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# PCR detection of pathogenic viruses in southern California urban rivers

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## ABSTRACT

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**Aims:** To investigate human viral contamination in urban rivers and its impact on coastal waters of southern California, USA.

**Methods and Results:** Three types of human viruses (adeno, entero and hepatitis A) were detected using nested- and RT-PCR from 11 rivers and creeks. Faecal 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 faecal 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 quality of coastal water than following storm events.

**Conclusions:** This study provides the first direct evidence that human viruses are prevalent in southern California urban rivers. Urban run-off impacts coastal water quality most significantly during the storm season.

**Significance and Impact of the Study:** To protect human health during water recreational activities, it is necessary to develop effective strategies to manage urban run-off during storm events.

**Keywords:** adenovirus, enterovirus, F-specific coliphage, hepatitis A virus, urban run-off.

## 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 programmes for faecal indicator bacteria, including total coliform, faecal coliform and enterococcus, at major bathing beaches. However, the relationship between the occurrence of faecal 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 it rarely rains 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 faecal 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. Lipp *et al.* (2001) showed that enteroviruses were detected at 75% of the sampling stations during the El Nino event with

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increased rainfall, while none were detected in other months of the year in Charlotte Harbor estuary (Florida). No study has investigated the seasonal distribution of human viruses in southern California urban rivers and their impact on the water quality of local beaches. With the rapid urban development of southern California, the volume of urban storm run-off will only increase in future years. Therefore, it is crucial to understand the impact of urban rivers on 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 faecal 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 US Environmental Protection Agency (USEPA) describes the enteric virus group (including norovirus, rotavirus, hepatitis A virus, adenovirus, 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 such as paralysis, meningitis, respiratory disease, epidemic vomiting and diarrhoea, 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 US. Although hepatitis A occurs in virtually every area of the US, 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 that contain DNA. Adenoviruses 40 and 41 have been recognized as important aetiological agents of gastroenteritis in children. Giordano *et al.* (2001) reported adenoviruses 40/41 were the major causative 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 five 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 because of adenovirus types 3 and 4 associated with swimming in contaminated recreational waters (Crabtree *et al.* 1997). Respiratory adenovirus and those causing eye infection are important aetiological 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 1/1000 for a single exposure (Crabtree *et al.* 1997). So far there has not been a microbial indicator for nongastroenteritis 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 because of their resistant characteristics to water treatment processes. Therefore, they are one of the priorities for monitoring and development of treatment strategies.

This study presents evidence that southern California urban rivers are a likely source of human viral contamination to beach waters. Three types of human viruses (adeno, entero and hepatitis A virus) together with coliphage and faecal indicator bacteria were tested in urban rivers of southern California. The seasonal variability of pollution input from urban rivers and their impacts to local beach water quality were also investigated.

## MATERIALS AND METHODS

### Urban river study sites

An intense water quality investigation of southern California urban rivers were conducted between 10 July and 30 August 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 (Fig. 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) *ca* 5–16 km from the first site. In general, the study area is highly urbanized, not impacted by agriculture run-off. At least one river, San Gabriel River, receives tertiary-treated sewage effluents from one of the Los Angeles sewage treatment facilities.

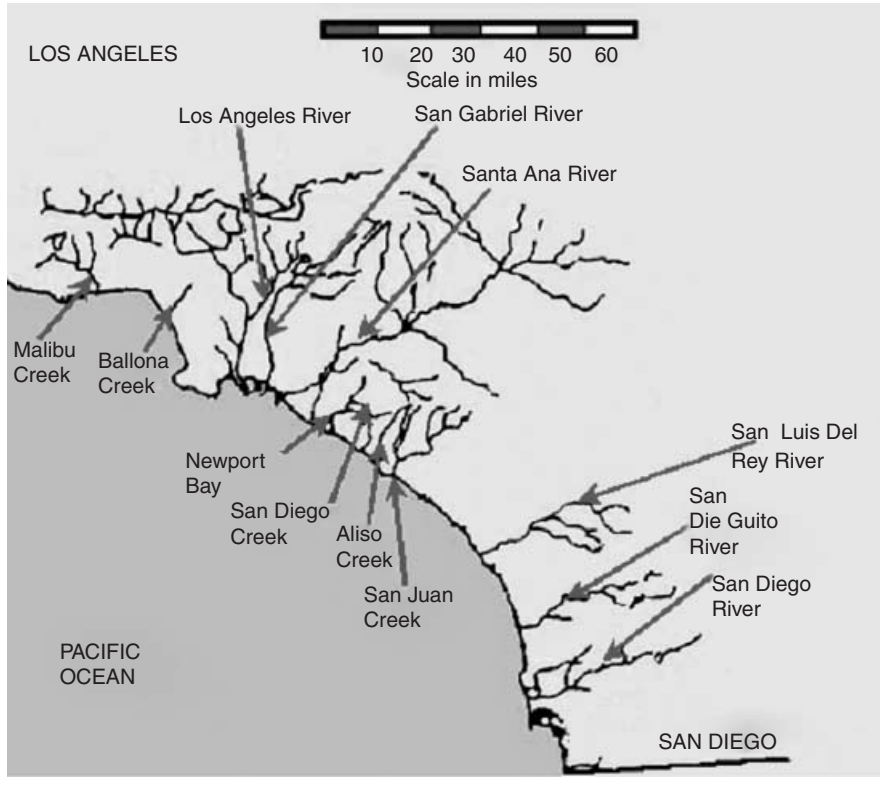


**Fig. 1** Map of southern California showing locations of rivers and creeks sampled during this study

## 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 *ca* 150 m 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., Pittsburgh, PA, USA). Beach samples were collected at ankle depth in accordance with the sampling protocol used for beach monitoring programme 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 h 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), faecal coliform (FC) and *Enterococcus* were determined using a membrane filtration method following standard protocols (Clesceri *et al.* 1998). In brief, samples were serially diluted, filtered onto 0·45-$\mu$m pore size 47 mm diameter sterile filters (Fisher Scientific, Inc.), which were incubated on solid medium for 24 (TC and FC) to 48 h (*Enterococcus*) 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 *Enterococcus*, respectively. EIA agar plates (Difco Lab.) were used to confirm *Enterococcus* colonies that grew on m-E medium after 48 h incubation at 41·5°C. The incubation temperature

for TC and FC was 37 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 100-kDa molecular weight cutoff filtration membrane or a Centriprep-100 centrifugal ultrafiltration device with a 100-kDa molecular weight cutoff membrane (Millipore, Bedford, MA, USA). 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-fold 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 was 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. *Escherichia 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 $\mu$g ml$^{-1}$ each of ampicillin and streptomycin to prevent background growth of indigenous bacteria. Coliphage densities were determined using either VFF-concentrated or unconcentrated water samples. One millilitre and 0·1 ml of sample were mixed with 1 ml of *E. coli* host in 1% soft agar, then overlayed on bottom nutrient agar. Plaques were enumerated after 12 h of incubation at 37°C. The number of phage was converted using sample volume and the concentration factor to plaque-forming unit (PFU) per litre 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 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 $\mu$l. Amplicons were confirmed by probing with internal oligonucleotide probes and/or labelled 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 (http://www.ipm.ucdavis.edu). Precipitation data from the CIMS Long Beach station were 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

**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′<br>Downstream, 5′-ACCGGATGGCCAATCCAA-3′<br>Probe, 5′-TACTTTGGGTGTCCGTGTTTC-3′ | 197-bp highly conserved 5′ untranslated region | DeLeon *et al.* 1990 |
| Hepatitis A virus | Upstream, 5′-CAGCACATCAGAAAGGTGAG-3′<br>Downstream, 5′-CTCCAGAATCATCTCCAAC-3′<br>Probe, 5′-TGCTCCTCTTTATCATGCTATG-3′ | 192-bp VP 1 and VP 2 capside protein interphase | Tsai *et al.* 1993 |
| Adenovirus | First upstream, 5′- GCCGCAGTGGTCTTACATGCACATC-3′<br>First downstream, 5′-CAGCACGCCGCGGATGTCAAAGT-3′ | 301-bp Hexon | Pina *et al.* 1998 |
| | Nested upstream, 5′-GCCACCGAGACGTACTTCAGCCTG-3′<br>Nested downstream 5′-TTGTACGAGTACGCGGTATCCTCGCGGTC-3′ | 143-bp Hexon | |

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). Run-off 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).

### Faecal indicator bacteria and coliphages in urban rivers

Table 3 shows the concentration of three indicator bacteria (TC, FC and *Enterococcus*), somatic and F-specific coliphage at 21 sampling sites. TC ranged from below the detection

**Table 2** Sampling sites and environmental conditions

| Sampling sites | Date and time | Salinity (‰) | Temp. (°C) | Weather | Location description |
|---|---|---|---|---|---|
| Malibu Creek I | 24 July 2000, 11:10 AM | 0·5 | 23 | Sunny | Dense green vegetation, no flow, birds and trash in water, storm drain nearby |
| Malibu Creek II | 24 July 2000, 10:50 AM | 0 | 24 | Sunny | Shallow, narrow, minimal flow, surrounding is not urbanized, no animal farms either |
| Ballona Creek I | 17 July 2000, 11:32 AM | 0 | 27 | Sunny | Minimal flow, storm drain nearby, concrete lined, freshwater marsh nearby |
| Ballona Creek II | 17 July 2000, 12:00 PM | 0 | 32 | Sunny | Minimal flow, shallow, narrow, concrete lined, sediment on the bottom |
| LA River I | 12 July 2000, 11:16 AM | 0 | 24·5 | Sunny | Tall vegetation, dense algae, murky, minimal flow, storm drain nearby |
| LA River II | 12 July 2000, 11:33 AM | 0 | 29 | Sunny | Shallow, concrete lined, dense algae, trash and birds in water, storm drains nearby |
| San Gabriel River I | 10 July 2000, 11:40 AM | 30 | 29·5 | Overcast | Deep, clear, wide, 1000 m from the ocean, tidal driven flow |
| San Gabriel River II | 10 July 2000, 11:08 AM | 1 | 27 | Overcast | Minimal flow, shallow, little vegetation, two storm drains nearby |
| Santa Ana River I | 19 July 2000, 11:18 AM | 36 | 31·5 | Sunny | Tidal flow only, upstream is completely dry |
| Newport Bay | 30 August 2000, 10:48 AM | 30 | 24 | Overcast | Murky, heavy vegetation, no flow, ducks in water |
| San Diego Creek II | 30 August 2000, 10:27 AM | 0 | 25 | Overcast | Shallow, algae and birds in water, storm drain nearby |
| Aliso Creek I | 26 July 2000, 10:52 AM | 0·5 | 28 | Sunny | Shallow, submerged vegetation, medium flow |
| Aliso Creek II | 26 July 2000, 10:37 AM | 0·5 | 26·5 | Sunny | Shallow, narrow, clear, fast flow, runs through a golf course |
| San Juan Creek I | 31 July 2000, 9:28 AM | 0 | 25 | Sunny | Narrow, concrete lined, clear, algae in water, storm drain nearby |
| San Juan Creek II | 31 July 2000, 9:48 AM | 0 | 27 | Sunny | Shallow, concrete lined, clear, algae in water, Del Obispo Park nearby |
| San Luis del Rey River I | 2 August 2000, 9:19 AM | 5 | 28·5 | Sunny | Murky, deep, medium flow, near a harbour, marsh vegetation |
| San Luis del Rey River II | 2 August 2000, 9:45 AM | 0 | 24 | Sunny | Shallow, narrow, murky, heavy vegetation |
| San Diequito River I | 21 August 2000, 9:35 AM | 15 | 27 | Sunny | Deep, wide, birds and fishes in water, heavy vegetation |
| San Diequito River II | 21 August 2000, 9:00 AM | 0 | 28 | Sunny | Deep, murky, no flow, nonurbanized agricultural land |
| San Diego River I | 23 August 2000, 9:15 AM | 4·5 | 26 | Sunny | No flow, narrow, algae in water, heavy vegetation |
| San Diego River II | 23 August 2000, 10:05 AM | 0 | 27 | Sunny | Murky, no flow, algae in water, lake-like setting |

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

| Sampling site | Total coliform | Faecal 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.

limit (<10 CFU per 100 ml) at San Gabriel River I and Santa Ana River I where urban run-off was highly diluted by ocean water as indicated by high salinity readings, to 230 000 CFU per 100 ml at Malibu Creek site I where stagnant water was colonized by a large number of birds. The geometric mean for all sites was 2653 CFU per 100 ml. 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 1587 CFU per 100 ml. Seven of 21 sites had FC levels >400 CFU per 100 ml (State of California single sample Rec-1 Water Quality Objective). *Enterococcus* did not correlate well with TC ($r = 0{\cdot}01$) and FC ($r = 0{\cdot}00$). High concentrations of *Enterococcus* were detected at Aliso Creek I and San Diequito River site II, where TC and FC at these sites were below geometric mean. Somatic coliphages were present at all sites, except Santa Ana River site I. However, they were poorly correlated with TC concentration at each site ($r = 0{\cdot}03$). F-specific coliphage were detected at 57% of the sites sampled and they were positively correlated with TC ($r = 0{\cdot}93$), FC ($r = 0{\cdot}83$), but poorly with *Enterococcus* ($r = -0{\cdot}03$).

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 that 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 100 ml.

## 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 4-month period. Samples taken during the wet season between 25 January and 27 February 2001 had three orders of magnitude greater concentrations of all indicator bacteria than those taken during the dry season between 17 April and 29 May 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 (Fig. 2). Enterovirus was found more frequently than adenovirus, which was found only once (5 February). 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 in for January and February, respectively, while only 0·62 in 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 programme (Fig. 3). The highest concentrations of TC and FC were found on 10 October 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 in at the CIMS Santa Ana station, south of the river. As 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 10 October 2000.

A dramatic decrease in all three indicators was recorded at the next sampling time (24 October). However, both samples at the point zero and surfzone remained positive for enteroviruses (Fig. 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 (26 October, 0·08 in; 27 October, 0·7 in and 29 October, 0·39 in). This may have resulted in the observed increase of indicator level and the detection of enteroviruses in the 1 November 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 because of these precipitation events (Fig. 3).

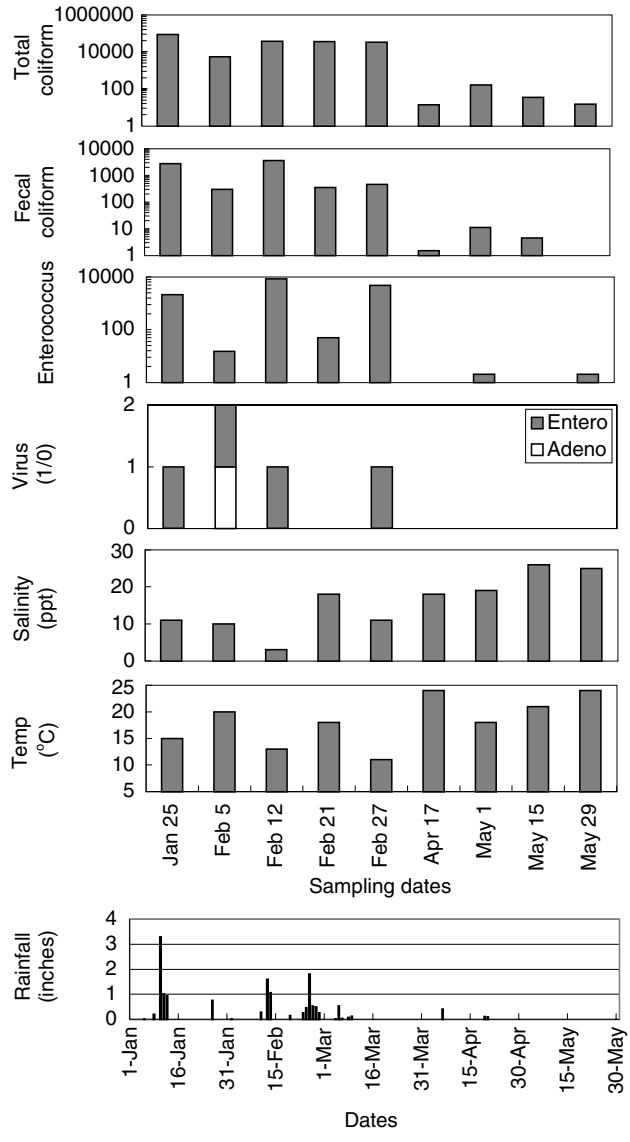


**Fig. 2** Seasonal variability of fecal indicator bacteria (total coliform, fecal coliform and enterococcus), human viruses (adeno and enteroviruses), salinity and temperature at the mouth of Los Angeles River. Fecal indicator bacteria are in CFU per 100 ml. Presence of a human virus was scored as 1,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

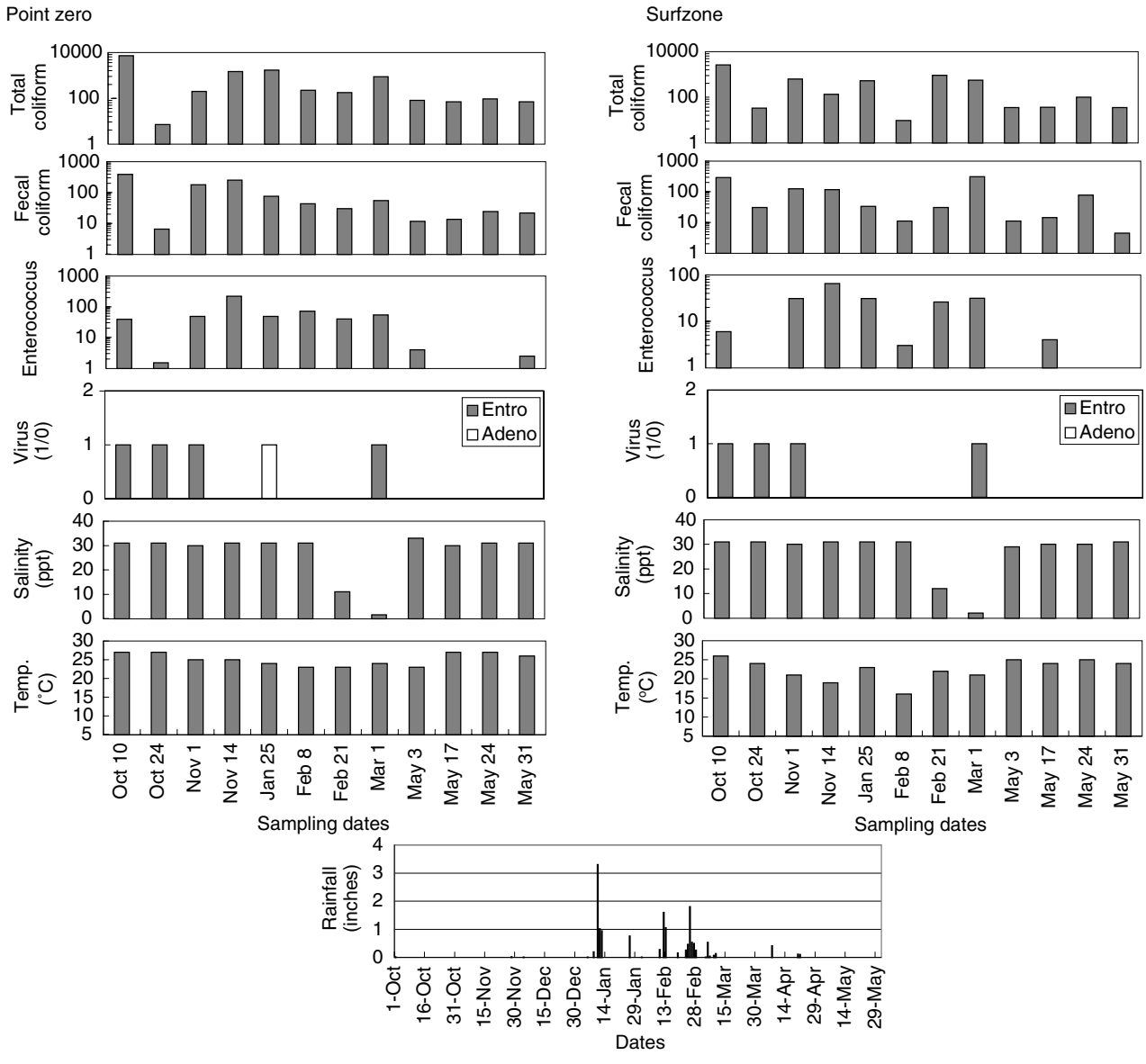


**Fig. 3** Seasonal variability of fecal indicator bacteria (total coliform, fecal coliform and enterococcus), human viruses (adeno and enteroviruses), salinity and water temperature at the mouth of San Gabriel River. Point zero station was located directly at the mouth of the river where river and coastline 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

Between January and February, a total precipitation of 13·92 in 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 1 March and adenoviruses were found on 25 January only at the point zero station (Fig. 3). Decreases in salinity were also recorded on 21 February and 1 March, 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 4 October (Fig. 4). Bottom dredging near the sampling site was noted during this sampling date, which

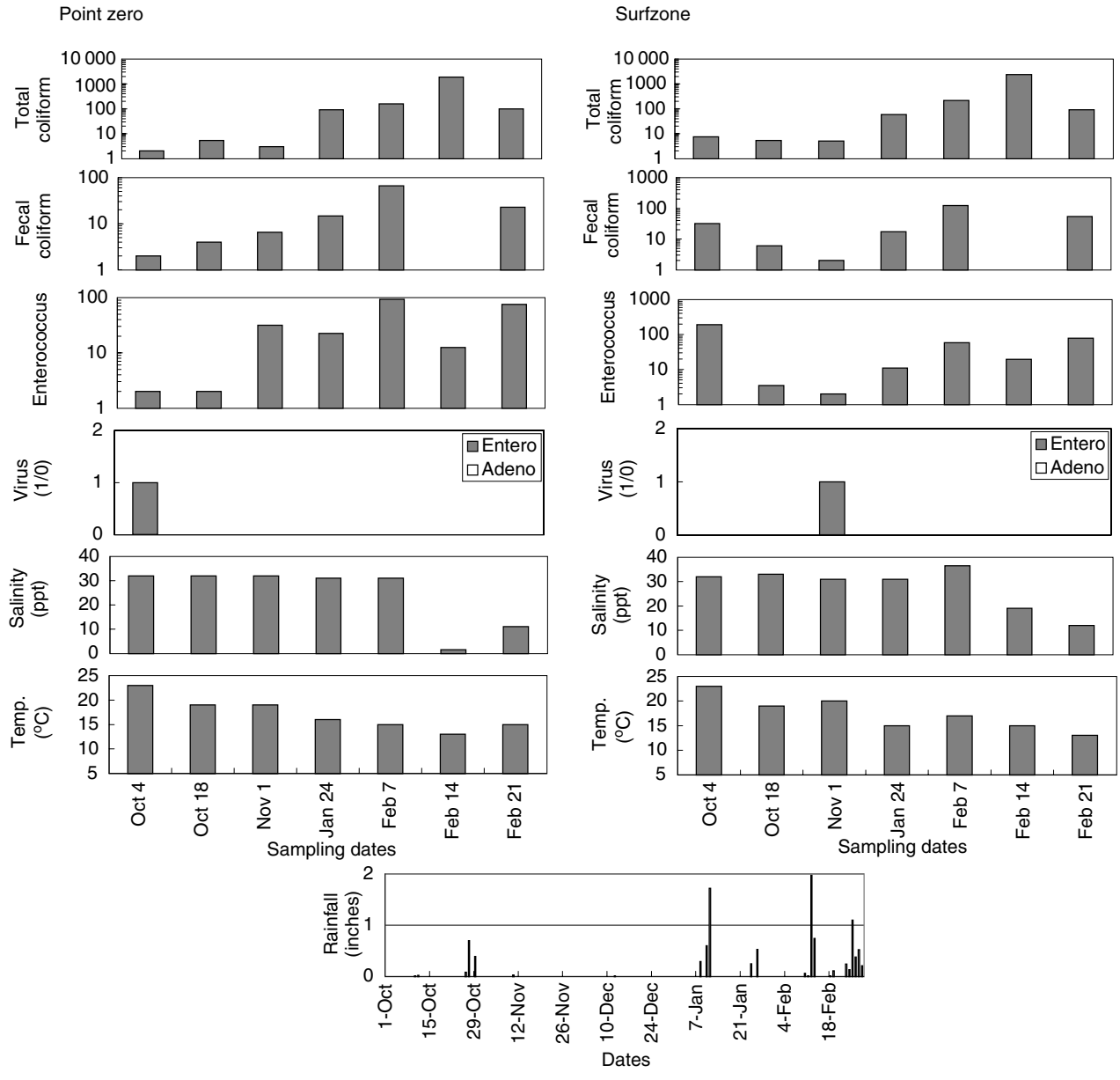


**Fig. 4** Seasonal variability of fecal indicator bacteria (total coliform, fecal coliform and enterococcus), human viruses (adeno and enteroviruses), salinity and water temperature at the mouth of Santa Ana River. Point zero and surfzone stations were the same as described in Fig. 2. Rainfall data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

may have contributed to this elevation in the surfzone. Samples taken between 4 October and 1 November had very low levels of TC and FC. However, enteroviruses were detected at point zero on 4 October and at surfzone on 1 November (Fig. 4). During the wet season between 24 January and 21 February, 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 stepwise 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 faecal 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 faecal indicator, suggesting faecal contamination from nonhuman sources, i.e. indigenous animal populations (i.e. birds, rodents) and natural in soil regrowth. 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 US. In the Florida Keys, Griffin *et al.* (1999) reported, using an 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 six 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, faecal 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 because only PCR method is used in our study, the viral signal detected may be noninfectious. The discharge of tertiary-treated sewage effluent into the urban river system may contribute to the noninfectious viral particles that only detectable by PCR method. This may explain the high incidence 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 faecal indicator data. When the viral signal is concurrent with high levels of faecal 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 faecal indicator bacteria, it may suggest an aged source of faecal 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 nonhuman 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′-nontranslated region that is highly conserved across enteroviruses including animal enteroviruses. Natural mutation among animal viruses may contribute to the false-positivity of human viral detection. Therefore, these PCR results represent our best knowledge of the target organisms. However, as 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-km 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.

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