Title
Is urban runoff a source of human pathogenic viruses to recreational beach waters?

Author
Jiang, Sunny

Publication Date
2004-07-01

Is Urban Runoff a Source of Human Pathogenic Viruses to recreational Beach Waters?

Sunny Jiang

Environmental Health, Science and Policy,

University of California, Irvine, California 92697

Tel: 949-824-5527, Fax: 949-824-2056, Email: sjiang@uci.edu

UC Water Resources Center Technical completion report Project No. W-943

July 2004

ABSTRACT

The Aim of this study is to investigate human viral contamination in urban rivers and its impact on coastal waters of southern California. During the first year study, three types of human viruses (adeno, entero and hepatitis A) were detected using nested- and RT-PCR at eleven rivers and creeks along southern California coast. Fecal indicator bacteria as well as somatic and F-specific coliphage were also tested. Approximately 50% of the sites were positive for human adenoviruses. However, there was no clear relationship between detection of human viruses and the concentration of indicator bacteria and coliphage. Both fecal indicator bacteria and human viral input at beaches near river mouths were associated with storm events. The first storm of the wet season seemed to have the greatest impact on the coastal water quality than following storm events. During the second year of study, a detailed seasonal assessment of microbial pollution was conducted in two major Southern California urban rivers, San Gabriel and Los Angeles. A total of 114 river samples from five different sample locations along these two rivers were collected over a one-year period and analyzed for fecal indicator bacteria, including total coliform, fecal coliform, and enterococcus and indicator viruses, including F-specific and somatic coliphage, and human entero and adenoviruses. Based on the California recreational water quality standards, 52.5% of the samples exceeded at least one fecal indicator bacteria standard. Both somatic and F-specific coliphage were detected in higher concentrations (up to $5x10^2$ PFU/100ml) during storm events. Enterovirus was detected in ~13% of the samples by reverse-transcription PCR. Adenovirus was detected by real-time PCR in approximately 50% of the samples, with concentrations ranging from 10 to $>10^6$ genomes per liter. However, plaque assay using two human tissue culture cell lines, HEK-293A and A549, yielded negative results suggesting adenoviruses detected by real-time PCR may not be infectious. Correlations between human adenoviruses and F-specific ($p=0.13$) and somatic coliphages ($p=0.06$) were found. However, these correlations were not statistically significant at 95% level. No significant correlation between human adenoviruses and fecal indicator bacteria (total coliform, fecal coliform, and enterococcus) was observed. This study presents the first quantitative measure of human adenoviruses in urban rivers and their statistical relationship to fecal indicator bacteria and coliphages. This study also provides the first direct evidence that human viruses are prevalent in southern California urban rivers.

INTRODUCTION

Southern California beaches are unique recreational and economical resources to the State of California. To protect human exposure from microbial pathogens during water recreations, the state implemented routine monitoring programs for fecal indicator bacteria, including total coliform, fecal coliform and enterococcus, at major bathing beaches. However, the relationship between the occurrence of fecal indicator bacteria and human pathogenic viruses is not clear. An early investigation of beach water quality along the coast of southern California indicated the presence of human viruses at several sites near the mouths of rivers, creeks and wetlands (Jiang et al. 2001), where bacterial indicators are at acceptable level for water contact recreation. Furthermore, this early study also suggested that urban rivers and streams are the most possible sources of human viral pollution although no direct investigation of the occurrence and distribution of human viruses in southern California urban rivers was conducted (Jiang et al. 2001).

Coastal southern California weather displays distinct wet (winter) and dry (summer) seasons. Over 90% of the precipitation occurs between November and April, while rain rarely occurs during the summer months. Several early studies (Boehm et al. 2002; Ackerman and Weisberg 2003; Noble et al. 2003) have shown correlations between the rainfall events and widespread pollution of fecal indicator bacteria at southern California coasts. Stanley et al. (2002) suggested that rainfall can be used as a predictor for near-real-time bathing beach bacterial water quality in areas where there are combined sewer overflow systems. Lipps et al. (2001) showed that enteroviruses were detected at 75% of the sampling stations during the El Nino event with increased rainfall, while none were detected in other months of the year in Charlotte Harbor estuary, Florida. There has not been a study to investigate the seasonal distribution of human viruses in southern California urban rivers and their impact to the water quality of the local beaches. With the rapid urban development of southern California, the volume of urban storm runoff will only increase in future years. Therefore, it is crucial to understand the impact of urban rivers to beach water quality in order to develop proper strategies for the management/remediation of storm water.

There are more than 100 different types of viruses found in human waste and all are potentially transmitted by water (Berg 1983). These viruses are more resistant to degradation than fecal bacterial indicators in the aquatic environment (Shuval 1971). Therefore the viral quality of natural waters cannot be accurately evaluated by monitoring for the presence or absence of bacterial indicators (Goyal et al. 1978). Human enteroviruses have been detected in coastal waters of southern California (Noble and Fuhrman 2001), the Sarasota Bay estuary, Florida (Lipp et al. 2001) and in residential canals in the Florida Keys (Griffin et al. 1999) where the level of bacterial indicators met current water quality standards.

The U.S. Environmental Protection Agency (USEPA) describes the enteric virus group (including norovirus, rotavirus, hepatitis A virus, adenovirus, and enterovirus etc.) as the most meaningful, reliable and effective virus index for environmental monitoring (Karaganis et al. 1983). These viruses, mostly RNA viruses, cause diseases include

paralysis, meningitis, respiratory disease, epidemic vomiting and diarrhea, myocarditis, congenital heart anomalies, infectious hepatitis, and eye infection mostly in children or elderly. Hepatitis A continues to be one of the most frequently reported vaccine-preventable diseases in the United States. Although hepatitis A occurs in virtually every area of the United States, western states including southern California have higher rates than rest of the country (http://www.cdc.gov/ncidod/diseases/hepatitis/a/vax/index.htm). Adenoviruses are the only human enteric viruses to contain DNA. Adenoviruses 40 and 41 have been recognized as important etiological agents of gastroenteritis in children. Giordano et al. (2001) reported adenoviruses 40/41 were the major causing agent of viral gastroenteritis in children in Cordoba City, Argentina following rotaviruses and astroviruses. Simpson et al. (2003) reported that the most commonly found single viral pathogen among children under the age of 5 in East Anglia was rotavirus (27.9%), followed by norovirus (13.4%), enteric adenoviruses (7.9%) and astrovirus (2.3%). Similarly, a survey of German children admitted to hospital with acute gastroenteritis showed rotavirus, norovirus and enteric adenoviruses were the major causes of infection (Oh et al. 2003). However, Rodriguez-Baez et al. (2002) concluded that adenoviruses played a limited role in gastroenteritis in hospitalized children in a study conducted at Stanford University Hospital, USA.

Adenoviruses are known to be substantially more stable than either polio or hepatitis A viruses in tap water and seawater (Enriquez et al. 1995). They are also reported to be more resistant to inactivation by UV than enteroviruses (Meng and Gerba 1996; Gerba et al. 2002). There are also documented outbreaks of conjunctivitis due to adenovirus types 3 & 4 associated with swimming in contaminated recreational waters (Crabtree et al. 1997). Respiratory adenovirus and those cause eye infection are important etiological agents for disease associated with water-contact activities because of their potential transmission via water spray. Based on the data obtained from human dose-response studies and monitoring data from recreational water, risks of adenoviral disease were calculated to be as high as 1/1,000 for a single exposure (Crabtree et al. 1997). So far there has not been a microbial indicator for non-gastroenteritis diseases, while eye, ear and respiratory infections were commonly reported among bathers at recreational beaches (Dwight 2002). Adenoviruses are currently included in the drinking water microbial contamination candidate list by the USEPA due to their resistant characteristics to water treatment processes. Therefore, they are one of the priorities for monitoring and development of treatment strategies.

OBJECTIVES

There are two major objectives of this study: 1. To investigate the prevalence of human pathogenic viruses in southern California urban waterways in order to understand their contributions to coastal water pollution; 2. To examine the seasonal and spatial distribution of pathogenic viruses in the Los Angeles River and San Gabriel River.

Objective 1. Prevalence Of Human Pathogenic Viruses In Southern California Rivers

MATERIALS AND METHODS

Urban River Study Sites: An intense water quality investigation of southern California urban rivers were conducted between July 10 and August 30, 2000. Water samples were collected from 11 rivers and creeks along the coast of southern California starting north of Malibu Creek to south of the San Diego River at 21 locations (Figure 1). Two sampling locations were selected from each river and creek; one located downstream (site I), near the mouth of the river, and the other upstream (site II) approximately 5 to 16 kilometers from the first site. In general, the study area is highly urbanized, not impacted by agriculture runoff. At least one river, San Gabriel River, receives tertiary treated sewage effluents from one of the Los Angeles sewage treatment facilities.

Seasonal Study at River Mouths: To determine the seasonal variability of pollution input from urban rivers at local beaches, water samples were collected repetitively from the mouths of three rivers: Los Angeles, San Gabriel and Santa Ana, over both wet and dry seasons. Sampling for Santa Ana and San Gabriel River mouths started in early October 2000 and collection from the mouth of Los Angeles River began in January 2001. Two sites from the mouths of the Santa Ana and San Gabriel Rivers were chosen, one located directly at the mouth where the river and the coastline meet (point zero), the other located at surfzone approximately 150 meters down the coast from the mouth of the river. Because the Los Angeles River empties into Long Beach Harbor only one sample was taken at the mouth of the river. Sampling at the mouth of the Santa Ana River was terminated in March 2001 due to dredging at the sampling sites and upstream divergence of water flow to local sewage treatment facilities (no river input to the beach). The remaining two sites were sampled periodically until the end of the May 2001.

Sampling Procedure: All samples were taken between 9 and 11:30 am using a bleach-sterilized, triple sample-rinsed bucket and collected in sterilized polypropylene carboys or sterile sampling bags (Wirlpak bag, Fisher Scientific, Inc.). Beach samples were collected at ankle depth in accordance with the sampling protocol used for beach monitoring program by local agencies. Samples from the mouth of the Los Angeles River were taken from a fishing pier using a sterilized-bucket hanging at the end of rope. All samples were transported back to the laboratory within 1-2 hours of collection for immediate processing. Water temperature and salinity were measured on-site using a calibrated thermometer and a hand-held refractometer, respectively. Environmental and weather conditions at each sampling site were also recorded.

Determination of Indicator Bacteria: Total coliform (TC), fecal coliform (FC) and enterococcus (Ent) were determined using a membrane filtration method following

standard protocols (Clesceri et al. 1998). In brief, samples were serially diluted, filtered onto 0.45 μm pore-size 47 mm diameter sterile filters (Fisher Scientific, Inc.), which were incubated on solid medium for 24 (TC and FC) to 48 hours (Ent) for the development of colonies. Commercially available m-Endo, m-FC and m-E medium (Difco Lab.) were used for cultivation of TC, FC and Ent, respectively. EIA agar plates (Difco Lab.) were used to confirm Ent colonies that grew on m-E medium after 48 hours incubation at 41.5°C. The incubation temperature for TC and FC was 37°C and 44.5°C, respectively. Each assay was performed using replicate samples from each site.

Concentration of Water Samples for Viral Detection: Water samples were concentrated using either a vortex flow filtration (VFF) system with a 100KD molecular weight cutoff filtration membrane or a Centriprep-100 centrifugal ultrafiltration device with a 100KD molecular weight cutoff membrane (Millipore). The efficiency of viral recovery determined by phage seeding study for both systems and detailed methods of viral concentration were described previously by Jiang et al. (1992) and Chu et al (2003), respectively. The viral recovery rates for both systems are comparable, ranging from 60% to 80%. The concentration factors ranged from 200 to 500 folds for different samples. Most of the lower concentration factors were from samples collected in rivers and creeks that contain high concentrations of suspended solids.

Approximately 5 ml each of the concentrate from river and creek samples were used for plaque assay of coliphage immediately after concentration and the rest of the concentrates were frozen at –70°C until used for PCR analysis of human viruses.

Determination of Coliphage Concentrations: Two *E. coli* hosts, ATTCC 15597 and HS (pFamp)R, were used. *E. coli* ATTCC 15597 is a general host for somatic coliphage; *E. coli* HS(pFamp)R contains a plasmid coding for both ampicillin and streptomycin resistance and is a specific host for F-specific coliphage (Debartolomeis and Cabelli 1991). When HS(pFamp)R was employed the bottom agar contained 15 μg/ml of each ampicillin and streptomycin to prevent background growth of indigenous bacteria. Coliphage densities were determined using either VFF concentrated or un-concentrated water samples. One milliliter and 0.1 ml of sample were mixed with one ml of *E. coli* host in 1% soft agar, then overlayed on bottom nutrient agar. Plaques were enumerated after 12 hours of incubation at 37 °C. The number of phage was converted using sample volume and the concentration factor to plaque forming unite (pfu) per liter of original water sample.

PCR Detection of Human Viruses: Viral nucleic acid from concentrated water samples was purified to remove PCR inhibitors using the method originally developed by Boom et al. (1999) with minor modifications by Jiang et al. (2001). Primers and probes for detection of adenoviruses, enteroviruses and hepatitis A viruses are listed in Table 1. Nested-PCR was performed following the protocol of Pina et al (1998) for detection of adenoviruses with minor modifications by Jiang et al. (2001). These primer sets was shown to amplify multiple serotypes of adenoviruses including enteric adenoviruses serotypes 40 and 41 (Pina et al. 1998). Reverse transcription PCR for enteroviruses and hepatitis A viruses was performed essentially as described by Tsai et al. (1993) with a modification of the total reaction volume to 50 μl. Amplicons were confirmed by probing

with internal oligonucleotide probes and/or labeled PCR products from positive control by southern transfer or in a dot-blotting format to increase the sensitivity of detection as well as confirmation of correct amplification products.

Precipitation Data: Rainfall data were retrieved from California Irrigation Management System (CIMS) rain stations managed by the California Department of Water Resources (www.ipm.ucdavis.edu). Precipitation data from the CIMS Long Beach station was used to correlate the results obtained from the mouths of the Los Angeles and San Gabriel Rivers, while the CIMS Santa Ana station was used to interpret results from the Santa Ana River because of the approximate location of the respective stations to the rivers.

RESULTS

Description of Study Sites. No rainfall was recorded in coastal Southern California during the summer of 2000. Most of the rivers and creeks sampled had minimal flow from inland to the ocean (Table 2). Runoff from urban irrigation, car wash and other domestic water usage is the major source of freshwater input to the rivers and creeks during the season. Dense vegetation along the river/creek bank and heavy algal blooms were observed at many inland sites where water was stagnant. Near the coastal zone, the rivers/creeks are influenced by tidal flushing. The salinity at the near-coast sites (site I) was generally higher than the upstream sites (site II) reflecting different degrees of tidal mixing (Table 2). The highest salinity, 36 ‰, was observed at Santa Ana River site I. This site was completely influenced by the ocean because no freshwater flow from upstream was observed. Salinity of 0 ‰ or near 0 ‰ was recorded for the inland portion of all rivers and creeks. Water temperature over all sites averaged 27°C. Higher temperatures were associated with sites having shallow water or stagnant flow (Table 2).

Fecal Indicator Bacteria and Coliphages in Urban Rivers Table 3 shows the concentration of three indicator bacteria (TC, FC and Ent), somatic and F-specific coliphage at 21 sampling sites. TC ranged from below the detection limit (<10 per 100ml) at San Gabriel River I and Santa Ana River I where urban runoff was highly diluted by ocean water as indicated by high salinity readings, to 230,000 CFU per 100ml at Malibu Creek site I where stagnant water was colonized by a large number of birds. The geometric mean for all sites was 2,653 CFU per 100ml. The concentration of FC correlated with that of the TC with a pearson correlation value of 0.76. The geometric mean for all sites was 298 and averaged 1,587 CFU per 100ml. Seven of 21 sites had FC levels greater than 400 CFU per 100 ml (State of California single sample Rec-1 Water Quality Objective). Ent did not correlate well with TC (r=0.01) and FC (r=0.00). High concentrations of Ent were detected at Aliso Creek I and San Diequito River site II, where TC and FC at these sites were below geometric means. Somatic coliphages were present at all sites, except Santa Ana River site I. However, they were poorly correlated with TC concentration at each site (r=0.03). F-specific coliphage were detected at 57% of the sites sampled and they were positively correlated with TC (r=0.93), FC (r=0.83), but poorly with Ent (r=-0.03).

Based on microbial indicator concentration, sites were ranked from best to worst for each indicator organism (Table 3). For example, both Santa Ana River site I and San Gabriel River site I were rank 1 for TC because they have the lowest concentration of TC among 21 sites. Malibu Creek I was rank 19 for the highest concentration of TC among all sites. The final rank of each site combined the ranking for individual microbial indicator. The highest total ranking number indicated the worst water quality among all sites (Table 3). Using this system, Malibu Creek I ranked the last among all sites, San Gabriel I and Santa Ana I where were highly influenced by ocean water ranked the best of water quality. Two sites at Los Angeles River ranked 18 and 19, respectively, towards the worst category of the classification. All sites ranked 15 and up exceed California REC-1 (water contact recreation) Water Quality Objectives for FC of 400 organisms per 100ml.

Occurrence of Human Viral Contamination in Urban Rivers. The occurrence and distribution of human pathogenic viruses in 21 samples collected from southern California urban rivers is also shown in Table 3. Adenoviruses were detected at 11 of the 21 sites (52%). Enteroviruses were found at two additional sites. Hepatitis A viruses were the most frequently detected, with 16 of the 21 samples (76%) tested positive. There was no apparent relationship between the occurrence of human viruses and the microbial quality of the water based on indicators. Interestingly, viruses were not detected at the worst ranked site, Malibu Creek I, but were found at San Gabriel River I which ranked the second best in water quality based on microbial indicator data.

Seasonal Variability of Pollution Input from Urban Rivers. Figure 2 shows the biological and physical parameters at the mouth of the Los Angeles River over a four-month period. Samples taken during the wet season between Jan. 25 to Feb. 27, 2001 had three orders of magnitude greater concentrations of all indicator bacteria than those taken during the dry season between Apr. 17 and May 29, 2001. Indicator concentrations were inversely related to salinity with pearson correlations ranging from -0.6 to -0.8 for three bacteria indicators. Human viruses were also detected in four of the five samples taken between January and February but were undetectable between April and May (Figure 2). Enterovirus was found more frequently than adenovirus which was found only once in a Feb. 5th sampling. The average water temperature at Los Angeles River mouth was 15.4°C for January and February, 3.6°C lower than that for April and May. Monthly total rainfall was 6.23 and 6.91 inches, respectively, for January and February, while only 0.62 inches of rainfall in April and no rain in May.

At the mouth of the San Gabriel River, both the point zero and surfzone stations displayed similar patterns for biological and physical parameters during the seasonal sampling program (Figure 3). The highest concentrations of TC and FC were found on Oct. 10, 2000 when the first rainfall of the season (first flash) occurred after a long summer drought. Although the level of precipitation was not recorded at the CIMS Long Beach station located north of the river, the rain event was recorded during our sampling trip and registered 0.01 inches at the CIMS Santa Ana station, south of the river. Since the drainage area for San Gabriel River is greater than a single rain station recorded area, rain event records for nearby stations should also be considered in the case of patchy rainfall over a large geographical area. Enteroviruses were found both at point zero and in the surfzone on Oct. 10th, 2000.

A dramatic decrease in all three indicators was recorded at the next sampling time (Oct. 24th). However, both samples at the point zero and surfzone remained positive for enteroviruses (Figure 3). This may be explained by the resistant nature of viruses to environmental degradation conditions, thus surpassing the viability of bacterial indicators in coastal ocean. Low levels of rainfall were again recorded at the Santa Ana rain station at the end of October (Oct. 26, 0.08 inches, 27, 0.7 inches and 29, 0.39 inches). This may have resulted in the observed increase of indicator level and the detection of enteroviruses in the Nov. 1st samples. The salinity at the mouth of the river did not decrease significantly at either sampling time suggesting there was not a large input of freshwater due to these precipitation events (Figure 3).

Between January and February, a total precipitation of 13.92 inches was recorded at the CIMS Long Beach rain station, accounting for more than 80% of the total rainfall for the year. Samples taken during this wet season had elevated levels of TC and FC at both the point zero and surfzone stations in most cases but were no greater than those after the first rainfall of the season (first flush). Enteroviruses were detected at both stations on March 1 and adenoviruses were found on Jan. 25 only at the point zero station (Figure 3). Decreases in salinity were also recorded on Feb. 21 and Mar. 1, correlating with the heavy rainfall event. No precipitation was recorded after April and no human virus was detected in any of the samples collected in May 2001.

Patterns of microbial and rainfall records similar to those at the San Gabriel River mouth were also observed at the mouth of the Santa Ana River point zero and surfzone stations with the exception of an unexpected peak of enterococcus in the surfzone found on Oct. 4th (Figure 4). Bottom dredging near the sampling site was noted during this sampling date, which may have contributed to this elevation in the surfzone. Samples taken between Oct. 4 and Nov. 1 had very low levels of TC and FC. However, enteroviruses were detected at point zero on Oct. 4th and at surfzone on Nov. 1 (Figure 4). During the wet season between Jan 24th and Feb. 21st, elevated levels of indicator bacteria were found at both the surfzone and point zero stations, yet human viruses were not found at any time.

DISCUSSION

The step-wised implementation of the total maximum daily load (TMDL) plan for rivers and creeks in California and elsewhere in the United States requires the identification of pollution sources in order to best manage contamination problems. Currently, only fecal coliform TMDL is used to evaluate the quality of inland water bodies in California. Research conducted in this study found no clear relationship between the concentration of indicator organisms and the presence of human viruses. No human viruses were detected at the site ranking the worst for fecal indicator, suggesting fecal contamination from non-human sources, i.e. indigenous animal populations (i.e. birds, rodents) and natural in soil re-growth. Nevertheless, viral contamination was detected in over 50% of southern California urban rivers and creeks sampled during this study. This result is generally in agreement with reports of viral contamination in coastal waters impacted by urbanization in the U.S. In the Florida Keys, Griffin et al. (1999)

reported, using a RT-PCR method, 79% of samples collected from various locations were positive for human enteroviruses, 63% were positive for Hepatitis A viruses, and 95% of sites were positive for at least one of the target viruses. Using tissue culture, similar results were reported for Sarasota Bay estuary, Florida, where infectious enteroviruses were found at 81.8% of the stations and 25% of the samples collected from 6 tidal influenced rivers/creeks (Lipp et al. 2001). However, study sites investigated here differ from Florida studies in terms of the urban setting. Both the Florida Keys and the Sarasota Bay estuary are influenced by on-site sewage disposal systems. In Florida, fecal indicator bacterial levels were very low compared with those found in southern California urban rivers, and F-specific coliphage was not found in the Florida Keys.

It is important to emphasize that since only PCR method is used in our study, the viral signal detected may be non-infectious. The discharge of tertiary treated sewage effluent into the urban river system may contribute to the non-infectious viral particles that only detectable by PCR method. This may explain the high incidences of human viruses detected by PCR in San Gabriel River. In addition, PCR-based virus detection methods offer greater sensitivity compared with culture-based assays. Application of nested procedures or probing with an internal probe further enhances the sensitivity and specificity of the PCR assay. Therefore, positive PCR detection of viral genomes may or may not represent the presence of infectious human viruses. This information has more utility in identifying the source of contamination and should be used in combination with fecal indicator data. When the viral signal is concurrent with high levels of fecal indicator bacteria, it suggests a recent human sewage contamination and potential health risk event. When the presence of the viral signal is not accompanied by an elevated level of fecal indicator bacteria, it may suggest an aged source of fecal contamination and the infectivity of the viral pathogens should also be questioned.

Research conducted in Europe showed that adenoviruses were frequently detected in coastal waters suggesting they be used as an index for human viral contamination (Pina et al. 1998). Therefore, we were surprised to find in our study that adenoviruses were detected less frequently than entero and hepatitis A viruses. These results may have several underlying reasons as follows. Method differences in detection of entero, hepatitis A and adenoviruses possibly provided greater sensitivity for detecting entero and hepatitis A viruses. Alternatively, entero and hepatitis A viruses may be shed from the southern California population in greater numbers than adenoviruses. Furthermore, amplification efficiency may differ for different sets of primers and target organisms. For example, the sensitivity of detection of hepatitis A virus may be higher than that for adeno and enteroviruses, therefore resulting in more frequent detection of this organism.

The design of PCR primers and probes is based on, and limited to, our current knowledge of existing sequences of human and animal viruses and related organisms. Although primers and probes used in this study have been previously tested by several investigators (i.e. Tsai et al. 1993; Schwab et al. 1996; Griffin et al. 1999) and periodically re-checked by submitting sequences to GenBank (NCBI) for matching with the most up-to-date sequence database to verify the specificity to human viruses, it is still difficult to completely rule out the possibility of amplifying non-human viral sequences because the vast majority of organisms in the environment have not been sequenced. For example, the primers used for detection of enteroviruses targeting at 5'-non translated

region that is highly conserved across enteroviruses including animal enteroviruses. Natural mutation among animal viruses may contribute to the false positive of human viral detection. Therefore, these PCR results represent our best knowledge of the target organisms. However, since none of the sampling sites included in this study are adjacent to animal farms, the possibility of detecting animal viruses is not one of the strong possibilities.

Seasonal data from the mouths of three rivers indicated that the pollution input was greater during rainy season. The first storm of the season (first flush) has a greater impact on recreational beach water quality than the following storm events. Detection of human viruses at the mouths of the Los Angeles and the San Gabriel rivers can be explained by the land source and related rainfall events. However, the occurrence of human viruses at the mouth of the Santa Ana River seemed to have no relationship to rainfall events and did not appear to result from land-based contamination sources. A secondary source of viral contamination at the Santa Ana River mouth may be the 1.6-kilometer sewage outfall-pipe that discharges mixed primary and secondary sewage directly offshore from this site. The impact of this sewage outfall to local coastal water quality requires further investigation.

REFERENCES

Ackerman, D., and Weisberg, S.B. (2003) Relationship between rainfall and beach bacterial concentrations on Santa Monica Bay beaches. *Journal of Water and Health* **1**: 85-89.

Berg, G. (1983) *Viral pollution of the environment*. Boca Raton, Fla.: CRC Press.

Boehm, A.B., Grant, S.B., Kim, J.H., Mowbray, S.L., McGee, C.D., Clark, C.D. et al. (2002) Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. *Environmental Science and Technology* **36**: 3885-3892.

Boom, R., Sol, C., Beld, M., Weel, J., Goudsmit, J., and Wertheim-Van Dillen, P. (1999) Improved silica-guanidiniumthiocyanate DNA isolation procedure based on selective binding of bovine alpha-casein to silica particles. *Journal of Clinical Microbiology* **37**: 615-619.

Chu, W., Choi, S., and Jiang, S. (2003) Concentration of viruses from aquatic environments by CentraMate TFF systems and Centricon Plus-80. In *Abstracts of the General Meeting of the American Society for Microbiology*. Washington D. C.

Clesceri, L.S., Greenberg, A.E., and Eaton, A.D. (eds) (1998) *Standard methods for the examination of water and wastewater*. Washington, D.C.: American Public Health Association.

Crabtree, K.D., Gerba, C.P., Rose, J.B., and Haas, C.N. (1997) Waterborne adenovirus: A risk assessment. *Water Science and Technology* **35**: 1-6.

Debartolomeis, J., and Cabelli, V.J. (1991) Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific bacteriophages. *Applied and Environmental Microbiology* **57**: 1301-1305.

Dwight, R.H. (2002) Ph.D. Dissertation. *University of California*.

Enriquez, C.E., Hurst, C.J., and Gerba, C.P. (1995) Survival of the enteric adenoviruses 40 and 41 in tap, sea, and waste water. *Water Research* **29**: 2548-2553.

Gerba, C.P., Gramos, D.M., and Nwachuku, N. (2002) Comparative inactivation of enteroviruses and adenovirus 2 by UV light. *Applied and Environmental Microbiology* **68**: 5167-5169.

Giordano, M.O., Ferreyra, L.J., Isa, M.B., Martinez, L.C., Yudowsky, S.I., and Nates, S.V. (2001) The epidemiology of acute viral gastroenteritis in hospitalized children in Cordoba City, Argentina: an insight of disease burden. *Revista do Instituto de Medicina Tropical de Sao Paulo* **43**: 193-197.

Goyal, S.M., Gerba, C.P., and Melnick, J.L. (1978) Prevalence of human enteric viruses in coastal canal communities. *Journal of Water Pollution Control Federation* **50**: 2247-2250.

Griffin, D.W., Gibson, C.J., Lipp, E.K., Riley, K., Paul, J.H., and Rose, J.B. (1999) Detection of viral pathogens by reverse transcriptase PCR and of microbial indicators by standard methods in the canals of the Florida Keys. *Applied and Environmental Microbiology* **65**: 4118-4125.

Jiang, S., Noble, R., and Chu, W. (2001) Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California. *Applied and Environmental Microbiology* **67**: 179-184.

Jiang, S., Thurmond, J., Pichard, S.L., and Paul, J.H. (1992) Concentration of microbial populations from aquatic environments by vortex flow filtration. *Marine Ecology Progress Series* **80**: 101-107.

Karaganis, J.V., Larking, E.P., Melnick, J.L., Scarpino, P.V., Schaub, S.A., Sorber, C.A. et al. (1983) Research priorities for monitoring viruses in the environment. In. Cincinnati: U.S. environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory.
Lipp, E.K., Farrah, S.A., and Rose, J.B. (2001) Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. *Marine Pollution Bulletin* **42**: 286-293.
Meng, Q.S., and Gerba, C.P. (1996) Comparative inactivation of enteric adenoviruses, poliovirus, and coliphage by ultraviolet irradiation. *Water Research* **30**: 2665-2668.
Noble, R.T., and Fuhrman, J.A. (2001) Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: Low correlation to bacterial indicator levels. *Hydrobiologia* **460**: 175-184.
Noble, R.T., Weisberg, S.B., Leecaster, M.K., McGee, C.D., Dorsey, J.H., Vainik, P.M., and Orozco-Borbon, V. (2003) Storm effects on regional beach water quality along the southern California shoreline. *Journal of Water and Health* **1**: 23-31.
Oh, D.Y., Gaedicke, G., and Schreier, E. (2003) Viral agents of acute gastroenteritis in German children: prevalence and molecular diversity. *Journal of Medical Virology* **71**: 82-93.
Pina, S., Puig, M., Lucena, F., Jofre, J., and Girones, R. (1998) Viral pollution in the environment and in shellfish: Human adenovirus detection by PCR as an index of human viruses. *Applied and Environmental Microbiology* **64**: 3376-3382.
Rodriguez-Baez, N., O'Brien, R., Qiu, S.Q., and Bass, D.M. (2002) Astrovirus, adenovirus, and rotavirus in hospitalized children: prevalence and association with gastroenteritis. *Journal of Pediatric Gastroenterology and Nutrition* **35**: 64-68.
Schwab, K.J., De Leon, R., and Sobsey, M.D. (1996) Immunoaffinity concentration and purification of waterborne enteric viruses for detection by reverse transcriptase PCR. *Applied and Environmental Microbiology* **62**: 2086-2094.
Shuval, H.I. (1971) Detection and control of enteroviruses in the water environment. In *Developments in Water Quality Research.* Shuval, H.I. (ed). Ann Arbor: Ann Arbor-Humphrey, p. 47.
Simpson, R., Aliyu, S., Iturriza-Gomara, M., Desselberger, U., and Gray, J. (2003) Infantile viral gastroenteritis: on the way to closing the diagnostic gap. *Journal of Medical Virology* **70**: 258-262.
Stanley, A.M., Coughlin, K.A., Shine, J., Coull, B.A., and Rex, A.C. (2002) Receiver operating characteristic analysis is a simple and effective tool for using rainfall data to predict bathing beach bacteria water quality. In *Abstracts of the General Meeting for the American Society for Microbiology*, p. 102.
Tsai, Y.L., Sobsey, M.D., Sangermano, L.R., and Palmer, C.J. (1993) Simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-polymerase chain reaction. *Applied and Environmental Microbiology* **59**: 3488-3491.

FIGURE LEDGENDS

Figure 1. Map of southern California showing locations of rivers and creeks sampled during this study.

Figure 2. Seasonal variability of fecal indicator bacteria (total coliform, fecal coliform and enteroccus), human viruses (adeno and enteroviruses), salinity and temperature at the mouth of Los Angeles River. Fecal indicator bacteria are in CFU per 100 ml. Presence of a human virus was scored as 1, absence of any human virus was scored as 0 (the same system is also used in figure 2 and figure 3). Rainfall data was collected at the Long Beach rain station managed by the California Department of Water Resources.

Figure 3. Seasonal variability of fecal indicator bacteria (total coliform, fecal coliform and enteroccus), human viruses (adeno and enteroviruses), salinity and water temperature at the mouth of San Gabriel River. Point zero station was located directly at the mouth of the river where river and coastline meet. Surfzone station was located 150 meters down coast from the river mouth. Rainfall data were collected at Long Beach rain station managed by California Department of Water Resources.

Figure 4. Seasonal variability of fecal indicator bacteria (total coliform, fecal coliform and enteroccus), human viruses (adeno and enteroviruses), salinity and water temperature at the mouth of Santa Ana River. Point zero and surfzone stations were the same as described in figure 2. Rainfall data was collected at the Santa Ana rain station managed by the California Department of Water Resources.

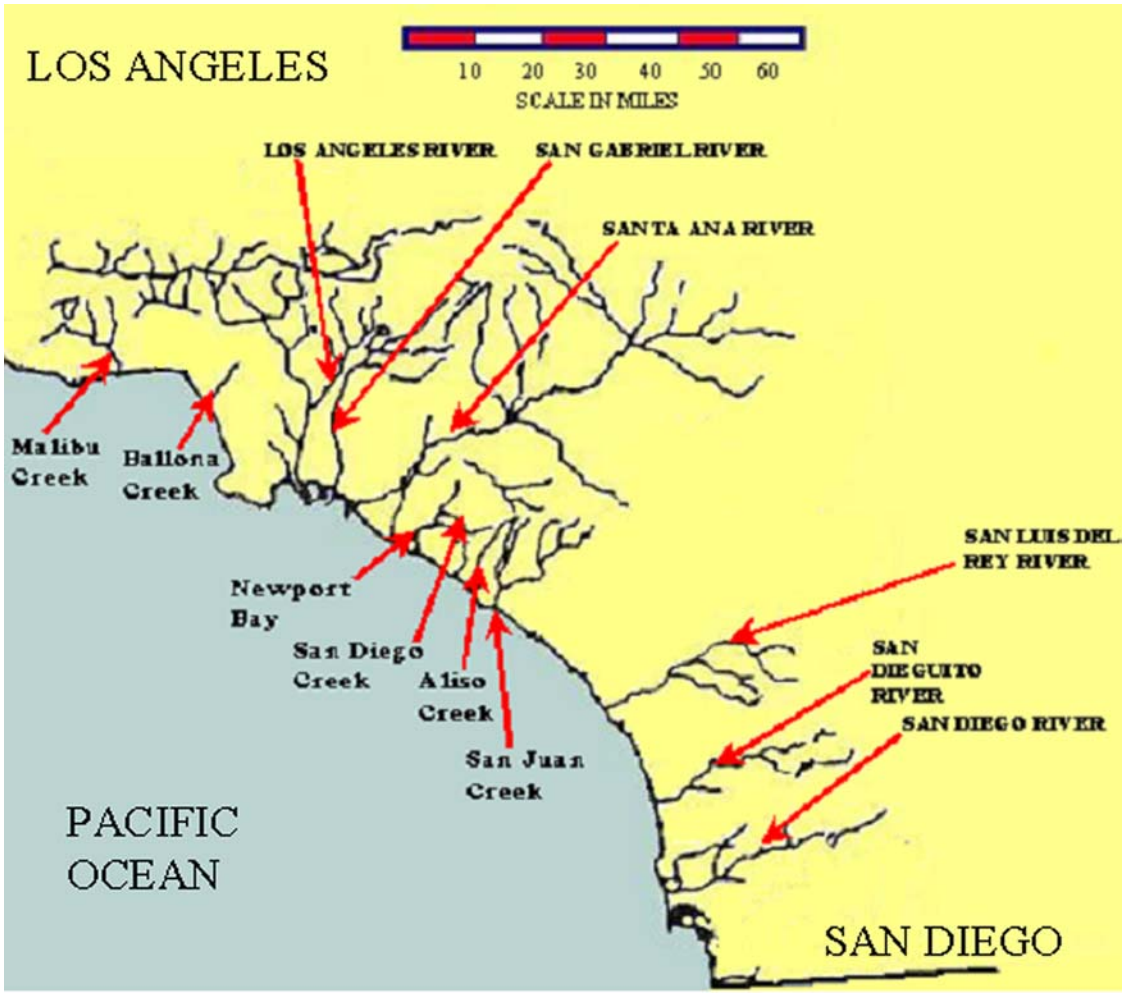
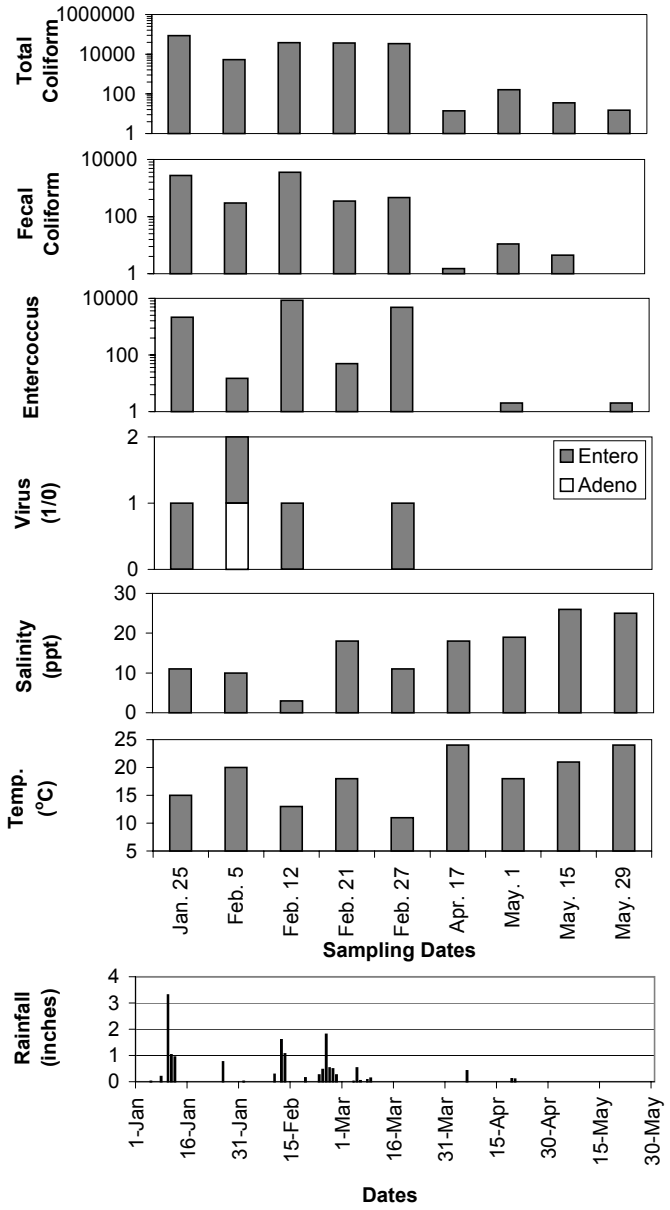
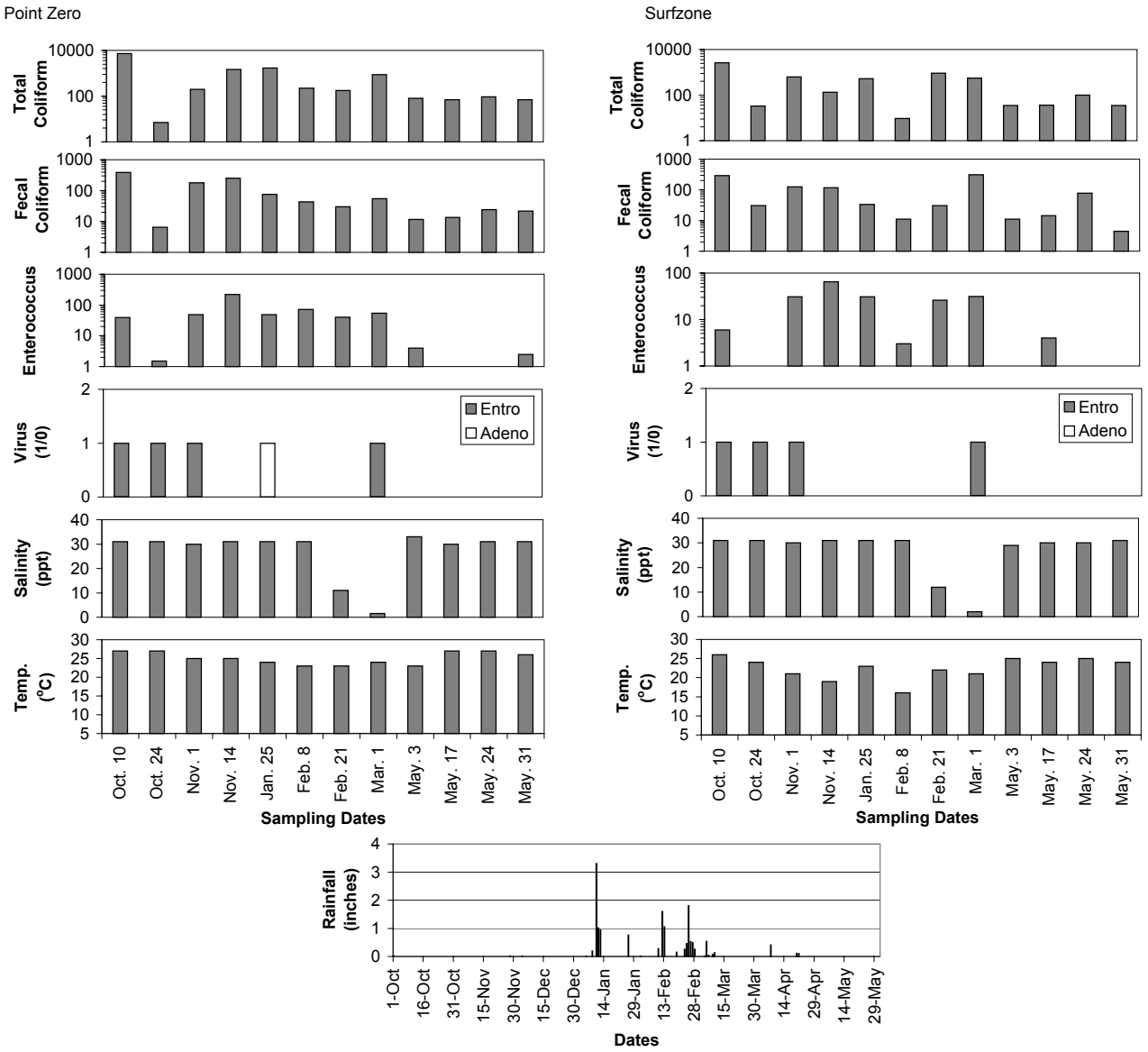

LOS ANGELES
10 20 30 40 50 60
SCALE IN MILES
LOS ANGELES RIVER
SAN GABRIEL RIVER
SANTA ANA RIVER
Malibu Creek
Ballona Creek
Newport Bay
San Diego Creek
Aliso Creek
San Juan Creek
SAN LUIS DEL REY RIVER
SAN DIEGUITO RIVER
SAN DIEGO RIVER
PACIFIC OCEAN
SAN DIEGO

Figure 1.


Total Coliform
1000000
10000
100
1
Fecal Coliform
10000
100
1
Entercoccus
10000
100
1
Virus (1/0)
2
1
0
Entero
Adeno
Salinity (ppt)
30
20
10
0
Temp. (°C)
25
20
15
10
5
Jan. 25
Feb. 5
Feb. 12
Feb. 21
Feb. 27
Apr. 17
May. 1
May. 15
May. 29
Sampling Dates
Rainfall (inches)
4
3
2
1
0
1-Jan
16-Jan
31-Jan
15-Feb
1-Mar
16-Mar
31-Mar
15-Apr
30-Apr
15-May
30-May
Dates


Point Zero
Surfzone
Total Coliform
Fecal Coliform
Enterococcus
Virus (1/0)
Entro
Adeno
Salinity (ppt)
Temp. (°C)
Oct. 10
Oct. 24
Nov. 1
Nov. 14
Jan. 25
Feb. 8
Feb. 21
Mar. 1
May. 3
May. 17
May. 24
May. 31
Sampling Dates
Rainfall (inches)
1-Oct
16-Oct
31-Oct
15-Nov
30-Nov
15-Dec
30-Dec
14-Jan
29-Jan
13-Feb
28-Feb
15-Mar
30-Mar
14-Apr
29-Apr
14-May
29-May
Dates

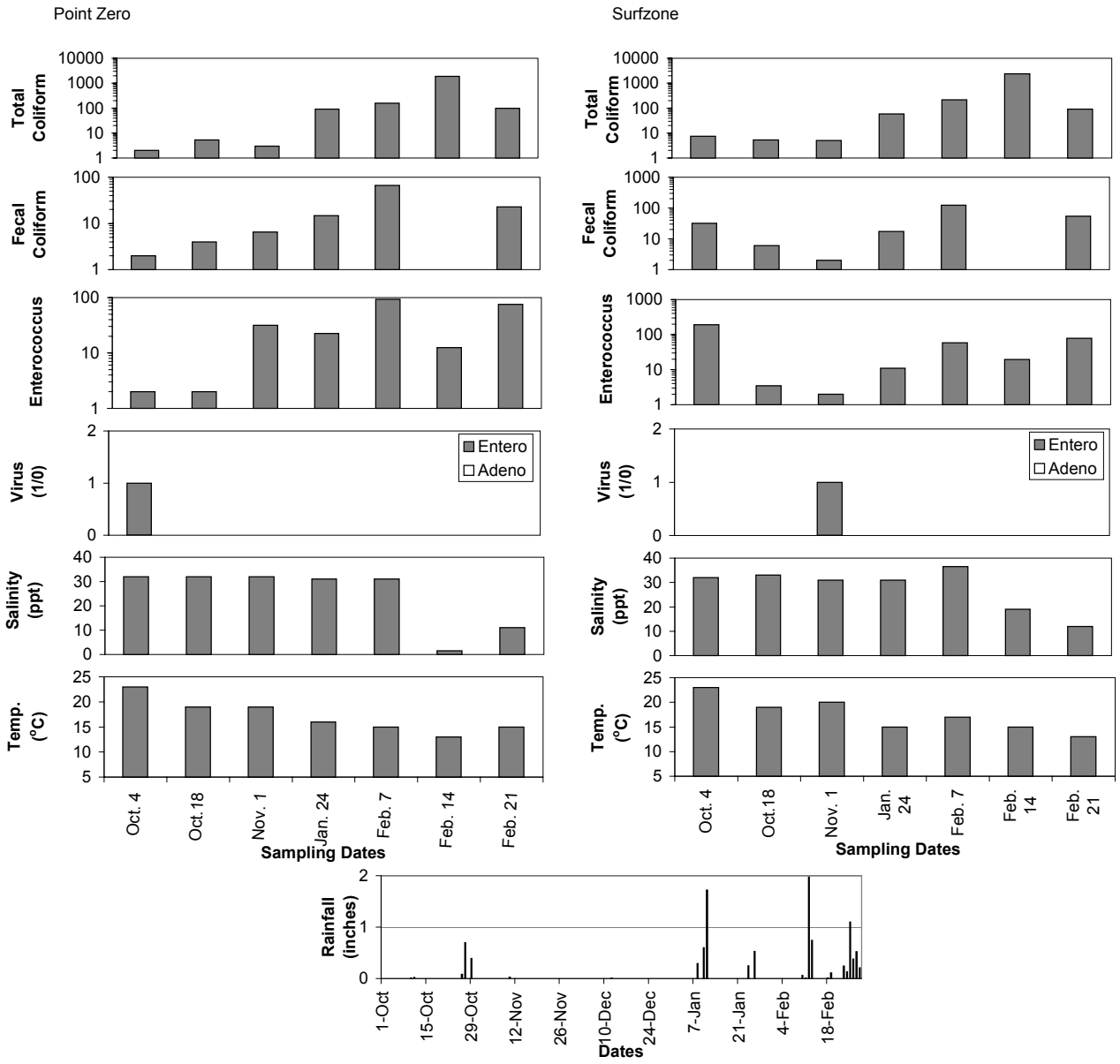

Point Zero
Surfzone
Total Coliform
Fecal Coliform
Enterococcus
Virus (1/0)
Entero
Adeno
Salinity (ppt)
Temp. (°C)
Oct. 4
Oct.18
Nov. 1
Jan. 24
Feb. 7
Feb. 14
Feb. 21
Sampling Dates
Rainfall (inches)
1-Oct
15-Oct
29-Oct
12-Nov
26-Nov
10-Dec
24-Dec
7-Jan
21-Jan
4-Feb
18-Feb
Dates

Table 1. Human virus primer sets and internal probe sequences.

Target viruses	Primer and probe sequences	Amplicon size and target	Reference
Pan-enterovirus	Upstream, 5'-CCTCCGGCCCTGAATG-3' Downstream, 5'-ACCGGATGGCCAATCCAA-3' Probe, 5'-TACTTTGGGTGTCCGTGTTTC-3'	197-bp highly conserved 5' untranslated region	DeLeon et al 1990
Hepatitis A virus	Upstream, 5'-CAGCACATCAGAAAGGTGAG-3' Downstream, 5'-CTCCAGAATCATCTCCAAC-3' Probe, 5'-TGCTCCTCTTTATCATGCTATG-3'	192-bp VP 1 and VP 2 capside protein interphase	Tsai et al 1993
Adenovirus	First upstream, 5'-GCCGCAGTGGTCTTACATGCACATC-3' First downstream, 5'-CAGCACGCCGCGGATGTCAAAGT-3'	301-bp Hexon	Pina et al 1990
	Nested upstream, 5'-GCCACCGAGACGTACTTCAGCCTG-3' Nested downstream 5'-TTGTACGAGTACGCGGTATCCTCGCGGTC-3'	143-bp Hexon	

Table 2. Sampling sites and environmental conditions

Sampling Sites	Date & Time	Salinity (‰)	Temp. (°C)	Weather	Location Description
Malibu Creek I	7/24/00, 11:10am	0.5	23	Sunny	Dense green vegetation, no flow, birds and trash in water, storm drain nearby
Malibu Creek II	7/24/00, 10:50am	0	24	Sunny	Shallow, narrow, minimal flow, surrounding is not urbanized, no animal farms either
Ballona Creek I	7/17/00, 11:32am	0	27	Sunny	Minimal flow, storm drain nearby, concrete lined, freshwater marsh nearby
Ballona Creek II	7/17/00, 12:00pm	0	32	Sunny	Minimal flow, shallow, narrow, concrete lined, sediment on the bottom
LA River I	7/12/00, 11:16am	0	24.5	Sunny	Tall vegetation, dense algae, murky, minimal flow, storm drain nearby
LA River II	7/12/00, 11:33am	0	29	Sunny	Shallow, concrete lined, dense algae, trash and birds in water, storm drains nearby
San Gabriel River I	7/10/00, 11:40am	30	29.5	Overcast	Deep, clear, wide, 1000 m from the ocean, tidal driven flow
San Gabriel River II	7/10/00, 11:08am	1	27	Overcast	Minimal flow, shallow, little vegetation, 2 storm drains nearby
Santa Ana River I	7/19/00, 11:18am	36	31.5	Sunny	Tidal flow only, upstream is completely dry
Newport Bay	8/30/00, 10:48am	30	24	Overcast	Murky, heavy vegetation, no flow, ducks in water
San Diego Creek II	8/30/00, 10:27am	0	25	Overcast	Shallow, algae and birds in water, storm drain nearby
Aliso Creek I	7/26/00, 10:52am	0.5	28	Sunny	Shallow, submerged vegetation, medium flow
Aliso Creek II	7/26/00, 10:37am	0.5	26.5	Sunny	Shallow, narrow, clear, fast flow, runs thru a golf course
San Juan Creek I	7/31/00, 9:28am	0	25	Sunny	Narrow, concrete lined, clear, algae in water, storm drain nearby
San Juan Creek II	7/31/00, 9:48am	0	27	Sunny	Shallow, concrete lined, clear, algae in water, Del Obispo Park nearby
San Luis del Rey River I	8/2/00, 9:19am	5	28.5	Sunny	Murky, deep, medium flow, near a harbor, marsh vegetation
San Luis del Rey River II	8/2/00, 9:45am	0	24	Sunny	Shallow, narrow, murky, heavy vegetation
San Dieguito River I	8/21/00, 9:35am	15	27	Sunny	Deep, wide, birds and fishes in water, heavy vegetation
San Dieguito River II	8/21/00, 9:00am	0	28	Sunny	Deep, murky, no flow, non-urbanized agricultural land
San Diego River I	8/23/00, 9:15am	4.5	26	Sunny	No flow, narrow, algae in water, heavy vegetation
San Diego River II	8/23/00, 10:05am	0	27	Sunny	Murky, no flow, algae in water, lake-like setting

Table 3. Indicator bacteria (CFU per 100ml), coliphage (PFU per 100ml) and human viruses in southern California rivers and site ranking based on indicator concentrations.

Sampling Site	Total coliform	Fecal coliform	Enterococcus	Coliphage	F-Coliphage	Final Rank*	Adenovirus	Enterovirus	Hepatitis A
Malibu Creek I	230000	9975	600	1178	853	21	-	-	-
Malibu Creek II	1425	95	120	9	<3	7	-	-	-
Ballona Creek I	100	67	5	5	<2	3	+	+	+
Ballona Creek II	4300	700	<10	1095	160	16	-	-	+
Los Angeles River I	17050	8525	2100	334	144	19	+	+	+
Los Angeles River II	46500	925	1425	119	74	18	-	-	+
San Gabriel River I	<10	<10	<10	6	<3	2	+	+	+
San Gabriel River II	3925	700	<10	90	50	11	+	+	-
Santa Ana River I	<10	<10	<10	<2	<2	1	-	-	-
Newport Bay	4100	450	325	10	<3	15	-	+	+
San Diego Creek II	20000	4100	400	1836	332	20	+	+	+
Aliso Creek I	1625	20	6250	3	<2	8	+	+	+
Aliso Creek II	1875	5	30	3000	98	12	+	+	+
San Juan Creek I	3675	300	105	295	22	13	-	-	+
San Juan Creek II	4075	432	170	7597	33	17	-	-	+
San Luis Rey River I	7875	220	183	8	5	10	+	+	+
San Luis Rey River II	338	358	50	7	<3	5	-	+	+
San Diequito River I	100	<10	50	2251	33	9	+	+	+
San Diequito River II	1725	<10	3350	502	18	14	+	+	+
San Diego River I	500	50	<10	96	<2	4	+	+	+
San Diego River II	300	50	50	131	<3	6	-	-	-
Geometric Mean	2653	298	222	119	64				
Average	18394	1587	951	929	152				

*Determined from combining the rank of each individual indicator.

Objective 2. Seasonal and Spatial distribution of pathogenic viruses in Los Angeles and San Gabriel Rivers

MATERIAL AND METHODS

Sampling Sites Between February 2002 and February 2003, water samples were collected from three different sampling sites along the Los Angeles River and two sites along the San Gabriel River (Figure 1)**.** The five sampling sites are designated LA1, LA2, and LA3 for the Los Angeles River, and SG1 and SG2 for the San Gabriel River. Sites SG1 and LA1 are located at the mouth of each river and LA2, LA3, and SG2 are located upstream approximately 5-35 miles apart. All sites are located in urban settings; the three upstream sites are adjacent to major highways while the 2 sites located at the mouth of the rivers are located in commercial settings. The water flow was fairly consistent for all sites at Los Angeles River, while water level and water turbidity varied in the San Gabriel River throughout the study period. In addition, recreational fishing activities were observed at both sites of the San Gabriel River. All sites of each river were sampled semi-monthly using 10L containers sterilized with bleach. The samples were transported back to the laboratory at UC Irvine within two hours of collection for immediate processing. Sample salinity was determined by refractometry, water temperature was measured on site, and rainfall events were recorded. During the study period, a total of 114, 10-liter samples were collected from both rivers.

Detection of Indicator Bacteria Analysis of total and fecal coliform and enterococcus was performed immediately after sample collection following the EPA's guidelines for bacterial indicator analysis by membrane filtration (9). In brief, environmental samples were filtered through 0.45 μm nitrocellulose membranes. Fecal coliform and total coliforms on the membrane were cultured using m-FC agar and m-Endo agar, and incubated for 24 hr at 44.5^{o}C and 37^{o}C, respectively. For entercoccus analysis, mE agar (Fisher Scientific Inc.) was used and incubated for 48 hr at 41.5^{o}C. Membranes were then transferred to EIA plates for 20 minutes for confirmation and recorded as cfu/100mL.

Concentration of Water Samples for Viral Analysis Ten liter samples were concentrated to ~115mL using a Tangential Flow Filtration system with a 30KD Omega cartridge at a flow rate of 350-450 mL/min under a constant pressure at <10 psi (TFF, Pall Life Science). The samples were spiked with bacteriophage ϕHSIC, and were titered in samples prior to and after TFF concentration by plaque assay to determine the rate of viral recovery. ϕHSIC was isolated from coastal waters off Hawaii, and were not found in urban river waters of Southern California (16). Therefore, there are no interfering effects of viral recovery assay. Immediately after concentration, one milliliter of the concentrated samples was used for coliphage plaque assay and the rest were stored at –80^{o}C for later use.

Detection of Somatic and F-specific Coliphages Densities of somatic and F-specific coliphage were determined by using both TFF-concentrated and unconcentrated water samples. Both 100µL and 1mL of the concentrated and unconcentrated samples were mixed with 1mL of bacterial host in 3mL of soft agar and poured over a LB agar plate. *E. coli ATCC* 15597 strain was used as the host for somatic coliphage, while *E. coli* Famp was the specific host used for F-specific coliphage. Plaques were enumerated after 12-24 hours of incubation at 37^{o}C.

Viral Nucleic Acid Purification by GuSCN-Silica Bead Extraction Method of Boom et al (4) was used for nucleic acid purification and removal of PCR inhibitors for all environmental concentrates. Briefly, 900µL of guanidinium thiocyanate lysis buffer (GuSCN) was added to a mixture of 40µL of silica particle and 50µL or 100µL of viral concentrate at room temperature for 10 minutes. The mixture was then centrifuged, supernatant discarded, and pellet washed. The pellet was dried briefly and nucleic acid eluted using 50µL of 1 X Tris–EDTA at 56^{o}C.

Detection of Human Enterovirus by RT-PCR and Probe/Hybridization The enterovirus-specific primer sequences used for reverse transcription-PCR (RT-PCR) were 5'-CCTCCGGCCCCTGAATG-3' and 5'- ACCGGATGGCCAATCCAA-3'. This primer pair amplifies a target at the highly conserved 5' untranslated region. The PCR protocol followed those previously described by Tsai *et al*. (1998). Briefly, reverse transcription was performed at 25^{o}C for 10 min, followed by 42^{o}C for 30 min and enzyme inactivation at 99^{o}C for 5 min. The PCR amplifications were performed at 95^{o}C for 2 min, followed by 30 cycles of 95^{o}C for 1 min, 55^{o}C for 1 min, and 72^{o}C for 1 min, a final extension of 72^{o}C for 10 min, following a hold step at 4^{o}C. The PCR products were then analyzed on a 2% agarose gel.

To confirm the amplicon, DNA in gel was transferred to positively charged MagnaGraph 0.45 Transfer nylon membrane (Osmonics Inc.) via Southern Transfer (24). In brief, the gel was washed in 0.5M NaOH/1.5M NaCl and 1.5M NaCl/0.5M Tris (pH8) twice with each solution for 20 minutes. The blot was set up using 3MM Whatman paper cut to the size of the gel and soaked overnight in 10 x SCC (pH7). The membrane was then dried and the DNA covalently linked to the membrane by UV-crosslinking at 1200 x 100µJ/cm^{2} twice.

After DNA transfer, nylon membranes were washed three times in 3 x SSC containing 0.1% SDS at room temperature for 10 minutes and at 60^{o}C for 1 hour. The membranes were prehybridized for 1 hour at 37^{o}C in solution containing 6x SSC, 5x Denhardt, 0.05% sodium phyrophosphate, 100mg/mL of salmon sperm, and 0.5% SDS. Hybridization was carried out overnight at 45^{o}C in solution containing 6x SSC, 1x Denhardt solution, 100mg/mL of salmon sperm, 0.05% sodium pyrophosphate, and 25ng/mL of the γ–[32^{p}] ATP labeled oligonucleotide probe. The sequence for the enterovirus probe is '5-TACTTTGGGTGTCCGTGTTTC3'. After hybridization, the membrane was washed three times at room temperature for 15 minutes and two more times at 55^{o}C for 1 hour each time with a solution containing 6x SSC, 1% SDS, and 0.05% sodium pyrophosphate to remove any unbound probe. The membrane was sealed in a plastic bag and exposed to x-ray film for 24-48 hours at –80^{o}C. A positive result was characterized by a signal on the film.

Detection of Human Adenovirus by Real-Time PCR For adenovirus analysis by real-time PCR, samples were further concentrated by ultracentrifugation. In brief, ten milliliters of TFF concentrate was centrifuged at 41,000 rpm using the Beckman rotor SW41 for 1.5 hours at 10^{o}C. The pellets were resuspended in ~200µL of the supernatant and 100µL was purified by GuSCN-Silica bead extraction (as described above). Either 1µL or 4µL of purified viral concentrate (TFF-ultracentrifugation) was used in triplicate reactions for real-time PCR. The standard curve used for quantitation was plotted from quadruplicate samples using C_t values of 6-fold dilution of template extracted from cloned adenovirus serotype 40. A standard curve was run for each set of assay.

Each real-time PCR reaction (25µL) contained 1x TaqMan Universal PCR Master Mix, 300nM of each primer, and 200nM TaqMan probe. Degenerative primers specific to human adenoviruses used were 5`-GACTCYTCWGTSAGYTGGCC-3` AND 5`-CCCTGGTAKCCRATRTTGTA-3`, and probe FAM-AACCAGTCYTTGGTCATGTTRCATTG~TAMRA as described by He and Jiang (submitted). The assay targets of the primers and probe were human adenovirus serotypes 1-5, 6, 7, 9, 17, 19, 21, 28, 37, 40, 41, and simian 25. The PCR cycle conditions consist of holding samples at 50^{o}C for 2 min and then 95^{o}C for 10min, followed by 45 cycles of 95^{o}C for 15 sec, 56^{o}C for 15 sec, and 62^{o}C for 30 sec with 1 sec increments between each cycle. An Applied Biosystems 7000 Sequence Detector System was used for real-time PCR, and the data was analyzed with the ABI Prism 7000 software.

Tissue Culture Analysis for Infectious Human Adenoviruses The infectivity of adenoviruses in environmental concentrates was analyzed using two cell lines, A549 and HEK-293A. The A549 human lung carcinoma cell line is currently the most commonly used cell line for adenovirus propagation and plaque titration while the 293 human kidney embryonic cells are mostly used as a package cell line for production of non-replicative adenovirus vectors (Giard et al. 1973, Grahm, et al. 1977, Smith et al. 1986). HEK-293A is a recombinant 293 cell line with an insertion of adenovirus E1 gene so E1 protein is constitutively expressed to enhance adenovirus replication (11). After TFF concentration and ultracentrifugation, approximately 200µL of viral concentrate was purified by chloroform extraction, filtered through 0.2µm low protein binding and retention Whatman filters (Whatman International Ltd), and overlaid onto monolayers of HEK-293A and A549 cells. After gentle shaking for 60mins at 37^{o}C, the monolayers were washed with pre-warm PBS, and overlaid with 1.25% agarose containing 5% DMEM, 50µg/mL gentamicin, and 2.5µg/mL amphotericin B. The cell culture was incubated for 7 to 8 days in 5% CO_2 at 37^{o}C, then a second overlay was carried out as described above, and incubated for an additional 7 to 10 days. Cell cultures were examined under an Olympus Microscope using a 10x magnification lens for plaque formation.

Statistical Analysis Spearman's rho correlation was used to determine correlations in seasonal distribution of fecal indicator bacteria, coliphages, and human viruses. Kruskal Wallis test was used to determine significant variability in parameters during rainy and non-rainy seasons. The SPSS program (SPSS Inc., Illinois) was used

for all statistical analyses and correlations were considered significant at a 95% confidence level.

RESULTS

Indicator Bacteria and Coliphages Distribution of fecal indicator bacteria, coliphages, and human viruses at both rivers are reported in geometric mean value for each month to determine the seasonal variability of each microorganism. All three fecal indicator bacteria were highest in concentration in November during a period of heavy rainfall (Fig. 2a,b, and c). Based on the California recreational water quality daily limits (10,000 CFU/100mL for total coliforms, 400 CFU/100ml for fecal coliforms, and 104 CFU/100mL for enterococcus), 28% to 52.5% of the samples tested exceeded at least one daily bacterial indicator limit. Higher concentrations of fecal bacterial contamination were detected in LA2, LA3, and SG2, while bacterial contamination was approximately one order of magnitude lower at site SG1 compared to the other sites (Fig. 2d).

The seasonal distribution of F-specific and somatic coliphage were similar to fecal indicator bacteria, with a major peak occurring within 48 hours of a rain storm during November (Fig. 3a and b). Increases in somatic-coliphage concentration surpassing $1x10^2$ PFU/100mL were also observed during the months of February, March, and June, while the lowest concentration was observed during the summer month of July. Concentrations of F-specific coliphage were generally consistent during the winter months, with the lowest concentration observed during the month of July similar to somatic coliphage. Higher concentrations of both somatic and F-specific coliphages were detected in the upstream sites of both rivers, similar to the distribution patterns of fecal indicator bacteria (Fig. 3c). The highest concentrations for both F-specific and somatic coliphages were observed in LA2. The annual geometric mean value of somatic coliphage ranged from 1 to 8,870 PFU/100mL at individual sampling sites. Statistical analysis by Spearman's rho showed significant correlations in seasonal distribution between F-specific, somatic coliphage, and all 3 fecal indicator bacteria ($p < 0.05$). Statistical analysis by the Kruskal Wallis test also showed a significant difference during rainy/non-rainy periods ($p = 0.053$) in F-specific and somatic coliphages, and all three fecal indicator bacteria concentrations.

Human Entero and Adenoviruses Adenoviruses were detected in approximately 50% of all samples analyzed by real-time PCR. A three-peak seasonal distribution pattern was apparent for adenoviruses with peaks observed in January, April, and November (Fig. 4a). The concentration of adenoviruses ranged from 10 to 10^6 genomes per liter for single samples. Geometric mean of adenoviruses exceeded 10^4 genomes/L in all sites except at SG2 (Fig. 4b). The adenovirus concentrations of the two sites located at the mouth of each river were higher compared to the concentration of the sites located upstream. Although the correlation value was not significant at $p < 0.05$ level, statistical analysis by the Spearman's rho showed a strong correlation in seasonal distribution of adenoviruses and F-specific coliphage ($p = 0.13$) and somatic coliphage ($p=0.06$). Analysis by Kruskal Wallis test showed no significant difference in adenovirus concentrations during rainy versus non-rainy periods. In addition, no significant

correlations were observed between adenoviruses and any of the fecal indicator bacteria, although enterococcus and fecal coliform showed stronger correlations to adenoviruses compared to total coliform.

Approximately 7% of all samples analyzed were positive for enterovirus by RT-PCR and confirmed by probe hybridization. The seasonal distribution of enterovirus was sporadic and no trend or correlation with any other parameters measured could be established (Fig. 5a). Enterovirus was slightly more prevalent in the Los Angeles River, with approximately 8.33% of the samples being positive, compared to the San Gabriel River, where approximately 5.88% of the samples were positive for enterovirus. However, enterovirus was detected in at least one environmental sample collected from each site during the study period (Fig. 5b).

Results from the infectivity assay by two different cell cultures showed no positive results in all of the 114 environmental samples analyzed. A few samples were randomly selected and retested by 2nd round of infection to ensure that the results were true negative. Cytotoxicity was observed in less than three percent of samples tested after purification.

DISCUSSION

Storm water and urban runoff is increasingly being considered as a major source of coastal water pollution. Health concerns for beach goers is clear as numerous studies suggest that Southern California shorelines receiving runoff fail to meet California water quality standards. Noble *et al.* (2003) showed in a study that the failure of water quality standards increased 90% for shoreline areas adjacent to urban runoff outlets following rainstorms in Southern California Bight. Similarly, Lipp *et al*. (2001) demonstrated that the highest concentrations of fecal indicator bacteria were observed near areas where estuaries receive urban streams in Charlotte Harbor, Florida. Most recently, Jiang and Chu (2003) showed that human virus contamination was wide spread in Southern California coastal rivers and streams. This current study further investigated the presence of human pollution in urban rivers by presenting the first quantitative assessment of human adenoviruses in urban rivers by real-time PCR and to explore their statistical relationships to fecal indicator bacteria and coliphages.

In accordance with previous observations, our current investigation shows that the Los Angeles and San Gabriel Rivers are contaminated with high levels of fecal indicator bacteria and human viruses. The concentrations of both fecal indicator bacteria and coliphages were higher at the upstream locations of both rivers while high concentrations of adenoviruses were observed at both the mouth and upstream sites. A greater abundance of adenoviruses was detected at the mouths of each river in comparison to fecal indicator bacteria and somatic and F-specific coliphage. This may be due to the possibility that enteric viruses are more resistant to environmental degradation and salt-water conditions, thus allowing them to maintain structural integrity for weeks and even months after initial release (USEPA 1985). Alternatively, samples from upstream sites contain higher concentrations of PCR inhibitors and interferences, due to the high

concentrations of humic material, therefore may have caused greater problems of PCR inhibition. In comparing the real-time PCR and tissue culture results, one possible explanation for the discrepancy is that natural or treatment processes such as sunlight or chlorination may have inactivated the adenoviruses while the genomes remain intact, thus persisting in the environment for much longer periods of time resulting in positive results by real-time PCR. Sobsey et al. (26) described this concern by showing that environmental samples tested by molecular methods such as RT-PCR yielded positive results for inactivated viruses.

In comparing the seasonal distributions of fecal indicator bacteria, somatic coliphage, and F-specific coliphage, the highest concentrations of all organisms were detected in November during a storm period. Similar trends were also observed during the month of February over a period of heavy rainstorms. However, no significant increase of concentration was observed during the month of January, indicating that light rain showers may not have a significant impact on urban runoff. In contrast, the seasonal distribution of adenoviruses showed a 3-peak trend, with an increase in concentration occurring during the months of January, April, and November. However, there was a decrease in adenovirus concentration during the month of February although heavy rain was observed. This observation may be attributed by the 1st flash phenomenon, where large load of contaminates are carried by the first few major storm events of the wet season.

The EPA suggests that enterococci may be the better bacterial indicator of water quality in marine water (USEPA 1986) because previous studies have indicated that enterococci have a higher survival rate in saltwater compared to total and fecal coliforms, and that they have a greater correlation with swimming-associated gastrointestinal illness than other bacterial indicator organisms (Anderson et al. 1997; Mcbride et al. 1998). However, all three fecal indicator bacteria showed no statistically significant correlation to adenoviruses, although the seasonal distribution of enterococcus more closely represented the distribution of adenovirus than total or fecal coliform.

Although the spatial distribution of both somatic and F-specific coliphages were different in comparison to adenoviruses, where higher densities were observed in the upper sites, the strong correlations in seasonal distribution suggest that coliphages are more similar to enteric viruses than to fecal indicator bacteria. These results suggest that both somatic and F-specific coliphage may be favorable as indicators of viral pollution. The results from this study support previous studies indicating that bacteriophages closely resemble enteric viruses in morphology and survival characteristics (Turner and Lewis, 1995).

No positive results for adenovirus from the infectivity assay by tissue culture were observed although approximately 50% of samples were positive for adenovirus by real-time PCR. It is conceivable that the adenoviruses detected in the environmental samples may not be infectious with any binding affinity to A459 and HEK-293A cells. In addition, the tissue cultures used in this study may not be sensitive for specific serotypes present in the environmental samples. Environmental inhibitors may also be a factor since viruses are known to adsorb to solids such as sediments (USEPA 1985), which may

impair their ability to bind to cell receptors potentially causing results to be false negative.

It is apparent that urban rivers such as the Los Angeles and San Gabriel Rivers are a source of coastal water pollution and that the quality of recreational waters may be influenced by seasonal variations. Similarly, the results from this study support previous observations suggesting that fecal indicator bacteria do not reflect the viral quality in our waters, and that supplementary surrogates be used in addition to current standards to successfully define the broad spectrum of illnesses associated to both bacterial and viral human pathogens. In particular, the detection of adenoviruses may be used as an index because of their ability to survive longer than most other enteric viruses, and because adenoviruses are more abundant and can be detected rapidly by real-time PCR.

REFERENCES

Giard, D. J. S., A. Aaronson, G. J. Todaro, P. Arnstein, J. H. Kersey, H. Dosik and W. P. Parks. 1973. In vitro cultivation of human tumors: Establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst. 51:1417–1423.

Goldsmith, K. T., L. D. Dion, D. T. Curiel, and R. I. Jr. Garver. 1998. Trans E1 component requirements for maximal replication of E1-defective recombinant adenovirus. Virology. 248:406-419.

Graham, F. L., J. Smiley, W. C. Russell and R. Nairn. 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. 36:59–74.

Harm, W. 1980. Biological effects of ultraviolet radiation. Cambridge University Press, Cambridge, United Kingdom.

Jiang, S., W. Chu. 2004. PCR detection of pathogenic viruses in southern California urban rivers. J. Appl. Microbiol. 97(1):17-28.

Lipp, E. K., R. Kurz, R. Vincent, C. Rodriguez-Palacios, S. R. Farrah, & J. B. Rose. 2001. The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. Estuaries. 24:66–276.

Noble, R.T., Weisberg S.B., M.K. Leecaster, C.D. McGee, J.H. Dorsey, P. Vinik, and V. Orozco-Borbon. 2003. Storm effects on regional water quality along the southern California shoreline. J. Water Health. 01:23-31.

Puig, M., J. Jofre, F. Lucena, A. Allard, G. Wadell, and R. Girones. 1994. Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. Appl. Environ. Microbiol. 60(8): 2963–2970.

Smith, C. D., D. W. Craft, R. S. Shiromoto and P. O. Yan. 1986. Alternative cell line for virus isolation. J. Clin. Microbiol. 24:265–268.

Sobsey, M.D., D. A. Battigelli, G.-A.Shin, and S. Newland. 1998. RT-PCR amplification detects inactivated viruses in water and wastewater. Water Sci. and Technol. 8(12):91-94.

Tsai, Y. L., M. D. Sobsey, L. R. Sangermano, and C. J. Palmer. 1993. Simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-polymerase chain reaction. Appl. Environ. Microbiol. 59:3488-3491.

Turner, S.J., and G.D. Lewis. 1995. Comparison of F-specific bacteriophage, enterococci, and faecal coliform densities through wastewater treatment process employing oxidation ponds. Water Sci. Technol. 31:85-89.

U.S. Environmental Protection Agency. 1985. Drinking water criteria document for viruses. Draft PB86-118270. National Technical Information Service, United States Portection Agency, Springfield, VA.

U.S. Environmental Protection Agency. 1986. Ambient water quality criteria for bacteria. 1986. U.S. Environmental Protection Agency, Washington D.C.

FIGURE LEGEND

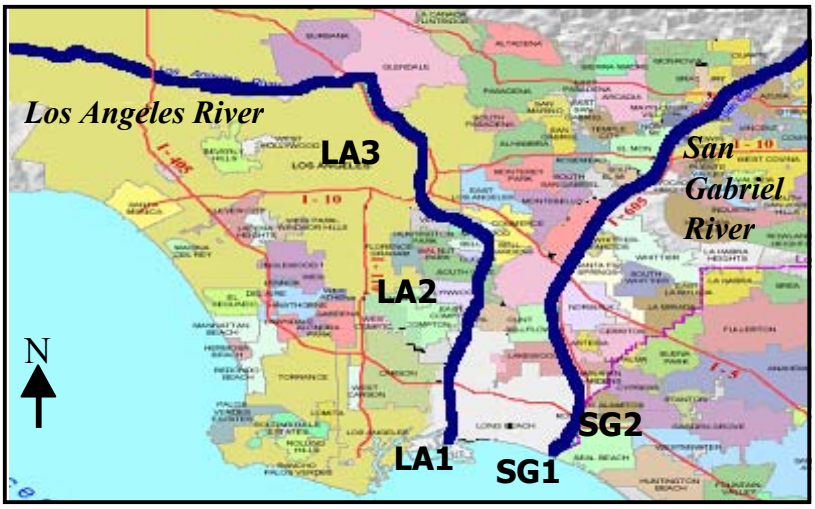
Figure 1. Sampling locations along Los Angeles River and San Gabriel River. LA1 located at the mouth of the Los Angeles River, LA2 located approximately 10 miles upstream, and LA3 located approximately 35 miles upstream from the mouth. SG1 located at the mouth of the San Gabriel River and SG2 located approximately 5 miles upstream.

Figure 2. Seasonal distribution of total coliform (a), fecal coliform (b), and enterococcus (c), and their annual geometric mean distribution at each sampling site (d). (■) Indicates storm event within 48 hr of sampling and (⊟) indicates light rain (<.05 inch).

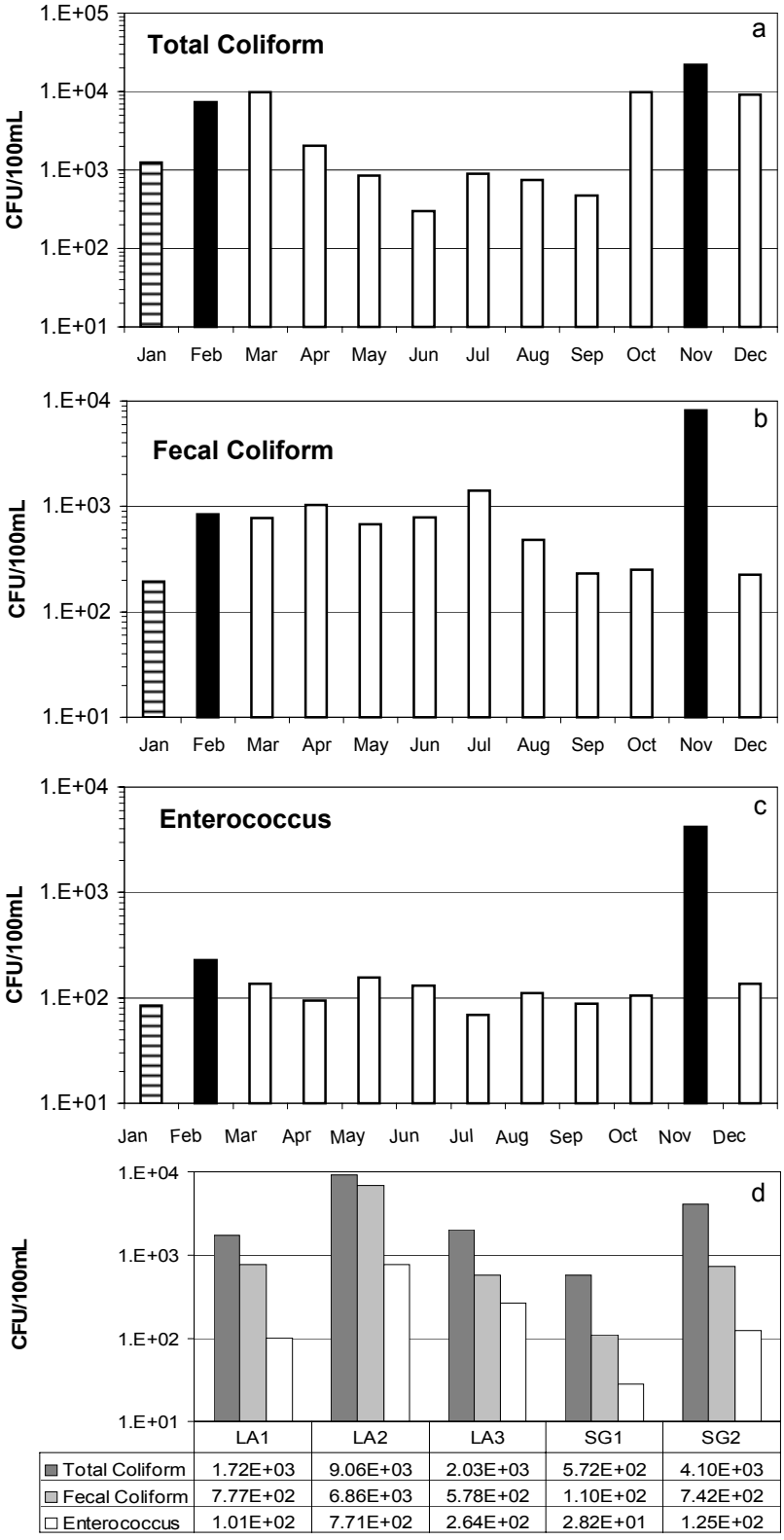
Figure 3. Seasonal distribution of somatic coliphage (a) and F-specific coliphage (b), and their annual geometric mean distribution at each sampling site (c). (■) Indicates storm event within 48 hr of sampling and (⊟) indicates light rain (<.05 inch).

Figure 4. Seasonal distribution of adenoviruses (a), and their annual geometric mean distribution at each sampling site (b). (■) Indicates storm event within 48 hr of sampling and (⊟) indicates light rain (<.05 inch).

Figure 5. Seasonal distribution of enteroviruses (a), and their annual geometric mean distribution at each sampling site (b). (■) Indicates storm event within 48 hr of sampling and (⊟) indicates light rain (<.05 inch).

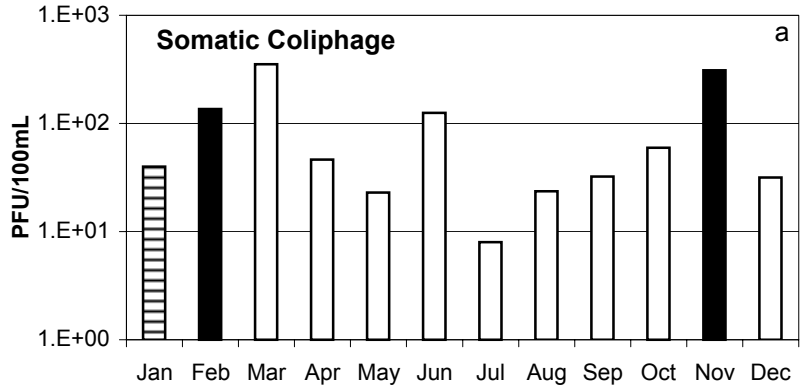


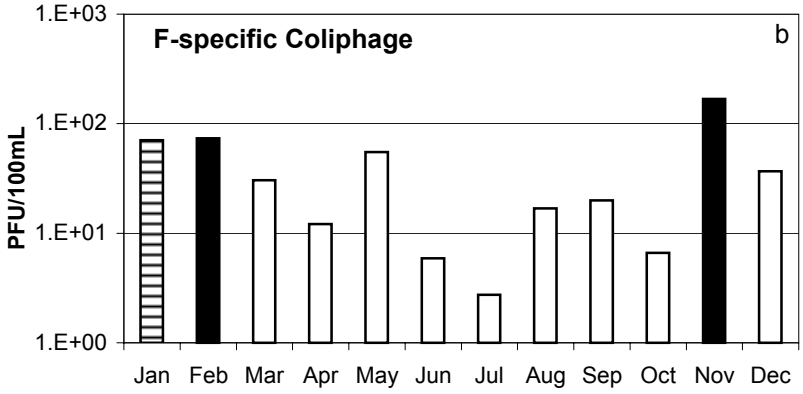
Choi, et al.
Figure 1

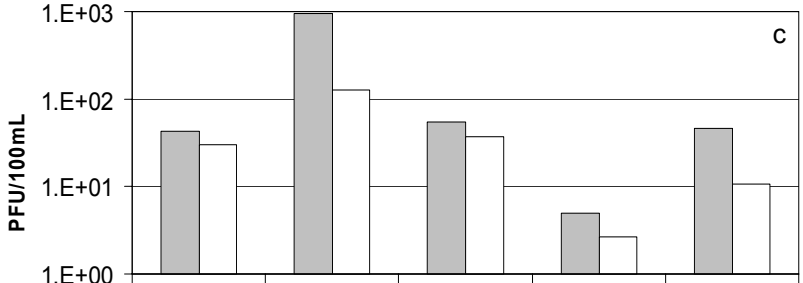


	LA1	LA2	LA3	SG1	SG2
Total Coliform	1.72E+03	9.06E+03	2.03E+03	5.72E+02	4.10E+03
Fecal Coliform	7.77E+02	6.86E+03	5.78E+02	1.10E+02	7.42E+02
Enterococcus	1.01E+02	7.71E+02	2.64E+02	2.82E+01	1.25E+02

Choi, et al.
Figure 2

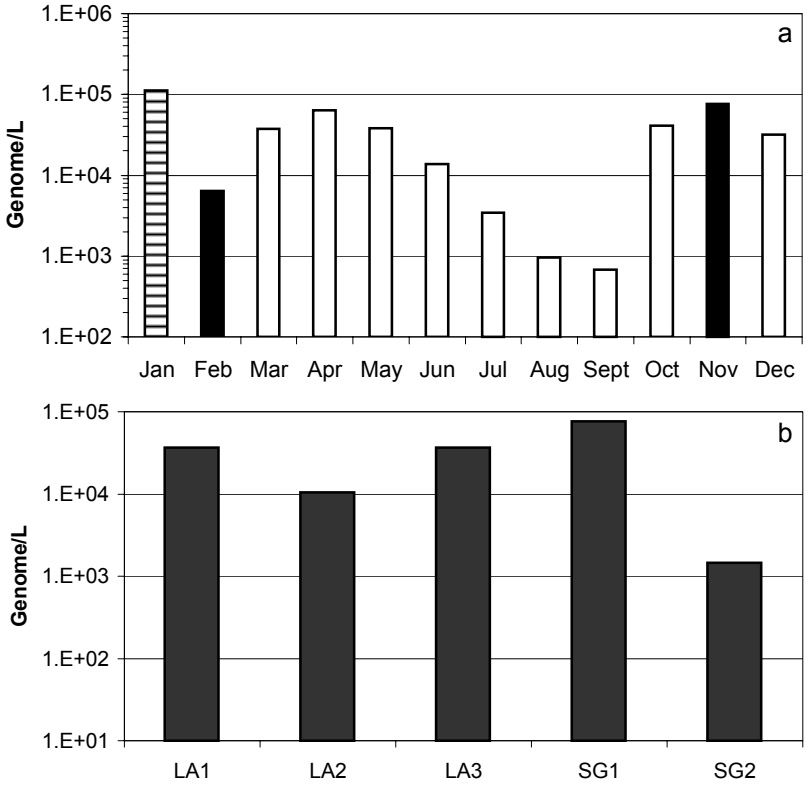




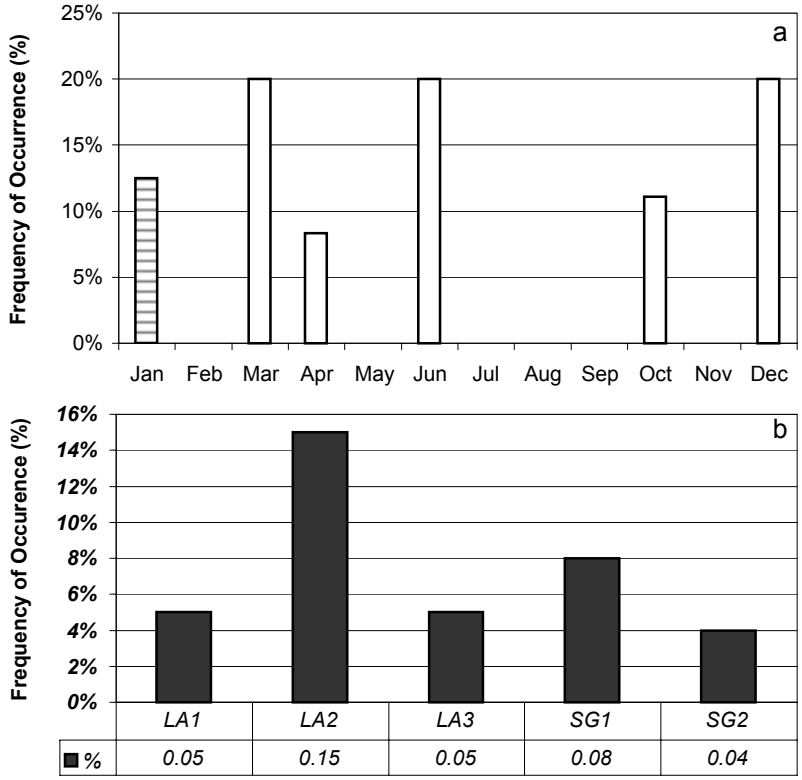


	LA1	LA2	LA3	SG1	SG2
Somatic	4.25E+01	9.52E+02	5.43E+01	4.97E+00	4.67E+01
F-specifc	3.00E+01	1.28E+02	3.73E+01	2.69E+00	1.07E+01

Choi, et al.
Figure 3



Choi, et al.
Figure 4



Choi, et al.
Figure 5

LIST OF PUBLICATIONS

Jiang S. and W. Chu 2003. PCR Detection of Pathogenic Viruses in Southern California Urban Rivers. J. Applied Microbiol. 97: 17-28