13	.5	1.6
11075620	01/25/00	1640	109,000	.2	.5	13	.5	1.9
11075620	01/25/00	2240	393,000	.5	.6	14	.4	1.4
11075620	01/26/00	0740	525,000	.4	.5	12	.4	1.2
11075620	01/26/00	1530	725,000	.4	.7	13	.4	1.0
11075620	02/12/00	0130	135,000	1.7	.9	13	.5	1.4
11075620	02/12/00	0300	146,000	1.8	.9	13	.5	1.5
11075620	02/12/00	0430	192,000	1.0	.9	12	.5	1.8
11075620	02/12/00	0440	123,000	1.3	.8	12	.5	1.4
11075620	02/12/00	0930	98,600	2.1	.9	12	.4	1.4
11075620	02/12/00	0930	113,000	2.0	.8	12	.5	1.4
11075620	02/12/00	1400	213,000	1.7	.9	12	.5	1.6
11075620	02/12/00	2130	189,000	1.8	.9	13	.5	1.6
11075620	2/22/00	2200	197,000	1.4	.8	11	.5	1.7
11075620	02/23/00	0730	181,000	1.5	.8	11	.5	1.9
11075620	02/23/00	1200	190,000	1.5	.8	12	.5	2.1
11075620	02/23/00	1430	178,000	1.3	.8	12	.6	2.1
11075620	02/23/00	1600	179,000	1.3	.7	12	.5	2.0
11075620	02/23/00	2200	155,000	1.0	.8	12	.6	2.2
11075620	02/24/00	0100	201,000	1.4	.9	12	.5	2.3
11075620	02/24/00	0700	177,000	1.4	.8	12	.6	2.4
11075620	02/27/00	0300	126,000	1.3	.9	12	.5	3.1
11075600	11/12/01	1800	18,200	1.9	.9	19	.8	2.5
11075600	11/12/01	2030	155,000	1.7	.8	14	.7	2.6
11075600	11/12/01	2240	44,900	1.4	.7	14	.6	2.4
11075600	11/13/01	0100	48,500	2.0	.8	16	.6	2.4
11075600	11/13/01	0340	38,100	1.3	.8	15	.7	2.4
11075600	11/13/01	1215	155,000	1.9	.7	14	.4	1.4
11075600	11/14/01	0800	55,900	2.3	.8	15	.5	1.8
11075600	03/06/02	1830	19,600	.9	.6	17	.7	3.7
11075600	03/07/02	0650	19,300	.3	.4	16	.9	2.7
11075600	03/07/02	1030	25,600	1.5	.6	17	.5	2.9
11075600	03/07/02	1330	37,000	.8	.4	14	.4	8.1
11075600	03/07/02	1630	22,100	1.1	.7	19	.6	2.1
11075600	03/07/02	2300	16,700	.4	.5	19	.7	2.7
11075600	03/08/02	0700	12,100	.0	.0	19	1.7	3.5

Appendix B3. Mid-chain branched saturated phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons

[Data collected by the U.S. Geological Survey and analyzed by Microbial Insights, Inc.; individual PLFA concentrations in percent of total PLFA; standard nomenclature used for fatty-acid names follows the form X:Y ω Z, where X is the number of carbon atoms, Y is the number of carbon-carbon double bonds, ω is the end of the molecule, and Z is the number of carbon atoms between the terminal double bond and the methyl end of the molecule, *a*, anteiso methyl branching, *me*, methyl branching; number in parentheses is effective carbon chain length; pmole/L, picomoles per liter; 11075620, Santa Ana River at the diversion downstream from Imperial Highway; 11075600, Santa Ana River at Imperial Highway; —, no data]

Station No.	Date	Time	Total PLFA (pmole/L)	14:0 <i>a</i> (14.41)	15:0 (15.48)	10 <i>me</i> 15:0 (15.57)	16:0 (16.07)	10 <i>me</i> 16:0 (16.43)	11 <i>me</i> 16:0 (16.47)	12 <i>me</i> 16:0 (16.5)
11075620	01/25/00	0915	99,100	—	—	—	—	0.9	—	—
11075620	01/25/00	1040	130,000	—	—	—	—	.9	—	—
11075620	01/25/00	1240	140,000	—	—	—	—	1.0	—	—
11075620	01/25/00	1440	193,000	—	—	—	—	.9	—	—
11075620	01/25/00	1640	109,000	—	—	—	—	.9	—	—
11075620	01/25/00	2240	393,000	—	—	—	—	.8	—	—
11075620	01/26/00	0740	525,000	—	—	—	—	.6	—	—
11075620	01/26/00	1530	725,000	—	—	—	—	.8	—	—
11075620	02/12/00	0130	135,000	—	—	—	—	1.0	—	0.0
11075620	02/12/00	0300	146,000	—	—	—	—	.9	—	.0
11075620	02/12/00	0430	192,000	—	—	—	—	1.2	—	.2
11075620	02/12/00	0440	123,000	—	—	—	—	1.0	—	.0
11075620	02/12/00	0930	98,600	—	—	—	—	1.1	—	.0
11075620	02/12/00	0930	113,000	—	—	—	—	.9	—	.0
11075620	02/12/00	1400	213,000	—	—	—	—	1.3	—	.1
11075620	02/12/00	2130	189,000	—	—	—	—	1.1	—	.0
11075620	02/22/00	2200	197,000	—	—	—	0.1	2.0	—	.1
11075620	02/23/00	0730	181,000	—	—	—	.1	1.8	—	.1
11075620	02/23/00	1200	190,000	—	—	—	.0	1.7	—	.1
11075620	02/23/00	1430	178,000	—	—	—	.1	1.7	—	.1
11075620	02/23/00	1600	179,000	—	—	—	.0	1.7	—	.2
11075620	02/23/00	2200	155,000	—	—	—	.1	1.8	—	.1
11075620	02/24/00	0100	201,000	—	—	—	.1	1.8	—	.1
11075620	02/24/00	0700	177,000	—	—	—	.7	1.7	—	.2
11075620	02/27/00	0300	126,000	—	—	—	.0	1.1	—	.0
11075600	11/12/01	1800	18,200	—	—	—	—	.8	0.4	—
11075600	11/12/01	2030	155,000	—	—	—	—	.9	.1	—
11075600	11/12/01	2240	44,900	—	—	—	—	.7	.0	—
11075600	11/13/01	0100	48,500	—	—	—	—	.5	.0	—
11075600	11/13/01	0340	38,100	—	—	—	—	.8	.2	—
11075600	11/13/01	1215	155,000	—	—	—	—	.5	.2	—
11075600	11/14/01	0800	55,900	—	—	—	—	.6	.2	—
11075600	03/06/02	1830	19,600	.7	—	1.3	.0	.3	.2	—
11075600	03/07/02	0650	19,300	.3	—	.5	.1	.4	.3	—
11075600	03/07/02	1030	25,600	1.2	—	1.0	.1	.3	.2	—
11075600	03/07/02	1330	37,000	.5	—	1.0	.1	.2	.1	—
11075600	03/07/02	1630	22,100	1.0	—	.8	.1	.3	.2	—
11075600	03/07/02	2300	16,700	.4	—	.7	.1	.4	.2	—
11075600	03/08/02	0700	12,100	.0	—	.0	.0	.0	.0	—

56 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix B3. Mid-chain branched saturated phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons—Continued

Station No.	Date	Time	17:0a (17.05)	17:0 (17.11)	10me17:0 (17.47)	11me17:0 (17.46)	18:2 (17.47)	12me17:0/1 8:2 (17.473)	10me18:0 (18.38)	12me18:00 (18.43)
11075620	01/25/00	0915	—	—	—	—	—	—	0.2	—
11075620	01/25/00	1040	—	—	—	—	—	—	.2	—
11075620	01/25/00	1240	—	—	—	—	—	—	.2	—
11075620	01/25/00	1440	—	—	—	—	—	—	.1	—
11075620	01/25/00	1640	—	—	—	—	—	—	.2	—
11075620	01/25/00	2240	—	—	—	—	—	—	.2	—
11075620	01/26/00	0740	—	—	—	—	—	—	.1	—
11075620	01/26/00	1530	—	—	—	—	—	—	.2	—
11075620	02/12/00	0130	—	—	1.0	—	—	—	.2	—
11075620	02/12/00	0300	—	—	.9	—	—	—	.2	—
11075620	02/12/00	0430	—	—	.9	—	—	—	.2	—
11075620	02/12/00	0440	—	—	1.0	—	—	—	.2	—
11075620	02/12/00	0930	—	—	.8	—	—	—	.2	—
11075620	02/12/00	0930	—	—	1.1	—	—	—	.2	—
11075620	02/12/00	1400	—	—	1.0	—	—	—	.3	—
11075620	02/12/00	2130	—	—	1.0	—	—	—	.3	—
11075620	02/22/00	2200	0.2	—	.6	—	1.0	—	.5	0.2
11075620	02/23/00	0730	.2	—	.5	—	1.0	—	.5	.2
11075620	02/23/00	1200	.1	—	.6	—	1.0	—	.5	.2
11075620	02/23/00	1430	.2	—	.6	—	1.1	—	.5	.1
11075620	02/23/00	1600	.2	—	.7	—	1.0	—	.6	.1
11075620	02/23/00	2200	.2	—	.6	—	1.0	—	.7	.2
11075620	02/24/00	0100	.2	—	.6	—	1.0	—	.7	.2
11075620	02/24/00	0700	.2	—	.6	—	1.1	—	.7	.2
11075620	02/27/00	0300	.0	—	1.0	—	1.0	—	.8	.0
11075600	11/12/01	1800	—	—	.0	—	—	0.7	.0	—
11075600	11/12/01	2030	—	—	.2	—	—	.4	.2	—
11075600	11/12/01	2240	—	—	.3	—	—	.4	.3	—
11075600	11/13/01	0100	—	—	.2	—	—	.4	.2	—
11075600	11/13/01	0340	—	—	.3	—	—	.5	.3	—
11075600	11/13/01	1215	—	—	.0	—	—	.3	.0	—
11075600	11/14/01	0800	—	—	.2	—	—	.5	.2	—
11075600	03/06/02	1830	—	0.0	.3	0.4	—	.8	.2	0.0
11075600	03/07/02	0650	—	.2	.4	.5	—	.9	.3	.1
11075600	03/07/02	1030	—	.1	.2	.5	—	.9	.2	.1
11075600	03/07/02	1330	—	.1	.2	.6	—	.9	.1	.0
11075600	03/07/02	1630	—	.2	.4	.5	—	.9	.2	.1
11075600	03/07/02	2300	—	.2	.3	.4	—	1.0	.3	.1
11075600	03/08/02	0700	—	.0	.0	.0	—	1.0	.0	.0

Appendix B4 Terminally branched saturated phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons

[Data collected by the U.S. Geological Survey and analyzed by Microbial Insights, Inc; individual PLFA concentrations in percent of total PLFA; standard nomenclature used for fatty-acid names follows the form $X:Y\omega Z$, where X is the number of carbon atoms, Y is the number of carbon-carbon double bonds, ω is the end of the molecule, and Z is the number of carbon atoms between the terminal double bond and the methyl end of the molecule, t , trans, i , iso methyl branching, a , anteiso methyl branching; number in parentheses is effective carbon chain length; pmole/L, picomoles per liter; 11075620, Santa Ana River at the diversion downstream from Imperial Highway; 11075600, Santa Ana River at Imperial Highway]

Station No.	Date	Time	Total PLFA (pmole/L)	$i14:0$ (13.62)	$i15:0$ (14.63)	$a15:0$ (14.70)	$i16:0$ (15.63)	$i17:0$ (16.62)	$a17:0/17:1\omega8c$ (16.70)
11075620	01/25/00	0915	99,100	0.0	0.9	0.7	0.7	0.4	1.2
11075620	01/25/00	1040	130,000	.0	1.1	.9	.8	.4	1.6
11075620	01/25/00	1240	140,000	.0	1.4	1.2	.9	.4	1.5
11075620	01/25/00	1440	193,000	.3	1.8	1.5	.9	.4	1.3
11075620	01/25/00	1640	109,000	.0	1.0	.9	.9	.4	1.5
11075620	01/25/00	2240	393,000	.1	1.6	1.5	.8	.3	1.3
11075620	01/26/00	0740	525,000	.1	1.9	1.8	.9	.3	1.5
11075620	01/26/00	1530	725,000	.1	2.0	1.7	1.0	.3	1.9
11075620	02/12/00	0130	135,000	.8	3.1	2.4	1.0	.5	1.2
11075620	02/12/00	0300	146,000	.9	3.3	2.6	1.0	.5	1.2
11075620	02/12/00	0430	192,000	.4	2.4	2.4	1.2	.5	1.7
11075620	02/12/00	0440	123,000	.5	2.7	2.4	1.1	.5	1.5
11075620	02/12/00	0930	98,600	1.0	3.0	2.4	1.1	.5	1.2
11075620	02/12/00	0930	113,000	1.2	3.1	2.5	1.0	.5	1.2
11075620	02/12/00	1400	213,000	.8	3.2	2.9	1.3	.6	1.6
11075620	02/12/00	2130	189,000	.9	3.3	2.9	1.3	.5	1.6
11075620	02/22/00	2200	197,000	.7	3.4	3.2	1.3	.8	1.6
11075620	02/23/00	0730	181,000	.6	3.3	3.2	1.2	.7	1.6
11075620	02/23/00	1200	190,000	.6	3.0	3.0	1.2	.7	1.6
11075620	02/23/00	1430	178,000	.6	2.9	2.7	1.4	.7	1.7
11075620	02/23/00	1600	179,000	.5	3.0	2.8	1.5	.8	1.8
11075620	02/23/00	2200	155,000	.4	2.8	2.9	1.4	.8	1.8
11075620	02/24/00	0100	201,000	.7	3.2	3.4	1.3	.8	1.7
11075620	02/24/00	0700	177,000	.6	3.0	3.4	1.3	.8	1.7
11075620	02/27/00	0300	126,000	.6	3.1	3.5	1.1	.8	1.7
11075600	11/12/01	1800	18,200	.6	2.8	2.1	2.2	.9	.8
11075600	11/12/01	2030	155,000	.9	3.0	2.7	1.6	.8	1.0
11075600	11/12/01	2240	44,900	.5	2.6	2.5	2.0	.8	1.0
11075600	11/13/01	0100	48,500	.6	2.9	2.8	2.4	.7	.9
11075600	11/13/01	0340	38,100	.5	2.6	2.3	1.9	.8	.8
11075600	11/13/01	1215	155,000	.6	2.9	2.6	1.8	.4	.6
11075600	11/14/01	0800	55,900	.9	3.2	2.5	2.0	.6	.7
11075600	03/06/02	1830	19,600	.2	1.8	1.3	.7	.5	.5
11075600	03/07/02	0650	19,300	.0	1.1	.7	.6	.6	.6
11075600	03/07/02	1030	25,600	.4	2.6	1.8	.8	.4	.4
11075600	03/07/02	1330	37,000	.1	1.6	1.2	.5	.3	.3
11075600	03/07/02	1630	22,100	.2	2.7	2.0	.9	.5	.9
11075600	03/07/02	2300	16,700	.0	1.4	.9	.8	.6	.9
11075600	03/08/02	0700	12,100	.0	.0	.0	.0	.0	.0

58 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix B5. Monoenoic phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons

[Data collected by the U.S. Geological Survey and analyzed by Microbial Insights, Inc.; individual PLFA concentrations in percent of total PLFA; standard nomenclature used for fatty-acid names follows the form $X:Y\omega Z$, where X is the number of carbon atoms, Y is the number of carbon-carbon double bonds, ω is the end of the molecule, and Z is the number of carbon atoms between the terminal double bond and the methyl end of the molecule, c , cis, cy , cyclopropane ring within the carbon chain, t , trans; number in parentheses is effective carbon chain length; pmole/L, picomoles per liter; 11075620, Santa Ana River at the diversion downstream from Imperial Highway; 11075600, Santa Ana River at Imperial Highway]

Station No.	Date	Time	Total PLFA (pmole/L)	15: 1 ω 6 c (14.80)	16: 1 ω 9 c (15.69)	16: 1 ω 7 c (15.75)	16: 1 ω 7 t (15.78)	16: 1 ω 5 c (15.83)	16: 1 ω 13 t (15.90)
11075620	01/25/00	0915	99,100	—	1.1	21	0.3	1.8	—
11075620	01/25/00	1040	130,000	—	1.2	21	.3	1.9	—
11075620	01/25/00	1240	134,000	—	1.3	23	.3	2.2	—
11075620	01/25/00	1440	193,000	—	1.2	28	.3	2.3	—
11075620	01/25/00	1640	109,000	—	1.0	26	.3	2.3	—
11075620	01/25/00	2240	393,000	—	1.0	34	.4	1.9	—
11075620	01/26/00	0740	525,000	—	.8	29	.3	1.6	—
11075620	01/26/00	1530	725,000	—	.8	35	.0	2.6	—
11075620	02/12/00	0130	135,000	0.4	1.5	28	.4	3.0	0.3
11075620	02/12/00	0300	146,000	.4	1.5	28	.4	3.0	.3
11075620	02/12/00	0430	192,000	.4	1.2	25	.4	2.3	.2
11075620	02/12/00	0440	123,000	.4	1.3	29	.4	2.9	.3
11075620	02/12/00	0930	98,600	.4	1.4	28	.4	2.8	.4
11075620	02/12/00	0930	113,000	.4	1.6	28	.4	2.8	.3
11075620	02/12/00	1400	213,000	.4	1.4	25	.4	3.0	.2
11075620	02/12/00	2130	189,000	.4	1.5	25	.4	3.0	.3
11075620	02/22/00	2200	197,000	.3	1.4	24	.5	2.7	—
11075620	02/23/00	0730	181,000	.4	1.3	26	.6	2.7	—
11075620	02/23/00	1200	190,000	.4	1.3	24	.5	2.5	—
11075620	02/23/00	1430	178,000	.3	1.2	21	.4	2.8	—
11075620	02/23/00	1600	179,000	.3	1.3	22	.4	3.1	—
11075620	02/23/00	2200	155,000	.3	1.3	22	.6	2.7	—
11075620	02/24/00	0100	201,000	.3	1.3	23	.6	2.7	—
11075620	02/24/00	0700	177,000	.3	1.3	22	.6	2.4	—
11075620	02/27/00	0300	126,000	.3	1.4	22	.7	2.0	—
11075600	11/12/01	1800	18,200	.0	1.5	17	.5	2.3	—
11075600	11/12/01	2030	155,000	.2	1.5	13	.4	2.3	—
11075600	11/12/01	2240	44,900	.0	1.3	16	.4	2.3	—
11075600	11/13/01	0100	48,500	.0	1.2	19	.5	2.3	—
11075600	11/13/01	0340	38,100	.2	1.3	17	.4	2.2	—
11075600	11/13/01	1215	155,000	.2	1.0	30	.0	1.8	—
11075600	11/14/01	0800	55,900	.2	1.3	25	.0	2.2	—
11075600	03/06/02	1830	19,600	.0	1.1	20	.4	1.8	—
11075600	03/07/02	0650	19,300	.0	.9	14	.3	1.4	—
11075600	03/07/02	1030	25,600	.1	1.1	21	.3	1.9	—
11075600	03/07/02	1330	37,000	.0	.9	20	.2	1.4	—
11075600	03/07/02	1630	22,100	.1	1.2	23	.4	2.3	—
11075600	03/07/02	2300	16,700	.0	1.1	18	.4	2.0	—
11075600	03/08/02	0700	12,100	.0	.0	18	.0	2.0	—

Appendix B5. Monoenoic phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons—Continued

Station No.	17: 1 ω 8c (16.74)	17: 1 ω 6c (16.78)	cy 17:0 (16.80)	17:1 (16.90)	17: 1 ω 6c (16.78)	18: 1 ω 7c (17.75)	18: 1 ω 7t (17.79)	18: 1 ω 6c (17.86)	18: 1 ω 5c (17.84)	19: 1 ω 6c (18.78)	cy 19:0 (18.81)
11075620	—	—	0.3	—	0.4	14	—	—	—	—	0.5
11075620	—	—	.3	—	.4	15	—	—	—	—	.6
11075620	—	—	.4	—	.4	14	—	—	—	—	.6
11075620	—	—	.3	—	.4	14	—	—	—	—	.5
11075620	—	—	.4	—	.5	17	—	—	—	—	.6
11075620	—	—	.3	—	.4	18	—	—	—	—	.4
11075620	—	—	.3	—	.4	15	—	—	—	—	.4
11075620	—	—	.5	—	.5	20	—	—	—	—	.5
11075620	—	0.5	.5	—	—	14	—	—	0.2	—	.6
11075620	—	.5	.5	—	—	14	—	—	.2	—	.5
11075620	—	.5	.4	—	—	14	—	—	.2	—	.6
11075620	—	.5	.5	—	—	15	—	—	.2	—	.6
11075620	—	.5	.5	—	—	13	—	—	.2	—	.6
11075620	—	.4	.4	—	—	14	—	—	.2	—	.5
11075620	—	.5	.6	—	—	15	—	—	.2	—	.8
11075620	—	.5	.6	—	—	15	—	—	.2	—	.6
11075620	—	.5	.9	—	—	14	0.3	—	.3	—	1.3
11075620	—	.5	.7	—	—	14	.4	—	.2	—	1.1
11075620	—	.5	.7	—	—	13	.4	—	.3	—	1.0
11075620	—	.4	.7	—	—	15	.3	—	.3	—	1.1
11075620	—	.4	.8	—	—	15	.3	—	.3	—	1.2
11075620	—	.5	.8	—	—	14	.4	—	.3	—	1.3
11075620	—	.5	.8	—	—	14	.4	—	.3	—	1.1
11075620	—	.5	.8	—	—	14	.2	—	.2	—	1.0
11075620	—	.4	.5	—	—	13	.5	—	.1	—	.6
11075600	0.5	.4	.6	—	—	14	—	0.0	.3	—	1.1
11075600	.7	.5	.6	—	—	18	—	.2	.5	—	1.8
11075600	.8	.4	.6	—	—	18	—	.0	.4	—	1.5
11075600	.7	.4	.6	—	—	15	—	.0	.2	—	1.0
11075600	.7	.4	.6	—	—	18	—	.0	.3	—	1.2
11075600	.5	.3	.4	—	—	18	—	.0	.2	—	.7
11075600	.5	.4	.6	—	—	19	—	.0	.2	—	.9
11075600	.3	.3	.3	0.0	—	11	—	.0	.3	—	.3
11075600	.4	.4	.8	.1	—	15	—	.0	.3	—	.7
11075600	.3	.3	.2	.1	—	10	—	.0	.2	—	.3
11075600	.2	.2	.2	.1	—	9	—	.0	.3	—	.2
11075600	.0	.3	.3	.1	—	12	—	.0	.4	—	.3
11075600	.0	.3	.3	.1	—	13	—	.2	.2	—	.4
11075600	.0	.0	.0	.0	—	21	—	.0	.0	—	.0

60 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix B6. Branched monoenoic phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons

[Data collected by the U.S. Geological Survey and analyzed by Microbial Insights, Inc.; individual PLFA concentrations in percent total PLFA; standard nomenclature used for fatty-acid names follows the form $X:Y\omega Z$, where X is the number of carbon atoms, Y is the number of carbon-carbon double bonds, ω is the end of the molecule, and Z is the number of carbon atoms between the terminal double bond and the methyl end of the molecule; number in parentheses is effective carbon chain length; pmole/L, picomoles per liter; 11075620, Santa Ana River at the diversion downstream from Imperial Highway; 11075600, Santa Ana River at Imperial Highway; —, no data]

Station No.	Date	Time	Total PLFA (pmole/L)	15:1 (14.35)	15:1 (14.41)	15:1 (14.46)	16:1 (15.41)	16:1 (15.41)	17:1 ω 7 (16.34)	19:1 (18.03)
11075620	01/25/00	0915	99,100	—	0.2	—	—	—	0.4	0.5
11075620	01/25/00	1040	130,000	—	.3	—	—	—	.5	.5
11075620	01/25/00	1240	140,000	—	.5	—	—	—	.5	.4
11075620	01/25/00	1440	193,000	—	.8	—	—	—	.5	.3
11075620	01/25/00	1640	109,000	—	.3	—	—	—	.5	.4
11075620	01/25/00	2240	393,000	—	.6	—	—	—	.6	.4
11075620	01/26/00	0740	525,000	—	.5	—	—	—	.5	.3
11075620	01/26/00	1530	725,000	—	.8	—	—	—	.7	.4
11075620	02/12/00	0130	135,000	—	1.6	0.3	—	—	.7	.4
11075620	02/12/00	0300	146,000	—	1.6	.3	—	—	.7	.4
11075620	02/12/00	0430	192,000	—	1.0	.4	—	—	.7	.4
11075620	02/12/00	0440	123,000	—	1.4	.3	—	—	.7	.4
11075620	02/12/00	0930	98,600	—	1.8	.3	—	—	.7	.4
11075620	02/12/00	0930	113,000	—	1.9	.3	—	—	.6	.4
11075620	02/12/00	1400	213,000	—	1.4	.4	—	—	.8	.5
11075620	02/12/00	2130	189,000	—	1.5	.4	—	—	.8	.4
11075620	02/22/00	2200	197,000	0.1	1.3	.3	—	0.2	1.1	.5
11075620	02/23/00	0730	181,000	.0	1.4	.4	—	.1	1.1	.5
11075620	02/23/00	1200	190,000	.0	1.2	.4	—	.1	1.0	.5
11075620	02/23/00	1430	178,000	.0	1.1	.3	—	.2	1.0	.6
11075620	02/23/00	1600	179,000	.0	1.1	.3	—	.3	1.1	.5
11075620	02/23/00	2200	155,000	.0	1.0	.3	—	.1	1.1	.6
11075620	02/24/00	0100	201,000	.0	1.4	.4	—	.1	1.1	.5
11075620	02/24/00	0700	177,000	.0	1.2	.4	—	.2	1.0	.2
11075620	02/27/00	0300	126,000	.0	1.5	.3	—	.2	.9	.0
11075600	11/12/01	1800	18,200	—	—	—	—	—	.6	.6
11075600	11/12/01	2030	155,000	—	—	—	—	—	.7	1.0
11075600	11/12/01	2240	44,900	—	—	—	—	—	.8	.8
11075600	11/13/01	0100	48,500	—	—	—	—	—	.6	.6
11075600	11/13/01	0340	38,100	—	—	—	—	—	.6	.7
11075600	11/13/01	1215	155,000	—	—	—	—	—	.6	.5
11075600	11/14/01	0800	55,900	—	—	—	—	—	.5	.6
11075600	03/06/02	1830	19,600	—	—	—	0.0	.0	.4	.3
11075600	03/07/02	0650	19,300	—	—	—	.2	.0	.5	.5
11075600	03/07/02	1030	25,600	—	—	—	.2	.1	.3	.3
11075600	03/07/02	1330	37,000	—	—	—	.1	.1	.2	.3
11075600	03/07/02	1630	22,100	—	—	—	.2	.1	.3	.4
11075600	03/07/02	2300	16,700	—	—	—	.1	.0	.4	.5
11075600	03/08/02	0700	12,100	—	—	—	.0	.0	.0	.0

Appendix B7. Polyenoic phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons

[Data collected by the U.S. Geological Survey and analyzed by Microbial Insights, Inc.; individual PLFA concentrations in percent total PLFA; standard nomenclature used for fatty-acid names follows the form $X:Y\omega Z$, where X is the number of carbon atoms, Y is the number of carbon-carbon double bonds, ω is the end of the molecule, and Z is the number of carbon atoms between the terminal double bond and the methyl end of the molecule, c , cis , a , anteiso methyl branching; number in parentheses is effective carbon chain length; pmole/L, picomoles per liter; 11075620, Santa Ana River at the diversion downstream from Imperial Highway; 11075600, Santa Ana River at Imperial Highway; —, no data]

Station No.	Date	Time	Total PLFA (pmole/L)	16:2 (15.58)	18:4 ω 3 (17.11)	poly18 (17.30)	18:3 ω 6 (17.41)	18:4 ω 3 (17.45)	18:2 (17.47)	18:2 <i>a</i> (17.52)	18:2 <i>b</i> (17.57)	18:2 ω 6 (17.59)	18:3 ω 3 (17.63)	18:10 ω 9 <i>c</i> (17.69)	18:2 <i>c</i> (18.53)
11075620	01/25/00	0915	99,100	0.8	—	0.3	0.4	2.0	1.1	—	—	6.0	3.7	8.0	—
11075620	01/25/00	1040	130,000	.7	—	.3	.4	1.5	.9	—	—	7.5	3.6	7.9	—
11075620	01/25/00	1240	140,000	.9	—	.2	.4	1.9	.9	—	—	5.2	3.9	7.0	—
11075620	01/25/00	1440	193,000	.8	—	.2	.4	1.5	1.1	—	—	4.6	3.0	5.5	—
11075620	01/25/00	1640	109,000	.7	—	.3	.5	1.6	1.3	—	—	4.3	2.9	5.6	—
11075620	01/25/00	2240	393,000	.4	—	.1	.3	.5	1.0	—	—	4.0	2.3	4.3	—
11075620	01/26/00	0740	525,000	.3	—	.1	.2	.4	.8	—	—	3.4	2.0	3.6	—
11075620	01/26/00	1530	725,000	.4	—	.1	.3	.6	1.0	—	—	2.6	1.7	3.3	—
11075620	02/12/00	0130	135,000	—	—	—	—	1.1	—	—	—	3.4	2.2	4.3	—
11075620	02/12/00	0300	146,000	—	—	—	—	1.1	—	—	—	3.4	2.2	4.4	—
11075620	02/12/00	0430	192,000	—	—	—	—	.7	—	—	—	8.3	2.6	5.7	—
11075620	02/12/00	0440	123,000	—	—	—	—	1.2	—	—	—	4.0	2.1	4.7	—
11075620	02/12/00	0930	98,600	—	—	—	—	2.0	—	—	—	2.8	2.5	4.0	—
11075620	02/12/00	0930	113,000	—	—	—	—	1.3	—	—	—	3.6	2.2	4.1	—
11075620	02/12/00	1400	213,000	—	—	—	—	.7	—	—	—	5.6	2.6	5.0	—
11075620	02/12/00	2130	189,000	—	—	—	—	1.0	—	—	—	4.5	2.5	4.8	—
11075620	02/22/00	2200	197,000	.6	0.2	—	1.0	—	—	—	—	3.1	1.9	4.4	—
11075620	02/23/00	0730	181,000	.7	.2	—	1.1	—	—	—	—	2.9	2.3	4.7	—
11075620	02/23/00	1200	190,000	.6	.2	—	1.0	—	—	—	—	4.5	2.6	5.8	—
11075620	02/23/00	1430	178,000	.5	.2	—	.8	—	—	—	—	5.9	2.7	5.7	—
11075620	02/23/00	1600	179,000	.6	.2	—	.9	—	—	—	—	5.0	2.7	5.7	—
11075620	02/23/00	2200	155,000	.6	.2	—	1.1	—	—	—	—	3.7	2.5	5.6	—
11075620	02/24/00	0100	201,000	.6	.2	—	1.0	—	—	—	—	3.4	2.2	5.3	—
11075620	02/24/00	0700	177,000	.6	.2	—	1.0	—	—	—	—	4.6	2.4	5.8	—
11075620	02/27/00	0300	126,000	.9	.3	—	1.0	—	—	—	—	2.7	2.1	5.5	—
11075600	11/12/01	1800	18,200	—	—	—	—	—	—	2.1	—	3.9	4.4	6.5	—
11075600	11/12/01	2030	155,000	—	—	—	—	—	—	1.6	—	9.5	2.9	7.8	—
11075600	11/12/01	2240	44,900	—	—	—	—	—	—	1.5	—	8.7	3.0	7.1	—
11075600	11/13/01	0100	48,500	—	—	—	—	—	—	1.5	—	7.5	3.0	6.2	—
11075600	11/13/01	0340	38,100	—	—	—	—	—	—	1.9	—	7.5	3.6	6.4	—
11075600	11/13/01	1215	155,000	—	—	—	—	—	—	1.4	—	5.1	2.3	4.2	—
11075600	11/14/01	0800	55,900	—	—	—	—	—	—	1.7	—	3.5	2.4	4.4	—
11075600	03/06/02	1830	19,600	—	—	—	—	—	—	1.5	—	4.5	2.2	6.0	—
11075600	03/07/02	0650	19,300	—	—	—	—	—	—	2.0	—	5.3	3.7	7.4	—
11075600	03/07/02	1030	25,600	—	—	—	—	—	—	1.3	—	6.3	3.2	5.8	—
11075600	03/07/02	1330	37,000	—	—	—	—	—	—	1.0	—	9.6	2.0	7.6	—
11075600	03/07/02	1630	22,100	—	—	—	—	—	—	1.8	—	3.8	2.2	5.1	—
11075600	03/07/02	2300	16,700	—	—	—	—	—	—	2.5	—	4.0	2.2	5.7	—
11075600	03/08/02	0700	12,100	—	—	—	—	—	—	2.3	—	4.6	3.2	7.9	—

62 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix B7. Polyenoic phospholipid fatty acids (PLFA) in stormflow in the Santa Ana River at Imperial Highway, southern California, 1999–2000 and 2001–02 rainy seasons—Continued

Station No.	Date	Time	20: 4ω6 (19.15)	20: 5ω3 (19.19)	20: 3 (19.98)	20: 3ω6 (19.40)	20: 2ω3 (19.60)	20: 1ω11c (19.66)	20: 1ω9c (19.68)	20: 1ω7c (19.75)	20:0 (20.00)	22: 4ω6 (21.18)	22: 5ω6 (21.06)	22: 5ω3 (21.23)	22: 6ω3 (21.14)	22:0 (22.00)	23:0 (23.00)	24:0 (24.00)
11075620	01/25/00	0915	1.2	11.9	—	0.4	0.2	—	0.4	—	0.3	—	—	0.2	—	0.3	—	0.3
11075620	01/25/00	1040	1.4	9.7	—	.4	.2	—	.4	—	.3	—	—	.2	—	.4	—	.3
11075620	01/25/00	1240	1.3	9.0	—	.3	.2	—	.4	—	.3	—	—	.2	—	.3	—	.2
11075620	01/25/00	1440	1.0	7.6	—	.2	.2	—	.3	—	.2	—	—	.2	—	.3	—	.2
11075620	01/25/00	1640	1.1	8.2	—	.2	.2	—	.4	—	.3	—	—	.1	—	.3	—	.2
11075620	01/25/00	2240	.9	3.2	—	.2	.1	—	.1	—	.2	—	—	.1	—	.2	—	.2
11075620	01/26/00	0740	.8	2.7	—	.2	.1	—	.1	—	.2	—	—	.1	—	.2	—	.1
11075620	01/26/00	1530	.8	3.1	—	.1	.1	—	.2	—	.1	—	—	.1	—	.1	—	.1
11075620	02/12/00	0130	1.1	6.1	0.1	.1	—	—	—	—	.2	0.1	—	—	—	.2	—	.1
11075620	02/12/00	0300	1.1	5.9	.1	.1	—	—	—	—	.2	.1	—	—	—	.3	—	.2
11075620	02/12/00	0430	1.1	4.9	.2	.2	—	—	—	—	.2	.2	—	—	—	.2	—	.2
11075620	02/12/00	0440	1.0	5.6	.1	.1	—	—	—	—	.2	.1	—	—	—	.2	—	.1
11075620	02/12/00	0930	.9	7.5	.1	.2	—	—	—	—	.2	.1	—	—	—	.1	—	.1
11075620	02/12/00	0930	1.0	6.7	.1	.1	—	—	—	—	.2	.1	—	—	—	.2	—	.1
11075620	02/12/00	1400	1.1	3.7	.1	.1	—	—	—	—	.3	.0	—	—	—	.3	—	.2
11075620	02/12/00	2130	1.1	4.6	.1	.1	—	—	—	—	.3	.0	—	—	—	.2	—	.2
11075620	02/22/00	2200	1.8	3.5	.1	.1	.1	0.5	—	0.1	.3	.1	—	—	—	.4	0.1	.3
11075620	02/23/00	0730	1.5	2.8	.1	.1	.0	.0	—	.1	.3	.1	—	—	—	.3	.0	.3
11075620	02/23/00	1200	1.7	3.0	.2	.1	.1	.0	—	.0	.3	.2	—	—	—	.4	.0	.3
11075620	02/23/00	1430	1.7	2.7	.2	.1	.1	.0	—	.0	.3	.2	—	—	—	.4	.2	.3
11075620	02/23/00	1600	1.9	3.0	.2	.1	.1	.0	—	.0	.4	.2	—	—	—	.4	.0	.3
11075620	02/23/00	2200	1.9	3.3	.2	.2	.1	.0	—	.0	.3	.2	—	—	—	.4	.1	.3
11075620	02/24/00	0100	1.8	3.2	.2	.2	.1	.0	—	.1	.3	.2	—	—	—	.3	.1	.3
11075620	02/24/00	0700	1.7	2.7	.2	.1	.1	.0	—	.0	.3	.1	—	—	—	.4	.1	.3
11075620	02/27/00	0300	5.9	4.0	.2	.2	.1	.0	—	.0	.2	.3	—	—	—	.2	.0	.2
11075600	11/12/01	1800	1.9	3.7	—	—	—	.0	.0	1.2	.4	—	—	—	—	—	—	—
11075600	11/12/01	2030	1.5	1.8	—	—	—	.2	.4	.3	.4	—	—	—	—	—	—	—
11075600	11/12/01	2240	2.2	2.8	—	—	—	.3	.5	.3	.4	—	—	—	—	—	—	—
11075600	11/13/01	0100	2.0	3.0	—	—	—	.3	.0	.9	.4	—	—	—	—	—	—	—
11075600	11/13/01	0340	1.8	2.6	—	—	—	.3	.0	1.0	.5	—	—	—	—	—	—	—
11075600	11/13/01	1215	1.2	2.2	—	—	—	.0	.0	.3	.2	—	—	—	—	—	—	—
11075600	11/14/01	0800	1.3	2.5	—	—	—	.2	.0	.7	.3	—	—	—	—	—	—	—
11075600	03/06/02	1830	2.1	10.8	—	—	.2	.2	.5	.3	.5	.0	0.5	.4	1.8	.3	.2	—
11075600	03/07/02	0650	2.7	11.2	—	—	.2	.4	1.1	.0	.3	.1	.4	.5	1.2	.3	.2	—
11075600	03/07/02	1030	1.4	7.6	—	—	.1	.2	.4	.2	.5	.0	.3	.2	1.0	.2	.2	—
11075600	03/07/02	1330	1.5	10.7	—	—	.1	.2	.4	.2	.7	.0	.2	.2	.6	.3	.1	—
11075600	03/07/02	1630	1.3	5.5	—	—	.3	.2	.8	.1	.3	.0	.3	.2	.9	.3	.2	—
11075600	03/07/02	2300	2.5	9.1	—	—	.4	.3	.7	.3	.3	.1	.5	.3	1.3	.4	.2	—
11075600	03/08/02	0700	2.8	9.8	—	—	.0	.0	1.8	.0	.0	.0	.0	.0	1.6	.0	.0	—

Appendix C. Dissolved organic carbon (DOC) and ultraviolet absorption (UVA) data for stormflow in the Santa Ana River at the diversion downstream from Imperial Highway and at the subsurface collection and recharge system (SCARS), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons

[11075620, Santa Ana River at the diversion downstream from Imperial Highway; 11075600, Santa Ana River at Imperial Highway; samples collected by the U.S. Geological Survey (USGS) and analyzed by the USGS Sacramento lab; DOC, dissolved organic carbon; UV₂₅₄, ultraviolet absorption at 254 nanometers; UV₂₈₅, ultraviolet absorption at 285 nanometers; mg/L, milligrams per liter; cm-1, absorbance per centimeter length; —, no data]

Station name	Station No.	Date	Time	DOC (mg/L)	UV ₂₅₄ (cm-1)	UV ₂₈₅ (cm-1)	
Santa Ana River at the diversion downstream from Imperial Highway	11075620	01/25/2000	0915	—	0.116	0.088	
	11075620	01/25/2000	1040	—	.187	.138	
	11075620	01/25/2000	1130	—	.188	.140	
	11075620	01/25/2000	1240	—	.157	.120	
	11075620	01/25/2000	1440	—	.150	.114	
	11075620	01/25/2000	1640	—	.145	.111	
	11075620	01/25/2000	1940	—	.189	.137	
	11075620	01/25/2000	2240	—	.269	.194	
	11075620	01/26/2000	0140	—	.319	.231	
	11075620	01/26/2000	0740	—	.377	.275	
	11075620	01/26/2000	1040	—	.423	.308	
	11075620	01/26/2000	1340	—	.389	.283	
	11075620	01/26/2000	1530	—	.368	.268	
	11075620	02/12/2000	0000	0000	6.3	.157	.115
	11075620	02/12/2000	0130	0130	5.8	.154	.114
	11075620	02/12/2000	0300	0300	5.3	.161	.119
	11075620	02/12/2000	0430	0430	5.5	.167	.123
	11075620	02/12/2000	0600	0600	5.4	.164	.125
	11075620	02/12/2000	0730	0730	6.7	.196	.147
	11075620	02/12/2000	0930	0930	5.3	.169	.125
	11075620	02/12/2000	1130	1130	6.1	.172	.128
	11075620	02/12/2000	1400	1400	5.7	.144	.106
	11075620	02/12/2000	1630	1630	6.1	.165	.129
	11075620	02/12/2000	1900	1900	5.9	.150	.111
	11075620	02/12/2000	2130	2130	5.5	.136	.102
	11075620	02/13/2000	0030	0030	5.8	.157	.121
	11075620	02/13/2000	0330	0330	5.7	.148	.112
	11075620	02/13/2000	0630	0630	5.5	.176	.132
	11075620	02/13/2000	0930	0930	6.4	.184	.139
	11075620	02/22/2000	2220	2220	6.1	.198	.154
	11075620	02/23/2000	0730	0730	5.9	.186	.146
	11075620	02/23/2000	0830	0830	5.8	.190	.147
	11075620	02/23/2000	0930	0930	5.9	.198	.155
	11075620	02/23/2000	1030	1030	5.8	.192	.149
	11075620	02/23/2000	1200	1200	5.7	.187	.145
	11075620	02/23/2000	1300	1300	5.2	.168	.132
	11075620	02/23/2000	1430	1430	4.7	.159	.127
	11075620	02/23/2000	1600	1600	5.1	.184	.145
	11075620	02/23/2000	1900	1900	5.3	.187	.146
	11075620	02/23/2000	2200	2200	6.4	.184	.142

64 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix C. Dissolved organic carbon (DOC) and ultraviolet absorption (UVA) data for stormflow in the Santa Ana River at the diversion downstream from Imperial Highway and at the subsurface collection and recharge system (SCARS), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—
Continued

Station name	Station No.	Date	Time	DOC (mg/L)	UV ₂₅₄ (cm-1)	UV ₂₈₅ (cm-1)
Santa Ana River at the diversion downstream from Imperial Highway	11075620	02/24/2000	0100	—	0.184	0.142
	11075620	02/24/2000	0400	6.2	.177	.138
	11075620	02/24/2000	0700	6.2	.178	.139
	11075620	02/24/2000	1000	5.9	.183	.143
	11075620	02/24/2000	1900	5.5	.351	.287
	11075620	02/25/2000	0300	5.4	.358	.296
	11075620	02/25/2000	1100	5.2	.346	.283
	11075620	02/25/2000	1900	5.9	.303	.244
	11075620	02/26/2000	0300	5.2	.316	.256
	11075620	02/26/2000	1100	6.0	.292	.234
	11075620	02/26/2000	1900	7.0	.271	.216
	11075620	02/27/2000	0300	6.7	.268	.212
	11075620	02/27/2000	1100	6.7	.240	.189
	11075620	01/10/2001	1540	4.6	.110	.076
	11075620	01/10/2001	1710	4.6	.114	.080
	11075620	01/10/2001	1900	6.6	.180	.124
	11075620	01/10/2001	2100	7.2	.234	.166
	11075620	01/10/2001	2300	6.6	.201	.143
	11075620	01/11/2001	0100	6.1	.184	.132
	11075620	01/11/2001	0300	5.1	.268	.211
	11075620	01/11/2001	0500	5.4	.267	.204
	11075620	01/11/2001	0700	8.2	.331	.253
	11075620	01/11/2001	1100	7.5	.355	.272
	11075620	01/11/2001	1400	8.5	.359	.272
	11075620	01/11/2001	1700	9.4	.306	.222
	11075620	01/11/2001	2000	11	.343	.249
	11075620	01/11/2001	2300	12	.557	.435
	11075620	01/12/2001	0030	10	.590	.467
	11075620	01/12/2001	0330	9.9	.649	.524
	11075620	01/12/2001	0630	9.7	.522	.404
	11075620	01/12/2001	0930	9.2	.591	.481
	11075620	01/12/2001	1230	8.7	.585	.466
	11075620	01/12/2001	1530	9.1	.545	.425
	11075620	01/12/2001	2130	8.8	.562	.445
	11075620	01/13/2001	0330	8.8	.452	.349
	11075620	01/13/2001	0930	8.5	.472	.367
	11075620	01/13/2001	1530	8.3	.429	.333
	11075620	01/13/2001	2130	8.2	.411	.313
	11075620	01/14/2001	0330	8.0	.400	.306
	11075620	01/14/2001	0930	8.1	.384	.295
	11075620	01/14/2001	1200	8.1	.368	.278
	11075620	02/11/2001	1900	5.7	.128	.088
11075620	02/12/2001	0200	5.5	.130	.090	

Appendix C. Dissolved organic carbon (DOC) and ultraviolet absorption (UVA) data for stormflow in the Santa Ana River at the diversion downstream from Imperial Highway and at the subsurface collection and recharge system (SCARS), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—Continued

Station name	Station No.	Date	Time	DOC (mg/L)	UV ₂₅₄ (cm-1)	UV ₂₈₅ (cm-1)
Santa Ana River at the diversion downstream from Imperial Highway	11075620	02/12/2001	0400	5.6	0.137	0.095
	11075620	02/12/2001	0600	5.3	.162	.114
	11075620	02/12/2001	0800	3.0	.192	.147
	11075620	02/12/2001	1000	4.6	.218	.162
	11075620	02/12/2001	1200	4.6	.211	.158
	11075620	02/12/2001	1500	4.3	.220	.168
	11075620	02/12/2001	1800	6.9	.341	.257
	11075620	02/12/2001	2100	5.6	.232	.172
	11075620	02/13/2001	0000	6.8	.319	.237
	11075620	02/13/2001	0300	5.6	.255	.187
	11075620	02/13/2001	0600	6.5	.292	.219
	11075620	02/13/2001	0900	6.7	.347	.264
	11075620	02/13/2001	1200	6.1	.384	.295
	11075620	02/13/2001	1700	5.8	.431	.340
	11075620	02/13/2001	2200	6.1	.404	.315
	11075620	02/14/2001	0300	6.8	.385	.298
	11075620	02/14/2001	0800	5.9	.416	.327
	11075620	02/14/2001	1300	6.0	.371	.289
	11075620	02/14/2001	1800	6.1	.415	.328
	11075620	02/15/2001	0800	5.7	.332	.258
	11075620	02/15/2001	1100	5.7	.328	.256
	11075620	03/5/2001	1100	7.6	.240	.175
	11075620	03/6/2001	0120	8.1	.251	.184
	11075620	03/6/2001	0230	7.1	.247	.179
	11075620	03/6/2001	0400	8.8	.259	.181
	11075620	03/6/2001	0530	7.3	.228	.165
	11075620	03/6/2001	0700	7.3	.224	.163
	11075620	03/6/2001	0830	7.6	.232	.169
	11075620	03/6/2001	1000	7.6	.236	.172
	11075620	03/6/2001	1230	8.0	.237	.173
	11075620	03/6/2001	1700	7.9	.232	.168
	11075620	03/6/2001	2300	8.1	.229	.168
	11075620	03/7/2001	0500	8.0	.232	.170
	11075620	11/12/2001	1545	4.8	.127	.089
	11075620	11/12/2001	1555	4.8	.124	.095
	11075620	11/12/2001	1745	6.1	.170	.113
	11075620	11/12/2001	1800	5.0	.124	.062
	11075620	11/12/2001	1845	5.0	.113	.076
	11075620	11/12/2001	1915	4.3	.114	.081
	11075620	11/12/2001	2015	12	.376	.254
	11075620	11/12/2001	2045	13	.418	.278
	11075620	11/12/2001	2145	12	.394	.267
	11075620	11/12/2001	2230	13	.412	.281

66 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix C. Dissolved organic carbon (DOC) and ultraviolet absorption (UVA) data for stormflow in the Santa Ana River at the diversion downstream from Imperial Highway and at the subsurface collection and recharge system (SCARS), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—Continued

Station name	Station No.	Date	Time	DOC (mg/L)	UV ₂₅₄ (cm-1)	UV ₂₈₅ (cm-1)
Santa Ana River at the diversion downstream from Imperial Highway	11075620	11/12/2001	2330	11	0.367	0.259
	11075620	11/13/2001	0030	12	.359	.248
	11075620	11/13/2001	0130	9.6	.261	.182
	11075620	11/13/2001	0230	7.8	.218	.151
	11075620	11/13/2001	0330	6.9	.182	.130
	11075620	11/13/2001	0430	7.0	.195	.158
	11075620	11/13/2001	0530	7.0	.182	.128
	11075620	11/13/2001	0630	6.5	.173	.121
	11075620	11/13/2001	0730	6.3	.166	.123
	11075620	11/13/2001	1015	13	.400	.266
	11075620	11/13/2001	1600	15	.522	.344
	11075620	11/13/2001	2200	14	.420	.278
	11075620	11/14/2001	0400	11	.366	.241
	11075620	11/14/2001	1000	11	.344	.229
Santa Ana River at Imperial Highway	11075600	11/12/2001	1800	4.2	.092	.062
	11075600	11/12/2001	2030	13	.318	.215
	11075600	11/12/2001	2240	8.6	.197	.140
	11075600	11/13/2001	0100	6.9	.153	.100
	11075600	11/13/2001	0340	6.3	.143	.097
	11075600	11/13/2001	1215	15	.473	.302
	11075600	11/14/2001	0800	11	.328	.204
Santa Ana River at the diversion downstream from Imperial Highway	11075620	03/06/2002	1830	4.3	.099	.069
	11075620	03/07/2002	0600	5.6	.141	.094
	11075620	03/07/2002	0800	5.7	.143	.097
	11075620	03/07/2002	1130	5.5	.140	.094
	11075620	03/07/2002	1330	5.3	.129	.088
	11075620	03/07/2002	1530	5.2	.129	.088
	11075620	03/07/2002	1730	4.8	.118	.081
	11075620	03/07/2002	2000	4.6	.108	.075
	11075620	03/07/2002	2300	4.5	.102	.070
	11075620	03/08/2002	0200	5.5	.129	.087
	11075620	03/08/2002	0500	6.7	.159	.104
	11075620	03/08/2002	0800	7.6	.195	.125
	11075620	03/08/2002	1100	7.6	.189	.122
Subsurface collection and recharge system	SCARS	11/12/2001	1950	1.4	.041	.028
	SCARS	11/12/2001	2100	1.4	.042	.024
	SCARS	11/12/2001	2250	2.1	.043	.028
	SCARS	11/13/2001	0140	2.1	.043	.030
	SCARS	11/13/2001	0410	2.4	.042	.029
	SCARS	11/13/2001	0905	2.2	.044	.027
	SCARS	11/13/2001	1242	1.5	.041	.029
	SCARS	11/13/2001	1620	2.2	.042	.029

Appendix C. Dissolved organic carbon (DOC) and ultraviolet absorption (UVA) data for stormflow in the Santa Ana River at the diversion downstream from Imperial Highway and at the subsurface collection and recharge system (SCARS), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—Continued

Station name	Station No.	Date	Time	DOC (mg/L)	UV ₂₅₄ (cm-1)	UV ₂₈₅ (cm-1)
Subsurface collection and recharge system	SCARS	11/14/2001	0915	1.7	0.052	0.032
	SCARS	03/07/2002	0715	1.6	.040	.027
	SCARS	03/07/2002	1100	1.6	.039	.026
	SCARS	03/07/2002	1330	1.6	.042	.028
	SCARS	03/07/2002	1700	1.6	.040	.027
	SCARS	03/07/2002	2330	1.6	.039	.026
	SCARS	03/08/2002	0700	1.5	.039	.027

68 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix D. Data for field parameters, major ions, and nutrients in the Santa Ana River at the diversion downstream from Imperial Highway (11075620), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons

[Samples analyzed by U.S. Geological Survey Geochemical lab in San Diego; 11075620, Santa Ana River at the diversion downstream from Imperial Highway; alkalinity measured as filtered incremental titration; ft³/s, cubic feet per second; μS/cm, microsiemens per centimeter at 25 degrees Celsius; mg/L, milligrams per liter; E, Estimated value; <, less than; —, no data]

Station No.	Date	Time	Stream-flow (ft ³ /s)	pH (standard units)	Specific conductance (μS/cm)	Alkalinity (mg/L as CaCO ₃)	Sulfate, dissolved (mg/L as SO ₄)	Chloride, dissolved (mg/L)	Nitrate, dissolved (mg/L as N)	Nitrite, dissolved (mg/L as N)	Bromide, dissolved (mg/L)	Phosphate, ortho, dissolved (mg/L as PO ₄)
11075620	01/25/2000	0915	492	6.8	807	152	95	100	3.7	<0.02	<0.4	0.5
11075620	01/25/2000	1040	565	6.9	768	137	110	87	3.3	<.02	<.4	<.3
11075620	01/25/2000	1130	512	6.9	744	138	95	82	3.2	<.02	<.4	.4
11075620	01/25/2000	1240	492	6.9	782	144	100	90	3.6	<.02	.4	.3
11075620	01/25/2000	1440	471	7.5	791	—	—	—	3.6	<.02	—	—
11075620	01/25/2000	1640	457	7.2	788	—	—	—	3.7	<.02	—	—
11075620	01/25/2000	1940	424	7.4	820	153	—	—	4.4	.03	—	—
11075620	01/25/2000	2240	431	7.2	551	102	—	—	3.1	.04	—	—
11075620	01/26/2000	0140	433	7.3	563	103	—	—	3.4	.05	—	—
11075620	01/26/2000	0740	426	7.3	758	130	95	79	4.2	.09	<.4	.5
11075620	01/26/2000	1040	437	7.2	807	131	130	87	4.3	.11	<.4	.4
11075620	01/26/2000	1340	432	7.2	779	131	100	84	4.2	.13	<.4	.6
11075620	01/26/2000	1530	426	7.9	799	133	120	84	4.3	.14	<.4	.4
11075620	02/12/2000	0000	321	7.9	756	157	95	80	5.4	.09	<.4	.4
11075620	02/12/2000	0130	319	7.9	766	160	—	78	5.1	.08	—	—
11075620	02/12/2000	0300	323	8.0	686	150	—	77	5.0	.08	—	—
11075620	02/12/2000	0430	620	7.8	495	94	75	47	2.9	.06	<.4	<.3
11075620	02/12/2000	0600	629	7.9	510	95	85	49	3.0	.06	<.4	<.3
11075620	02/12/2000	0730	460	8.0	603	110	90	58	3.6	.08	<.4	.3
11075620	02/12/2000	0930	415	8.0	670	130	90	67	4.2	.09	<.4	<.3
11075620	02/12/2000	1130	385	7.3	690	140	90	68	4.2	.09	<.4	<.3
11075620	02/12/2000	1400	366	8.0	763	150	100	79	4.4	.10	<.4	.5
11075620	02/12/2000	1630	346	8.0	731	150	90	77	4.4	.10	.7	2
11075620	02/12/2000	1900	333	8.0	743	150	85	79	4.6	.10	.4	<.3
11075620	02/12/2000	2130	324	7.9	758	160	95	81	4.8	.09	.9	1
11075620	02/13/2000	0030	340	7.9	709	140	90	75	4.4	.09	<.4	<.3
11075620	02/13/2000	0330	334	7.9	728	150	95	79	4.5	.10	<.4	.5
11075620	02/13/2000	0630	334	7.9	711	150	95	75	4.2	.10	1.0	.4
11075620	02/13/2000	0930	357	7.8	702	140	95	72	4.0	.10	<.4	.3
11075620	02/22/2000	2220	—	7.8	462	99	50	44	2.0	.08	<.4	.5
11075620	02/23/2000	0730	—	7.9	422	89	50	45	2.2	.08	<.4	.5
11075620	02/23/2000	0830	—	7.9	422	91	45	38	1.9	.07	<.4	.4
11075620	02/23/2000	0930	—	—	—	—	50	42	2.0	.08	<.4	.4
11075620	02/23/2000	1030	—	7.9	424	91	50	40	1.9	.07	<.4	.5
11075620	02/23/2000	1200	—	7.9	362	76	50	35	1.6	.06	<.4	.3
11075620	02/23/2000	1300	—	7.8	336	66	45	30	3.3	1.1	<.4	.3
11075620	02/23/2000	1430	—	7.9	301	59	50	29	1.3	.04	<.4	.3
11075620	02/23/2000	1600	—	7.9	381	70	60	34	.07	<.02	<.4	.5
11075620	02/23/2000	1900	—	7.1	518	94	3	1.7	2.5	.07	<.4	<.3
11075620	02/23/2000	2200	—	7.2	552	110	100	60	2.4	.07	<.4	<.3
11075620	02/24/2000	0100	—	7.4	474	100	70	49	2.6	.09	<.4	.3
11075620	02/24/2000	0400	—	7.3	501	100	70	54	2.5	.08	.5	.2
11075620	02/24/2000	0700	—	7.3	486	110	70	51	2.5	.06	<.4	.2

Appendix D. Data for field parameters, major ions, and nutrients in the Santa Ana River at the diversion downstream from Imperial Highway (11075620), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—Continued

Station No.	Date	Time	Stream-flow (ft ³ /s)	pH (standard units)	Specific conductance (μS/cm)	Alkalinity (mg/L as CaCO ₃)	Sulfate, dissolved (mg/L as SO ₄)	Chloride, dissolved (mg/L)	Nitrate, dissolved (mg/L as N)	Nitrite, dissolved (mg/L as N)	Bromide, dissolved (mg/L)	Phosphate, ortho, dissolved (mg/L as PO ₄)
11075620	02/24/2000	1000	—	7.6	475	98	60	47	<0.02	<0.02	<0.4	<0.3
11075620	02/24/2000	1900	464	7.8	469	99	55	43	2.3	.06	<.4	.5
11075620	02/25/2000	0300	460	8.0	477	100	55	42	2.2	.07	<.4	.4
11075620	02/25/2000	1100	460	7.9	473	100	60	43	2.2	.10	<.4	.5
11075620	02/25/2000	1900	452	7.9	505	110	70	46	2.3	.10	<.4	.3
11075620	02/26/2000	0300	450	7.7	556	120	75	51	2.4	.14	<.4	<.3
11075620	02/26/2000	1100	456	7.9	547	120	70	50	2.4	.14	<.4	.6
11075620	02/26/2000	1900	452	7.8	576	120	75	53	2.5	.16	<.4	.3
11075620	02/27/2000	0300	447	7.9	597	130	75	56	2.5	.18	<.4	.4
11075620	02/27/2000	1100	443	7.9	607	130	75	56	2.6	.17	<.4	.3
11075620	01/10/2001	1540	275	8.5	971	—	100	110	6.6	.04	<.4	.5
11075620	01/10/2001	1710	284	8.3	965	—	100	100	7.5	<.04	<.4	<.6
11075620	01/10/2001	1900	386	8.3	860	—	91	88	5.2	<.04	<.4	<.6
11075620	01/10/2001	2100	499	8.2	629	—	76	63	3.8	<.02	<.2	<.3
11075620	01/10/2001	2300	466	8.2	648	—	87	64	3.7	<.02	<.2	<.3
11075620	01/11/2001	0100	456	8.4	628	—	90	70	3.9	.04	<.2	<.3
11075620	01/11/2001	0300	1570	8.0	244	—	36	21	1.5	.02	<.2	<.3
11075620	01/11/2001	0500	1550	8.0	306	—	53	27	1.3	.02	<.2	<.3
11075620	01/11/2001	0700	957	7.8	418	—	82	40	1.5	.02	<.2	<.3
11075620	01/11/2001	1100	700	8.1	475	—	80	38	2.1	.04	<.2	<.3
11075620	01/11/2001	1400	600	7.9	472	—	78	39	2.4	.04	<.2	<.3
11075620	01/11/2001	1700	500	7.8	541	—	84	48	3.3	.05	<.2	.4
11075620	01/11/2001	2000	1450	7.7	543	—	78	49	3.5	.03	<.2	<.3
11075620	01/11/2001	2300	3410	7.8	425	—	53	40	2.6	.05	<.2	<.3
11075620	01/12/2001	0030	3840	7.8	402	—	47	37	2.6	.04	<.2	<.3
11075620	01/12/2001	0330	4110	7.8	371	—	41	32	2.4	.02	<.2	<.3
11075620	01/12/2001	0630	2340	7.8	401	—	51	35	2.3	.04	<.2	<.3
11075620	01/12/2001	0930	1410	7.9	408	—	51	36	2.6	.05	<.2	<.3
11075620	01/12/2001	1230	1170	7.9	417	—	48	37	2.7	.04	<.4	.03
11075620	01/12/2001	1530	1030	7.8	415	—	50	37	2.6	.04	.3	<.3
11075620	01/12/2001	2130	434	7.6	457	—	61	42	2.8	.08	<.2	.3
11075620	01/13/2001	0330	414	7.8	577	—	85	59	2.9	<.02	<.2	<.3
11075620	01/13/2001	0930	395	7.8	574	—	85	58	2.8	<.02	<.2	<.3
11075620	01/13/2001	1530	384	7.8	571	—	82	56	2.8	.06	<.2	.3
11075620	01/13/2001	2130	376	7.9	573	—	78	57	2.8	<.02	<.2	<.3
11075620	01/14/2001	0330	372	8.0	603	—	83	64	2.9	.04	<.4	<.3
11075620	01/14/2001	0930	371	7.9	572	—	77	57	2.9	<.02	<.2	<.3
11075620	01/14/2001	1200	367	7.9	572	—	75	56	2.9	<.02	<.2	<.3
11075620	02/11/2001	1900	401	8.3	985	—	100	110	5.5	.16	<.4	<.6
11075620	02/12/2001	0200	395	8.2	987	—	100	100	5.9	.15	<.4	<.6
11075620	02/12/2001	0400	401	8.3	989	—	—	100	—	.15	—	—
11075620	02/12/2001	0600	691	8.1	606	—	65	61	3.3	.10	<.4	<.6
11075620	02/12/2001	0800	2100	7.8	184	—	25	15	.79	.02	<.2	<.3
11075620	02/12/2001	1000	1550	7.9	289	—	44	25	1.2	<.02	<.2	<.3
11075620	02/12/2001	1200	1030	8.0	415	—	58	38	2.1	.06	<.2	<.3
11075620	02/12/2001	1500	1690	7.8	196	—	32	16	.65	<.02	<.2	<.3
11075620	02/12/2001	1800	756	8.0	443	—	86	40	1.3	.03	<.2	<.3
11075620	02/12/2001	2100	1800	8.1	404	—	65	35	1.5	.04	<.2	<.3

70 Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River, Southern California

Appendix D. Data for field parameters, major ions, and nutrients in the Santa Ana River at the diversion downstream from Imperial Highway (11075620), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—Continued

Station No.	Date	Time	Stream-flow (ft ³ /s)	pH (standard units)	Specific conductance (μS/cm)	Alkalinity (mg/L as CaCO ₃)	Sulfate, dissolved (mg/L as SO ₄)	Chloride, dissolved (mg/L)	Nitrate, dissolved (mg/L as N)	Nitrite, dissolved (mg/L as N)	Bromide, dissolved (mg/L)	Phosphate, ortho, dissolved (mg/L as PO ₄)
11075620	02/13/2001	0000	854	8.0	487	—	90	43	1.4	0.03	<0.2	<0.3
11075620	02/13/2001	0300	2480	8.1	634	—	100	60	2.8	.05	<.4	<.6
11075620	02/13/2001	0600	4510	8.0	499	—	65	47	2.5	.04	<.2	<.3
11075620	02/13/2001	0900	5050	8.0	480	—	56	44	2.6	.06	<.2	<.3
11075620	02/13/2001	1200	5400	8.0	386	—	46	34	2.0	.05	<.2	<.3
11075620	02/13/2001	1700	4970	8.0	326	—	36	28	1.9	.05	<.2	<.3
11075620	02/13/2001	2200	4550	8.0	321	—	35	27	1.8	.04	<.2	<.3
11075620	02/14/2001	0300	2570	8.0	344	—	40	28	1.9	.03	<.2	<.3
11075620	02/14/2001	0800	2480	8.0	344	—	38	28	1.8	.03	<.2	<.3
11075620	02/14/2001	1300	1080	8.0	401	—	48	33	1.8	.04	.3	<.3
11075620	02/14/2001	1800	593	8.1	413	—	50	35	1.9	.04	<.2	<.3
11075620	02/15/2001	0800	611	8.0	498	—	66	43	1.9	.05	<.2	<.3
11075620	02/15/2001	1100	601	7.9	489	—	63	41	1.9	.04	<.2	<.3
11075620	03/05/2001	1100	537	8.1	635	140	77	62	2.8	.12	<.2	.4
11075620	03/06/2001	0120	530	8.0	655	140	75	63	2.6	.25	<.4	<.6
11075620	03/06/2001	0230	542	8.1	623	130	68	59	2.7	.18	<.4	.5
11075620	03/06/2001	0400	615	8.1	614	130	73	58	2.7	.13	<.4	.2
11075620	03/06/2001	0530	618	8.1	577	120	70	52	2.5	.15	<.2	.3
11075620	03/06/2001	0700	610	8.1	588	120	69	54	2.6	.11	<.2	.3
11075620	03/06/2001	0830	600	8.1	641	140	74	61	3.4	.11	<.2	.4
11075620	03/06/2001	1000	591	8.1	672	140	78	68	3.0	.01	<.2	.3
11075620	03/06/2001	1230	530	8.1	696	140	79	65	3.1	.13	<.2	.5
11075620	03/06/2001	1700	536	8.3	719	150	92	70	3.0	<.1	<.4	.4
11075620	03/06/2001	2300	538	8.3	720	160	81	73	3.5	.23	<.2	.6
11075620	03/07/2001	0500	574	8.2	723	150	84	72	3.4	.26	<.2	.5
11075620	11/12/2001	1545	234	8.7	960	—	120	110	6.0	<.05	<.2	.3
11075620	11/12/2001	1555	234	8.7	962	—	110	110	5.3	<.05	<.2	.8
11075620	11/12/2001	1800	231	8.6	865	—	120	110	5.3	<.05	<.2	.8
11075620	11/12/2001	1845	232	8.5	993	—	120	110	5.4	<.05	<.2	.5
11075620	11/12/2001	1915	245	8.5	978	—	110	110	4.8	<.05	<.2	.7
11075620	11/12/2001	1945	249	—	—	—	120	110	4.8	<.05	<.2	.7
11075620	11/12/2001	2015	269	8.3	656	—	93	78	4.7	<.05	<.2	<.3
11075620	11/12/2001	2045	383	8.2	648	—	80	66	4.2	<.05	<.2	<.3
11075620	11/12/2001	2115	388	—	—	—	81	64	4.1	<.05	<.2	.3
11075620	11/12/2001	2145	383	8.2	669	—	87	69	4.3	<.05	<.2	<.3
11075620	11/12/2001	2230	380	8.1	684	—	96	70	4.0	<.05	<.2	<.3
11075620	11/13/2001	0030	296	8.1	751	—	110	77	4.2	<.05	<.2	<.3
11075620	11/13/2001	0130	295	8.2	814	—	110	89	4.3	<.05	.4	.7
11075620	11/13/2001	0230	293	—	—	—	120	98	4.6	<.05	<.2	.8
11075620	11/13/2001	0330	290	8.2	922	—	120	100	4.5	<.05	<.2	1.0
11075620	11/13/2001	0430	287	8.3	939	—	120	100	4.6	<.05	<.2	.8
11075620	11/13/2001	0530	290	8.2	939	—	120	100	4.6	<.05	<.2	.8
11075620	11/13/2001	0630	291	8.2	946	—	120	110	4.7	<.05	<.2	.7
11075620	11/13/2001	0730	308	8.2	920	—	--	110	4.8	<.05	<.2	.8
11075620	11/13/2001	1015	330	8.1	886	—	100	100	5.8	<.05	.2	.7
11075620	11/13/2001	2200	263	8.2	947	—	100	76	4.4	<.05	<.2	.3

Appendix D. Data for field parameters, major ions, and nutrients in the Santa Ana River at the diversion downstream from Imperial Highway (11075620), southern California, 1999–2000, 2000–01, and 2001–02 rainy seasons—Continued

Station No.	Date	Time	Stream-flow (ft ³ /s)	pH (standard units)	Specific conductance (μS/cm)	Alkalinity (mg/L as CaCO ₃)	Sulfate, dissolved (mg/L as SO ₄)	Chloride, dissolved (mg/L)	Nitrate, dissolved (mg/L as N)	Nitrite, dissolved (mg/L as N)	Bromide, dissolved (mg/L)	Phosphate, ortho, dissolved (mg/L as PO ₄)
11075620	03/06/2002	1830	255	8.5	1,040	—	130	120	6.7	0.05	<1	<1
11075620	03/07/2002	0630	224	8.2	1,030	—	120	110	5.7	.07	<1	1.0
11075620	03/07/2002	0800	229	8.2	1,000	—	120	110	5.7	.06	<1	<2
11075620	03/07/2002	0930	234	8.3	994	—	110	110	5.8	.07	<1	<2
11075620	03/07/2002	1130	236	8.5	1,000	—	120	120	5.9	.06	<1	<.5
11075620	03/07/2002	1330	240	8.6	997	—	110	120	7.2	.07	<1	.5E
11075620	03/07/2002	1530	238	8.8	1,000	—	120	120	6.4	.06	<1	.4E
11075620	03/07/2002	1730	237	8.7	1,020	—	120	120	6.5	.06	<1	.5
11075620	03/07/2002	2000	237	8.6	1,020	—	120	120	6.8	.06	<1	.5
11075620	03/07/2002	2300	238	8.3	1,020	—	120	120	6.9	.06	<1	.6
11075620	03/08/2002	0200	243	8.3	994	—	120	120	6.6	.06	<1	.4E
11075620	03/08/2002	0500	244	8.2	987	—	120	120	6.4	.07	<1	.4E
11075620	03/08/2002	0800	244	8.2	983	—	120	110	6.3	.08	<1	.4E
11075620	03/08/2002	1100	247	8.4	984	—	120	110	6.2	.10	<1	.6