Comment Letters Received from Academia

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- Dr. Richard Horner
- UCLA La Kretz Center

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Mr. Sam Unger

Executive Officer and Members of the Board

California Regional Water Quality Control Board, Los Angeles Region

320 West 4th Street, Suite 200

Los Angeles, CA 90013

July 22, 2012

RE: MS4 Permit for Los Angeles County

Dear Mr. Unger,

At the request of the Natural Resources Defense Council, I am submitting the following comments on the Draft Municipal Separate Storm Sewer System (MS4) Permit for Los Angeles County.

I am an Associate Professor with tenure in the Civil and Environmental Engineering Department at the University of California Los Angeles. I earned my B.S., M.S., and Ph.D. from the Civil and Environmental Engineering Department at the Massachusetts Institute of Technology. At UCLA, I have taught the following courses: Applied Environmental Microbiology, Aquatic Chemistry, Chemical Fate and Transport, Climate Change, Water Quality, and Ecosystem Functioning: A Service Learning Course, and Environmental Applications of Geochemical Modeling.

I run an active research program spanning several areas of environmental engineering: coastal water quality, mercury methylation, and arsenic contamination of groundwater. Our coastal water quality work includes source identification of fecal pollution in local watersheds, and the development of an in-field rapid method for detection of fecal indicator bacteria (FIB). I have co-authored 32 peer-reviewed journal articles (see Appendix A for list of peer-reviewed, published articles,) one book chapter, and one peer-reviewed conference proceedings. Of my published journal articles, five are directly related to FIB and source identification. I I have also co-authored three additional papers to be included in a special issue of Water Research on source identification this summer, and two additional manuscripts in this research area currently in review with other journals.

I have received the National Science Foundation Early CAREER award (2003), the Presidential Early Career Award in Science and Engineering (PECASE) (2003), the Northrup Grumman Award to Excellent in Teaching (2005), a Carnegie Fellowship for Service Learning (2007), and a Pritzker Fellowship for innovative courses in sustainability (2011 and 2012.)

Stormwater in Los Angeles

Santa Monica Bay receives significant year-round inputs of runoff from Los Angeles County, reaching volumes of well over a billion gallons during some storms. Some fraction is treated by small treatment facilities before discharge and another fraction is diverted to the wastewater treatment plant at Hyperion Wastewater Treatment Plant (the largest facility providing treatment of municipal wastewater in the Los Angeles Metropolitan Area,) while the remainder flows untreated into the ocean.

Runoff can contain elevated levels of many types of contaminants including fecal indicator bacteria (FIB), oils and greases, heavy metals, and volatile organic compounds. While FIB are not themselves the organisms that in most cases make swimmers ill, they are used as proxies due to their prevalence in fecal matter and ease of measurement. Elevated FIB levels are commonly observed in stormwater in Los Angeles, and have been observed up to 100 yards from flowing stormdrains (Gold 1994).

Noble et al. (2006) studied microbial contamination in Ballona Creek, which drains the largest watershed contributing runoff to Santa Monica Bay (Noble et al. 2006). Over a six-hour period, they sampled six main-stem and four major tributaries to Ballona Creek. *FIB levels were ubiquitous and high, with all tributaries contributing significant FIB loading.*

My lab has recently investigated the distribution of FIB (and human-associated *Bacteroidales* (HF183), discussed below) in Santa Monica Canyon in both wet and dry weather. Water samples were collected on 20 days from January 2008 to February 2009 (17 dry days, three wet days). There were a total of 11 sampling sites throughout the channel system, and a total of eight storm drain samples were also collected. *Widespread FIB contamination was observed in both channels throughout the sampling period.* Marine water quality standards were exceeded in 94% of samples for enterococci (maximum values > 62,700 MPN 100 mL^{-1}) and 81% of samples for *E. coli* (maximum values up >24,100 MPN 100 mL^{-1}). Exceedance rates were higher during wet weather than dry; wet-weather samples exceeded 100% of the time (12/12) for both enterococci and *E. coli*. During dry weather, *E. coli* exceeded 73% of the time (47/64) and enterococci exceeded 83% of the time (39/47) (Mika et al. 20xx).

Elevated levels of FIB in stormwater are not only observed in Los Angeles. For example, work by Noble and colleagues in North Carolina showed FIB levels in stormwater often exceeding water quality guidelines by orders of magnitude (Parker et al. 2010). Work by Jody Harwood (another well-respected expert in water quality) and colleagues showed high levels in stormwater in Florida (Brownell et al. 2007).

Impacts on health due to recreational coastal water use in Southern California

Previous studies have shown that there is a correlation between the levels of FIB in recreational waters and incidence of illness. Landmark studies (Cabelli et al. 1982; Kay et al. 1994) provide dose-response curves between levels of FIB and observed ailments in swimmers. At both of these study sites, there was a likely source of human fecal contamination.

At beaches in Santa Monica Bay, Haile et al conducted a carefully designed study on health effects due to swimming in coastal water impacted by storm drain runoff (as opposed to a likely human source as in the previous studies) (Haile et al. 1999). The study took place at three beaches that all have a high density of swimmers: Santa Monica, Will Rogers, and Surfrider. "Swimming" was defined as immersing one's head in the water, rather than simply walking in the water. While most epidemiological studies compare swimmers with non-swimmers, this study compared only swimmers, and took into account the distance from a storm drain and the water quality at that time and location. Water quality parameters included traditional FIB (total coliform, fecal coliform, *Escherichia coli* and entercocci) as well as human enteric viruses.

Swimmers within 100 m of a stormdrain and those swimming greater than 400 m from a storm drain were targeted for the study, except those who swam on multiple days. 15,492 swimmers were determined eligible and interviewed at the beach. Nine to 14 days later, a phone interview was conducted to inquire about the occurrence of a wide variety of ailments. Highly credible gastrointestinal illness I (HCGI 1) was defined as experiencing one or more of the following: 1) vomiting, 2) diarrhea and fever, or 3) stomach pain and fever. Highly credible gastrointestinal illness 2 (HCGI 2) was defined as experiencing vomiting and fever. The classification of significant respiratory disease (SRD) was used for cases of: 1) fever and nasal congestion, 2) fever and sore throat, or 3) coughing with phlegm.

The Haile et al. study had three major findings:

1) **The risk of many ailments was higher among subjects swimming near the storm drain.** A relative risk (RR) was calculated by comparing the risk at a particular distance within 100 m of the storm drain (grouped into 0 m, 1-50 m, and 51-100 m) with the risk calculated for individuals swimming greater than 400 m away. RR indicates the factor by which the background risk (that observed at greater than 400 m) is exceeded at the distance of

interest; for example, a RR of 2 for a particular ailment indicates swimmers were twice as likely to experience that health outcome. A RR greater than 1 (meaning that there was an increased risk of a particular ailment) was observed for numerous ailments, including both HCGI 1 and HCGI 2 for those swimming right at the storm drain. The entire 95% confidence interval for RR was exceeded at 0 m for fever (RR 1.61 (95% CI 1.16-2.24),) chills (RR 1.60 (95% CI 1.03-2.50),) ear discharge (RR 2.09 (95% CI 1.01-4.33),) coughing with phlegm (RR 1.65 (95% CI 1.11-2.46),) and SRD (RR 1.78 (95% CI 1.29-2.45).) Of these five outcomes, SRD had the highest magnitude of risk at the storm drain (risk of 7.6% compared to 4.6% at the background location.)

2) **There was a positive association between adverse health outcome and the levels of bacterial indicators.** The RR of occurrence of ailments was also compared at different levels of measured FIB. For example, at levels of total coliform above 400 colony forming units (cfu)/100 mL, the RR (when compared with the risk calculated for waters with less than 200 cfu/100 mL) was greater than 1 for eleven of nineteen ailments; the entire 95% confidence interval was greater than 1 for skin rash (RR of 1.86 with the 95% confidence range between 1.17 and 2.95.) Similarly, at levels of fecal coliform between 200 and 400 cfu/100 mL, the RR (compared with less than 200 cfu/100 mL) was greater than 1 for fifteen of nineteen outcomes, with the 95% confidence range greater than 1 for cough (RR 1.34 (CI 1.03-1.74)) and sore throat (RR 1.4 (CI 1.07-1.82).)

Exposure to enterococci levels above 104 cfu/100 mL resulted in RRs greater than 1 (indicating increased risk relative to the background of less than 35 cfu/100 mL) for eleven of nineteen ailments (the highest RR was observed for diarrhea with blood (RR 2.90 (CI 0.66 – 12.68).) *E. coli* levels above 320 cfu/100 mL also resulted in RR greater than one in fourteen of the nineteen ailments studied; for skin rash, the entire 95% CI was above 1 (RR 2.04 (CI 1.11 – 3.76).)

3) The RR was in general higher for swimming in water containing observable levels of enteric viruses. While there was high variability observed in the 95% confidence interval, the most notably high RRs (indicating increased risk after swimming in water with detectable viruses compared with water with no detectable viruses) were observed for diarrhea with blood (RR 5.82 (CI 0.45-75.72),) HCGI 2 (RR 2.32 (CI0.91-5.88),) eye discharge (RR 1.86 (CI 0.85-4.09),) and vomiting (RR 1.86 (CI 0.92-3.90).)

Another very important local epidemiological study was conducted at Doheny Beach in Orange County (which would have similar conditions to Los Angeles County) in 2007 and 2008 (Colford et al. 2012). San Juan Creek is the largest source of FIB to this beach. In this study, 9,525 individuals were studied to determine the relative illness rates at various levels of exposure (non-swimming, body immersion, head immersion, and swallowed water.) Water quality parameters were measured traditionally; in addition, enterococci were measured by three rapid methods. Some notable findings from this study include:

1) The risk of diarrhea was significantly increased among all swimming groups compared to non-swimmers.
2) Eye infections and earaches occurred at higher rates for swimmers.
3) FIB levels were strongly positively associated with diarrhea. The strongest association was observed for those swallowing water on days San Juan Creek was flowing into the ocean.

Markers of human contamination present in southern California stormwater

Notably, markers of human waste have also been observed in storm drains, even when levels of FIB were low, indicating that pathogens may be present even in storm water that meets safety standards for FIB. Prof. Sunny Jiang at UCI has investigated the presence of human-associated viruses in Southern California stormwater. She is an expert in detection of pathogens in environmental samples and her laboratory is well-versed in various quantitative polymerase chain reaction (qPCR) methods. Ahn et al (2005) studied how stormwater from the Santa Ana River, which drains a large Southern California watershed, impacted water quality at 17 stations along the shoreline as well as offshore sites (Ahn et al. 2005). These authors found the runoff to be a significant source of turbidity, FIB, fecal indicator viruses, and human pathogenic adenovirus and enterovirus to the coastal ocean. In some cases, FIB levels were low even when observable levels of human pathogenic viruses were present in offshore samples. *In my opinion this demonstrates that while the level of FIB in storm water is associated with illness, the lack of FIB in storm water does not indicate that it's safe.*

In the Noble et al. study of Ballona Creek (mentioned above), the authors, well-known experts in detection of microbial markers, saw evidence of widespread contamination with human markers (Noble et al. 2006). The HF183 marker was present in 86% of the samples analyzed, and enteroviruses were quantifiable in 39%; the Cochran storm drain had the highest levels of enterovirus present (up to 3255 genomes/L), with 86% if the samples having quantifiable levels. In my opinion, this demonstrates that there appears to be widespread FIB and human contamination in Los Angeles storm water.

As in the Ballona creek study, in my lab's investigation of FIB and human-associated <u>Bacteroidales</u> (HF183) in Santa Monica Canyon, the presence of human-associated marker was widespread in both channels of Santa Monica canyon. Human-associated marker was observable on every sampling day throughout both channels in Santa Monica Canyon. Of 78 samples tested for this marker, 45 (58%) had detectable levels (Mika et al. 20xx).

Occurrence of illness and related costs due to swimming in Southern California beaches

Given, Pendleton and Boehm studied the occurrence of gastrointestinal illness due to swimming at Southern California Beaches and the associated costs of such illnesses (Given et al. 2006). Dr. Boehm is a highly-respected expert in coastal water quality and Dr. Pendleton is a well-regarded economist specializing in issues pertaining to water quality. The authors combined water quality data obtained for 28 beaches in Los Angeles and Orange Counties with daily attendance data for each beach to estimate the number of cases of illness. Using the two most widely-known dose-response models for the risk of illness (Cabelli et al and Kay and Fleischer), the authors estimate between 427,800 and 993,000 cases of illness occurring due to swimming at the beaches in Los Angeles County alone (the total region including Orange County had between 627,800 and 1,479,200 cases of swimming-related illness.) While the dose-response curves used in this study were not attained in Southern California, the Haile et al study, conducted in local water, is in general agreement with these models.

Using a conservative estimate of the cost per illness, this occurrence of illness translates to an annual economic cost ranging from $14 to $35 million, depending on which dose-response curve was applied, for Los Angeles County alone (the total region including Orange County had costs between $21 to $51 million.)

The work by Given et al. presents an underestimate of the actual cost of illness occurrence in these counties because: 1) only a subset of beaches were considered, 2) only gastrointestinal illness was considered, while other ailments have been shown to be important at Southern California beaches, and 3) the cost per illness only included direct medical costs and lost work. The willingness-to-pay to not get sick and losses due to decreased tourism were not considered.

Importance of human-markers versus animal sources

It is often stated that human feces likely pose a larger risk to humans than animal feces. This is widely believed because viruses are almost always host-specific; thus, viral pathogens in animal hosts would pose low risk to humans.

However, more research is needed before we can assess overall risk due to exposure to non-human feces. Although many in the waterborne illness field believe that the majority of illnesses seen in swimmers is due to exposure to human viruses, the literature demonstrates that non-viral infections are quite common. In fact, a review of illness outbreaks due to recreational water found that fully three-fourths of outbreaks, and incidences of disease, had either bacterial or protozoan etiology; only 7% of outbreaks and illnesses had known viral causes (Craun et al. 2005). In addition, recent research indicates that animal-human transmission of illness occurs more frequently than had been believed, and often with more significant health outcomes.

There are numerous bacterial and protozoan pathogens with animal hosts that do pose a serious health threat to humans, such as enterohemorrhagic *E. coli* (EHEC). And importantly, animal waste is often discharged to receiving water without treatment. *Leptospira* species, bacterial pathogens, are frequently found in wild animal urine, and can cause illness in humans through contact, ingestion, or inhalation of contaminated water, especially in tropical areas (Narita et al. 2005). Animal urine was the likely source of contamination leading to outbreaks of leptospirosis associated with recreational water use in Japan (Narita et al. 2005) and among participants in an adventure race in Guam (Lee et al. 2002). While some species of the pathogen protozoan *Cryptosporidium* are host-specific, and many outbreaks are clearly from human fecal contamination, other species are truly zoonotic (Dillingham et al. 2002; King and Monis 2007). Runoff contaminated by cattle is the likely source for outbreaks of cryptosporidiosis in Indiana and Nebraska (Levy et al. 1998).

Health risk from swimming in mostly non-human impacted water is impossible to assess accurately until the data gap surrounding health impacts due to animal fecal sources is addressed. Determination of particular pathogens of concern at animal-influenced sites is an important future research need; however, epidemiological studies are the preferred approach to risk determination at this time.

Sincerely,

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APPENDIX A

Jennifer A. Jay, Associate Professor

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Education

Ph.D. 1999 Environmental Engineering, Massachusetts Institute of Technology, Camb., MA

M.S. 1993 Civil and Environmental Eng., Massachusetts Institute of Technology, Camb., MA
B.S. 1991 Civil and Environmental Eng., Massachusetts Institute of Technology, Camb., MA

Appointments

2002-present Assistant then Associate Professor, Civil and Environmental Engineering Department, University of California, Los Angeles.

2000-2002 Postdoctoral fellow, Tufts and MIT Collaboration, Cambridge, MA.

1999-2000 Postdoctoral fellow, Harvard School of Public Health, Boston, MA.

Selected Awards and Honors

Northrop Grumman Award for Excellence in Teaching (2007)

Carnegie Foundation Faculty Fellow for Service Learning for Political Engagement (2007-2008)

Presidential Early Career Award in Science and Engineering (PECASE, 2004)

Martin Fellow for Global Sustainability (1999), NSF Superfund Graduate Fellowship, (1993 – 1998)

NSF Traineeship at Harvard School of Public Health (2000)

Parsons Fellowship (1991)

National In View Award for Outstanding Contribution to the Preservation of the Environment (1991)

Chi Epsilon

Professional Affiliations

Society for Environmental Toxicology and Contamination (SETAC)

Board member for SoCal SETAC Chapter

Association of Environmental Engineering and Science Professor (AEESP), member

American Society for Microbiology (ASM)

American Chemical Society (ACS)

Technical Advisory Committee for the Santa Monica Bay Restoration Commission

member of Clean Beach Task Force organized by Southern California Coastal Water Research Project (SCCWRP)

Teaching experience

CEE 254 Aquatic Chemistry (2002 – present)

CEE M166/CEE 266 Environmental Microbiology (2003 – present)

CEE M166L Environmental Microbiology Laboratory (2004 – present)*

CEE 154 Fate and Transport of Chemicals in Aqueous Environments (2003 – present)*

CEE 58SL Wetlands and water quality: service learning course (2004)*

* Developed

Courses CEE 154 and CEE M166/266 have an innovative service learning component in which UCLA students work with middle school students to conduct community-based research projects. Middle school students visit UCLA to present their results.

In all classes, I pursue varied teaching methods to maximize engagement and active learning:

1) create many opportunities for in-class small group problem solving; 2) incorporate numerous demonstrations as well as inquiry-based field activities into normally lecture-based classes; and 3) draw heavily from the primary literature.

Papers published in peer-reviewed journals

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Coastal Water Quality Impact of Stormwater Runoff from an Urban Watershed in Southern California

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Field studies were conducted to assess the coastal water quality impact of stormwater runoff from the Santa Ana River, which drains a large urban watershed located in southern California. Stormwater runoff from the river leads to very poor surf zone water quality, with fecal indicator bacteria concentrations exceeding California ocean bathing water standards by up to 500%. However, cross-shore currents (e.g., rip cells) dilute contaminated surf zone water with cleaner water from offshore, such that surf zone contamination is generally confined to <5 km around the river outlet. Offshore of the surf zone, stormwater runoff ejected from the mouth of the river spreads out over a very large area, in some cases exceeding 100 km^2 on the basis of satellite observations. Fecal indicator bacteria concentrations in these large stormwater plumes generally do not exceed California ocean bathing water standards, even in cases where offshore samples test positive for human pathogenic viruses (human adenoviruses and enteroviruses) and fecal indicator viruses (F^+ coliphage). Multiple lines of evidence indicate that bacteria and viruses in the offshore stormwater plumes are either associated with relatively small particles (<53 μm) or not particle-associated. Collectively, these results demonstrate that stormwater runoff from the Santa Ana River negatively impacts coastal water quality, both in the surf zone and offshore. However, the extent of this impact, and its human health significance, is influenced by numerous factors, including prevailing ocean currents, within-plume processing of particles and pathogens, and the timing, magnitude, and nature of runoff discharged from river outlets over the course of a storm.

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Introduction

Oceans adjacent to large urban areas, or “urban oceans”, are the final repositories of pollutants from a myriad of point and nonpoint sources of human waste (*1*). Pollutants are transported to the urban ocean by surface water runoff (*1*−*4*), discharge of treated sewage through submarine outfalls (*5*), wet and dry deposition of airborne pollutants (*6*), and submarine discharge of contaminated groundwater (*7*). Until recently, effluent from sewage treatment plants was often the primary source of urban coastal pollution, including nutrients, pathogens, pesticides, and heavy metals (*8*). However, pollutant loading from many sewage treatment plants has declined over the past several decades because of improvements in civil infrastructure (e.g., separation of the storm and sanitary sewer systems to prevent combined sewer overflows), pollutant source control, and disposal/treatment technology (*9*). As a result, surface water runoff, in many cases, has supplanted sewage treatment plants as the primary source of pollutant loading to the urban ocean (*3*, *10*).

The focus of this study is the coastal water quality impact of surface water runoff during storms, or “stormwater runoff”, from an urban watershed in southern California. The study was motivated by several considerations. First, beneficial use designations for the coastal ocean in southern California apply year-round and, consequently, watershed managers are legally required to develop stormwater management plans for reducing wet-weather impairments of the coastal ocean (*11*). The impact of stormwater runoff on coastal water quality is of particular concern in arid regions such as southern California because, on an annual basis, a large percentage (>99.9% according to Reeves et al. (*2*) and >95% according to Schiff et al. (*10*)) of the surface water runoff and associated pollution flows into the ocean during a few storms in the winter. Second, while recreational use of the coastal ocean in southern California is lighter in the winter, compared to the summer, winter ocean recreation is still very common, particularly among surfers who surf the large waves that often accompany storm events (R. Wilson, personal communication). Third, to the extent that particles in stormwater runoff are associated with pathogens and other contaminants, their discharge to the ocean during storms may serve as a source of near-shore pollution that persists long after the storm season is over (*10*, *12*). Finally, in many urban watersheds in southern California and elsewhere, the flow of stormwater runoff is highly regulated by civil infrastructure (e.g., dams) designed to minimize flood potential and maximize water reclamation. As will be demonstrated later in this paper, the regulated nature of stormwater runoff implies that the ocean discharge of stormwater runoff from urban watersheds can occur days after the cessation of rain, when the potential for human exposure to pathogens by marine recreational contact is significant.

This paper describes how stormwater runoff from several major rivers in southern California, with particular focus on the Santa Ana River in Orange County, impacts coastal water quality, as measured by turbidity, particle size spectra, total organic carbon, fecal indicator bacteria, fecal indicator viruses, and human pathogenic viruses. The present study is unique in the combination of data resources utilized, including data and information from routine surf zone water quality and wave field monitoring programs, an automated in-situ ocean observing sensor, shipboard sampling cruises, and satellite sensors. Further, this is the first wet weather study to examine the linkage between water quality in the surf zone, where routine monitoring samples are collected

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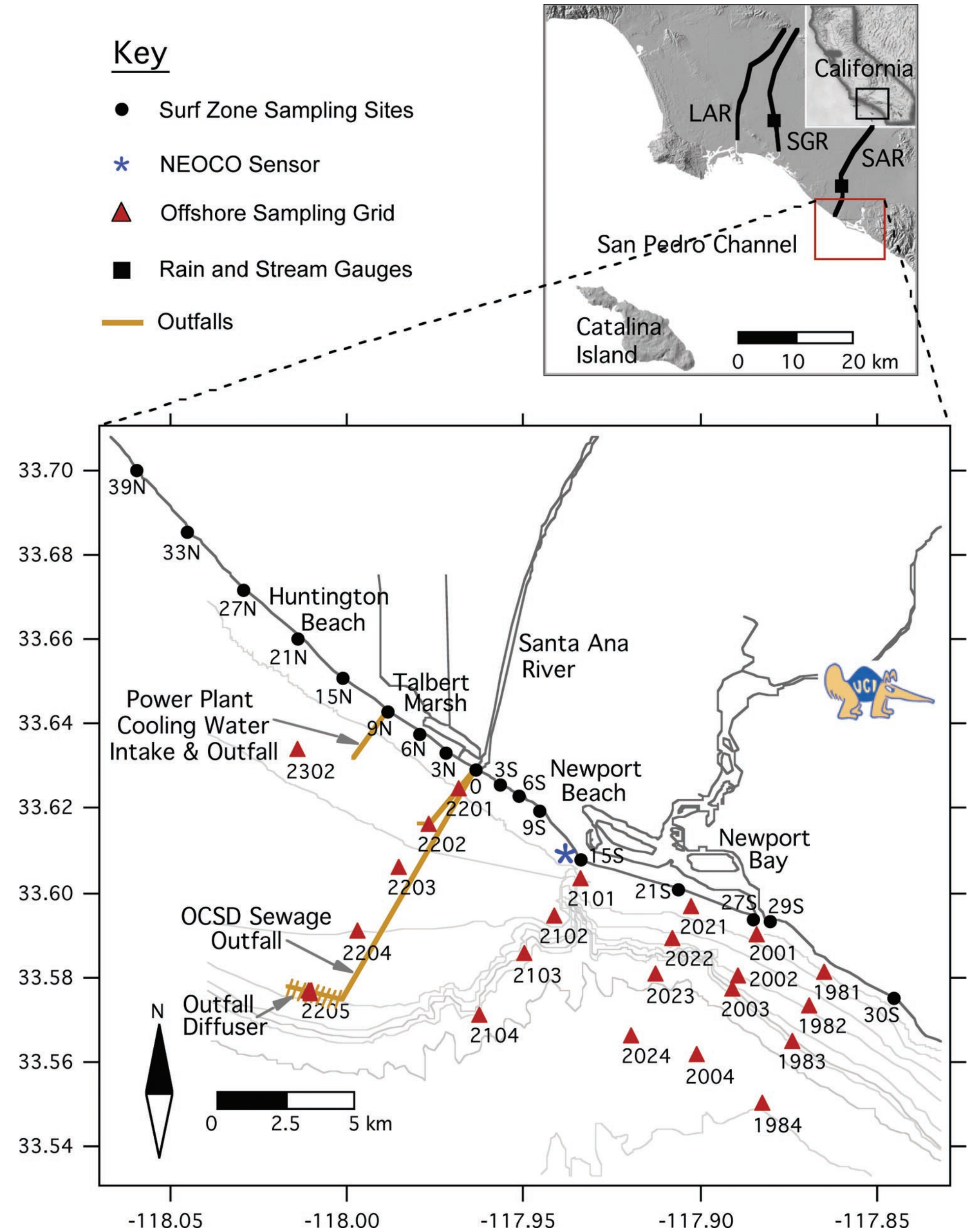

FIGURE 1. Map showing location of field site and sampling sites in the surf zone and offshore. Also shown are the locations of the NEOCO sensor on the end of the Newport Pier and the rain and stream gauges located on the Santa Ana River and the San Gabriel River. Abbreviations are Los Angeles River (LAR), San Gabriel River (SGR), Santa Ana River (SAR), Orange County Sanitary District (OCSD), and University of California, Irvine (UCI).

and most human exposure occurs, and water quality offshore of the surf zone. The work described in this study was carried out in parallel with a watershed-focused study that examined the spatial variability of fecal indicators, and the relationship between suspended particle size and fecal indicators, in storm runoff from the Santa Ana River watershed (*13*). Background information is available elsewhere on coastal water quality impairment at our Orange County field site (*2*, *14*–*18*) and the transport and mixing dynamics of sediment plumes as they flow into the coastal ocean from river outlets in southern California (*4*, *19*, *20*).

Materials and Methods

Rainfall and River Discharge. Weather information and Next Generation Radar (NEXRAD) images for planning the field studies and interpreting rainfall patterns were obtained online from the National Weather Service (http://www.nwsla.noaa.gov/). Precipitation and stream discharge data were obtained at two sites, one located where the Santa Ana River crosses 5th Street in the City of Santa Ana and another located where the San Gabriel River crosses Spring Street in the City of Long Beach (black squares in inset, Figure 1). These data were obtained, respectively, from the U.S. Army Corps of Engineers and the Los Angeles County Department of Public Works. Both of these gauge sites are located relatively close (within 11 km) to the rivers' respective ocean outlets, and hence streamflow measured at these sites will likely make its way to the ocean.

Surf Zone Measurements: NEOCO Data. Time series of water temperature, conductivity, chlorophyll, and water depth were obtained from an instrument package deployed at the end of the Newport Pier, where the local water depth is between 6.5 and 9 m (blue star in Figure 1). This instrument package is part of a recently deployed network of coastal sensors in southern California called the Network for Environmental Observations of the Coastal Ocean (NEOCO). The NEOCO sensor package contains an SBE-16plus CTD (Sea-Bird Electronics, Inc., Bellevue, WA) and a Seapoint Chlorophyll Fluorometer (Seapoint Sensors, Inc.). These instruments are mounted on a pier piling at a depth of approximately 1 m (below mean lower low water) and are programmed to acquire data at a sampling frequency of 0.25 min^{-1}.

Surf Zone Measurements: Fecal Indicator Bacteria and Breaking Waves. The concentration of fecal indicator bacteria in the surf zone was measured at 17 stations (black circles along shoreline in Figure 1) by personnel at the Orange County Sanitation District (OCSD). The stations are designated by OCSD according to their distance (in thousands of feet) north or south of the Santa Ana River outlet (e.g., station 15N is located approximately 15 000 ft, approximately 5 km, north of the Santa Ana River outlet). Water samples were collected 5 days per week (not on Friday and Sunday) from 5:30 to 10:00 local time at ankle depth on an incoming wave, placed on ice in the dark, and returned to the OCSD (Fountain Valley, CA) where they were analyzed within 6 h of collection for total coliform (TC), fecal coliform (FC), and enterococci bacteria (ENT) using standard methods 9221B and 9221E and EPA method 1600, respectively. Results are reported in units of colony forming units per 100 mL of sample (CFU/100 mL). Wave conditions, including both the direction and height of breaking waves, were recorded by lifeguards at the Newport Beach pier (near surf zone station 15S, Figure 1) twice per day, once at 7:00 and again at 14:00 local time.

Offshore Measurements: Satellite Ocean Color Imagery. The satellite images used in this study were collected by NASA's Moderate-Resolution Imaging Spectroradiometer (MODIS) instruments. These instruments operate onboard two near-polar sun-synchronous satellite platforms orbiting at 705 km altitude: Terra (since February 24, 2000) and Aqua (since June 24, 2002). Terra passes across the equator from north to south at ~10:30 local time, while Aqua passes the equator south to north at ~13:30 local time. As such, all the images were acquired within 2 h before or after local noon or between 18:00 and 22:00 UTC. The MODIS sensors collect data in 36 spectral bands, from 400 to 14 000 nm. We utilized bands 1 (250-m spatial resolution, 620−670 nm), 3, and 4 (500-m resolution, 459−479 and 545−565 nm, respectively) to produce "true color" (i.e., RGB) images, with band 1 used for the red channel, band 4 for the green channel, and band 3 for the blue channel. Using a MATLAB program, the 500-m green (band 4) and blue (band 3) monochrome channels were "sharpened" to 250-m resolution using fine details from the higher resolution red channel (band 1). Then, the contrast of each of these monochrome channels was increased to emphasize maximum details in the coastal ocean region of interest. Finally, all three monochrome channels (i.e., red, green, and blue) were combined to form a single true color image. In all, 16 satellite images from February 23 to March 5 were acquired and processed for this study; four of them were selected as most illustrative, on the basis of their quality and observed features. The timing of these satellite acquisitions relative to the storms and sampling periods is indicated at the top of Figure 2.

Offshore Measurements: Sampling Cruises. The offshore monitoring grid (red triangles in Figure 1) was sampled during three separate cruises on February 23, February 28, and March 1, 2004, coinciding with a sequence of storm events in late February 2004. Table 1 provides a summary of activities performed during each cruise. A short description of the offshore sampling and analysis protocols is presented here; details can be found in the Supporting Information for this paper. All offshore water samples were analyzed for salinity and fecal indicator bacteria, specifically, total coliform (TC), *Escherichia coli* (EC, a subset of FC), and enterococci bacteria (ENT), using the defined substrate tests known commercially as Colilert-18 and Enterolert (IDEXX, Westbrook, ME) implemented in a 97-well quantitray format; results are reported in units of most probable number of bacteria per 100 mL of sample (MPN/100 mL). A subset of the offshore water samples was analyzed for total organic carbon (TOC) by U.S. EPA Method 415.1, fecal indicator viruses (F^+ coliphage) by a two-step enrichment method (U.S. EPA Method 1601), and human pathogenic viruses (human adenovirus and human enterovirus) by real-time quantitative polymerase chain reaction (Q-PCR), nested PCR, and reverse-transcriptase (RT)-PCR using published protocols (*21*−*25*). Details on the PCR protocols used here can be found in the Supporting Information for this paper.

Coincident with the collection of the offshore water samples, temperature, particle size spectra, and light transmissivity were measured using an LISST-100 (laser in situ scattering and transmissometry) analyzer (Sequoia Scientific, Inc., Bellevue, WA). The LISST-100 estimates the particle volume per unit fluid volume (ΔV) resident in 32 logarithmically spaced particle diameter bins ranging in size from d_p = 2.5 to 500 μm. At least 10 replicates of the particle size spectra were collected at each offshore station. Following the recommendation of Mikkelsen (*26*), ΔV was taken as the median of all replicate measurements. The LISST-100 data are presented in this paper in one of three ways: (1) particle size spectra represented by plots of $\Delta V/\Delta \log d_p$ against log d_p, (2) the number of particles per unit fluid volume or total number concentration (TNC), and (3) the number-averaged particle size, $\bar{d}$. The last two parameters were computed from the particle size spectra as follows (*26*, *27*):

$$\mathrm{TNC} = \sum_{i=1}^{32} \frac{6\Delta V_i}{\pi d_{p,i}^{\ 3}} \tag{1a}$$

$$\bar{d} = \sqrt[3]{\frac{6}{\pi} \frac{\sum_{i=1}^{32} \Delta V_i}{\mathrm{TNC}}} \tag{1b}$$

Results and Discussion

Rainfall and River Discharge. Over the period of study (February 18 through March 3, 2004), four rain events were recorded by the rain gauge on the Santa Ana River in the City of Santa Ana (black curve, top panel, top axis, Figure 2). The first event accumulated 16.0 mm of rain in the afternoon of February 21 (RE_1 in Figure 2), the second event accumulated 23.4 mm of rain in the afternoon of February 22 (RE_2), the third event accumulated 51.3 mm of rain in the evening of February 25 (RE_3), and the fourth event accumulated 6.8 mm of rain in the evening of March 1 (RE_4). The rain gauge located on the San Gabriel River in the City of Long Beach did not record RE_2 but recorded a fifth rain event on February 18 (red curve, top panel, top axis, Figure 2). The difference in rainfall recorded at the Santa Ana River and the San Gabriel River sites is a consequence of the spatial variability of rainfall near the coast (see Figures S1 and S2, Supporting Information, for NEXRAD maps acquired during RE_1 and RE_2). Records of stream discharge (in units of m^3/s) at the Santa Ana River and the San Gabriel River sites are also quite different (black and red curves, top panel, bottom axis, Figure 2). While rainfall and stream discharge are coupled at the San Gabriel River site (i.e., stream discharge increases shortly after locally recorded rain events, compare set of red curves in top panel, Figure 2), rainfall and stream discharge are frequently uncoupled at the Santa Ana River site. For example, the Santa Ana River discharge events DE_3 and DE_4 do not obviously correlate with records of local rainfall. Instead, these two discharge events can be traced to stormwater runoff generated from inland regions of the Santa Ana River watershed

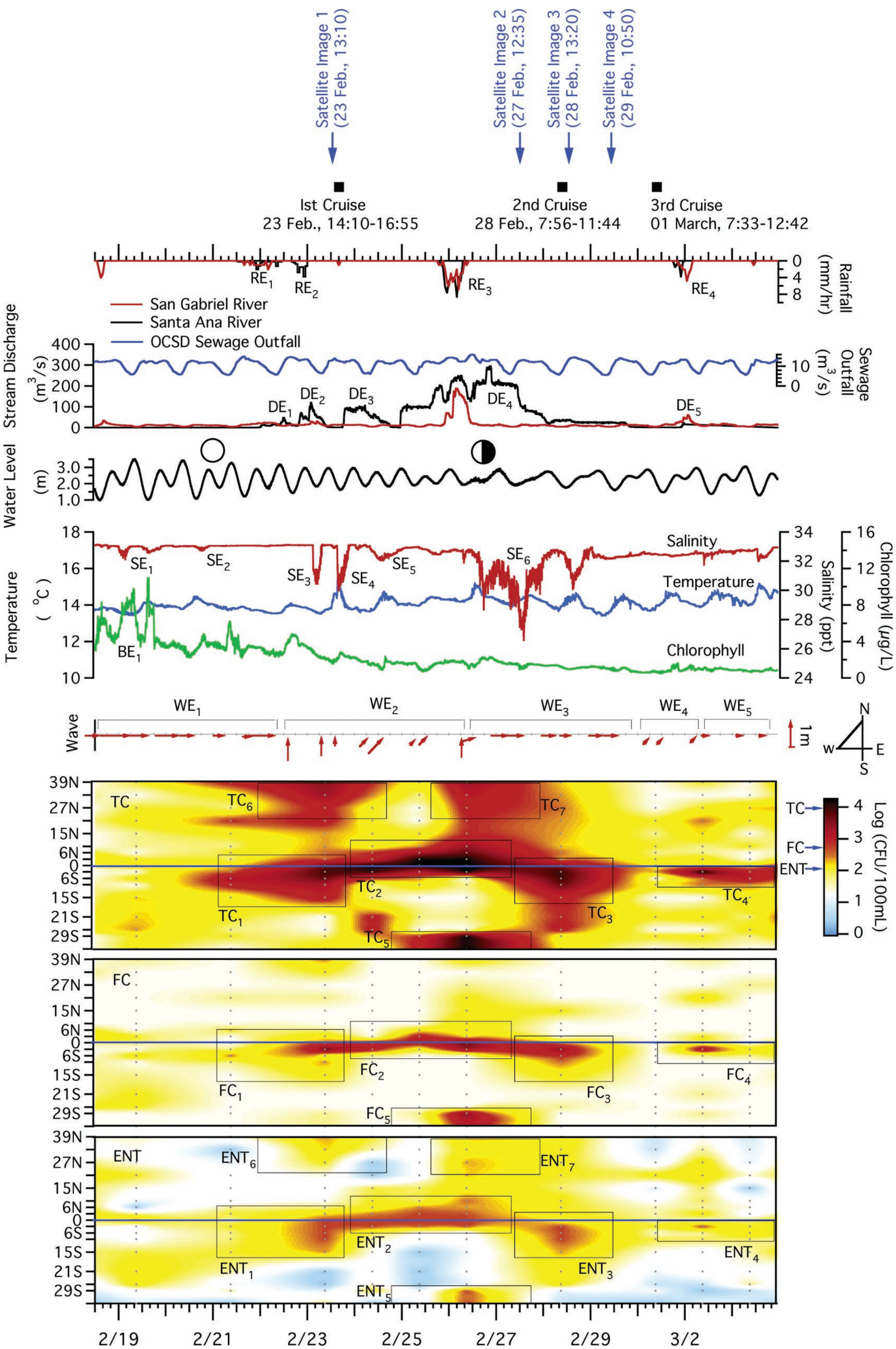

FIGURE 2. Time series measurements of rainfall, stream discharge at the Santa Ana River and San Gabriel River, and discharge of treated sewage from the OCSD outfall (top panel); water level, salinity, temperature, and chlorophyll measured at the NEOCO sensor (second and third panels); the direction and height of breaking waves at the Newport Beach Pier (fourth panel); and the concentration of fecal indicator bacteria in the surf zone (color contour plots, fifth through seventh panels). Shown at the top of the figure is the timing of the satellite images (blue lettering) and the offshore sampling cruises (black squares).

that was released from inland dams after the cessation of rain (*13*). For comparison, we have also included in the plot hourly volume discharge records (unit of m^3/s, blue curve, top panel, Figure 2) of treated sewage discharged from the Orange County Sanitation District (OCSD) sewage outfall (courtesy of OCSD).

TABLE 1. Summary of Analyses Performed during the Sampling Cruises

sampling parameters	methods	number of offshore sites sampled: February 23, 2004	February 28, 2004	March 1, 2004
conductivity[a]	Thermo Orion162A or CTD (SBE-32)	20	21	21
temperature[b]	thermocouple w/ LISST-100 or CTD (SBE-32)	20	21	21
total coliform, *Escherichia coli*, enterococcus[c]	Colilert and Enterolert (IDEXX)	20 (+2 sets of fractionated samples)	21 (+6 sets of fractionated samples)	21
total organic carbon[d]	EPA 415.1	17 (+2 sets of fractionated samples)		
human adenoviruses & enteroviruses[5]	nested PCR RT-PCR	2	6	
fecal indicator viruses (F^+coliphage)[e]	two-step enrichment	2	6	
particle size spectra	LISST-100 (light diffraction)	20	16	21
transmissivity	LISST-100	20	16	21

[a] Measured using a Thermo Orion 162A conductivity meter on February 23 and a CTD instrument (SBE-32) on February 28 and March 1. [b] Measured using a thermocouple bundled with an LISST-100 on February 23 and a CTD instrument (SBE-32) on February 28 and March 1. [c] Samples collected by UCI and analyzed by OCSD on February 23 and collected and analyzed by OCSD on February 28 and March 1. Fractionated samples collected and analyzed by UCI on February 23 and 28. [d] Collected by UCI and analyzed by Del Mar Analytical (Irvine, CA). [e] Carried out on the fractionated samples and measured using a real-time PCR for enterovirus and a nested PCR for adenovirus.

Surf Zone Measurements: NEOCO Data. Water level, salinity, temperature, and chlorophyll measurements at the NEOCO sensor, located on the end of the Newport Pier at the offshore edge of the surf zone, are presented in Figure 2 (second and third panels). The largest rain event (RE_3) and the largest discharge of stormwater runoff from the Santa Ana River (DE_4) occurred during a neap tide when the daily tide range was small (see quarter moon and water level measurements in the second panel, Figure 2). The other rainfall and stream discharge events occurred during periods of time when the daily tide range was larger, either during the transition from spring to neap tide (RE_1, RE_2, DE_1, DE_2, DE_3) or during the transition from neap to spring tide (RE_4, DE_5).

Salinity recorded at the NEOCO sensor is characterized by a series of low salinity events, relative to ambient ocean water salinity of 32.5−33.0 ppt (salinity events SE_1−SE_6, Figure 2). These low salinity events may be caused, at least in part, by stormwater discharged from the Santa Ana River (e.g., SE_6 appears to be related to DE_4). However, correlating discharge and the low salinity events is complicated by the fact that once river water is discharged to the ocean, its offshore transport is controlled by a complex set of near-shore currents (*28*). These near-shore currents, and their impact on the spatial distribution of stormwater runoff plumes, are explored in the next several sections. Temperature and chlorophyll records at the NEOCO sensor appear to be relatively unaffected by rainfall or discharge from the Santa Ana River. Surf zone temperature exhibits a diurnal pattern consistent with solar heating (i.e., temperatures are higher during the day and lower at night). Chlorophyll measurements indicate a bloom event occurred early in the study period (bloom event 1, BE_1), but this bloom event mostly dissipated prior to the rain and discharge events that occurred later. While the chlorophyll fluorometer was being maintained during this period, we cannot rule out the possibility that the downward trend in the chlorophyll signal is related to instrument fouling.

Surf Zone Measurements: Wave Data and Along-Shore Currents. Wave conditions, including the direction and height of breaking waves, were recorded twice per day by lifeguards stationed at the Newport Pier (surf zone station 15S, Figure 1). These wave data, which are plotted in the fourth panel of Figure 2, can be divided into five events, depending on whether waves approach the beach from the west (WE_1, WE_3, and WE_5) or from the south to southwest (WE_2 and WE_4). Because this particular stretch of shoreline strikes northwest−southeast (see Figure 1), waves approaching the beach from the west are likely to yield a down-coast surf zone current (i.e., directed to the southeast). Likewise, waves approaching the beach from the south are likely to yield an up-coast surf zone current (i.e., directed to the northwest) (*28*, *29*).

This expectation is consistent with the salinity signal measured at the NEOCO sensor, which is located approximately 5 km down-coast of the Santa Ana River ocean outlet. The onset of low salinity event SE_6 at the NEOCO sensor coincides very closely in time with the change in wave conditions from WE_2 to WE_3 and a likely change in the direction of the surf zone current from up-coast to down-coast (Figure 2). Discharge from the Santa Ana River was particularly high during this period (discharge event DE_4 overlaps wave events WE_2 and WE_3). Hence, the onset of SE_6 was probably triggered by a change in the direction of wave-driven surf zone currents from up-coast during WE_2 to down-coast during WE_3 and a consequent down-coast transport of stormwater runoff entrained in the surf zone from the Santa Ana River during DE_4.

Employing the same logic, low salinity events SE_3−SE_5, which occurred during a period when waves were out of the south to southwest, may have originated from stormwater discharged by river outlets or embayment located down-coast of the NEOCO sensor (e.g., the Newport Bay outlet). Low salinity events SE_1 and SE_2, which occurred during a period when waves were out of the west, may have originated from stormwater discharged by outlets located up-coast of the NEOCO sensor, although no significant discharge from the Santa Ana River was recorded during this period of time.

Some of these low salinity events may have originated from the cross-shore transport of lower salinity water from offshore, perhaps from surface runoff plumes or submarine wastewater fields associated with local sewage outfalls (*16*), or from the submarine discharge of low salinity groundwater (*7*). While the power-plant cooling water intake and outfall appear to affect local circulation patterns offshore of Huntington Beach (*30*), the power-plant effluent consists of pure ocean water and therefore is very unlikely to be a source of the low salinity events documented in Figure 2. It is theoretically possible that the OCSD sewage outfall is a source of SE_1 and SE_2, although there is nothing unusual about the sewage discharge rates observed during these two periods of time (compare SE_1 and SE_2 with the blue curve, top panel, Figure 2).

Surf Zone Measurements: Fecal Indicator Bacteria. The concentrations of the three fecal indicator bacteria groups (TC, FC, and ENT) in the surf zone are presented as a set of color contour plots in Figure 2 (bottom three panels). Fecal indicator bacteria concentrations were log-transformed to visualize the temporal and spatial variability associated with these measurements. For comparison, the California single-sample standards for the three fecal indicator bacteria (10^4 for TC, $10^{2.602}$ for FC, and $10^{2.017}$ for ENT, all CFU or MPN/100 mL) are indicated by a set of arrows on the scale bar in the figure. The concentration of fecal indicator bacteria was frequently elevated around the ocean outlet of the Santa Ana River (near surf zone station 0), particularly during and after rain events when stormwater was discharging from the river. For example, during stormwater discharge events (DE_3 and DE_4), water quality around the Santa Ana River outlet was very poor (see water quality events TC_2, FC_2, and ENT_2 in Figure 2). During this period of time, fecal indicator bacteria concentrations around the Santa Ana River outlet frequently exceeded one or more state standards, in some cases by as much as 300−500% (depending on the fecal indicator group).

The spatial distribution of fecal indicator bacteria in the surf zone around the Santa Ana River outlet appears to be controlled by local wave conditions, in a manner consistent with the earlier discussion of wave-driven surf zone currents. When waves approach the beach from the west and down-coast currents are likely to prevail, the concentration of fecal indicator bacteria in the surf zone is higher on the down-coast side of the ocean outlet (compare WE_1 with TC_1, FC_1, ENT_1 and WE_3 with TC_3, FC_3, ENT_3). Likewise, when waves approach the beach from the south and up-coast currents are likely to prevail, the concentration of fecal indicator bacteria in the surf zone is higher on the up-coast side of the ocean outlet (compare WE_2 with TC_2, FC_2, ENT_2). The exception is a short period of time when relatively small waves (wave height < 0.5 m) approach the beach from the southwest and the concentration of fecal indicator bacteria is higher on the down-coast side of the river (compare WE_4 with TC_4, FC_4, ENT_4). This exception can be rationalized by noting that waves out of the southwest break with their crests parallel to the beach, and hence the direction of long-shore transport in the surf zone is likely to be unpredictable under these conditions. The apparent time delay between change in wave direction (e.g., from WE_1 to WE_2) and change in the spatial distribution of fecal indicator bacteria around the Santa Ana River outlet (e.g., from TC_1 to TC_2) is, at least in part, a sampling artifact. Wave height and direction were recorded twice per day while fecal indicator bacteria concentrations in the surf zone were sampled at most once per day (the gray dots in the color contour plots indicate the timing of surf samples at each station).

Stormwater runoff discharged from the Santa Ana River appears to severely impact water quality in the surf zone over a fairly limited stretch of the beach (<5 km either side of the river between surf zone stations 15N and 15S). This spatial confinement of stormwater plumes in the surf zone, which is particularly evident for FC and ENT, could be the result of physical transport processes (e.g., dilution by rip cell mediated exchange of water between the surf zone and offshore) or nonconservative processes (e.g., the removal of fecal indicator bacteria from the surf zone by die-off or sedimentation) (*28*, *29*). An analysis of historical fecal indicator bacteria measurements at Huntington Beach concluded that the length of surf zone impacted by point sources of fecal indicator bacteria, such as the Santa Ana River, is influenced more by rip cell dilution and less by nonconservative processes such as die-off (*31*). The decay length scale reported here of 5 km is very close to the length scale predicted by rip cell dilution alone (2−4 km, assuming a rip cell spacing of 0.5 km) (*31*). Hence, die-off probably plays a secondary role, compared to dilution, in limiting the distance over which water quality is impaired in the surf zone by stormwater runoff from the Santa Ana River.

Fecal indicator bacteria events also occur in the surf zone at the northern (events TC_6, TC_7, ENT_6, ENT_7) and southern (events TC_5, FC_5, and ENT_5) edges of our study area. Possible sources of these fecal indicator bacteria events include stormwater discharged from the Huntington Harbor and Newport Bay Harbor located at the extreme northern (5 km up-coast of station 39N) and southern (stations 27S and 29S) ends of the study site and, possibly, from river outlets located outside of the study area (e.g., the Los Angeles River and San Gabriel River, see inset in Figure 1). Boehm and co-workers (*32*, *33*) suggested that the OCSD sewage outfall might be a source of fecal indicator bacteria in the surf zone at Huntington Beach, particularly during dry weather summer periods. However, compared to the Santa Ana River, the sewage outfall probably had a negligible impact on surf zone water quality at Huntington Beach and Newport Beach during the storm events sampled in this study. This conclusion is based on the following evidence. First, during our study period, sewage effluent discharged by OCSD was chlorinated and the fecal indicator bacteria concentrations in the final effluent (mean of 6000, 400, and 100 MPN/100 mL for TC, EC, and ENT, $n = 17$, C. McGee, personal communication) were significantly below the concentration of fecal indicator bacteria measured in stormwater runoff from the Santa Ana River (mean 17000, 5000, and 8000 MPN/100 mL for TC, EC, and ENT, $n = 30$, Surbeck et al. (*13*)). Second, the peak discharge rate from the OCSD outfall (ca. 13 m^3/s) is much smaller than the peak discharge rate of stormwater runoff from the Santa Ana River (ca. 300 m^3/s) (compare blue and black curves, second panel, Figure 2). Third, the sewage effluent is discharged 6 km offshore of the surf zone through a 1-km-long diffuser located at the end of OCSD's submarine outfall at a water depth of approximately 60 m (hatched region of the outfall pipe in Figure 1). By contrast, stormwater runoff from the Santa Ana River is discharged into the ocean directly at the surf line.

Offshore Measurements: Satellite Ocean Color Imagery. The spatio-temporal distributions of offshore stormwater runoff plumes sampled during this study are revealed by MODIS true color satellite imagery of a 100-km stretch of the coastline centered around our field site (Figure 3). The monitoring grid sampled during the offshore cruises is depicted on the satellite images by yellow dots. The timing of the satellite passes, relative to rain events, discharge events,

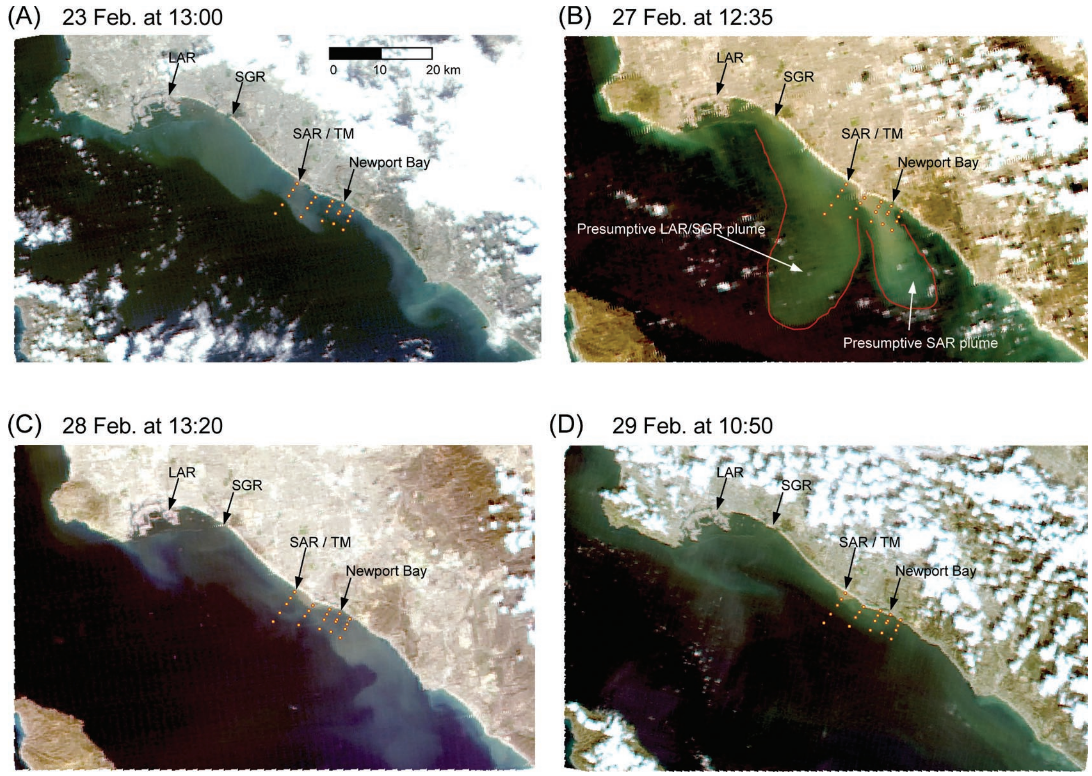

FIGURE 3. MODIS Terra and Aqua true color satellite imagery of stormwater runoff plumes along the San Pedro Channel, California, with nominal spatial resolution of 250 m. Yellow dots indicate location of field sampling stations offshore of Huntington and Newport Beach; black arrows denote the Los Angeles River (LAR) outlet, San Gabriel River (SGR) outlet, Santa Ana River/Talbert Marsh (SAR/TM) outlet, and Newport Bay outlet. (A) MODIS-Aqua, February 23, 2004, at 21:00 UTC (13:00 local time), (B) MODIS-Aqua, February 27, 2004, at 20:35 UTC (12:35 local time), (C) MODIS-Aqua, February 28, 2004, at 21:20 UTC (13:20 local time), (D) MODIS-Terra, February 29, 2004, at 18:50 UTC (10:50 local time).

wave events, surf zone water quality events, and offshore sampling cruises, is indicated at the top of Figure 2.

Generally speaking, in this collection of true color imagery the stormwater runoff plumes appear to be characterized by a band of turbid water turquoise to brown in appearance that is observed along the entire imaged region, although both cross-shelf and along-shore gradients in the color signature are evident. Following the rain events on February 21−22 (total of 39.4 mm, see RE_1 and RE_2 in Figure 2), a MODIS Aqua imagery from February 23 demonstrates the cross-shelf extent of the runoff plume to be variable, ranging from under 1 km in some places to more than 10 km offshore of the Los Angeles River and San Gabriel River (Figure 3A). At our study site, which is centrally located within this broad region, a distinct and apparently heavily particulate-laden runoff plume was observed in the vicinity of the Santa Ana River outlet and nearby station 2201 (see Figure 1 for numerical designation of offshore sampling sites). The Santa Ana River plume extended offshore past station 2203, with an apparent turn down-coast (i.e., southeast), continuing past stations 2104 and 2024. During this time, breaking waves were out of the south and the transport direction of fecal indicator bacteria in the surf zone was directed up-coast, opposite the apparent transport direction of stormwater plumes offshore of the surf zone (compare timing of satellite image 1 with WE_2 and fecal indicator bacteria events TC_2, FC_2, and ENT_2, Figure 2). It also appears that a portion of the Los Angeles River and the San Gabriel River stormwater plumes may have advected south and comingled with the Santa Ana River stormwater plume. Further south, offshore particulate loadings off the Newport Bay outlet (station 2001) do not appear to be as large as those off the Santa Ana River outlet.

A MODIS image on February 27 revealed two distinct plumes of considerable size and offshore extent (Figure 3B). This satellite acquisition preceded by 1 day the sampling cruise on February 28 (described in the next section), followed the large precipitation event on February 25−26 (total of 51.3 mm, see RE_3 in Figure 2), and followed the large discharge event from the Santa Ana River (DE_4, in Figure 2). The plume to the northwest in this image appears to be associated with the Los Angeles River or the San Gabriel River outlets, with an approximate areal extent of 450 km^2. The plume to the southeast appears to be distinct from the former plume and likely originated from the Santa Ana River outlet, with an approximate areal extent of 100 km^2 (the presumptive Los Angeles River, San Gabriel River, and Santa Ana River plumes are delineated by red lines in Figure 3B). The February 27 Santa Ana River stormwater plume is considerably larger in size than the one observed on February 23 (compare Figure 3A and 3B), consistent with the very large volume of water discharged from the Santa Ana River just prior to this satellite acquisition (approximately 4×10^7 m^3, see DE_4 in Figure 2). Further, the Los Angeles River, San Gabriel River, and Santa Ana River runoff plumes on February 27 differed from those on February 23 in that they penetrated farther offshore (30 km compared to 10 km) and thus potentially transported more sediments into the deep waters of the San Pedro Channel.

The jetlike appearance of the presumptive Los Angeles River, San Gabriel River, and Santa Ana River stormwater runoff plumes in Figure 3B has been observed elsewhere in the Southern California Bight, for example, off the Santa Clara River discharge (*4*, *29*), and is potentially the result of inertia-driven flow. At the time of this second satellite acquisition, breaking waves out of the west, and along-shore transport in the surf zone and offshore of the surf zone, appear to be directed down-coast (compare timing of satellite image 2 with WE_3 and fecal indicator events TC_3, FC_3, and ENT_3).

Subsequent MODIS true color imagery on February 28 (Figure 3C) and February 29 (Figure 3D) indicates that both

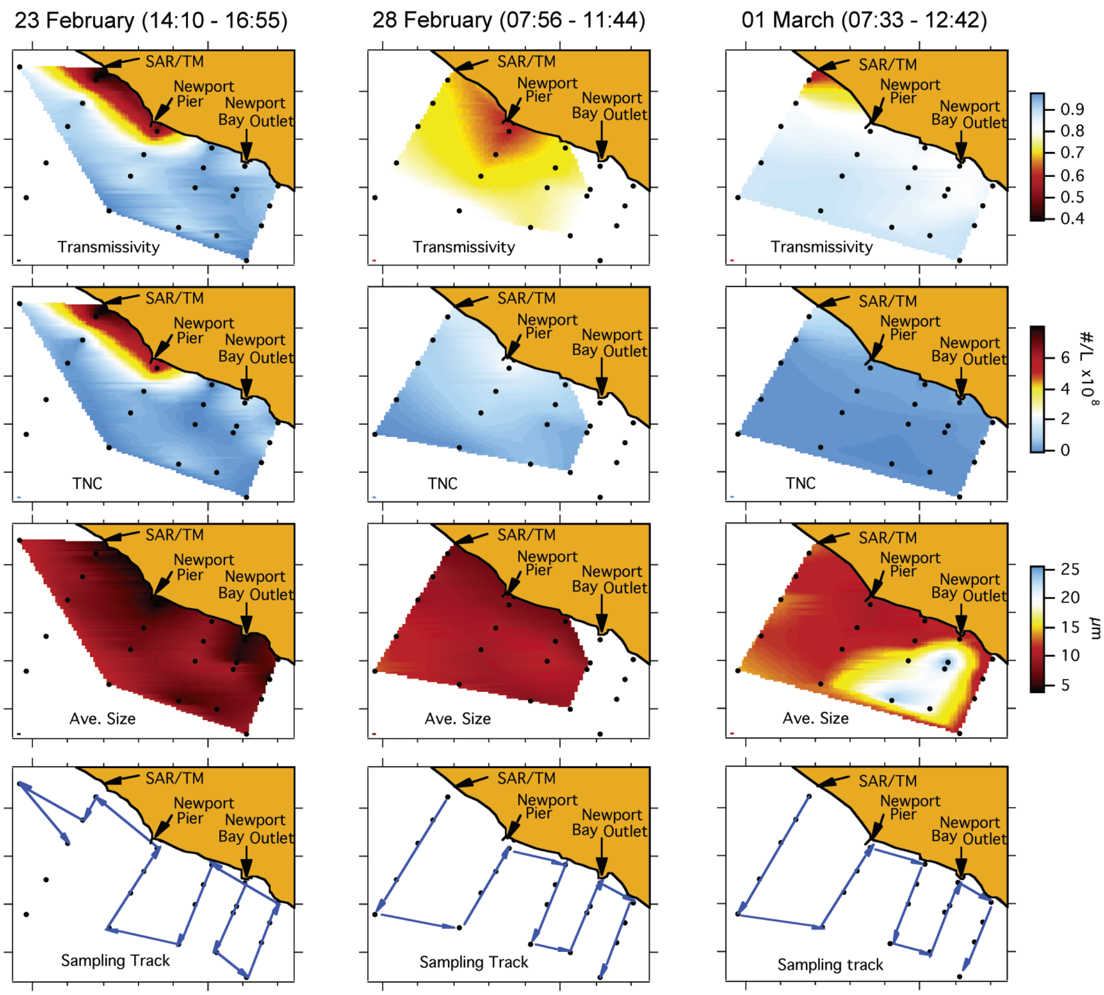

FIGURE 4. Particle measurements collected during the three sampling cruises. The bottom row of panels indicates the sampling track. TNC is an abbreviation for total particle number concentration. TNC and number-averaged particle size were calculated from measured particle size spectra using eq 1a, b.

the Los Angeles River/San Gabriel River and the Santa Ana River runoff plumes had significantly decreased in size, consistent with reduced flow out of the respective rivers (compare stream discharge curves with timing of satellite images 2 and 3, Figure 2). However, particulate matter appeared to remain high in the general vicinity of the Santa Ana River outlet. Whereas this zone of elevated particulate matter extended south to at least station 2021 on February 27–28, by February 29 it had receded somewhat and was fairly localized around station 2201. Unfortunately, no satellite imagery was available the following day (March 1) to complement the third sampling cruise, given persistent regional cloud cover that day.

Offshore Measurements: In-Situ Turbidity and Number-Averaged Particle Size. In-situ turbidity measurements collected during the three offshore cruises are presented as a series of color contour plots in Figure 4. During the February 23 cruise, a region of high turbidity, as evidenced by low transmissivity and high TNC, is evident offshore of, and to the south of, the Santa Ana River outlet (left-hand column of panels, Figure 4). The number-averaged particle size is depressed in this same region, as well as in the region offshore of the Newport Bay outlet. During subsequent cruises, the ocean became progressively less turbid closer to shore (although not necessarily offshore), as evidenced by increasing transmissivity and decreasing TNC, and the number-averaged particle size progressively increased (second and third columns, Figure 4). These results suggest that, offshore of the surf zone, particle size was steadily increasing and particle concentrations were steadily decreasing following the rain and stream discharge events that ended on, or before, the evening of February 27. The above turbidity patterns are generally consistent with the plume signatures and gradients observed in the true color satellite imagery (Figure 3), although some differences exist which could result from the offset timing (up to several hours) between the acquisition of the satellite images and the field measurements. As a technical aside, the number-averaged particle size ($\bar{d}$, see eq 1b) and the median particle size (d_{50}) follow similar trends (i.e., they both rise and fall together), although the magnitude of d_{50} was approximately 16-fold larger (Figure S3, Supporting Information). For the results presented here, $\bar{d}$ was chosen because it emphasizes changes in the small end of particle size spectra.

Offshore Measurements: Fecal Indicator Bacteria. Water quality test results from the three offshore cruises are presented as a set of color contour plots in Figure 5. During the February 23 cruise, the concentration of fecal indicator bacteria exceeded the California single-sample standards for TC, ENT, and EC in several samples collected just offshore, and to the south, of the Santa Ana River and Newport Bay outlets (left-hand column of panels in Figure 5). Nevertheless, the highest concentrations measured offshore of the surf zone are generally lower, in many cases by several orders of magnitude, compared to the highest concentrations measured in the surf zone (compare concentration scales for EC, FC, and ENT in Figures 2 and 5). The difference in offshore and surf zone fecal indicator bacteria concentrations is even

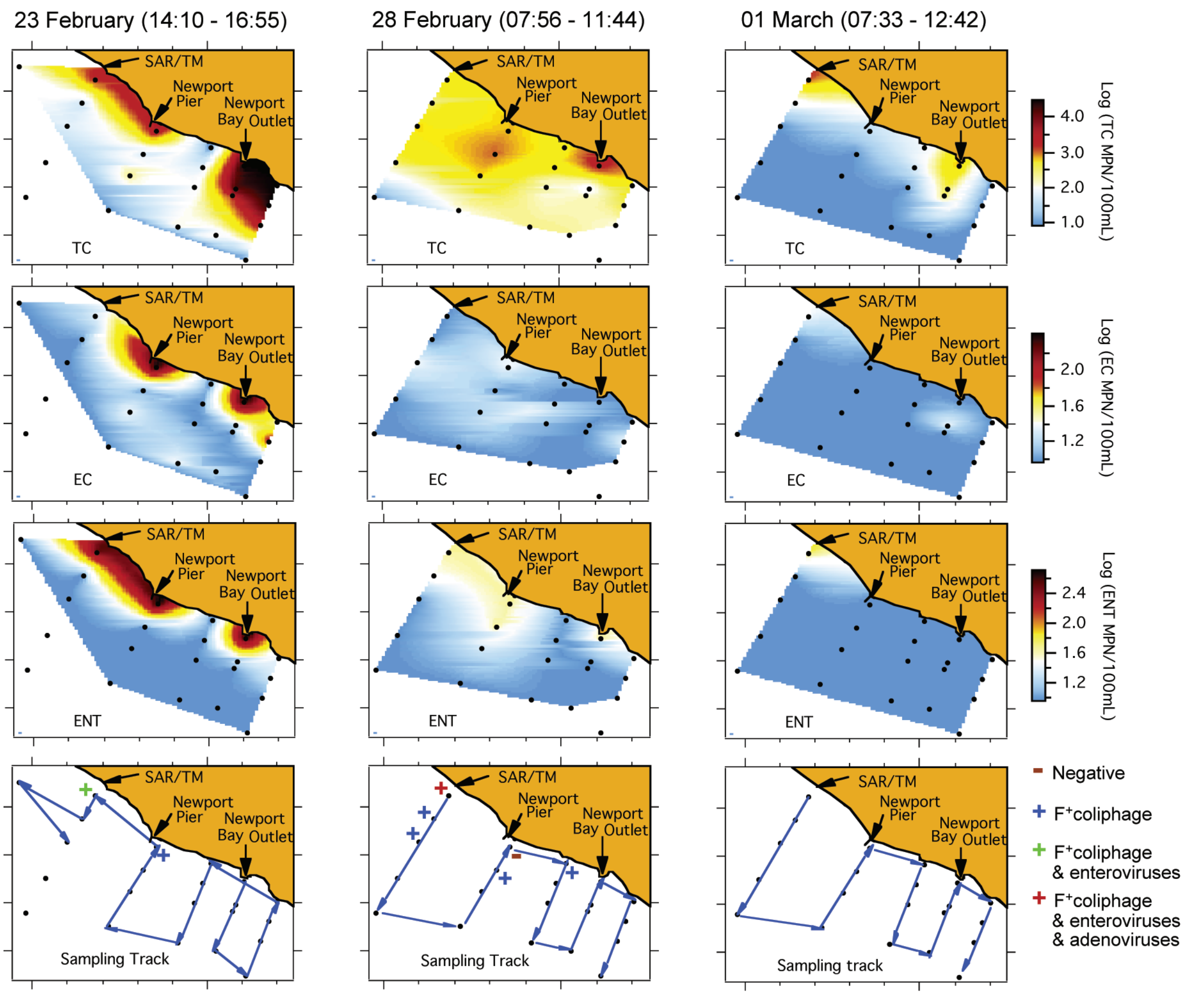

FIGURE 5. Fecal indicator bacteria concentrations measured during the three sampling cruises. The bottom row of panels indicates the sampling track (blue arrows) and the detection of F^+ coliphage and human viruses. SAR/TM is an abbreviation for the outlet of the Santa Ana River and Talbert Marsh.

more pronounced during the later cruise dates. For example, none of the samples collected during the February 28 and March 1 cruises exceeded state standards for fecal indicator bacteria, yet several of the samples collected from the surf zone during the same time period exceeded single-sample standards for one or more fecal indicator bacteria groups (compare concentrations measured during the second cruise date with TC_3, FC_3, and ENT_3 and concentrations measured during the third cruise date with TC_4, FC_4, and ENT_4, Figures 2 and 5).

Offshore Measurements: F^+ Coliphage and Human Viruses. Offshore samples tested positive for F^+ coliphage (n = 8, see Table 1), with the exception of a single sample collected on the February 28 cruise from offshore of the Newport Pier (blue, green, and red plus symbols, bottom panels, Figure 5). Human adenoviruses and enteroviruses were detected by real time Q-PCR, nested PCR, and RT-PCR in a sample collected from station 2201 located directly offshore of the Santa Ana River outlet during the February 28 cruise (red plus, middle bottom panel, Figure 5). The concentration of human adenoviruses in this sample is estimated to be 9.5×10^3 genomes per liter of water, which is approximately equivalent to 10 plaque forming units per liter of water, according to a laboratory study comparing Q-PCR results with plaque assay (*35*). Human enteroviruses were also detected in a sample collected directly offshore of the Santa Ana River outlet (station 2201) on the February 23 cruise (green plus, bottom left panel, Figure 5). While relatively few samples were tested for human viruses (n = 8), these results demonstrate that human viruses are present in surface water offshore of the Santa Ana River outlet following storm events, even when the fecal indicator bacteria concentrations are below state standards (e.g., station 2201 during the February 28 cruise, Figure 5). These results are consistent with previous observations that human pathogenic viruses and fecal indicator viruses persist longer than fecal indicator bacteria in ocean water (*36*). Direct PCR measurement of pathogenic viruses in highly turbid water is challenging because of PCR inhibition (*35*).

Offshore Measurements: Relationship between Fecal Indicator Bacteria, Turbidity, and Number-Averaged Particle Size. Turbidity has been suggested as a possible proxy for water quality (*37, 38*). However, on the basis of our offshore data, turbidity per se appears to be an inconsistent proxy for the concentration of fecal indicator bacteria. For example, during the February 23 cruise, there is good coherence between turbidity and TC, EC, and ENT concentrations off the Santa Ana River outlet and Newport Pier (compare transmissivity and TNC with fecal indicator bacteria results, left-hand column of panels, Figures 4 and 5). However, turbidity is low off of the Newport Bay outlet where the bacteria concentrations are particularly high. In addition, there are no consistently robust relationships between shipboard measurements of fecal indicator bacteria and shipboard measurements of TOC, temperature, or salinity (see Figure S4, Supporting Information). The number-averaged particle size, on the other hand, comes close to matching the along-shore spatial pattern of fecal indicator

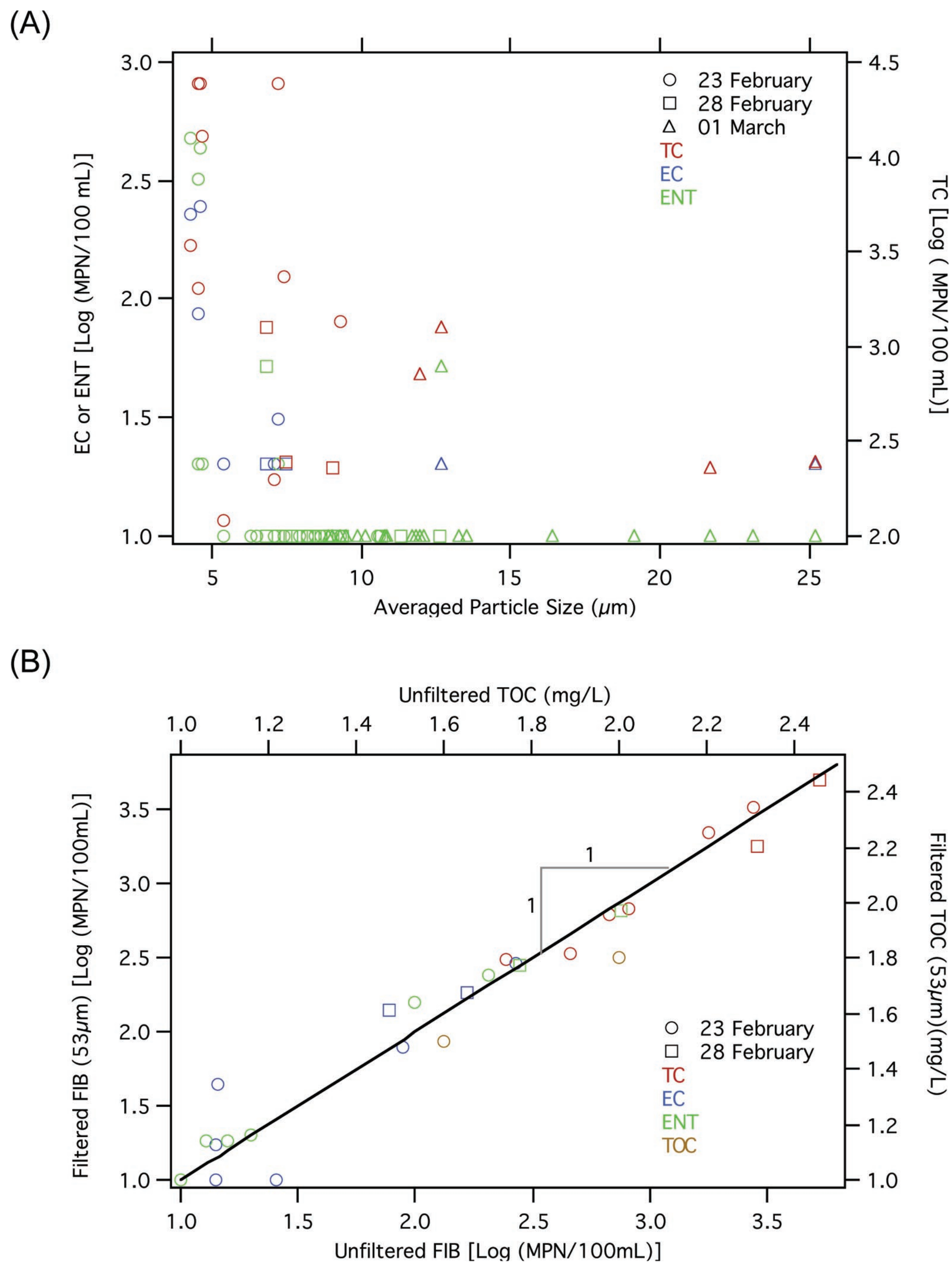

FIGURE 6. (A) Cross plots of log-transformed fecal indicator bacteria concentrations measured in samples collected during the three offshore cruises, against the corresponding number-averaged particle size. (B) Cross plots of log-transformed fecal indicator bacteria concentrations and TOC concentrations measured in samples collected during the three offshore cruises, before and after filtration through a 53-μm sieve. The one-to-one line corresponds to the case where the concentrations are the same before and after filtration.

bacteria measured during the February 23 cruise. Specifically, elevated fecal indicator bacteria concentration appears to correlate with depressed number-averaged particle size (compare fecal indicator bacteria and number-averaged particle size results for the February 23 cruise, left-hand column of panels, Figures 4 and 5). When all of the fecal indicator bacteria data collected during the three cruises are aggregated and plotted against number-averaged particle size, an inverse relationship between these two parameters emerges; specifically, samples with elevated fecal indicator bacteria concentrations also exhibit small number-averaged particle size (Figure 6A). Moreover, the concentration of fecal indicator bacteria in water samples collected during the first two cruises is the same, within error, before and after filtration through a 53-μm sieve (Figure 6B), implying that fecal indicator bacteria are either adsorbed to particles smaller than 53 μm or are not particle-associated. TOC also appears to pass through the 53-μm sieve (Figure 6B) as do human viruses and fecal indicator viruses (data not shown). The co-occurrence of small particles and indicators of fecal pollution (fecal indicator bacteria, fecal indicator viruses, and human pathogenic viruses) does not necessarily imply that the latter are adsorbed to the former. The inverse relationship evident in Figure 6A, for example, may reflect a temporal evolution of stormwater plumes as they age, from a predominance of small particles and high concentrations of fecal indicators initially, to larger particles and lower concentrations of fecal indicators later.

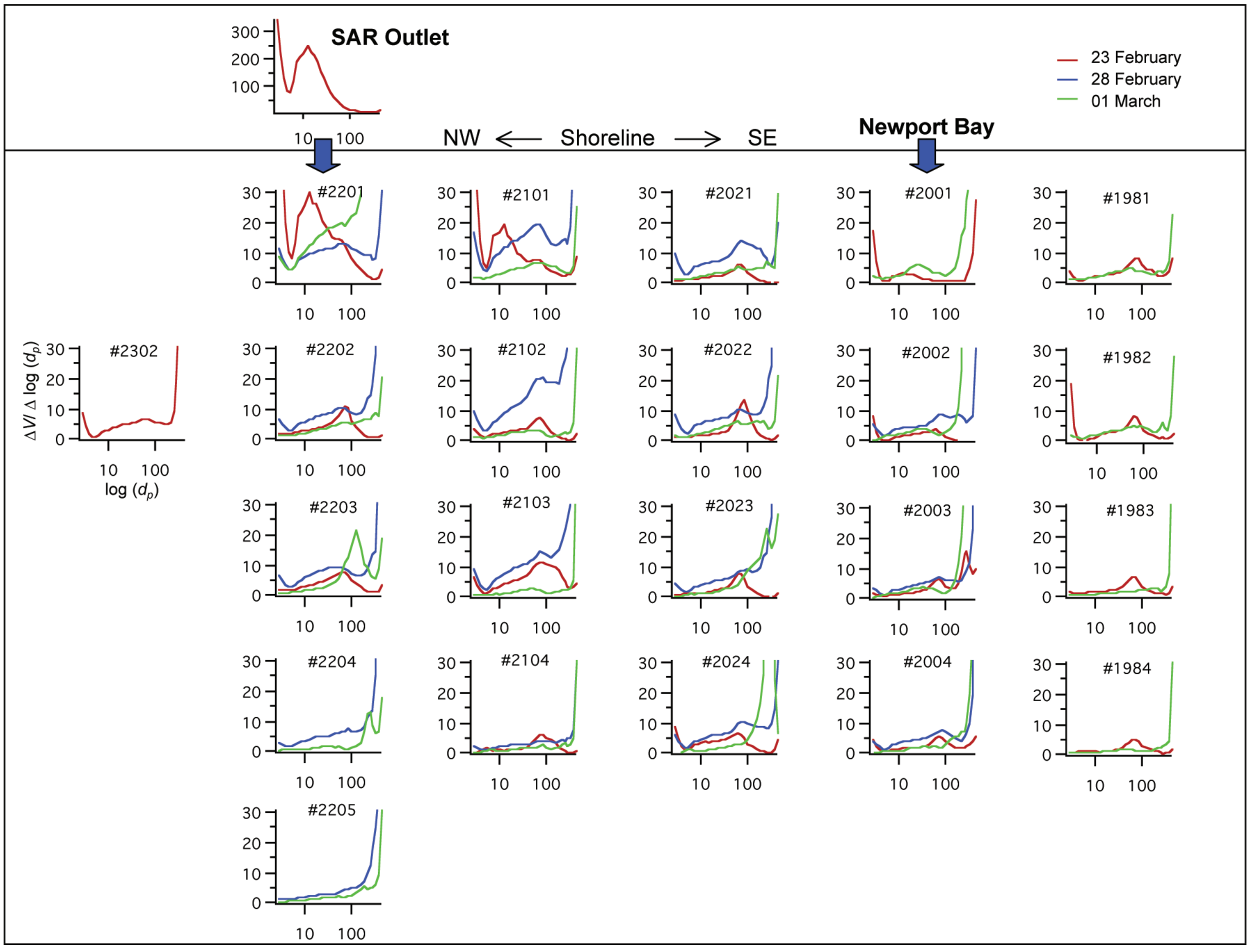

FIGURE 7. Particle size spectra measured during the three offshore cruises; numbers at the top of each panel denote the station number where the particle size spectra were measured (see Figure 1). The vertical axis in each plot represents the particle volume resident in logarithmically spaced particle diameter bins; the horizontal axis represents the diameter of the particles (in μm). These plots are arranged so that the stations progress from onshore to offshore (top to bottom) and up-coast to down-coast (left to right). The single plot labeled "SAR Outlet" corresponds to a particle size spectrum measured in stormwater runoff flowing out of the Santa Ana River outlet, just upstream of where it flows over the beach and into the ocean.

Offshore Measurements: Particle Size Spectra. Particle size spectra acquired during the three cruises are presented in Figure 7. Each plot displays the normalized particle volume (vertical axis) detected in 32 logarithmically spaced particle diameter bins ranging in size from 2.5 to 500 μm (horizontal axis). The particle size spectrum measured at a particular offshore location and time appear to be related to the specific stormwater plume the particles are associated with and, possibly, the elapsed time stormwater has spent in the ocean. Stormwater flowing out of the Santa Ana River during the February 23 cruise, for example, is characterized by two modes at the small end of the size spectrum, one in the <5 μm bin and another in the 10−50 μm bins (set of red curves, Figure 7). These modes are present in stormwater runoff sampled at several locations in the Santa Ana River watershed (*13*), in samples collected at the ocean outlet of the Santa Ana River (panel labeled "SAR Outlet" at top of Figure 7), and in samples collected just offshore (red curve at station 2201, Figure 7) and down-coast (red curve at station 2101, Figure 7) of the Santa Ana River outlet. Particles discharged from the Santa Ana River appear to dilute and merge into a background turbidity characterized by a single broad mode in the 50−300 μm size range (evident in the red curves at most stations, Figure 7).

Referring to Figure 3A and the earlier discussion of this satellite image, the 50−300 μm mode observed on February 23 may be characteristic of a large runoff plume originating from one or more up-coast sources of stormwater runoff, most likely the Los Angeles River or the San Gabriel River. Several factors can lead to artifacts in the particle size spectra estimated from the light-scattering instrument deployed in this study (*39*). However, in our case this caveat is mitigated somewhat by the observation that particle volume fractions calculated from the particle size spectra are strongly correlated (Spearman's rank correlation $Sp = 0.90$, $p = 0.02$) with independent measurements of total suspended solids (data not shown).

During the second and third cruises, the particle size spectra progressively coarsen with the result that, by March 1, virtually all of the particle volume is associated with the largest size bin (>500 μm, green curves in Figure 7). The observed temporal evolution in particle size spectra, from high turbidity and multiple modes at the lower end of the particle size spectrum to low turbidity and a single mode at the large end of the particle size spectrum, may reflect decreasing particle supply (i.e., reduced stormwater discharge from major river outlets) coupled with within-plume coagulation of particles into larger size classes and, ultimately, removal of the largest particles by gravitational sedimentation. Coagulation time scales estimated from these particle size spectra measurements are short (minutes to hours or longer) compared to time scales associated with the generation and offshore transport of stormwater plumes (hours to days), and hence coagulation cannot be ruled out as an important mechanism at our field site (see Supporting Information for details on the time scale calculations).

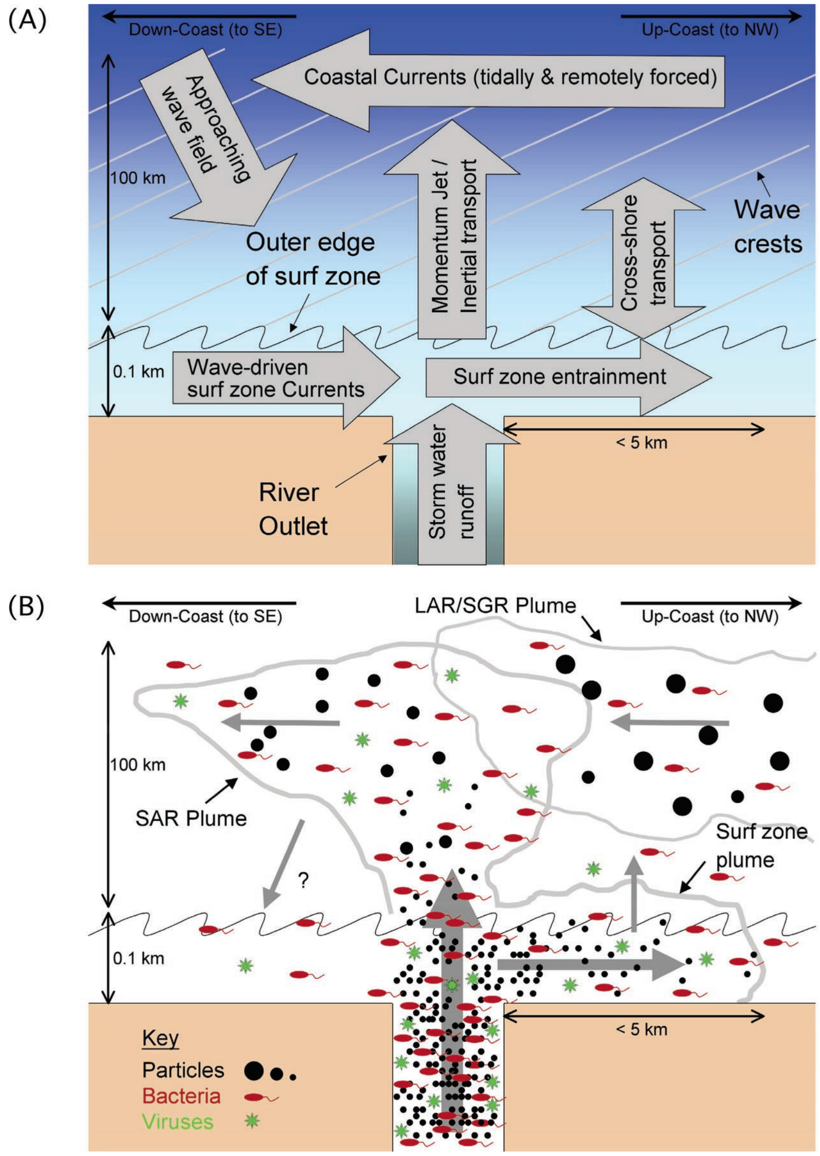

FIGURE 8. (A) Transport mechanisms that can affect the offshore distribution of contaminants discharged from river outlets. (B) Schematic representation of the spatial distribution of particles (black circles of varying size), fecal indicator bacteria (red symbols), and F^+ coliphage and human pathogenic viruses (green symbols). Abbreviations are SAR (Santa Ana River), SGR (San Gabriel River), and LAR (Los Angeles River).

Whether coagulation, in fact, plays a role in the fate and transport of particles and particle-associated contaminants in stormwater plumes will likely depend on the coagulation efficiency (i.e., the fraction of particle−particle collisions that result in sticking events) and shear rates present at a given location and time (*40*, *41*). Alternatively, the observed temporal coarsening of particles in the offshore may reflect changes in the particle size spectra of the stormwater runoff before it enters the ocean, from a predominance of smaller particles during the peak of the hydrograph, to a predominance of coarser particles during the falling limb of the hydrograph. Further studies are needed to determine whether observed coarsening of the offshore particle size spectra is caused by within-plume coagulation or by temporal evolution of the particle size spectra in stormwater runoff before it enters the ocean.

Data Synthesis. Results presented in this paper are represented schematically in Figure 8, including potential offshore transport mechanisms (panel A) and the resulting distribution of particles, bacteria, and viruses (panel B). As stormwater is discharged from the river outlet and flows over the beach, a fraction is entrained in the surf zone and the rest is ejected offshore in a momentum jet. Measurements of fecal indicator bacteria in the surf zone suggest that, once entrained, contaminants are transported parallel to shore by wave-driven currents, in a direction (i.e., up- or down-coast) controlled by the approaching wave field. When waves strike the beach so that a component of wave momentum is directed up-coast (the scenario pictured in Figure 8), fecal indicator bacteria in the surf zone are carried up-coast of the river outlet. Conversely, when waves strike the beach so that a component of wave momentum is directed down-coast, fecal indicator bacteria in the surf zone are carried down-coast of the river outlet. The buildup of water in the surf zone from breaking waves drives a cross-shore circulation cell, which can transport material between the surf zone and offshore of the surf zone. At our field site, this cross-shore circulation appears to limit the length of beach severely polluted with fecal indicator bacteria to <5 km around the river outlet, by diluting contaminated surf zone water with cleaner water from offshore. While the transport processes described here are based on measurements of fecal indicator bacteria in the surf zone, it is likely that other contaminants in stormwater runoff, in particular, human viruses and toxic contaminants associated with suspended particles (*13*, *42*), will behave similarly.

Further offshore, stormwater runoff plumes are common and readily detected through a variety of geophysical parameters (e.g., salinity, transmissivity, surface color). A clear linkage between these parameters and fecal indicator bacteria could not be established here. However, fecal indicator bacteria did appear to be associated with the smallest particle sizes, on the basis of both fractionation studies (Figure 6B) and the inverse relationship observed between fecal indicator bacteria concentrations and number-averaged particle size (Figure 6A). Particle size spectra in the offshore plumes coarsen with time post-release, and fecal indicator bacteria concentrations steadily drop (see the schematic representation of particle size in the various offshore plumes, Figure 8B). These results have several implications. First, they suggest that high concentrations of fecal indicator bacteria in the surf zone at our field site are probably not brought into the study area by coastal currents from distal sources (e.g., the Los Angeles river or the San Gabriel river). Second, cross-shore transport of water between the surf zone and offshore of the surf zone, for example, by rip cell currents, is likely to improve surf zone water quality by diluting dirty river effluent entrained in the surf zone with relatively clean ocean water from offshore.

While the concentrations of fecal indicator bacteria in the offshore plumes are generally below surf zone water quality standards, particularly during the latter two cruises, fecal indicator viruses (F^+ coliphage) were detected in nearly all offshore samples tested, and human adenoviruses and enteroviruses were detected in several offshore samples, including two collected offshore of the Santa Ana River outlet (station 2201 on February 23 and 28, see Figure 5). It is likely that the virus results presented here represent a conservative estimate of viral prevalence, because a limited numbers of samples were tested ($n = 8$). In addition, the presence of PCR inhibitors in stormwater reduces the efficiency of PCR detection of human pathogenic viruses, as mentioned earlier. At present, there are no water quality standards for fecal indicator viruses and human pathogenic viruses, largely because epidemiological data are not available to link adverse human health outcomes (e.g., gastrointestinal disease) to recreational ocean exposure to these organisms. However, the offshore detection of human pathogenic viruses begs several questions: First, do these viruses constitute a human health risk, either by contaminating the surf zone directly (see arrow with question mark, indicting the possible transfer of contaminants from offshore into the surf zone, Figure 8B) or by sequestering in offshore sediments? Second, given the fact that the Santa Ana River has separate storm and sanitary sewer systems, what is the source of human fecal pathogens in the wet weather water runoff? Many studies have shown that human fecal pathogens are associated with storm runoff from urban areas located throughout the United States (*25*, *43*−*45*), so the association between stormwater runoff and human fecal pathogens observed here is certainly not unique. Possible sources of human pathogens in stormwater runoff from urban areas include leaking sewer pipes, illicit sewage connections to the stormwater sewer system, homeless populations, and so forth.

Taken together, the results presented in this paper demonstrate that stormwater runoff from the Santa Ana River is a significant source of near-shore pollution, including turbidity, fecal indicator bacteria, fecal indicator viruses, and human pathogenic viruses. However, relationships between variables (e.g., between turbidity and fecal indicator bacteria and between fecal indicator bacteria and human viruses) vary from site to site (at the same time) and from time to time (at the same site) suggesting that the sources, fate, and transport processes are contaminant specific. The apparent exception is the inverse relationship observed between fecal indicator bacteria and number-averaged particle size, although further studies are needed to determine if this result is generalizable to other storm seasons and coastal sites and, if so, to determine the underlying mechanism at work. The relationship between water quality parameters (e.g., fecal indicator bacteria), turbidity, and other field proxies, such as number-averaged particle size, salinity, and colored dissolved organic matter, are the focus of ongoing and future regional studies, including as part of a coastal water quality observing program within the Bight '03 Project (http://www.sccwrp.org/regional/03bight/bight03_fact_sheet.html), as well as other investigations being carried out as part of the Southern California Coastal Ocean Observing System (SCCOOS).

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Supporting Information Available

Sampling and analysis protocols, calculation of the orthokinetic coagulation time scales, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Confirmation of putative stormwater impact on water quality at a Florida beach by microbial source tracking methods and structure of indicator organism populations

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ABSTRACT

The effect of a stormwater conveyance system on indicator bacteria levels at a Florida beach was assessed using microbial source tracking methods, and by investigating indicator bacteria population structure in water and sediments. During a rain event, regulatory standards for both fecal coliforms and *Enterococcus* spp. were exceeded, contrasting with significantly lower levels under dry conditions. Indicator bacteria levels were high in sediments under all conditions. The involvement of human sewage in the contamination was investigated using polymerase chain reaction (PCR) assays for the *esp* gene of *Enterococcus faecium* and for the conserved T antigen of human polyomaviruses, all of which were negative. BOX-PCR subtyping of *Escherichia coli* and *Enterococcus* showed higher population diversity during the rain event; and higher population similarity during dry conditions, suggesting that without fresh inputs, only a subset of the population survives the selective pressure of the secondary habitat. These data indicate that high indicator bacteria levels were attributable to a stormwater system that acted as a reservoir and conduit, flushing high levels of indicator bacteria to the beach during a rain event. Such environmental reservoirs of indicator bacteria further complicate the already questionable relationship between indicator organisms and human pathogens, and call for a better understanding of the ecology, fate and persistence of indicator bacteria.

1. Introduction

Stormwater runoff can cause an influx of indicator bacteria to receiving waters. Previous studies in southern California have demonstrated increased indicator bacteria levels in coastal waters influenced by stormwater runoff (Noble et al., 2003; Reeves et al., 2004; Ahn et al., 2005). Reeves et al. (2004) observed that during dry conditions, total coliforms, *Escherichia coli* and *Enterococcus* spp. were highly concentrated in runoff from forebays (underground storage tanks), which was transported to coastal water during storm events.

Underground storage of stormwater runoff may well provide favorable conditions for bacterial persistence, as sediments in the stormwater conveyance systems may act as a reservoir. Both *E. coli* and *Enterococcus* spp. can persist in a culturable state in sediments for weeks or months (Byappanahalli, 1998; Desmarais et al., 2002; Anderson et al., 2005; Jeng et al., 2005). Studies of indicator bacterial survival have shown lower decay rates in sediment compared to water (Sherer et al., 1992; Howell et al., 1996; Anderson et al., 2005), indicating that sediments provide protection from harmful stressors (e.g., high temperatures and sunlight). Both

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sediments and underground storage systems provide protection from these abiotic influences, and in addition supply inorganic and organic nutrients, promoting survival and possible regrowth.

Areas with widely different land-use practices, including agricultural, commercial, rural or residential, can contribute stormwater to environmental waters. The possibility also exists of cross-connections from sewer pipes, or leakage from sewer or septic systems delivering human sewage to the stormwater conveyance system. Both human health risk and strategies for remediation of microbial pollution from stormwater are influenced by the host source of microorganisms, but measurement of indicator bacteria alone does not provide information on this important parameter. Microbial source tracking (MST) is a group of methods whose goal is to define the source(s) of indicator bacteria. Such methods may be library-dependent; relying on a reference database of patterns, or fingerprints, of organisms from fecal material of known source (Wiggins, 1996; Hagedorn et al., 1999; Parveen et al., 1999; Dombek et al., 2000; Harwood et al., 2000; Moore et al., 2005). Library-independent methods do not require a database of patterns for comparison, but instead have a specific target which, when present, indicates fecal contamination from a particular source. The target could be a gene (Martellini et al., 2005), virus (Hsu et al., 1995; McQuaig et al., 2006) or a bacterium (Bernhard et al., 2003; Scott et al., 2005) associated with a specific host, and is frequently detected by a molecular method such as the polymerase chain reaction (PCR) (USEPA, 2005).

Two library-independent MST methods for detection of human-associated markers were used in this study to determine whether human sewage was impacting the stormwater system: the enterococcal surface protein (*esp*) gene of *Enterococcus faecium*, and the conserved T antigen of human polyomavirus strains JC and BK. The *Ent. faecium* strain(s) containing the *esp* gene is present throughout the US and other countries (Willems et al., 2001; Rice et al., 2003). At least two published studies detected the *esp* marker in 100% of sewage influent samples tested in the US (Scott et al., 2005; Soule et al., 2006). Furthermore, 100% of sewage samples tested in New Zealand ($n = 4$) were also positive (Harwood, unpublished data); and all the sewage samples representing greater than half of the states in the US have also tested positive (T. Scott, unpublished data). Human polyomaviruses are estimated to infect up to 80% of the human population, and are shed in urine and feces (Knowles et al., 2003; Behzad-Behbahani et al., 2004). In Florida, 36 sewage influent samples from three wastewater treatment facilities and 14 samples from different septic tanks were all positive for human polyomaviruses (McQuaig et al., 2006). Detection of *esp* and human polyomavirus markers were significantly correlated in Florida surface waters that were suspected of contamination from sewage (McQuaig et al., 2006). These markers were therefore considered good candidates for detection of human sewage contamination in this study, and were further confirmed during the study.

This study investigated the source of indicator bacteria contaminating waters at a Florida beach by combining library-independent MST methods (human-associated markers) and a MST tool previously used as a library-dependent method (BOX-PCR of indicator bacteria strains) to investigate the population structure of these bacteria under varying hydrological conditions. The goals of the study were threefold: (1) to determine whether the stormwater conveyance system contributed high indicator bacteria numbers to Gulf of Mexico waters; (2) determine whether human sewage was contributing to the indicator bacteria contamination and (3) determine whether survival in the stormwater conveyance system might contribute to elevated indicator bacteria levels. Characteristics of the *E. coli* and *Enterococcus* populations, including population diversity and population similarity, were used to explore the hypothesis that the microbial contamination at Siesta Key Beach originated from the stormwater system.

2. Materials and methods

2.1. Study site and sampling strategy

Siesta Key Beach is located on a barrier island on the west coast of Florida in Sarasota County. A stormwater conveyance system runs parallel to the beach underneath a paved thoroughfare (Fig. 1). The stormwater system receives runoff from an urban, residential area of approximately 0.24 km^2 (60 acres). The stormwater remains in the underground system, which runs southward to an underground concrete vault on the west side of the road, approximately 300 m from the beach. Water may be retained in the vault for many days until a rain event causes overflow, which is pumped into an adjacent retention pond. Surface runoff from the road and overflow from the pond enter a ditch, which flows ~300 m before it empties onto the beach. During heavy rain, the ditch outfall reaches the Gulf waters.

Two sampling events were conducted during this study; one within 48 h of heavy rainfall and one after a dry period (6 days of no precipitation). Water and sediment samples were taken at various points, i.e., access was obtained via a manhole to sample the stormpipe that feeds the vault, the vault was sampled through a metal-covered access portal, and the ditch and its beach outfall were sampled from the surface. The land around the ditch and the ditch itself was heavily vegetated, and therefore shaded, with Brazilian pepper trees and mangroves. More surface sampling sites were added (retention pond and Gulf of Mexico) for the second sampling (dry period) in order to obtain a more complete picture of the possible sources and sinks of microorganisms in the drainage system.

For genetic diversity studies, *Enterococcus* spp. were also isolated from sewage and a pristine water site. Untreated sewage samples were obtained from lift stations in the Florida counties of Duval and Wakulla. Water samples from a pristine site were collected at Deer Prairie Slough in the Myakka River, Myakka River State Park (Sarasota County; N Latitude 27°10.543′ and W Longitude 82°12.705′). This site was chosen due to the absence of known human impact and urban stormwater runoff.

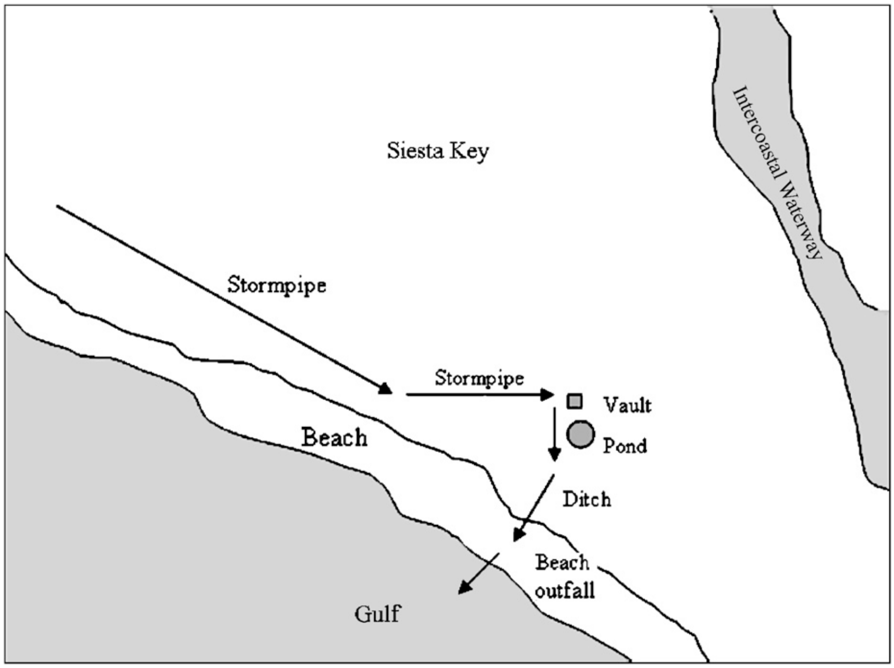

Fig. 1 – Sampling locations within the stormwater system draining to Siesta Key Beach.

2.2. Isolation and enumeration of indicator bacteria

Water and sediment samples were collected in sterile containers (in duplicate), immediately placed on ice and processed within 4 h of collection at the USF (Tampa, FL) laboratory. Water samples and supernatant from sonicated sediments were filtered through sterile nitrocellulose membranes (0.45 μm pore size, 47 mm diameter) using standard methods for fecal coliforms (APHA, 1998) and enterococci (USEPA, 2000). Twenty grams (wet weight) of sediment were added to 200 ml of sterile buffered water (0.0425 $g L^{-1}$ KH_2PO_4 and 0.4055 $g L^{-1}$ $MgCl_2$) and sonicated as modified from Anderson et al. (2005). The power was increased to 16 W for 30 s based on experiments that determined the highest recovery of indicator bacteria from contaminated sediments (data not shown).

Fecal coliforms were enumerated on mFC agar (Difco) and incubated for 24 h at 44.5 °C in a water bath (APHA, 1998). Blue colonies were counted as fecal coliforms and then inoculated into microtiter plates containing EC broth amended with 4-methylumbelliferyl-β-D-glucuronide (MUG) (50 μg ml^{-1}) in order to determine the percentage of the colonies that were *E. coli*. After incubation for 24 h at 37 °C, the microtiter plates were exposed to ultraviolet (UV) light. Fluorescence indicated strains that had β-glucuronidase activity (MUG+), a characteristic of *E. coli*. For further confirmation, 25% of the MUG+ isolates were profiled biochemically using API 20E strips (BioMerieux), and 100% were identified as *E. coli*. MUG+ fecal coliforms were therefore designated *E. coli* and fingerprinted by BOX-PCR for the similarity/diversity study.

Enterococci were enumerated by Method 1600 (USEPA, 2000), in which filters were incubated on mEI agar (base medium from Difco; indoxyl β-D-glucoside from Sigma Aldrich) at 41 °C for 24 h. All resultant colonies with a blue halo were inoculated into Enterococcosel Broth (Becton Dickinson) to confirm esculin hydrolyzation. Concentrations for all indicators were $\log_{10}$-transformed and recorded as CFU 100 ml^{-1} (water samples) or 100 g wet weight^{-1} (sediment samples).

2.3. Human-associated genetic markers

The method used for detection of an enterococcal surface protein (*esp*) gene of *Ent. faecium* was carried out as previously described (Scott et al., 2005). Briefly, 300 ml of each water sample was filtered using 0.45 μm pore-size membrane filters. Sediment samples were diluted 1:6 with sterile distilled water, vortexed for 2 min and allowed to stand for 2 min (Byappanahalli et al., 2003). The supernatant was filtered through sterile nitrocellulose membranes (0.45 μm pore size, 47 mm diameter). Filters were incubated on mEI agar at 41 °C for 48 h in a water bath. Filters were suspended in tryptic soy broth (Difco), vortexed and incubated for 3 h at 41 °C. Two milliliters of culture were used for DNA extraction, which was performed using a QIAamp DNA extraction kit (Qiagen, Inc.) according to manufacturer's instructions.

The forward primer (Scott et al., 2005), which is specific for the *Ent. faecium esp* gene (5′-TAT GAA AGC AAC AGC ACA AGT T-3′), and a conserved reverse primer (5′-ACG TCG AAA GTT CGA TTT CC-3′) (Hammerum and Jensen, 2002) were both previously published. PCR reactions were performed in a 50 μl reaction mixture containing 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each of the four deoxyribonucleotides, 0.3 μM of each primer, 2.5 U of HotStarTaq DNA polymerase (Qiagen, Inc.) and 5 μl of template DNA. Amplification consisted of an initial denaturation at 95 °C for 15 min (to activate Taq polymerase), followed by 35 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min. The amplicon (680 bps) was stained with GelStar nucleic acid stain (BioWhittaker) on a 1.5% agarose gel and viewed under UV light. The reliable presence of the *esp* marker in human sewage was determined in sewage samples collected throughout the State of Florida, including the study area (Sarasota County).

The method to detect human polyomaviruses (HPyVs) in water samples was carried out as previously described (McQuaig et al., 2006). Briefly, each water sample (600 ml) was adjusted to 9.5 pH using 1 M NaOH, and then prefiltered using a 47 mm filter (Millipore Cat. No. RW0304700). The filtrate was adjusted to 3.5 pH using 2.0 N HCl, and then filtered through a 0.45 μm pore size, 47 mm diameter nitrocellulose filter. The filter was placed into a 30 ml polypropylene tube with 2 ml of beef extract (pH 9.3) and vortexed for 30 s to elute viruses from filter. DNA was extracted from the resulting eluate using the QIAamp Blood Midi Kit (Qiagen, Inc., Valencia, CA). Previously published primers specific for the homologous T-antigen of both JC virus and BK virus were used to amplify HPyVs DNA (Fwd: 5′-AGT CTT TAG GGT CTT CTA CC-3′ and Rev: 5′-GGT GCC AAC CTA TGG AAC AG-3′) (Askamit, 1993). PCR reactions were prepared using 45 μl of Platinum® Blue PCR SuperMix (Invitrogen, Inc., Carlsbad, CA), 200 nM of each primer, and 4 μl of DNA template. The final reaction volume was adjusted to 50 μl using reagent-grade water (Whiley et al., 2004). The PCR reaction conditions were as follows: initial denaturation at 94 °C for 2 min, followed by 45 cycles of: 94 °C for 20 s, 55 °C for 20 s and 72 °C for 20 s, then a final elongation at 72 °C for 2 min. The nested PCR was run under the same reaction conditions as above, with 1 μl of the first reaction used as the template. PCR products were separated by agarose gel electrophoresis (1.5%). DNA was viewed using GelStar nucleic acid stain under UV light. Bands appearing at 172 bp were recorded as a positive PCR result. Throughout the course of the study, 17 sewage influent samples were tested for the presence of HPyVs, and all were positive.

Controls used for all PCR assays included method blanks (sterile buffer carried through the entire filtration/extraction/PCR procedure) and matrix spikes (water samples spiked with sewage to insure PCR performance, including lack of inhibition).

2.4. BOX-PCR of *E. coli* and *Enterococcus* spp.

E. coli strains were grown overnight in microcentrifuge tubes containing 750 μl of BHI broth (Becton Dickinson). After centrifugation at 14,000 RPM for 1 min, pellets were washed with sterile buffered water 2 times and resuspended in 500 μl of deionized sterile water. The cell suspension was boiled for 5 min to lyse the cells and then centrifuged again at 14,000 RPM for 1 min. Two μl of supernatant was used as template for each PCR reaction. BOX-PCR patterns (fingerprints) were generated using the previously published BOX-A1R primer (Koeuth et al., 1995), which has the following sequence: 5′-CTA CGG CAA GGC GAC GCT GAC G-3′. Reagents and volumes for each 25 μl reaction were: 2.5 μl 10X Buffer B (Fisher Scientific); 3.0 μl 25 mM $MgCl_2$; 1.0 μl 10 mM dNTPs (Fisher Scientific); 2.5 μl 2% bovine serum albumin (Sigma); 1.3 μl 10 μM BOXA1R primer (IDT, Coralville, IA); 1.0 μl Taq polymerase (5000 u ml^{-1}) (Fisher Scientific); and 11.7 μl PCR-grade water. Amplification contained three steps: (1) initial denaturation at 95 °C for 5 min; (2) 35 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min; and (3) final extension at 72 °C for 10 min. The preceding protocol was provided by correspondence with Dr. Cindy Nakatsu (2004), Purdue University, West Lafayette, IN.

Enterococci were grown overnight in microcentrifuge tubes containing 1.5 ml of BHI broth (Becton Dickinson). DNA was extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA) and the manufacturer's protocol for Gram-positive bacteria. BOX-PCR fingerprints for enterococci were generated using the BOXA2R primer (Koeuth et al., 1995), which has the following sequence: 5′-ACG TGG TTT GAA GAG ATT TTC G-3′. PCR reagents and conditions used were from previously published protocols with modifications (Versalovic et al., 1991; Malathum et al., 1998). Each 25 μl PCR reaction contained: 5 μl of 5 × Gitschier Buffer (Kogan et al., 1987); 2.5 μl of 10% dimethyl sulfoxide; 0.4 μl bovine serum albumin(10 mg ml^{-1}); 2.0 μl 10 mM dNTPs; 1.0 μl Taq polymerase (5000 u ml^{-1}); 11.6 μl PCR-grade water; 1.5 μl 10 μM BOXA2R primer; and 1.0 μl of DNA template, containing between 30 and 100 ng μl^{-1}. Amplification contained three steps: (1) initial denaturation at 95 °C for 7 min; (2) 35 cycles of 90 °C for 30 s, 40 °C for 1 min and 65 °C for 8 min; and (3) final extension at 65 °C for 16 min.

Fragments were separated by electrophoresis through a 1.5% agarose gel for 4 h at 90 V (*E. coli* fingerprints), or 6 h at 60 V (*Enterococcus* spp. fingerprints). Gels were stained with ethidium bromide (1% solution). Gels were digitally documented under UV light using a FOTO/Analyst Archiver (Fotodyne, Hartland, WI).

2.5. Statistical analysis

Fingerprint patterns of *E. coli* and *Enterococcus* spp. subtypes generated by BOX-PCR were analyzed with BioNumerics 4.0 software (Applied Maths, Belgium). Samples from which fewer than 10 isolates were recovered were not included in the analysis. Dendrograms were created using a densiometric curve-based algorithm (Pearson correlation coefficient, optimization 1%) and UPGMA to cluster patterns by similarity. Repeated runs of the control strains, ATCC 9637 for *E. coli* and ATCC 19433 (*E. faecalis*) for *Enterococcus* spp., were 86% and 93% similar, respectively. Therefore, as a first approximation, patterns showing ⩾ the similarity value established by the control strains were considered identical. The relationship of patterns considered similar was also visually confirmed.

The relationships among indicator bacteria populations isolated from the various sites were based on comparison of BOX-PCR genotypes. The relationships were visualized by dendrograms constructed using a population similarity coefficient (S_p), previously published by Kuhn et al. (1991). The algorithm ($S_p = (\sum qx_i/Nx + \sum qy_i/Ny)/2$) is based on the proportion of identical BOX-PCR patterns between two populations, *x* and *y*. For example, if two populations *x* and *y* have 10 isolates each and isolate ($i = 1$) in population *x* is repeated 2 times in population *x* and 3 times in population *y*, then the qx_1 would be 2/10 divided by 3/10. The qx_i is calculated for every isolate in population *x*, and then the $\sum qx_i$ is divided by the number (*N*) of isolates in population *x*. The same process is applied to every isolate in population *y*. Therefore, if two populations have no identical subtypes $S_p = 0$, and as the number of identical subtypes increases between two populations, the S_p increases to a maximum of 1.0 (Kuhn et al., 1991). The population similarity coefficient was used to compare *E. coli* and *Enterococcus* populations at Siesta Key after a rain event and during dry conditions, and to further compare *Enterococcus* populations at Siesta Key to *Enterococcus* populations in sewage and in a sampled site on Myakka River.

The population characteristics (assessed by BOX-PCR) were also assessed by accumulation curves and the Shannon–Weiner diversity index, which were calculated using EcoSim 7 software (Acquired Intelligence Inc. & Kesey-Bear, Jericho, VT). An accumulation curve measures the diversity of a sampled population (in this case, the number of unique BOX-PCR types) by plotting new subtypes as a function of sampling effort. As the curve approaches an asymptote (slope = 0), the probability of obtaining new subtypes with additional sampling diminishes. The Shannon–Weiner index of diversity, $(H') = -\Sigma p_i \ln(p_i)$, considers the frequency of the various subtypes in a population as well as the total number of subtypes; p_i being the number of isolates with pattern i divided by total number of isolates. H' was calculated with approximately the same number of isolates (19 or 20) per site population for Siesta Key, as this was the largest sample size that was common to all treatments. Data sets containing more isolates were randomly subsampled for inclusion in the diversity values. Both the accumulation curve and the Shannon–Weiner index were used to compare the relative diversities of *E. coli* and *Enterococcus* populations after a rain event and during dry conditions at Siesta Key, and to further compare *Enterococcus* populations at Siesta Key to *Enterococcus* populations in sewage and in a sampled site on Myakka River. Paired *t* tests, nonparametric tests (Mann-Whitney), and ANOVA were used to determine significant difference in the comparisons. GraphPad Prism version 4.02 (GraphPad Software, San Diego, CA) was used for the statistical analyses.

3. Results and discussion

3.1. *Indicator organism concentrations and human-associated markers*

The indicator bacterial concentrations and the absence/presence of the human-associated markers are summarized in Table 1A for samples collected after a rain event, and in Table 1B for samples collected during dry conditions. High levels of indicator bacteria in the stormwater drainage system, and stormwater flow during the rain event demonstrated the ability of the stormwater system to contaminate beach waters, and also raised the possibility of a sewage influence. Tests for human polyomaviruses and the enterococcal surface protein gene (*esp*) for *Ent. faecium*, which have previously been used to determine the presence of human sewage in environmental waters (Scott et al., 2005; McDonald et al., 2006; McQuaig et al., 2006) were carried out at all sites. All tests for human-associated markers were negative (Table 1A and B). These human-associated markers are very reliably present in human sewage in Florida and in all other geographic areas in which the markers have been tested, which suggests that human sewage input was not involved in the contamination. Furthermore, the wastewater collection system was examined for connections or leaks into the stormwater system by the Siesta Key Utility Authority several months prior to this study, and no connections were found.

While the negative results for human-associated suggest the absence of sewage contamination in the stormwater system, other factors can influence these results. In particular, little is known about the inactivation rates of most of the bacteria and viruses used in MST studies (Stoeckel and Harwood, 2007). The survival of *Ent. faecium* C68, which carries the *esp* gene, was assessed in the laboratory and was found to be 9 days in simulated freshwater and 10 days in simulated seawater (Scott et al., 2005). HPyVs in sewage could be detected by PCR in laboratory mesocosms for over 3 months (Bofill-Mas et al., 2001). These data should be interpreted in light of the knowledge that many factors influence the inactivation rates of microorganisms in aquatic environments that cannot be adequately simulated in the laboratory (Anderson et al., 2005); environmental stresses such as radiation, temperature variations, salinity and predators are among the factors that influence persistence outside the host. As MST methods continue to mature and the organisms are better characterized, this will be an important point to address. Chemical methods for MST, such as fluorometry, can provide additional or corroborating

Table 1A – Sites sampled at Siesta Key Beach after a rain event (A) and during dry conditions (B)

	Stormpipe water	Vault water	Ditch water	Ditch sediment	Standing water on beach	Beach sediment
Fecal coliform concentration	2.12	3.55[a]	3.72[a]	3.24	3.29[a]	3.64
Enterococci concentration	4.01[a]	4.23[a]	4.72[a]	4.37	3.86[a]	3.63
esp gene of *Ent. Faecium*	—	—	—	—	—	—
Human polyomaviruses	—	—	—	N/A	—	N/A
BOX-PCR *E. coli*	■	■	■	■	■	■
BOX-PCR *Enterococcus* spp.	■	■	■	■	■	■

Concentrations for all indicators were $\log_{10}$-transformed and recorded as CFU 100 ml^{-1} (water samples) or 100 g wet weight^{-1} (sediment samples). Human-associated markers are recorded as present/absent (+/–), and BOX-PCR analyses were conducted (■), or not conducted (□).

[a] Concentrations exceeded state regulatory standards. The Florida Department of Health, which monitors the beaches of Florida, considers a water sample of 100 ml containing ⩾104 *Enterococcus* spp. and/or⩾400 fecal coliforms an indicator of poor water quality (http://esetappsdoh.doh.state.fl.us/irm00beachwater/terms.htm). N/A, sediments were not tested for human polyomaviruses.

Table 1B

	Stormpipe water	Stormpipe sediment	Vault water	Pond water	Pond sediment	Ditch water	Ditch sediment	Standing water on beach	Beach sediment	Gulf water	Gulf sediment
Fecal coliform concentration	0.70	3.85	1.37	0.40	1.30	1.36	1.60	2.86[a]	1.74	1.62	0.0
Enterococci concentration	3.05[a]	4.44	2.31[a]	0.81	2.97	1.71	4.29	3.16[a]	2.36	0.98	2.0
esp gene of *Ent. Faecium*	—	—	—	—	—	—	—	—	—	—	—
Human polyomaviruses	—	N/A	—	—	N/A	—	N/A	—	N/A	—	N/A
BOX-PCR *E. coli*	□	■	■	□	□	■	□	■	□	■	□
BOX-PCR *Enterococcus* spp.	■	■	■	□	□	■	□	■	□	■	□

□ Fewer than 10 isolates recovered.
[a] Concentrations exceeded state regulatory standards. The Florida Department of Health, which monitors the beaches of Florida, considers a water sample of 100 ml containing ≥104 *Enterococcus* spp. and/or ≥400 fecal coliforms an indicator of poor water quality (http://esetappsdoh.doh.state.fl.us/irm00beachwater/terms.htm). N/A, sediments were not tested for human polyomaviruses.

evidence of human sewage (or the lack thereof) in stormwater systems and other environmental waters (McDonald et al., 2006). Because MST markers targeting only human sewage were used here, the results do not point toward a specific source of contamination (only away from one). This limitation of library-independent methods can be addressed by the use of multiple markers; however, reliable markers are now available for only a handful of target species, e.g., human, ruminant, horse and pig (Bernhard and Field, 2000; Dick et al., 2005; Scott et al., 2005; Layton et al., 2006; McQuaig et al., 2006).

All water samples collected after the rain event had fecal coliforms and *Enterococcus* concentrations above the regulatory level for recreational waters, with the exception of fecal coliforms in the stormpipe water (Table 1A). *Enterococcus* spp. concentrations were significantly higher than fecal coliforms ($P = 0.041$, paired t test). The mean concentrations ($\log_{10}$-transformed) were 3.17 ± 0.72 and 4.20 ± 0.37 for fecal coliforms and *Enterococcus* spp., respectively. Indicator bacterial levels were also high in sediments collected during the rain event, at $>10^3$ CFU 100 g^{-1}, although there are no regulatory standards for indicator bacteria concentrations in sediment.

During dry conditions (Table 1B), *Enterococcus* spp. concentrations also exceeded regulatory levels in the stormwater conveyance system (stormpipe and vault), which provided protection from such stressors as sunlight and high temperatures. Only the standing water on beach, where water pools and does not reach the Gulf, was in violation for fecal coliform concentrations (as well as *Enterococcus* spp.). The beach area may have been impacted by another source of indicator bacteria such as seagulls, which were observed flocking to the standing water on the beach. Indicator bacteria concentrations remained high in sediment samples during dry conditions; e.g., levels in stormpipe sediment were highest at $>10^{3.5}$ CFU 100 g^{-1} for both fecal coliforms and *Enterococcus* spp. *Enterococcus* spp. concentrations in sediments during dry conditions were significantly higher than fecal coliform concentrations (paired t test, $P = 0.020$). The mean concentrations ($\log_{10}$-transformed) were 1.70 ± 1.38 and 3.21 ± 1.11 for fecal coliforms and *Enterococcus* spp., respectively. The fact that indicator bacteria concentrations remained high in sediments during dry conditions when the overlaying water column (retention pond, ditch and Gulf) had concentrations below regulatory standards supports previous reports in the literature (Craig et al., 2002; Anderson et al., 2005; Ferguson et al., 2005), and indicates that the sediments act as a reservoir for these organisms.

Indicator bacteria concentrations in water samples at sites sampled in rainy and dry conditions (i.e., stormpipe water, vault water, ditch water and beach water) were compared by calculating the mean of $\log_{10}$-transformed concentrations at all sites. In general, indicator bacteria concentrations were significantly higher during the rain event than during dry conditions. The mean fecal coliform concentration during the rain event was 3.17 CFU 100 ml^{-1}, while it was 1.57 CFU 100 ml^{-1} under dry conditions. Corresponding means for *Enterococcus* spp. were 4.20 and 2.55 CFU 100 ml^{-1}. The difference in mean concentrations for rainy versus dry conditions was statistically significant for *Enterococcus* spp.

($P = 0.028$) and nearly significant for fecal coliforms ($P = 0.057$) at the $\alpha = 0.05$ level.

3.2. Population diversity

BOX-PCR patterns of *E. coli* and *Enterococcus* spp. isolated during the rain event were compared to those isolated during the dry sampling in order to determine whether the population structure was influenced by these conditions. The two methods used to estimate indicator bacteria population diversity were: (1) the Shannon–Weiner index, which has been previously used to measure microbial population diversity in habitats such as rhizospheres, artesian spring sediments and microbial mats (McCaig et al., 1999; Nubel et al., 1999; Elshahed et al., 2003); and (2) the accumulation curve, used to estimate diversity in animal populations (Bohannan and Hughes, 2003) and bacterial populations (Anderson et al., 2006).

Accumulation curves for vault water samples (Fig. 2) illustrate a tendency toward greater diversity during rainy conditions for both *E. coli* and *Enterococcus* populations. The Shannon–Weiner index of diversity corroborated these data, showing significantly greater diversity in *E. coli* and *Enterococcus* populations in the rain event versus dry conditions (Table 2). The increased population diversity during the rain event suggests that higher diversity is due to recent inputs into the stormwater system, undoubtedly from multiple sources.

The diversity of *Enterococcus* populations during the rain event and during dry conditions was compared to the diversity of *Enterococcus* populations sampled from sewage and from water samples collected from the Myakka River, considered to be a relatively unimpacted site with no direct human or agricultural input, or urban runoff (Fig. 3). Averaged accumulation curves were constructed for *Enterococcus* populations for the rain event ($n = 4$ sites), dry conditions ($n = 4$ sites), sewage samples ($n = 6$) and samples collected at Myakka River ($n = 3$). Similar diversity levels were observed in Siesta Key *Enterococcus* populations during the rain event and *Enterococcus* populations in sewage samples. In contrast, significantly lower diversity was found in *Enterococcus* populations during dry conditions and at Myakka River (unimpacted site) (Fig. 3). Previous studies have shown that *Enterococcus* populations in domestic sewage display higher diversity than those sampled from river water (Vilanova et al., 2002), and animal feces (Manero et al., 2002; Kuhn et al., 2003). The Shannon–Weiner index for these four groups reveals a significant difference in the diversity levels of Siesta Key rain event and sewage populations versus Siesta Key dry conditions and Myakka River populations (Table 3). These results suggest that stormwater can influence the diversity of indicator bacteria populations in waters it impacts to approach that of sewage input. Interestingly, the *Enterococcus* population from sewage shares very few subtypes with that of Siesta Key or Myakka River (see Section 3.3).

Table 2 – Comparison of the population diversity (Shannon-Weiner index, H') of *E. coli* and *Enterococcus* spp. during the rain event versus dry conditions

Indicator organism	Mean H'	P value
E. coli[a]	Rain event = 2.39 ± 0.22 Dry conditions = 1.12 ± 0.34	$P = 0.047$
Enterococcus spp.[b]	Rain event = 2.65 ± 0.13 Dry conditions = 1.88 ± 0.28	$P = 0.008$

[a] Site populations averaged for *E. coli*: beach water, ditch water and vault water.
[b] Site populations averaged for *Enterococcus* spp.: beach water, ditch water, stormpipe water and vault water.

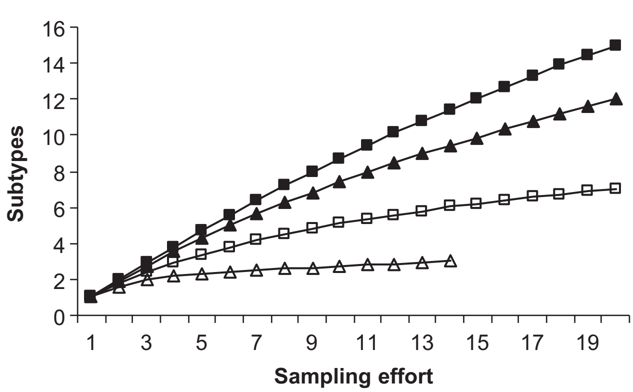

Fig. 2 – Accumulation curves of BOX-PCR fingerprint patterns of indicator bacteria from vault water samples. *E. coli* sampled after a rain event (▲) and during dry conditions (△); *Enterococcus* spp. sampled after a rain event (■) and during dry conditions (□).

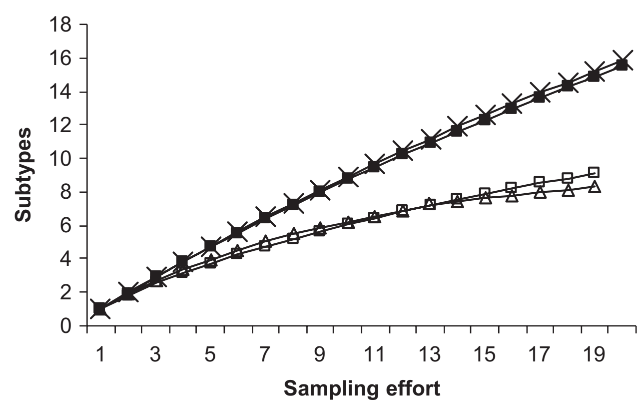

Fig. 3 – Accumulation curves based on averaged richness estimates for *Enterococcus* spp. sampled at Siesta Key sites after a rain event (■), at Siesta Key sites during dry conditions (□), from wastewater influent (x) and from Myakka River water (△).

3.3. Population similarity

The population similarity of indicator bacteria (*E. coli* or *Enterococcus* spp.) was compared by site for the rain event and dry conditions. A similarity coefficient was used to express the proportion of identical BOX-PCR subtypes sampled from the various sites (Kuhn et al., 1991). This strategy has been used to compare phenotypic subtypes of coliforms in environmental water samples (Kuhn et al., 1991), and phenotypic subtypes of fecal coliforms and enterococci in sewage (Manero et al., 2002; Vilanova et al., 2002, 2004), and in the feces of livestock, seabirds and dogs (Kuhn et al., 2003; Wallis and Taylor, 2003).

Higher similarity between sites was observed for *E. coli* populations sampled during dry conditions compared to the rain event (Fig. 4). Higher similarity also existed between sites for *Enterococcus* populations during dry conditions (Fig. 5), but the difference was less pronounced than for the *E. coli* populations. Increased population similarity for *E. coli* and *Enterococcus* spp. during dry conditions suggests that a greater portion of the population is composed of "survivor" isolates (Anderson et al., 2005) that have survived under the selective pressure of this secondary habitat. Interestingly, the increased population similarity for both *E. coli* and *Enterococcus* spp. under dry conditions was not observed in Gulf waters. A possible explanation is that under dry conditions, the standing water on the beach did not reach the Gulf, demonstrating the physical and microbiological disconnect between the stormwater drainage system and the Gulf, which contrasts with the direct stormwater flow observed during the rain event.

BOX-PCR patterns of enterococci from all stormwater sites used for the above analysis were pooled for a comparison of population similarity between rainy conditions and dry conditions, as well as sewage populations (positive control for human contamination) and Myakka River populations (control for unimpacted water) (Fig. 6). The two *Enterococcus* populations with the highest similarity were Myakka River and Siesta Key (dry), and the population with the least similarity to all other groups was sewage. Thus, the populations in the environments with relatively low levels of recent inputs of indicator bacteria (Myakka River and Siesta Key Dry) contain more identical *Enterococcus* genotypes, suggesting that survival in this secondary habitat is a form of selective pressure.

Even though human sewage input was not detected at Siesta Key Beach, one cannot rule out risk to human health from contact with the water. The health risks associated with exposure to recreational waters impacted by stormwater runoff have not been as well studied as the risks associated with sewage-impacted waters. One study found that respiratory and gastrointestinal symptoms increased as the distance decreased between swimmers and a stormwater outlet in Santa Monica Bay, CA (Haile et al., 1999). During an El Nino year, surfers in Orange County, CA reported twice as many symptoms as surfers in Santa Cruz County, considered to be less impacted by urban runoff (Dwight et al., 2004). These studies suggest that adverse heath outcomes are associated with stormwater impact of recreational waters.

Table 3 – Comparison of the population diversity (Shannon–Weiner index, H') of *Enterococcus* spp. in sewage, Myakka River water and Siesta Key water during rain event and dry conditions

Site	Mean H'
Sewage	2.69 ± 0.09 (a)
Myakka	1.96 ± 0.22 (b)
Siesta key (dry)	1.88 ± 0.28 (b)
Siesta key (rain)	2.65 ± 0.13 (a)

Values that share the same letter within columns are not significantly different. ANOVA ($P = 0.0001$, $\alpha = 0.05$).

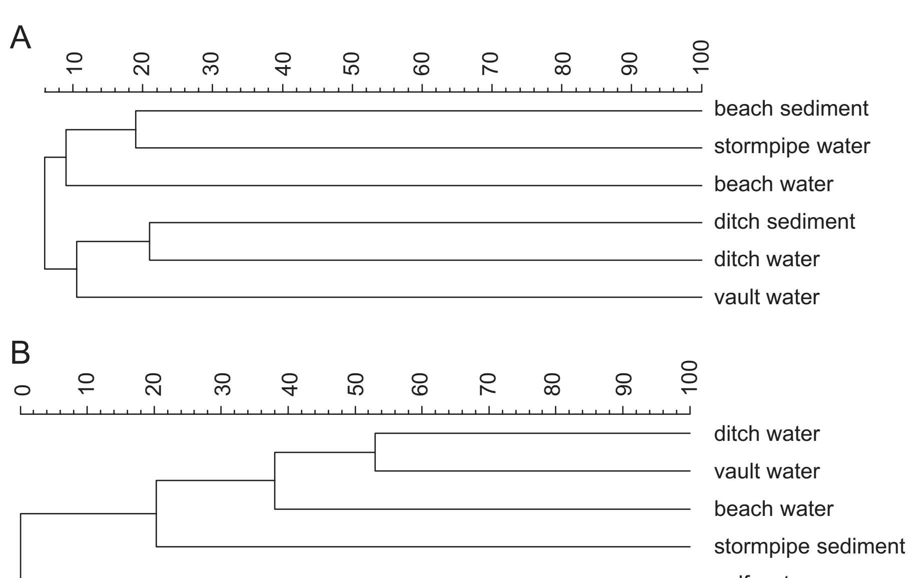

Fig. 4 – Similarity of *E. coli* populations by site at Siesta Key during the rain event (A), and during dry conditions (B), based on BOX-PCR fingerprint patterns.

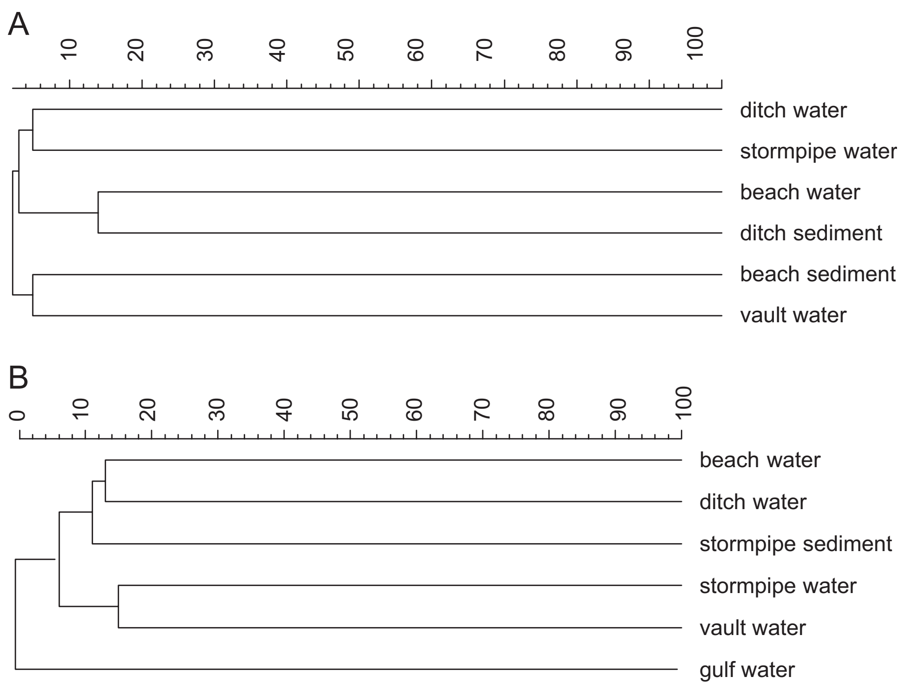

Fig. 5 – Similarity of *Enterococcus* populations by site at Siesta Key during the rain event (A), and during dry conditions (B), based on BOX-PCR fingerprint patterns.

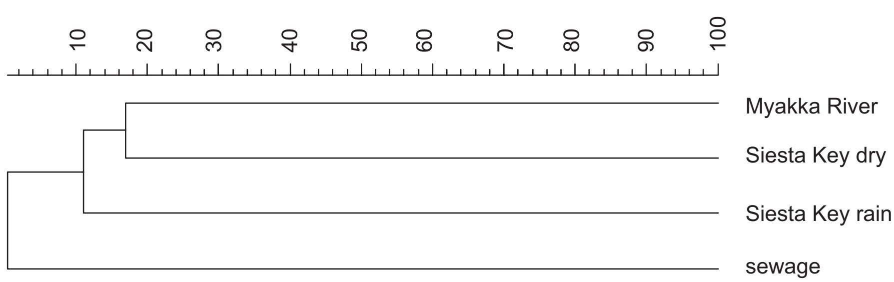

Fig. 6 – Similarity of *Enterococcus* populations sampled at Siesta Key during the rain event and dry conditions, compared to populations from sewage and from Myakka River, based on BOX-PCR fingerprint patterns.

The population dynamics of indicator bacteria in the storm drainage system at Siesta Key Beach are evidently affected by the hydrological changes caused by rain events. A change in bacterial concentrations and diversity, as well as similarity, of the populations extending from the stormpipe to the Gulf was observed. The transport of urban runoff collecting for days in the stormpipe and vault, and the persistence of survivor isolates in the sediments, suggests a reservoir for indicator bacteria that can be flushed through the system to the Gulf during a rain event, causing high levels of indicator bacteria. The design of the stormwater conveyance system, in which sediments collect during low-flow conditions, ultimately contributes to contamination of receiving waters under high-flow conditions, when collected material is flushed through the system. These observations suggest that better handling and treatment of stormwater is needed, particularly when it impacts areas that receive heavy human use. Such environmental reservoirs of indicator bacteria further complicate the already questionable relationship between indicator organisms and human pathogens (Byappanahalli, 1998; Anderson et al., 2005), and call for a better understanding of the ecology, fate and persistence of indicator bacteria in water.

4. Conclusions

Although indicator bacteria levels during the rain event were above state standards for recreational waters, no evidence of human fecal contamination was found by library-independent MST methods. The persistent high bacterial concentrations in stormwater sediment samples during dry conditions suggest that sediments are a reservoir of these organisms. During high flow (rainy) conditions indicator bacteria diversity was high and population similarity was low, suggesting fresh inputs of bacteria from many sources. These observations contrasted with results from dry conditions, where indicator bacteria diversity was lower and population similarity was higher, suggesting that a group of “survivor” strains may form a significant portion of the population when fresh inputs of indicator bacteria are minimal. The combination of ecological and hydrological approaches with MST methods allowed assessment of the source of *E. coli* and *Enterococcus* populations, where the library-independent methods alone would not have been sufficient.

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SWIMMING-ASSOCIATED GASTROENTERITIS AND WATER QUALITY[1]

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Cabelli, V. J. (Dept. of Microbiology, U. of Rhode Island, Kingston, RI 02881), A. P. Dufour, L. J. McCabe and M. A. Levin. Swimming-associated gastroenteritis and water quality. *Am J Epidemiol* 1982;115:606-16.

A direct, linear relationship between swimming-associated gastrointestinal illness and the quality of the bathing water was obtained from a multi-year, multiple-location prospective epidemiologic-microbiologic research program conducted in New York City, 1973–1975, Lake Pontchartrain, Louisiana, 1977–1978, and Boston, Massachusetts, 1978. Several microbial indicators were used in attempting to define the quality of the water; and, of those examined, enterococci showed the best correlation to total and "highly credible" gastrointestinal symptoms. The frequency of gastrointestinal symptoms also had a high degree of association with distance from known sources of municipal wastewater. A striking feature of the relationship was the very low enterococcus and *Escherichia coli* densities in the water (10/100 ml) associated with appreciable attack rates (about 10/1000 persons) for "highly credible" gastrointestinal symptoms. Moreover, the ratio of the swimmer to nonswimmer symptom rates indicated that swimming in even marginally polluted marine bathing water is a significant route of transmission for the observed gastroenteritis.

gastroenteritis; swimming; water microbiology

In an earlier report (1), the authors presented evidence from a prospective epidemiologic-microbiologic study that there are measurable health effects associated with swimming in sewage-polluted waters. In some cases, these effects were observed even in waters that were in compliance with existing recreational water quality guidelines and standards (2). The swimming-associated illness observed was an acute, relatively benign gastroenteritis which had a short incubation period and duration. The accompanying symptoms, as pointed out in another report (3), suggested that the etiologic agent might be the human rotaviruses or Norwalk-like viruses. The water-related nature of one of these agents, the Norwalk-like virus, recently has been confirmed in a shellfish-associated outbreak of gastroenteritis in Australia of some 2000 cases (4) and in several outbreaks associated with drinking water (5).

The objective of the overall research program was to determine if there are illnesses associated with swimming in sewage-polluted water and, if so, whether their rates can be quantitatively related to some measure of the quality of the bathing water. This question has been the subject of controversy since the 1950s

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when Stevenson (6) and Moore (7) obtained seemingly contradictory results. Therefore, studies similar to those reported for New York City beaches (8, 9) were conducted at two other locations in the United States: Lake Pontchartrain, Louisiana and Boston, Massachusetts. The results obtained at these two sites were essentially the same as those found in the New York City study.

This report describes the quantitative relationship of the swimming-associated gastroenteritis to the mean enterococcus density of the water as obtained from all the epidemiologic-microbiologic studies conducted in the United States.

MATERIALS AND METHODS

Study sites. Studies were conducted at three general locations: New York City (beaches on Coney Island and the Rockaways) in 1973–1975; Lake Pontchartrain, Louisiana (Levee Beach and Fontainbleau Beach) in 1977 and 1978; and Boston, Massachusetts (Revere Beach and Nahant Beach) in 1978. The beaches were chosen because they were near large metropolitan areas and, hence, used by large numbers of individuals who swam on weekends but not during midweek days. This was an essential requirement of the experimental design for reasons to be given.

The sources of pollution reaching the beaches in the New York City study were reasonably well defined as those emerging from the mouth of the Hudson River. They were less defined in the Boston study, and least defined in the Lake Pontchartrain study. Moreover, in order to determine which, if any, symptoms or groups of symptons were both swimming-associated and pollution-related (a major objective of the first two years of the New York City study), a relatively unpolluted beach at the Rockaways was paired with a barely acceptable beach on Coney Island. The latter was adjacent to a beach area classified by local authorities as unsafe for swimming. The paired beaches were also chosen so that they would have demographically similar populations. The results obtained with reference to this objective of the New York City study have been published (1).

Study design. A prospective cohort design was used in all the studies. The essential features of the design, which have been described previously (1, 9, 10), are as follows:

1. Discrete trials were conducted only on Saturdays and Sundays. Potential participants were recruited at the beach, preferably as family groups. Trials were limited to weekend days to maximize the size of the beachgoing population, especially the portion that comes to the beach only on weekends. By excluding from the study those individuals who swam in the five midweek days before and after the weekend in question or at other locations on either weekend day, exposure to bathing water was limited to that at the specific beach during a single day, or two days at the most. This decreased the confounding effect of beach-to-beach and day-to-day variability in pollution levels on the illness-pollution relationships sought. In addition, it allowed the analyses of the data by trials (study days) or by groups of trials when the pollution levels as indicated by the mean indicator densities in the water were similar.

2. Demographic information was obtained at the initial beach interview and during the subsequent telephone follow-up survey. The information included age, sex, ethnicity and socioeconomic status, as determined from a persons-to-rooms ratio.

3. Information on bathing activity was obtained at the initial beach interview. Swimming was stringently defined as complete exposure of the head to the water. This characteristic was determined by direct inquiry and by observation, i.e., whether or not the hair of the subject was wet. Individuals who did not

immerse their heads in the water were considered nonswimmers. The validity of the respondents' information on bathing activity was evaluated at the New York City beaches in 1972 by comparing their responses to information collected by teams of observers. Good agreement was obtained with regard to immersion of the head in the water, but not the duration of immersion or even the time spent in the water with the head not necessarily immersed. Recruitment of the participants at the beach as family groups and their designation as swimmers and nonswimmers provided a nonswimming but beach-going control (cohort) population which, in general, came from the same family groups as the swimmers.

4. One or two days after the initial contact, participating families were sent a reminder letter asking them to note any illnesses that might occur in the week following the beach activity. In the first year (1973) (pretest) of the New York City study, a telephone number was provided so that follow-up medical or laboratory examinations could be obtained by individuals with subsequent illnesses. Very few individuals called the number, and this approach toward confirming the reported symptomatology was abandoned. A substitute for the approach is described below.

5. Eight to 10 days after the initial beach interview, the participants were contacted by telephone and information on symptomatology and demographic characteristics was obtained. Participants were questioned about gastrointestinal, respiratory, "other" and disabling symptomatology. The categorized symptoms are shown in table 1. Follow-up inquiries for information on a variety of symptoms were consistent with another requirement of the experimental design, that no prejudgment would be made as to which are the "important" illnesses, diseases or symptoms. In fact, only gastrointestinal symptoms (vomiting, diarrhea, nausea or stomachache) were consistently both swimming-associated and pollution-related (1, 9). Therefore, only gastrointestinal symptomatology was examined relative to the indicator densities in the bathing water. Disability was estimated by asking whether the respondents remained home, remained in bed or sought medical advice. Hospitalization was not reported by any of the subjects, and the observation period was too short to identify illness with long incubation periods.

TABLE 1

Symptoms for which queries were made in studies of the relationship between illnesses and swimming in sewage-polluted waters in New York City, 1973–1975, Lake Pontchartrain, Louisiana, 1977–1978 and Boston, Massachusetts, 1978

Gastrointestinal
Vomiting
Diarrhea
Stomachache
Nausea
Respiratory
Sore throat
Bad cough
Chest cold
Runny or stuffy nose
Earache or runny ears
Sneezing, wheezing, tightness in chest
"Other"
Fever (over 37 78 C)
Headache (more than a few hours)
Backache
Skin rash, itchy skin, welts
Disabling
Home because of symptoms
In bed because of symptoms
Medical help because of symptoms

6. As an alternative to follow-up medical and laboratory examinations, the credibility of the information obtained on gastrointestinal symptomatology was confirmed by comparing the rates and trends for total gastrointestinal symptoms to those in a subset designated "highly credible." Highly credible gastrointestinal symptoms included all cases of vomiting, instances of diarrhea that were accompanied by a fever or that were disabling, or cases of nausea or stomachache that were accompanied by a fever. Henceforth, highly credible gastrointes-

tinal symptoms and gastroenteritis will be used synonomously.

Water quality monitoring. Water samples were collected periodically on trial days during the time of maximum swimming activity at the beaches. This was generally between the hours of 11 a.m. and 5 p.m. Usually, three to four samples were collected at two or three sites from each beach at chest depth approximately four inches below the surface of the water. Upon collection, the samples were iced and delivered to the laboratory, where they were assayed within eight hours of collection. Potential water quality indicators that were examined are shown in table 2. Total coliform and fecal coliform densities were obtained using the most probable number or membrane filter methods, as described (11). The densities of total coliforms and the component genera of that group (*Escherichia, Klebsiella, Citrobacter-Enterobacter*) were also measured using the membrane filter procedure for coliforms (mC) of Dufour and Cabelli (12). After 1974, *Escherichia coli* densities were determined by the membrane filter method for thermotolerant *E. coli* (mTEC) (13). Enterococci (14), *Pseudomonas aeruginosa* (15), *Aeromonas hydrophila* (16), *Clostridium perfringens* (17) and *Vibrio parahemolyticus* (18) were assayed using membrane filter methods.

TABLE 2

Potential water quality indicators used at the New York City, Lake Pontchartrain, Louisiana and Boston, Massachusetts beaches, 1973–1978

Indicators	New York City	Lake Pontchartrain	Boston
Enterococci	+*	+	+
Escherichia coli	+	+	+
Klebsiella sp	+	+	
Enterobacter sp	+		
Citrobacter sp.	+		
Total coliforms	+		
Clostridium perfringens	+		
Pseudomonas aeruginosa	+	+	
Fecal coliforms	+	+	
Aeromonas hydrophila	+		
Vibrio parahemolyticus	+		

* + shows that measurements were made for this indicator at the specified location

Analysis. The relationship of swimming-associated (swimmer minus nonswimmer) gastrointestinal symptom rates to the mean indicator densities in the water was examined by regression analysis. Because the participants were recruited at the beach on weekends and individuals who were swimming in the midweeks before and after the one in question were eliminated from the study, the symptom rates for a given weekend day (trial) and the associated mean indicator density could have been analyzed as a point on the regression line. In fact, this was not possible with most of the trials because the number of nonswimming participants was too small. This problem was circumvented by grouping the trials. Single-day trials were arrayed according to increasing indicator densities. Groups were selected by utilizing "natural breaks" in the array; in this way, those trial days with similar indicator densities formed a group of data from which a geometric mean density and the associated rates for gastrointestinal and highly credible gastrointestinal symptoms could be calculated. This arbitrary grouping of trials was done for each of the indicator organisms.

The attack rates for gastrointestinal and highly credible gastrointestinal symptoms were regressed against the mean indicator density. The log-linear regression equation

$$Y = a + b \log X \qquad (1)$$

was used in which X was the mean indicator density and Y the gastrointestinal symptom rate.

RESULTS

Studies were conducted over several years at three locations in the United States. The locations, beaches, study years, follow-up percentages and number of usable responses are shown in table 3.

TABLE 3

Location of beaches and the number of usable responses by beach and study year, 1973–1978

Location	Beaches	% Follow-up during study year			No. of usable responses during study year		
		1	2	3	1	2	3
New York City*	Coney Island	82.3	78 3	78.3	641	3146	6491
	Rockaways	86.6	82 9		681	4923	
Lake Pontchartrain, LA†	Levee	77.2	77 9		3432	2768	
	Fontainbleau		—§			551	
Boston, MA‡	Revere	81.2			1824		
	Nahant	81.2			2229		

* Coney Island, 1973–1975, Rockaways, 1973–1974.
† Levee, 1977–1978, Fontainbleau, 1978.
‡ Revere, 1978; Nahant, 1978.
§ Included with Levee Beach.

The degree of association of the mean indicator densities to swimming-associated gastrointestinal symptoms in the three years of the New York City study was used to reduce the number of indicators examined in subsequent studies. The correlation coefficients for the various indicators obtained from these regression analyses are shown in table 4. It can be seen that enterococci was the best indicator of those examined. Equally important, fecal coliform densities, the basis for most federal and state guidelines and standards (2), correlated very poorly with swimming-associated gastrointestinal symptoms.

The rates for total and highly credible gastrointestinal symptoms among swimmers and nonswimmers and the residuals (swimmer minus nonswimmer rates) for the grouped trials are given in table 5. Also included are the corresponding means and ranges of the enterococcus densities and the number of trials (days) in each group. Similar data for *E. coli* are given in table 6 by way of contrast. In a number of instances, the swimmer and nonswimmer rates were significantly different from each other. This was more frequent for residual rates associated with high enterococcus densities and with total gastrointestinal symptoms.

The regression lines obtained from the data given in tables 5 and 6 are shown in figure 1 along with their correlation coefficients (r). In addition to having higher r values, the enterococcus regression lines differ from those for *E. coli* in two other ways. The *E. coli* lines have shallower slopes and intercept the X axis at much lower densities. However, in the regression lines for both indicators, rather low densities are associated with appreciable attack rates. Attack rates for highly credible gastrointestinal symptoms of about

TABLE 4

Correlation coefficients for total gastrointestinal (GI) symptoms and the "highly credible" gastrointestinal (HCGI) portion against the mean indicator densities for studies at New York City beaches, 1973–1975

Indicator	Correlation coefficients (r)		No. of points
	HCGI	GI	
Enterococci	0.96	0.81	9
Escherichia coli	0.58	0.51	9
Klebsiella	0.61	0.47	11
Enterobacter-Citrobacter	0.64	0 54	13
Total coliforms	0.65	0.46	11
*Clostridium perfringens**	0.01	−0.36	8
Pseudomonas aeruginosa	0.59	0.35	11
Fecal coliforms	0 51	0.36	12
Aeromonas hydrophila	0.60	0.27	11
*Vibrio parahemolyticus**	0.42	0.05	7

* No data for 1973.

TABLE 5

Summary of the mean enterococcus density—gastrointestinal (GI) symptom rate relationships among swimmers, nonswimmers and residuals obtained from clustered trials in studies on swimming-associated gastrointestinal illness, 1973–1978

Study	Beach	Year	Enterococcus density/100 ml		Trials (days) clustered	No of swimmers	No of non-swimmers	Symptom rates in cases/1000					
								Total GI			Highly credible GI		
			Mean	Range				Swimmers	Non-swimmers	Residuals	Swimmers	Non-swimmers	Residuals
New York City	Rockaways	1973†	21.8	1.2–59	8	484	197	81	46	35	30.4	15.2	15.2
	Coney Island		91.2	6–186	8	474	167	72	24	48*	46.4	18.0	28.4
		1974	3.6	2–5	3	1391	711	27	23	4	7 6	4.2	3 4
			7 0	7	3	951	1009	38	34	4	10 5	6 9	3.6
			13.5	10–17	2	625	419	42	17	25*	16.0	2.4	13.6
			31 5	30–33	2	831	440	43	23	20	18 1		18.1*
		1975	5 7	2–11	14	2232	935	63	55	8	18 8	19 3	−0.5
			20.3	14–38	10	1896	678	59	37	22*	14 8	7.4	7.4
			154	86–298	4	579	191	60	31	29	34 5		34.5*
Lake Pont-chartrain, LA	Levee	1977	44	9 7–88	8	874	451	86	51	35*	32 0	11.1	20.9*
			224	190–249	4	720	456	108	50	58**	31.9	8.8	23 1*
			495	344–711	2	895	464	108	54	54**	35 8	8.6	27.2**
	Levee	1978	11.1	3–30	8	1230	415	75	34	41**	36 6	14.5	22 1*
	Fontainbleau		14.4	3–33	5	248	303	81	63	18	44.3	23 1	21.2
	Levee		142	67–303	4	801	322	112	50	62**	42 4	15 5	26.9*
Boston, MA	Revere	1978	4.3	2–6	3	697	529	83	66	17	23.0	11.0	12.0
	Nahant		7.3	6–9	2	1130	1099	71	67	4	33.0	28.0	5 0
	Revere		12 0	12	1	222	376	108	74	34*	41 0	13.0	28.0*

* $p < 0.05$, ** $p < 0.01$.

† Study population too small to cluster trials by similar indicator densities

TABLE 6

Summary of the mean E. coli density—gastrointestinal (GI) symptom rate relationships among swimmers, nonswimmers and residuals obtained from clustered trials in studies on swimming-associated gastrointestinal illness, 1973–1978

Study	Beach	Year	*E. coli* density/100 ml		Trials (days) clustered	No of swimmers	No of non-swimmers	Symptom rates in cases/1000: Total GI			Highly credible GI		
			Mean	Range				Swimmers	Non-swimmers	Residuals	Swimmers	Non-swimmers	Residuals
New York City	Rockaways	1973†	24.8	3–34	8	484	197	81	46	35	30.4	15.2	15.2
	Coney Island		174	50–708	8	474	167	72	24	48*	46.4	18.0	28.4
		1974	2.2	1–4	6	2514	1641	25	34	−9	8.0	3.7	4.3
			13.3	9–19	4	1304	1045	38	29	9	14.1	5.7	8.4*
			30.5	26–35	2	600	425	65	33	32*	23.3	2.4	20.9
		1975	46.8	22–89	10	1945	1099	55	51	4	13.4	17.8	−4.4
			142	115–169	6	775	194	76	41	35	24.5	10.3	14.2
			278	208–356	8	1049	330	55	24	31*	21.0	3.0	18.0*
			514	441–659	4	937	271	68	55	13	24.5	7.4	17.1
Lake Pont-chartrain, LA	Levee	1977	44	33–54	2	372	222	132	45	87**	32.3	9.0	23.3
			161	112–221	3	910	306	120	65	55**	52.7	22.8	29.9*
			497	435–556	4	574	307	85	45	40*	32.8	13.0	19.8
			3091	1030–4270	5	419	204	88	83	5	31.0	4.9	26.1
	Fontainbleau	1978	9.0	1–23	8	248	303	81	63	18	44.3	23.1	21.2
	Levee		32.6	17–87	5	1123	382	78	44	34*	38.3	20.9	17.4
			93.7	53–177	4	918	355	103	36	67**	39.2	8.5	30.7
Boston, MA		1978	5.5	4–7	2	541	874	72	63	9	39.0	29.0	10.0
			7.0	5–9	2	477	410	86	68	18	23.0	10.0	13.0
			17.5	13–22	2	589	225	70	67	3	27.0	27.0	0.0
			29.5	28–31	2	442	495	93	71	22	32.0	14.0	18.0

* $p < 0.05$, ** $p < 0.01$

† Study population too small to cluster trials by similar indicator densities

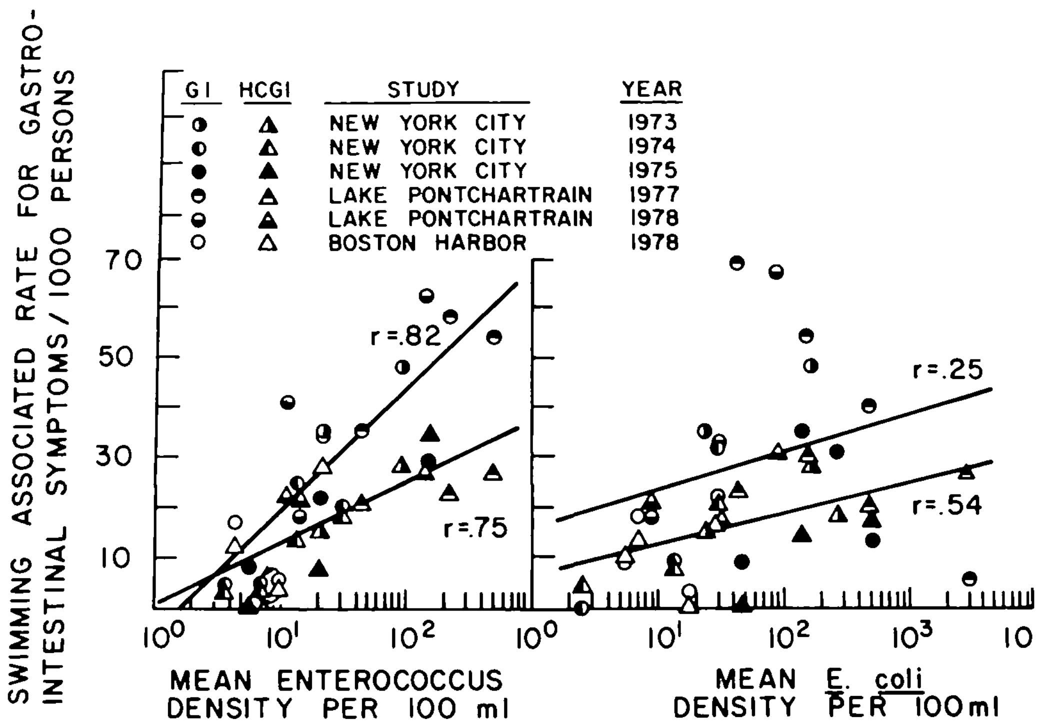

FIGURE 1 Regression of swimming-associated (swimmer minus nonswimmer) rates for gastrointestinal (GI) symptoms on the mean enterococcus and *E. coli* densities in the water Coordinate points are from tables 5 and 6. Correlation coefficients (r) are as given HCGI, highly credible gastrointestinal.

10/1000 (1 per cent) are associated with enterococcus densities of about 10/100 ml.

The enterococcus regression lines, their formulae, the r and p values and 95 per cent confidence limits for the lines are shown in figure 2. These relationships predict the illness rates from the mean enterococcus densities.

The relative importance of swimming in sewage-polluted water as a route of transmission for enteric illness was determined by examining the ratio of swimmer to nonswimmer gastroenteritis rates against the mean enterococcus density. It was assumed that all the cases acquired by all the routes other than swimming in sewage-polluted waters were included in the nonswimmer rates. The regression lines obtained for the trials clustered by indicator densities are shown in figure 3. It can be seen that the rates for both total and highly credible gastrointestinal symptoms were equal at a mean enterococcus density of about 1/100 ml. At a level of 10/100 ml, the rates for total and highly credible gastrointestinal symptoms were 1.5 times for swimmers and twice those for nonswimmers, respectively. The higher ratios for highly credible than for total gastrointestinal symptoms are of interest because of their implications concerning the reliability of the respondents' information to the illness queries.

DISCUSSION

The results clearly show that the risk of gastroenteritis associated with swimming in marine waters impacted with municipal wastewaters is related to the quality of the water as indexed by the mean enterococcus density in the water. More-

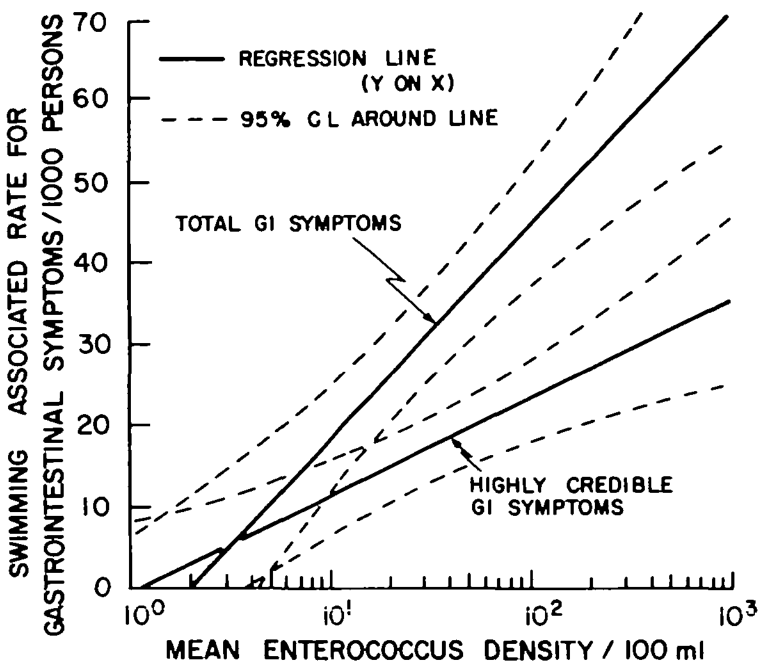

FIGURE 2. Regression of swimming-associated gastrointestinal (GI) symptom rates on the mean enterococcus densities in the water Data are from all US studies. The 95 per cent confidence limits (CL) for the lines are as shown. The slopes, intercepts, r and p values for GI symptoms are, respectively, 24 2, −5.1, 0.82 and <0 001. For highly credible GI symptoms, they are, respectively, 12 2, 0.2, 0 75 and <0.001.

over, the risk is detectable at extremely low levels of pollution. According to the criteria suggested by Hill (19), there is a strong suggestion of causality. First, the association is a good one; in some trials, the swimming-associated gastroenteritis rate was three to four times greater than the nonswimming rate. Second, there was a consistency in the association in that it was observed at multiple locations over multiple years. Third, the association between enteric disease and fecal contamination is a reasonable one by its very nature. Fourth, the association is a coherent one since there is a precedent for such a relationship by other waterborne routes of transmission, i.e., in shellfish (20) and potable water (21).

It was also understandable that, of the indicators examined, enterococcus densities in the water correlated best with the rates for the swimming-associated gastroenteritis. The two salient indicator characteristics required for the specific association obtained are a consistent fecal source and "good" survival during sewage treatment and transport in the aquatic environment. Of the indicators examined, enterococci and *E. coli* best satisfy the first requirement (22, 23); and, of the two, enterococci have the best survival characteristics (24), although their densities in raw or treated sewage are 1–2 orders of magnitude less than those of *E. coli* (25). These two differences are consistent with those observed in the slopes and X axis intercepts of the regression lines for the two indicators. That is, the slopes of the regression lines should become shallower and the lines should cross the X axis at lower indicator densities as the survival

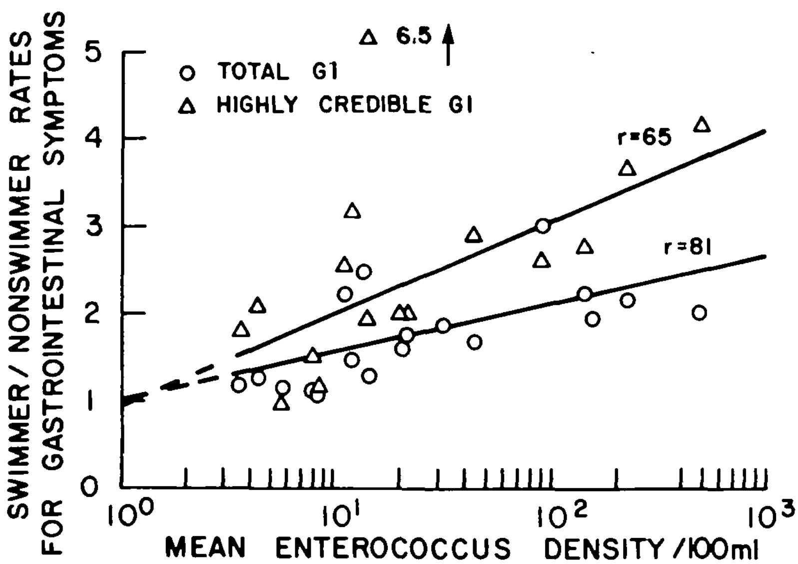

FIGURE 3 The relative risk of swimming in sewage-polluted waters as shown by the regression of the ratio of swimmer to nonswimmer (background) rates for gastrointestinal (GI) symptoms on the mean enterococcus density in the water. Data are from table 5

characteristics of the indicator become poorer relative to those of the etiologic agent(s).

There are two implications from the finding of rather high gastroenteritis rates (1 per cent) associated with the ingestion of one to five enterococci (the accidental ingestion of 10–15 ml of water (26) whose enterococcus density was about 10/100 ml). The first is that even enterococci may not survive as well as the etiologic agent for the gastroenteritis. The second is that the agent must be present in the bathing waters and, hence, municipal wastewaters in very large numbers, be highly infectious, survive very well in the marine environment or, most probably, a combination of all three.

The analysis of the ratios of the swimmer to nonswimmer gastroenteritis rates would suggest that, for individuals of "swimming age," swimming in sewage-polluted waters is not an insignificant route of transmission for the disease. Moreover, the risk of gastroenteritis is present even at relatively low pollution levels, as seen from the indicator densities. The higher ratio of the swimmer to nonswimmer rates observed with highly credible as opposed to total gastrointestinal symptoms suggests that nausea, stomachache and even diarrhea are disproportionately reported by nonswimmers. This, in turn, suggests that the swimming-associated rates for total gastrointestinal symptoms are underestimated.

Finally, the finding of swimming-related rates of gastroenteritis associated with very low indicator densities, i.e., the ingestion of one to five enterococci, has some interesting implications with regard to the existence of sporadic cases of this illness by the other potential water-associated routes of transmission, e.g., shellfish, drinking water and even aerosols generated from municipal sewage and its receiving waters. These possibilities should be pursued by prospective epidemiologic investigations.

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Using rapid indicators for *Enterococcus* to assess the risk of illness after exposure to urban runoff contaminated marine water

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ABSTRACT

Background: Traditional fecal indicator bacteria (FIB) measurement is too slow (>18 h) for timely swimmer warnings.
Objectives: Assess relationship of rapid indicator methods (qPCR) to illness at a marine-beach impacted by urban runoff.
Methods: We measured baseline and two-week health in 9525 individuals visiting Doheny Beach 2007–08. Illness rates were compared (swimmers vs. non-swimmers). FIB measured by traditional (*Enterococcus* spp. by EPA Method 1600 or Enterolert™, fecal coliforms, total coliforms) and three rapid qPCR assays for *Enterococcus* spp. (Taqman, Scorpion-1, Scorpion-2) were compared to health. Primary bacterial source was a creek flowing untreated into ocean; the creek did not reach the ocean when a sand berm formed. This provided a natural experiment for examining FIB-health relationships under varying conditions.
Results: We observed significant increases in diarrhea (OR 1.90, 95% CI 1.29–2.80 for swallowing water) and other outcomes in swimmers compared to non-swimmers. Exposure (body immersion, head immersion, swallowed water) was associated with increasing risk of gastrointestinal illness (GI). Daily GI incidence patterns were different: swimmers (2-day peak) and non-swimmers (no peak). With berm-open, we observed associations between GI and traditional and rapid methods for *Enterococcus;* fewer associations occurred when berm status was not considered.

Abbreviations: FIB, fecal indicator bacteria; qPCR, quantitative polymerase chain reaction; GI, gastrointestinal illness; HCGI, highly credible gastrointestinal illness; UTI, urinary tract infection; HCRESP, highly credible respiratory illness; CI, confidence interval; OR, odds ratio; CFU, colony forming unit; WQS, water quality standard; LOD, level of detection; MGD, million gallons per day.

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Conclusions: We found increased risk of GI at this urban runoff beach. When FIB source flowed freely (berm-open), several traditional and rapid indicators were related to illness. When FIB source was weak (berm-closed) fewer illness associations were seen. These different relationships under different conditions at a single beach demonstrate the difficulties using these indicators to predict health risk.

1. Introduction

Current methods for monitoring beach water quality involve the enumeration of fecal indicator bacteria (FIB) using culture-based methods, such as membrane filtration or defined substrate kits. These methods are widely accepted because of relative ease of use, low cost, and demonstrated relationship to health risk (Wade et al., 2003; Zmirou et al., 2003). However, the time required for FIB enumeration ranges from 18 to 24 h, with confirmation steps adding 1+ days. Each beach is unique, but FIB concentrations can change substantially on time scales of less than a day (Boehm et al., 2002). Thus, contaminated beaches remain open during the enumeration period and the contamination event may have passed by the time warnings are posted (Leecaster and Weisberg, 2001).

Technological advances provide opportunities to measure bacterial water quality more rapidly (Bushon et al., 2009; Haugland et al., 2005; Noble et al., 2010; Noble and Weisberg, 2005). Whereas current EPA-approved methods rely on bacterial growth and metabolic activity, these new rapid molecular methods directly quantify intracellular molecules, such as ATP, DNA, or RNA. Eliminating the enrichment and incubation steps associated with culture-based methods reduces assay time to as little as two hours and provides the opportunity for public health warnings to be issued on the same day that samples are collected (Griffith and Weisberg, 2011). The best developed of these methods is quantitative polymerase chain reaction (qPCR), such as the *Enterococcus* spp. (herein referred to as *Enterococcus*) assay developed by Haugland et al. (2005).

Quantitative PCR has been found to correlate with traditional culture-based methods (Griffith et al., 2009; Haugland et al., 2005; Lavender and Kinzelman, 2009; Noble et al., 2010), even though the measurement endpoint is different. Given the inherent differences between the two classes of methods, epidemiology studies are needed to establish health-risk relationships before establishing qPCR-based standards. Several studies have developed this relationship for waters affected by wastewater effluent (Wade et al., 2006, 2008, 2010), but few have assessed it for beaches affected by urban runoff (Sinigalliano et al., 2010). Here we report results from an epidemiologic study comparing health-risk relationships between qPCR-based (three different assays) and culture-based quantification of *Enterococcus* at a marine recreational beach affected by urban runoff.

2. Materials and methods

2.1. Study site

The study was conducted at Doheny State Beach in Dana Point, California, USA. Based on the frequency and magnitude of FIB water quality standards exceedances, Doheny Beach is chronically listed as one of the most polluted beaches in California (www.healthebay.org). Several potential sources of beach FIB exist including an adjacent small craft harbor and a 21 MGD secondary treated wastewater outfall 2.1 km offshore, but modeling and current measurement studies suggest that these sources are too distant to have a consistent effect on water quality at this beach (Jones, 2009). The largest and most direct FIB source to Doheny State Beach is San Juan Creek, which drains the adjacent 347 km^2 watershed. However, southern California has a Mediterranean climate and San Juan Creek does not flow to the ocean year-round because a sand berm forms and effectively dams the creek when creek flow is low. When the berm is open, the untreated creek-flow discharges directly to the surf zone and dramatically increases FIB concentration; when closed, water quality generally improves. There was no measurable rain during this 12-week study, as is typical in the summer, and the berm was open for three weeks.

2.2. Study design

The study was designed as a prospective cohort, similar to prior studies (Coford et al., 2007; Wade et al., 2006, 2008, 2010). Participants were recruited each sampling day with current health and degree of water exposure recorded. Ten to 14 days later interviewers contacted participants by phone and recorded illness occurring after their visit. We used regression models to evaluate the association of illness between swimmers and non-swimmers and between FIB and illness.

2.3. Water quality data collection and analysis

Surface water samples were collected in sterilized containers at 0.5 m depth on incoming waves. We collected samples three times (8 AM, 12 Noon, 3 PM) at each of five beach sites, three of which were within 400 m of the creek mouth (sites A, B, and D), one that was in the creek (site C), and one that was a reference site located about 3000 m to the south (site E; see Supplemental Material, Figure 1). Samples were analyzed for traditional culture-based FIB (*Enterococcus*, fecal coliforms, total coliforms) and three qPCR assays for *Enterococcus*. Total and fecal coliform bacteria were enumerated by membrane filtration on m-Endo and m-FC media, respectively (APHA, 2009). Culture methods for *Enterococcus* included EPA Method 1600 (USEPA, 2006) and Enterolert^{TM} (IDEXX, Westbrook ME; APHA, 2009) a defined substrate technology. All culture methods were processed immediately, while filters for the three qPCR methods were frozen for later processing. Two of the qPCR methods, here referred to as TaqMan and Scorpion-1 targeted the same broad species range of the genus *Enterococcus*, but differed in their probe chemistries and the manner in which final quantitative results were calculated (Haugland et al., 2005; Noble et al., 2010). The third method, here referred to as Scorpion-2, was identical to Scorpion-1 except that the primer-probe complex was slightly modified for more specific amplification of *Enterococcus faecium* and *Enterococcus faecalis*, two of the more common *Enterococcus* spp. commonly found in human fecal contamination (Layton et al., 2010). Taqman qPCR results were reported as calibrator cell equivalents per 100 ml based on the delta-deltaCt method described in Haugland et al. (2005), whereas Scorpion-1 and Scorpion-2 results were reported in cell equivalents (CE) per 100 ml using the deltaCt method outlined in Pfaffl (2001) and used by Noble et al. (2010).

2.4. Beach recruitment and follow-up interviews

The Committee for Protection of Human Subjects at the University of California, Berkeley approved all protocols. Eligibility criteria included: 1) no previous participation in the study; 2) at least one household member at the beach $\geq$18 years old; 3) home address in United States, Canada, or Mexico; and 4) verbal consent. Interviewers recorded the closest water-sampling site to the recruit. Participants were given an incentive (beach ball) and a questionnaire to complete prior to departure. The questionnaire assessed possible confounding exposures at the beach and exposures/illnesses experienced the previous three days. Participants failing to complete the beach survey on-site were contacted within 3 days by telephone. Ten to 14 days following their visit, participants were telephoned for a 10–15 min interview. This interview collected demographic information, swimming and exposures since the beach day, pre-existing health problems (e.g., chronic diarrhea), and acute health conditions since the beach visit. The head of household answered questions on behalf of the family.

2.5. Health outcomes

Health outcomes included gastrointestinal, respiratory, dermatologic symptoms, and non-specific symptoms. Gastrointestinal outcomes included nausea, vomiting, diarrhea, and stomachache or abdominal cramping. Diarrhea was defined as $\geq$3 loose or watery stools in 24 h (Baqui et al., 1991). Highly credible gastrointestinal illness (HCGI) was defined as: i) diarrhea; or ii) vomiting; or iii) nausea and stomach cramps; or iv) nausea and missed daily activities due to gastrointestinal illness, or (v) stomach cramps and missed daily activities due to gastrointestinal illness (identical to "GI illness" defined in Wade et al., 2010). Respiratory outcomes included cough and sore throat symptoms. Highly credible respiratory illness was defined as any 2 of the following symptoms: cough, runny nose, sore throat, fever or cold. Dermatologic outcomes included skin rashes and infected cuts. Non-specific symptoms included fever, ear infection, allergies, watery eyes, eye infection, and urinary tract infection. Respondents who reported a symptom at baseline (within 72 h before the beach visit) were excluded from analysis for that outcome, but not other outcomes.

2.6. Definition of swimming

We used four graded definitions of "swimmer" based on an individual's reported minimum exposure: i) any water contact; ii) body immersion; iii) head immersion; and iv) swallowed water. We defined body immersion as water contact above the waist, head immersion as head below the water line, and swallowed water as ingestion of any ocean water.

2.7. Statistical methods and data analysis

For swim exposure analyses, we modeled the probability of illness, p, with a logistic regression:

$$\ln\left[p/(1-p)\right] = a + \beta_1 A + \beta_2 S + \gamma \mathbf{X} \tag{1}$$

where A is an indicator variable for any water contact, S is a dichotomous indicator variable for exposure greater than or equal to some level of water contact (body immersion, head immersion, swallowed water), and **X** is a vector of potentially confounding covariates (see below). We estimated the relative risk of illness due to swim exposure using the odds ratio (OR), estimated as OR $=\exp(\hat{\beta}_1+\hat{\beta}_2)$. Thus the comparison group for these analyses was non-swimmers: individuals who had no contact with ocean water during their day at the beach.

In our analyses of the relationships between FIB concentrations and health outcomes, our goal was first to identify a set of conditions under which the traditional indicators appeared to have the expected relationships to health outcomes, especially gastrointestinal symptoms, as reported in prior studies (Wade et al., 2006, 2008, 2010). The conditions we examined included berm status (open, closed, and all days combined), level of participant exposure to water (body immersion, head immersion, swallowed water), specific health symptoms (detailed in 2.5 above) and indicator averaging method. Based on these exploratory analyses, we chose to use a site-specific daily average (one of nine averaging methods that we considered). We estimated site-specific daily averages by calculating the geometric mean of the indicator concentration levels over the 8:00 AM, 12 Noon, and 3 PM samples for each of the five sampling sites. Each swimmer was assigned the average indicator value for the sampling site nearest to where the individual reported swimming.

FIB concentrations were $\log_{10}$ transformed for the analysis because they were right-skewed. When indicator values were below the level of detection (LOD) for a given assay results were set equal to 10 per 100 ml. We also explored other imputation methods by substituting the LOD, the LOD/2, and LOD/SQRT(2). We restricted the population for each analysis to swimmers with a defined level of water contact. The probability of illness, p, was modeled for all berm days combined using logistic regression:

$$\ln\left[p/(1-p)\right] = \alpha + \beta \mathbf{I} = \gamma \mathbf{X} \tag{2}$$

where I is a continuous measure of the site-specific daily average for the indicator of interest and **X** is a vector of potentially confounding covariates. All ORs were estimated as OR $=\exp(\hat{\beta})$ and, thus estimate the increase in risk for a one unit change on the $\log_{10}$ scale of the indicator concentration among swimmers with a defined water exposure level.

The probability of illness, p, on berm-open and berm-closed days was modeled using logistic regression with a berm-indicator interaction term:

$$\ln\left[p/1-p\right] = \alpha + \beta \mathbf{I} + \gamma \mathbf{X} + \delta B + \varphi(I * B) \tag{3}$$

where I and $\mathbf{X}$ are equivalent to equation (2), B is a dichotomous indicator of berm status (open = 1, closed = 0) and $I*B$ is an interaction term between indicator concentration and berm status. ORs for berm-closed days were estimated from equation (3) as OR = exp ($\hat{\beta}$) and for berm-open days as OR = exp ($\hat{\beta}+\hat{\varphi}$), and estimate the increase in risk for a one unit change on the $\log_{10}$ scale of the indicator concentration among swimmers with a defined water exposure level. The coefficient ($\hat{\varphi}$) and associated p-value were used to test whether the interaction term differed from 0, and thus whether the association between an indicator concentration and health differed by berm status. Both models (2) and (3) assume that the association between indicators and illness is linear on the log-odds scale.

Models were adjusted for covariates, $\mathbf{X}$, that were associated with the outcome or judged to be potential confounders: study year, age, sex, race, swimming on multiple days, allergies, contact with animals, contact with other sick people, frequency of beach visits, digging in the sand, and consumption of raw or undercooked eggs or meat. All covariates, except age and frequency of beach visits, were categorized as 1 or 0. Race was dichotomized as white or nonwhite. Consistent with prior recreational water analyses (Coford et al., 2007; Wade et al., 2006, 2008, 2010), we selected a subset of these covariates for each model using a change in estimate algorithm, which retains covariates that change the estimated OR by at least 5% when removed from a multivariable specification (Rothman and Greenland, 1998). We estimated the 95% confidence intervals (CIs) for the ORs using robust standard errors (Freedman, 2010) that allow for correlated observations within household, but assume that households are independent. The decision to examine the health-indicator relationships stratified by berm status (berm-open, berm-closed, and all days combined) was planned prior to the initiation of the study. The "berm-open" analyses provide estimates of indicator-health relationships under poor water quality conditions; the "combined" analyses provide estimates of the indicator-health relationships averaged over the mix of berm conditions as would be typical for use of FIB at this beach.

3. Results

3.1. Water quality

A total of 481 water samples were collected and analyzed. Overall, *Enterococcus* concentrations by EPA 1600 ranged from <2 to 41,000 colony forming units (CFU)/100 mL. Overall, 17% of the samples exceeded the single sample marine water quality standard (WQS) of 104 CFU/100 ml for *Enterococcus* as determined by EPA Method 1600. At least 10% of the samples exceeded the standard at each of the three sampling sites located near the creek (see sites A, B, and D in Supplemental Material, Figure 1). Water quality at Doheny Beach differed significantly when the sand berm restraining San Juan Creek was closed compared to when open and the creek flowed untreated into beach waters (see Supplemental Material, Figure 2). Examining the site directly in front of the creek, median *Enterococcus* concentrations as measured by EPA1600 were 316 CFU/100 mL on berm-open days compared to 10 CFU/100 mL on berm-closed days. Similarly, 5% of samples from the same site exceeded single sample WQS on berm-closed days compared to 71% on berm-open days (data not shown).

3.2. Population characteristics

We approached 6686 eligible households. Of these, 4499 households (67%) agreed to participate and completed the beach interview, and 3587 households completed the two-week follow-up interview. Of 9525 individuals completing the study, 62% were swimmers (Table 1). Among individuals completing the study, 21% failed to complete beach interviews on-site (while at the beach) and were contacted by phone within 3 days of their visit, consistent with Coford et al. (2007). No differences were found in reported swim exposures by beach interview format (on-site vs. phone) or in the basic demographics of the two groups (data not shown). We collected limited data on those who enrolled but could not be located for follow-up; we did not observe notable differences ("lost to follow-up" in Tables 1 and 2).

3.3. Health outcomes for swimmers compared to non-swimmers

Among the 3585 non-swimmers at Doheny Beach, 3.49% had an episode of diarrhea in the 10–14 days following their visit (Table 3); this is comparable to the estimated 3.26% endemic 12-day prevalence of diarrhea in the United States (Scallan et al., 2005). The incidence of diarrhea following the beach visit was significantly higher for body immersion (4.58%), head immersion (4.59%) and those who swallowed water (6.13%) than among those with no contact. The adjusted odds ratio (aOR) for diarrhea among swimmers compared to non-swimmers increased with increasing water exposure: body immersion (aOR = 1.38, 95% CI 1.03–1.86); head immersion (aOR 1.46, 95% CI 1.07–1.99); and swallowed water (aOR 1.90, 95% CI 1.29–2.80). Similar patterns were observed for HCGI. We also collected information on non-gastrointestinal outcomes (see Supplemental Material, Tables 1–4 and 6–9). Generally these symptoms were less frequently observed than diarrhea and HCGI.

3.4. Associations of indicators with diarrhea and HCGI

The strongest associations between levels of FIB and diarrhea among swimmers were seen among those with highest level of water exposure ("swallowed water") on berm-open days (Table 4). For example, $\log_{10}$ increases in *Enterococcus* CFU measured by EPA Method 1600 were associated with an aOR of 2.50 (95% CI 1.52–4.11), fecal coliforms had an aOR of 2.30 (95% CI 1.48–-3.59) and TaqMan qPCR had an aOR of 2.34 (95% CI 1.13–4.84) when swimmers swallowed water on berm-open days. Berm-open ORs were consistently higher than berm-closed and berm-combined ORs. For each indicator, we report P-values for a test of interaction between indicator concentration and berm status (comparing open and closed estimates from the interaction model). The tests of interaction suggest that indicator-health associations differ by berm status, in particular among swimmers that swallowed

Table 1 – Doheny beach demographics by swimmer exposure status, individual level.

Variable	Lost to follow-up	Completed follow-up	Non-swimmers	Body immersion	Head immersion	Swallowed water
Individuals	2194	9525	3585	4335	3290	1219
Households	912	3587	913	2159	1784	769
Age (years)						
0–5	12.7%	12.5%	9.7%	13.3%	10.3%	16.5%
5.1–10	13.6	14.2	2.9	24.1	25.2	29.1
10.1–20	15.6	15.2	7.5	23.3	26.1	26.2
20.1–30	13.9	9.1	10.2	7.9	7.7	7.5
30.1–40	18.0	18.4	24.2	12.6	11.9	9.4
40.1–50	16.8	18.2	26.1	12.1	12.0	7.2
>50	9.4	12.0	19.0	6.3	6.5	3.8
Missing	0.4	0.4	0.4	0.3	0.3	0.4
Sex						
Male	45.9%	47.4%	38.0%	58.3%	62.1%	59.8%
Female	54.1	52.2	61.8	41.3	37.4	39.7
Missing	0.4	0.3	0.3	0.4	0.5	0.5
Race/Ethnicity						
White	58.0%	66.8%	68.8%	66.8%	67.4%	66.9%
White, Hispanic	0.0	4.4	4.4	4.3	4.4	5.7
Non-White, Hispanic	0.0	10.8	10.5	10.3	9.9	8.9
Black	2.4	1.3	1.3	1.2	1.1	1.0
Asian	7.3	4.9	5.4	4.3	3.6	3.7
Indian	0.0	0.5	0.6	0.5	0.4	0.3
Multiple	0.0	7.1	5.3	8.9	9.8	10.2
Other	8.8	2.0	1.9	1.9	1.7	1.9
Missing	23.5	2.0	1.9	1.8	1.6	1.4

water. Similar patterns (stronger, significant effects on berm-open days, among those who swallowed water) were seen for the association of traditional and rapid measurements of FIB with gastrointestinal illness (Table 5). Alternate LOD imputation methods were explored for indicator analyses, but did not alter conclusions (see Supplemental Material, Tables 10–12 for LOD/2; other results not shown.)

Table 2 – Doheny beach demographics, household level.

Variable	Lost to follow-up (%)	Completed follow-up (%)
Number of household residents		
1	14.9	9.5
2	17.7	19.8
3	24.6	22.3
4	26.1	27.9
5	11.9	13.4
6	3.3	5.2
7	1.3	1.0
8	0.4	0.8
Household income		
<$10,000–$25,000	–	5.50
$25,001–$50,000	–	10.90
$50,001–$75,000	–	14.50
$75,001–$100,000	–	15.80
$100,001–$150,000	–	22.70
>$150,000	–	19.10
Missing	–	11.50
Citizenship		
US	99.5	99.6
Canada	0.2	0.03
Mexico	0.3	0.4

3.5. *Lagged analysis (EPA 1600)*

In current beach monitoring practice, the 24 h incubation time needed for culture-based methods means that water quality results are not available until the day following collection. We therefore repeated our epidemiological analyses lagging culture-based exposure by one day to account for laboratory processing time (i.e. measuring the association between FIB on a given day and illness among swimmers the following day). In these analyses (Supplemental Material, Table 13) we found no significant associations between prior-day FIB and illness. For example, with berm-open the aOR for diarrhea was 1.30 (95% CI, 0.66–2.52) among swimmers with head immersion.

3.6. *Dichotomized analysis (EPA 1600)*

In current practice, single samples measuring EPA 1600 are typically reported as values above or below 104 CFU/100 ml. As a further check on the internal consistency of our findings, we dichotomized site-specific daily average values for $\log_{10}$ EPA 1600 at 2.017, corresponding to a concentration of 104 CFU/ml. We then took this dichotomized variable and measured the association with diarrhea and HCGI. We found strong associations between exposure and illness when specifying *Enterococcus* in this manner (see Supplemental Material, Tables 14 and 15). For example, among the small subsample of those who swallowed water ($N = 181$) on berm-open days, the aOR for diarrhea was 8.66 (95% CI 1.89–39.81) for those exposed to EPA 1600 levels above 104 CFU/100 ml compared to those exposed to levels below 104 CFU/100 ml.

Table 3 – Associations between gastrointestinal illness and swimming for various levels of water exposure and different berm conditions.

Health outcome	No contact (N = 3585)	Body immersion (N = 4335)		Head immersion (N = 3290)		Swallowed water (N = 1219)	
	% Ill	% Ill	Adjusted OR[a] [95% CI]	% Ill	Adjusted OR[a] [95% CI]	% Ill	Adjusted OR[a] [95% CI]
Berm-combined							
Diarrhea	3.49	4.58	1.38 [1.03–1.86]	4.59	1.46 [1.07–1.99]	6.13	1.90 [1.29–2.80]
HCGI	5.37	6.82	1.16 [0.90–1.50]	6.92	1.25 [0.96–1.63]	8.07	1.32 [0.96–1.79]
Berm-open							
Diarrhea	3.65	4.13	1.27 [0.64,2.51]	4.71	1.61 [0.82,3.16]	6.28	1.92 [0.77,4.78]
HCGI	6.41	6.80	1.00 [0.59,1.67]	7.50	1.21 [0.72,2.01]	8.97	1.31 [0.67,2.56]

HCGI, highly credible gastrointestinal illness.
a Odds Ratio calculated using non-swimmers as the reference group.

3.7. *Indicator-illness associations among non-swimmers: "negative controls"*

Our *a priori* assumption was that there should be only random associations between FIB concentrations and gastrointestinal illness among the non-swimmers. Because our study was observational rather than randomized and involved a multiplicity of analyses (i.e. multiple hypothesis testing), we carried out an additional step to investigate the robustness of the associations we observed. We used non-

Table 4 – Associations between diarrhea and exposure to specific indicators for various levels of water exposure and berm conditions.

Indicators[a]	Berm-combined Adjusted OR (95%)[b]	Berm-closed Adjusted OR (95%)[c]	Berm-open Adjusted OR (95%)[c]	Test of interaction P-value[d]
Body immersion				
Traditional				
EPA 1600	1.33 [1.07,1.64]	1.20 [0.94,1.53]	1.70 [1.17,2.46]	0.12
Enterolert	1.25 [1.03,1.50]	1.20 [0.99,1.46]	1.46 [0.94,2.26]	0.42
Fecal coliform	1.14 [0.93,1.40]	1.02 [0.82,1.28]	1.52 [1.05,2.19]	0.07
Total coliform	1.11 [0.93,1.31]	1.08 [0.9,1.29]	1.40 [0.81,2.41]	0.38
Rapid				
Taqman qPCR (delta delta)	1.03 [0.78,1.35]	0.92 [0.69,1.22]	1.50 [0.92,2.44]	0.09
Scorpion-1 qPCR	1.05 [0.82,1.33]	0.99 [0.74,1.33]	1.20 [0.76,1.91]	0.34
Scorpion-2 qPCR	1.03 [0.82,1.30]	1.01 [0.79,1.29]	1.15 [0.71,1.88]	0.64
Head immersion				
Traditional				
EPA 1600	1.33 [1.03,1.73]	1.12 [0.83,1.51]	1.87 [1.28,2.72]	0.04
Enterolert	1.29 [1.02,1.62]	1.20 [0.95,1.51]	1.54 [0.97,2.45]	0.35
Fecal coliform	1.18 [0.92,1.52]	1.04 [0.79,1.38]	1.61 [1.12,2.31]	0.06
Total coliform	1.12 [0.91,1.37]	1.03 [0.84,1.28]	1.49 [0.85,2.59]	0.23
Rapid				
Taqman qPCR (delta delta)	1.05 [0.76,1.45]	0.87 [0.62,1.22]	1.66 [1.02,2.68]	0.03
Scorpion-1 qPCR	1.12 [0.84,1.49]	1.07 [0.75,1.53]	1.24 [0.74,2.06]	0.65
Scorpion-2 qPCR	1.04 [0.79,1.36]	0.93 [0.67,1.27]	1.30 [0.82,2.04]	0.23
Swallowed water				
Traditional				
EPA 1600	1.74 [1.25,2.43]	1.42 [0.93,2.18]	2.50 [1.52,4.11]	0.09
Enterolert	1.38 [0.99,1.93]	1.07 [0.77,1.49]	2.17 [1.35,3.49]	0.02
Fecal coliform	1.29 [0.89,1.87]	0.96 [0.65,1.43]	2.30 [1.48,3.59]	0.00
Total coliform	1.29 [0.93,1.80]	1.13 [0.82,1.56]	2.15 [0.91,5.13]	0.17
Rapid				
Taqman qPCR (delta delta)	1.28 [0.82,2.01]	0.90 [0.56,1.44]	2.34 [1.13,4.84]	0.03
Scorpion-1 qPCR	1.34 [0.89,2.03]	1.16 [0.72,1.87]	2.02 [0.73,5.60]	0.34
Scorpion-2 qPCR	1.49 [1.14,1.95]	1.25 [0.90,1.73]	2.30 [1.46,3.61]	0.03

a Indicator exposure assigned based on site-specific daily average.
b Odds Ratio for diarrhea associated with a 1 unit increase in the $\log_{10}$ indicator concentration using non-interaction model.
c Odds Ratio for diarrhea associated with a 1 unit increase in the $\log_{10}$ indicator concentration using interaction model.
d P-value associated with interaction term comparing open to closed berm conditions.

Table 5 – Associations between HCGI and exposure to specific indicators for various levels of water exposure and berm conditions.

Indicators[a]	Berm-combined Adjusted OR (95%)[b]	Berm-closed Adjusted OR (95%)[c]	Berm-open Adjusted OR (95%)[c]	Test of interaction P-value[d]
Body immersion				
Traditional				
EPA 1600	1.16 [0.97,1.39]	1.08 [0.88,1.32]	1.36 [0.98,1.89]	0.24
Enterolert	1.10 [0.94,1.30]	1.09 [0.92,1.29]	1.15 [0.79,1.66]	0.79
Fecal coliform	1.11 [0.95,1.31]	1.03 [0.87,1.23]	1.36 [1.00,1.84]	0.13
Total coliform	1.10 [0.96,1.27]	1.09 [0.94,1.27]	1.19 [0.83,1.72]	0.66
Rapid				
Taqman qPCR (delta delta)	0.97 [0.79,1.20]	0.90 [0.71,1.13]	1.23 [0.80,1.91]	0.21
Scorpion-1 qPCR	1.02 [0.84,1.24]	1.00 [0.79,1.28]	1.06 [0.75,1.50]	0.80
Scorpion-2 qPCR	0.96 [0.79,1.16]	0.95 [0.77,1.17]	0.98 [0.66,1.45]	0.91
Head immersion				
Traditional				
EPA 1600	1.16 [0.94,1.45]	1.01 [0.79,1.29]	1.54 [1.10,2.16]	0.04
Enterolert	1.13 [0.93,1.36]	1.07 [0.87,1.30]	1.26 [0.85,1.86]	0.45
Fecal coliform	1.15 [0.94,1.39]	1.03 [0.83,1.29]	1.49 [1.09,2.03]	0.06
Total coliform	1.16 [0.99,1.36]	1.09 [0.91,1.31]	1.38 [0.95,2.01]	0.27
Rapid				
Taqman qPCR (delta delta)	0.94 [0.74,1.21]	0.83 [0.63,1.09]	1.26 [0.78,2.03]	0.14
Scorpion-1 qPCR	1.11 [0.89,1.39]	1.02 [0.77,1.36]	1.25 [0.85,1.82]	0.41
Scorpion-2 qPCR	1.00 [0.80,1.23]	0.93 [0.73,1.18]	1.12 [0.75,1.67]	0.42
Swallowed water				
Traditional				
EPA 1600	1.52 [1.12,2.06]	1.29 [0.88,1.88]	1.94 [1.23,3.05]	0.18
Enterolert	1.20 [0.88,1.63]	0.93 [0.69,1.26]	1.75 [1.16,2.64]	0.02
Fecal coliform	1.15 [0.84,1.59]	0.89 [0.63,1.27]	1.95 [1.29,2.97]	0.00
Total coliform	1.32 [1.01,1.72]	1.16 [0.88,1.53]	2.01 [1.06,3.83]	0.12
Rapid				
Taqman qPCR (delta delta)	1.21 [0.83,1.75]	0.95 [0.65,1.39]	1.95 [1.05,3.59]	0.05
Scorpion-1 qPCR	1.28 [0.92,1.77]	1.17 [0.79,1.71]	1.55 [0.80,3.00]	0.46
Scorpion-2 qPCR	1.35 [1.03,1.75]	1.19 [0.88,1.61]	1.70 [1.10,2.63]	0.18

HCGI, highly credible gastrointestinal illness.
a Indicator exposure assigned based on site-specific daily average.
b Odds Ratio for HCGI associated with a 1 unit increase in the $\log_{10}$ indicator concentration using non-interaction model.
c Odds Ratio for HGCI associated with a 1 unit increase in the $\log_{10}$ indicator concentration using interaction model.
d *p*-value associated with interaction term comparing open to closed berm conditions.

swimmers as "negative controls" (Lipsitch et al., 2010): we explored the association between average FIB concentrations at the beach for a given day and gastrointestinal illness among non-swimmers who visited the same day (who did not contact water, and were unlikely to be exposed to waterborne pathogens). In comparison with the indicator-illness associations seen among swimmers (Tables 4 and 5) there appear to be no patterns in the associations between FIB concentrations and gastrointestinal outcomes among non-swimmers (see Supplemental Material, Table 16). This suggests that health associations with FIB concentrations (both traditional and rapid) observed among *swimmers* are unlikely to be an artifact of unmeasured confounding, or our estimation approach.

3.8. *Daily incidence of diarrhea*

Swimmers reported a markedly different pattern of diarrhea incidence than non-swimmers following their beach visit (see Supplemental Material, Figure 3). Among swimmers, diarrhea rates were strongly elevated two days post-exposure relative to non-swimmers. Furthermore, these increases among swimmers were consistent with a dose–response relationship; the greatest elevation seen among swimmers who swallowed water, followed by swimmers with head immersion, and finally swimmers with body immersion.

3.9. *Morning vs. afternoon sampling*

As described in Methods (Section 2.7), we assigned indicator values to swimmers using the site-specific daily average of all morning and afternoon sample-values for the site nearest to the swimmer's area of immersion. To evaluate the impact of the timing of water sampling on indicator-health relationships, we analyzed the morning and afternoon samples separately (see Supplemental Material, Tables 17–20). Across all point estimates for the indicators, there appeared to be a stronger relationship to health when analyzing the morning rather than the afternoon samples.

3.10. Associations of indicators with other (non-gastrointestinal) symptoms

Although the principal goal of our study was to measure associations between FIB concentrations and gastrointestinal illnesses, we also measured associations between FIB concentrations and non-gastrointestinal health outcomes, including respiratory, eye, ear, and skin complaints. Because these outcomes were less frequently reported, we show only the data for swimmers who placed their heads under water (see Supplemental Material, Tables 21–27). Unlike associations seen for the indicators with diarrhea and highly credible gastrointestinal illness, there were no clear patterns of indicator-illness associations.

4. Discussion

4.1. Summary

We found that swimmers at Doheny Beach in the summers of 2007 and 2008 experienced diarrhea at a significantly increased rate compared to non-swimmers. Additionally, although it was not a primary focus of our study, we found increased rates of eye infections and earaches among swimmers. We found strong associations between several FIB quantification approaches and diarrhea, with evidence that these associations differed by berm status. Additionally, the data suggest an increasing dose–response relationship; the strongest associations were seen for those who reported swallowing water, especially on berm-open days. The associations of the FIB concentrations, using rapid molecular assays, with gastrointestinal health outcomes were similar to those of the traditional culture-based assays when examined under the same berm conditions. The pattern of time to diarrhea onset among swimmers (strong peak at 2 days) appears to be different from that seen among non-swimmers. Using non-swimmers as "negative controls" we saw no relationship between FIB and diarrhea among individuals with no water contact, further strengthening the suggestion that the associations observed between traditional and rapid indicators and illness among swimmers were not spurious findings related to our observational design.

4.2. Berm status: open, closed and all days combined

Our observation of a large difference in the associations between measures of *Enterococcus* and illness when the berm was open compared to berm-closed days, and all days combined could indicate a different FIB source between the different conditions. Boehm et al. (2004) suggested that FIB can transport through sand, but the transport of contaminated material to the beach is more rapid when the berm is open, reducing time for degradation and inactivation of FIB and pathogens alike. Additionally when the berm is closed, sand can filter out pathogens and *Enterococcus*, and appears to be impacting the association between *Enterococcus* densities and adverse health effects often seen among swimmers proximate to direct, flowing sources. More research is needed on the differential fate and transport impacts of pathogens and FIB through sand, and the potential cause of the breakdown of FIB density-illness relationships.

4.3. Lagged analyses

The associations we observed were similar between the culture-based and qPCR methods, but this is based on analyses assessing health relationships with samples collected on the same day that swimmers were in the water. We found that the indicator-health associations for the culture-based methods were no longer significant (nor was there a pattern of increasing odds ratios with increasing swimmer exposure) when the results were lagged by one day, typical of current beach monitoring practice. Thus, while these methods theoretically provide comparable levels of health protection, qPCR could provide a substantial advantage in practical application if rapid results were used to make decisions about health-risk management on the same day.

4.4. Morning vs. afternoon results

The processing lag for qPCR is less than for culture-based methods, but there is still about six hours from morning sample collection to when warnings are issued in the afternoon. Our results suggest that the effect of this 6-hr lag would be minimal, though, as we found that the odds ratios for samples collected in the morning were more likely to be statistically significant than those for samples collected in the afternoon (see Supplemental Material, Tables 17–20). This is in apparent contrast with rapid changes in bacterial concentrations that have been observed at some beaches (Boehm et al., 2002). A likely explanation is that the morning samples better represent the average swimmer's exposure compared to afternoon samples. This may be due to solar inactivation, which alters the relationship between FIB and the pathogens with which they co-occur (Davies-Colley et al., 1994; Noble et al., 2004; Sinton et al., 2007). This is consistent with our observation of consistently lower *Enterococcus* concentrations in the afternoon samples compared to morning samples (data not shown).

4.5. Differences in rapid indicators

We evaluated three qPCR assays that utilize primer-probe sets specific for *Enterococcus* and found little difference in their associations with illness. Two (TaqMan and Scorpion-1) used primer-probe sets targeting a gene sequence similar to that of Ludwig and Schleifer (2000) and are intended to quantify the broad range of *Enterococcus* species enumerated by EPA 1600 (Moore et al., 2008). The similarity in odds ratios between these two methods is consistent with several studies finding they yield similar *Enterococcus* concentrations (Griffith et al., 2009; Noble et al., 2010). The third primer-probe set (Scorpion-2) was a modified design intended to more specifically amplify *E. facaelis* and *E. faecium*, species thought to be important in human fecal contamination. The lack of difference in health relationships for this third method may result from the fecal sources at the site already having high concentrations of these species. Alternatively, it may result from the Scorpion-2 primer-probe design not being exclusionary and still amplifying a wide array of species, as suggested by the concentration correlations with the other two methods observed over a range of sample types (Noble et al., 2010).

4.6. Previous studies

Most bathing water epidemiology studies investigating municipal wastewater effluent-impacted waters, and studies examining the health risks from exposure to land-based runoff are equivocal. Schoen and Ashbolt (2010) used quantitative microbial risk assessment to show that non-point source runoff-affected beaches present considerably less health risk than those affected by wastewater, which is consistent with several studies that found no relationship between GI illness and increasing levels of *Enterococci* at beaches without known sources of sewage (Calderon et al., 1991; Coford et al., 2007; Fleisher et al., 2010). In contrast, McBride et al. (1998) found health risk from human and animal fecal material were not substantially different. Similarly, Haile et al. (1999) found increased health risk for several health outcomes (including fever, chills, cough, ear discharge and respiratory disease although not for HCGI-1 and HCGI-2) from swimming in proximity to urban runoff sources; these runoff source were known to contain human sources of fecal contamination based on the presence of human enteric viruses. Despite the separation of sanitary from storm-water runoff pipes and conduits in southern California, our study also provides an equivocal answer. When the berm was open, we observed associations between *Enterococcus* and health outcomes that were consistent with those seen in studies conducted near wastewater effluent (Wade et al., 2010). In contrast, these associations were weak when the berm status was not taken into account. The United States Environmental Protection Agency has committed to a new water quality standard by October 2012. Boehm et al. (2009) noted that some have suggested the potential establishment of different standards for beaches without direct impact from human fecal sources. Findings from our study suggest that while this option may be possible, the contamination source and delivery must be well understood, as FIB-illness relationships can vary between conditions even within a beach.

4.7. Limitations

There are potential limitations when evaluating our results. Although multiple attempts were made to contact all participants, 22% of participants could not be reached. We have no data to suggest that this introduced a systematic bias into our findings as the baseline enrollment characteristics of those who completed the study and those who did not are similar. The final number of participants completing the study (9525) was less than the 12,230 we had initially hoped to enroll. Enrollment was impacted by weather conditions that reduced beach usage during the months of our study and conceivably could have limited our ability to detect indicator-health associations for less frequently observed outcomes. We assigned exposure to each participant based on the FIB concentrations collected at the site closest to where that participant swam. Although this may not represent each individual's actual exposure, the internal consistency of the results (increased illness when water quality was poor during open-berm conditions, markedly different daily incidence pattern for swimmers and non-swimmers, increasing illness with increasing exposure) does not suggest a systematic bias. Although indicator exposure was not randomly assigned in our study, neither participants nor investigators had knowledge of water quality results during water exposure. Finally, our results must be interpreted with the understanding that we estimated and report numerous (indicator and health outcome) associations.

5. Conclusions

Our data suggest an increased risk of swimming-associated gastrointestinal illness at this urban runoff contaminated beach. When the source of FIB consistently exceeded water quality standards (berm-open), traditional and rapid methods for *Enterococcus* were both strongly related to illness. When the source of FIB was diffuse (berm status not adjusted for), fewer significant associations were measured. These differences in relationships between FIB and illness, even at a single beach, demonstrate that it can be difficult to consistently predict FIB-health associations at urban runoff impacted beaches using currently available indicators.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2012.01.033.

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Outbreaks associated with recreational water in the United States

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Outbreaks associated with recreational water in the United States

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Abstract
In this article, we review the causes of outbreaks associated with recreational water during 1971 – 2000. A bacterial or protozoan etiology was identified in three-quarters of the outbreaks; 23% of the outbreaks were of undetermined etiology. The most frequently identified agents were *Cryptosporidium* (15%), *Pseudomonas* (14%), *Shigella* (13%), *Naegleria* (11%), *Giardia* (6%), and toxigenic *E. coli* (6%). Outbreaks attributed to *Shigella*, *E. coli* O157:H7, and *Naegleria* were primarily associated with swimming in fresh waters such as lakes, ponds, and rivers. In contrast, outbreaks caused by *Cryptosporidium* and *Giardia* were primarily associated with treated water in swimming and wading pools. Important sources of contamination for both treated and untreated recreational waters were the bathers themselves. Contamination from sewage discharges and wild or domestic animals were also important sources for untreated waters. Contributing factors in swimming-pool outbreaks were inadequate attention to maintenance, operation, disinfection, and filtration. Although not all waterborne outbreaks are recognized nor reported, the national surveillance of these outbreaks has helped identify important sources of contamination of recreational waters and the etiologic agents. This information can affect prevention recommendations and research priorities that may lead to improved water quality guidelines.

Keywords: *Waterborne disease, waterborne pathogens, swimming and bathing, water quality*

Introduction

In 1971, the U.S. Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention (CDC), and the Council of State and Territorial Epidemiologists initiated a surveillance system for reporting the occurrence and causes of waterborne outbreaks in the United States (Craun et al. 1990). State and local health agencies have primary responsibility for detecting and investigating waterborne outbreaks which are then voluntarily reported to the CDC and EPA. The surveillance system provides information about outbreaks associated with drinking water systems and other types of water. Recently, national statistics were reported for outbreaks associated with recreational water during 2001 – 2002 (Yoder et al.

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2004). To supplement these recent statistics, we reviewed the historical information for waterborne outbreaks reported during 1971 – 2000. Recreational waters encompass swimming and wading pools, thermal and other natural springs, fresh and marine waters, water parks, interactive fountains, and other venues where water contact may take place. Fresh and marine waters are classified as untreated water; treated waters refer to the remaining recreational venues. Occasionally, an outbreak may be reported in a private swimming or wading pool that is a drain and fill type with no additional disinfection or filtration.

Yoder et al. (2004) included statistics for outbreaks associated with the use of whirlpools or hot tubs. In our review, we include these outbreaks only when persons also had a swimming pool exposure that was associated with illness. Also not included in this review are outbreaks on cruise ships operating from U.S. ports and wound infections associated with water recreation.

Methods

Information about the outbreaks reviewed here were obtained from the CDC-EPA database and surveillance summaries that have been published biennially or annually since 1973 (CDC 1973, 1974, 1976a, 1976b, 1977, 1979, 1980, 1981, 1982a, 1982b, 1983, 1984, 1985, St. Louis 1988; Levine and Craun 1990; Herwaldt et al. 1991; Moore et al. 1993; Kramer et al. 1996; Levy et al. 1998; Barwick et al. 2000; Lee et al. 2002). In 1978, the CDC began to specifically request information about recreational water outbreaks from state health agencies. Before then, recreational water outbreaks were sporadically reported, and in the early years, the database included several outbreaks reported in the literature.

Waterborne outbreak surveillance

Information available from the surveillance system pertains to outbreaks voluntarily reported. The outbreaks are classified according to the strength of the epidemiologic evidence implicating water as the vehicle of transmission. At least two persons must experience a similar illness after the ingestion of or contact with water, and epidemiologic evidence must implicate water as the probable source of the illness. Exceptions are single cases of laboratory-confirmed, primary amebic meningoencephalitis (PAM) and chemical poisoning if water-quality data indicate contamination by the chemical.

Since resources available for outbreak investigations differ from locality to locality and not all outbreaks are rigorously investigated (Craun et al. 2001; Yoder et al. 2004), the reported outbreaks do not always contain complete information about the number of ill or exposed persons, duration of illness, etiologic agent, or suspected sources of contamination. Outbreaks without water quality data or with incomplete information about sources of contamination are included in the surveillance data if the epidemiologic evidence implicates water (Lee et al. 2002; Yoder et al. 2004).

The surveillance system does not include endemic illnesses that may be associated with recreational water. In addition, not all outbreaks are recognized, investigated, or reported, and not all outbreaks are sufficiently investigated to estimate the total number of persons who may have been affected. The likelihood that individual cases of illness will be detected and investigated is dependent on many factors including: (a) public awareness of waterborne illnesses, (b) local requirements for reporting cases of particular diseases, (c) the surveillance and investigative activities of state and local public health and environmental agencies, and (d) availability of and extent of laboratory facilities. Outbreaks associated with recreational water are difficult to recognize and investigate because they usually result from persons who congregate in one venue and then disperse among the community or to other localities. Ill persons may consult different health-care providers, and different health districts may have jurisdiction for surveillance and investigation. Reliable estimates of the number of unrecognized and unreported waterborne outbreaks or illnesses are not available (Yoder et al. 2004).

Results

Outbreak statistics

During 1971 – 2000, the surveillance system contained information about 259 outbreaks and an estimated 21,740 cases of illness, 36 emergency room visits, 206 hospitalizations, and 28 deaths (Table I). Most (58%) illnesses were associated with protozoan outbreaks, and most (67%) hospitalizations and emergency room visits were associated with bacterial outbreaks. Most frequently, exposure to contaminated recreational waters resulted in acute gastroenteritis and dermatitis (Table II). Gastroenteritis was reported in 61% of the outbreaks and 85% of the illnesses. Dermatitis was reported in 20% of the outbreaks and 8% of the illnesses. PAM was reported in 28 single-case outbreaks. Occasionally, outbreaks of typhoid fever, infectious hepatitis, leptospirosis, and other illnesses were reported.

Outbreaks were primarily associated with recreational activities in lakes or ponds and swim pools (Table III). Other venues included wading pools, water slides, wave pools, interactive water fountains, rivers, canals, springs, and creeks. In one outbreak, 61 persons who volunteered for a dunking booth at a local fair became ill after accidentally swallowing water (Moore et al. 1993).

Reporting trends. Almost two-thirds of the outbreaks were reported during 1991 – 2000 (Figure 1). The recent increase in the number of outbreaks may be due, in part, to increased recreational activities and increased exposure to contaminated water; however, improved surveillance and outbreak detection, investigation, and reporting by public health agencies are also important. There has been a greater public awareness of waterborne pathogens such as *Giardia, Cryptosporidium*, and *E. coli* O157:H7 and more prompt reporting of these illnesses to health agencies in recent years. Reporting was inconsistent during the first half of the 30-year surveillance period when fewer than five outbreaks were reported in each of 11 years. In three of these years, no outbreaks were reported. The increase in the number of outbreaks was primarily due to outbreaks associated with gastroenteritis symptoms (Figure 2). An increased

Table I. Etiology, recreational water outbreaks, USA, 1971—2000.

	Outbreaks		Cases of Illness	
Etiology	(*n*)	%	(*n*)	%
Protozoa	97	37.5	12 701	58.4
Bacteria	97	37.5	4 548	20.9
Unidentified agents	40	15.4	2 966	13.6
Viruses	18	6.9	1 433	6.6
Chemicals	5	1.9	40	0.2
Bacteria and protozoa	1	0.4	38	0.2
Algae	1	0.4	14	0.1
Totals	259	100.0	21 740	100.0

Table II. Disease or Symptoms, Recreational Water Outbreaks, USA, 1971—2000.

	Outbreaks		Cases of illness	
Disease or symptoms	(*n*)	%	(*n*)	%
Gastroenteritis	157	60.6	18 584	85.5
Non-chemical dermatitis[a]	49	18.9	1 761	8.1
Primary amebic meningoencephalitis	28	10.8	28	0.1
Leptospirosis	7	2.7	426	2.0
Conjunctivitis[b], pharyngitis, or aseptic meningitis (adenovirus or enterovirus)	6	2.3	746	3.4
Otitis externa	3	1.2	118	0.5
Chemical dermatitis	3	1.2	34	0.1
Hepatitis	2	0.8	26	0.1
Typhoid fever	2	0.8	11	0.1
Chemical bronchial irritation	1	0.4	3	< 0.1
Chemical keratitis	1	0.4	3	< 0.1

[a] includes one outbreak (35 cases) of dermatitis with otitis externa. [b] includes one outbreak (5 cases) of dermatitis with conjunctival irritation.

Table III. Water source, recreational water outbreaks, USA, 1971—2000.

	Outbreaks		Cases of illness	
Recreational water	(*n*)	%	(*n*)	%
Lake or pond	116	44.8	7 559	34.8
Swimming pool only	72	27.8	11 692	53.8
Swimming pool and other waters[a]	17	6.6	431	2.0
River, stream, creek, or canal	12	4.6	80	0.4
Wading pool	10	3.9	195	0.9
Water slide, wave pool, or interactive water fountain	7	2.7	1 247	5.7
Spring	7	2.7	137	0.6
Ditch or puddle	6	2.3	22	0.1
Swimming pool and wading pool	5	1.9	268	1.2
Lake, pond, or river and other waters[b]	3	1.2	3	< 0.1
Ocean	2	0.8	44	0.2
Dunking booth	1	0.4	61	0.3
Unknown	1	0.4	1	< 0.1
Totals	259	100.0	21 740	100.0

[a] Swimming pool and whirlpool (7 outbreaks), swimming pool and hot tub (9 outbreaks), and swimming pool and sauna (1 outbreak). [b] Pond and swimming pool (1 outbreak), lake and swimming pool (1 outbreak), and river and wastewater holding pond (1 outbreak).

number of outbreaks were reported for both treated and untreated recreational water (Figure 3). In 2000, 20 outbreaks were associated with swimming, wading, and wave pools, water slides, and interactive fountains, whereas only eight and nine outbreaks were associated with

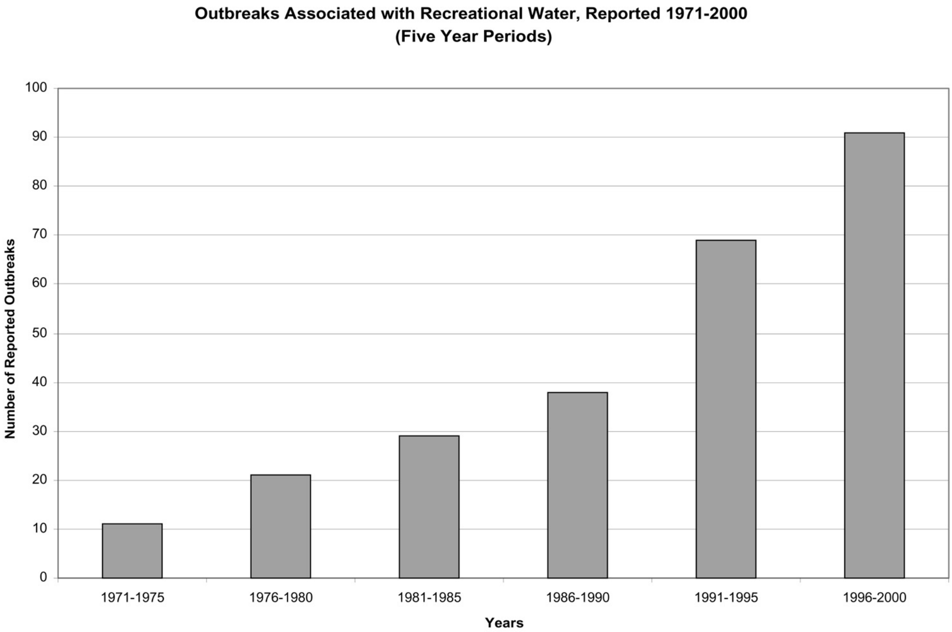


Figure 1.

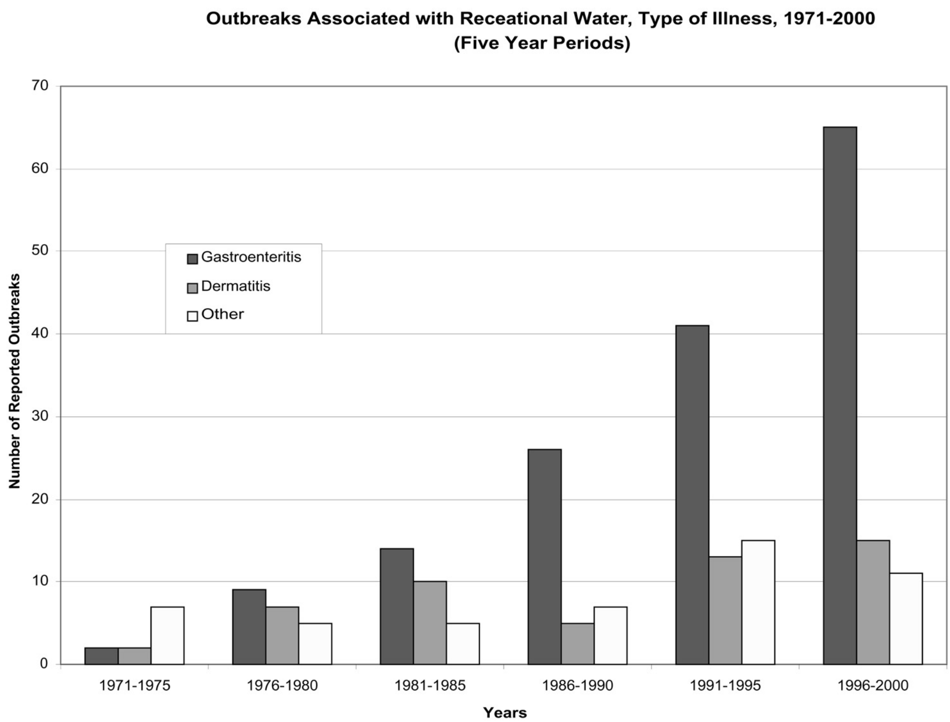


Figure 2.

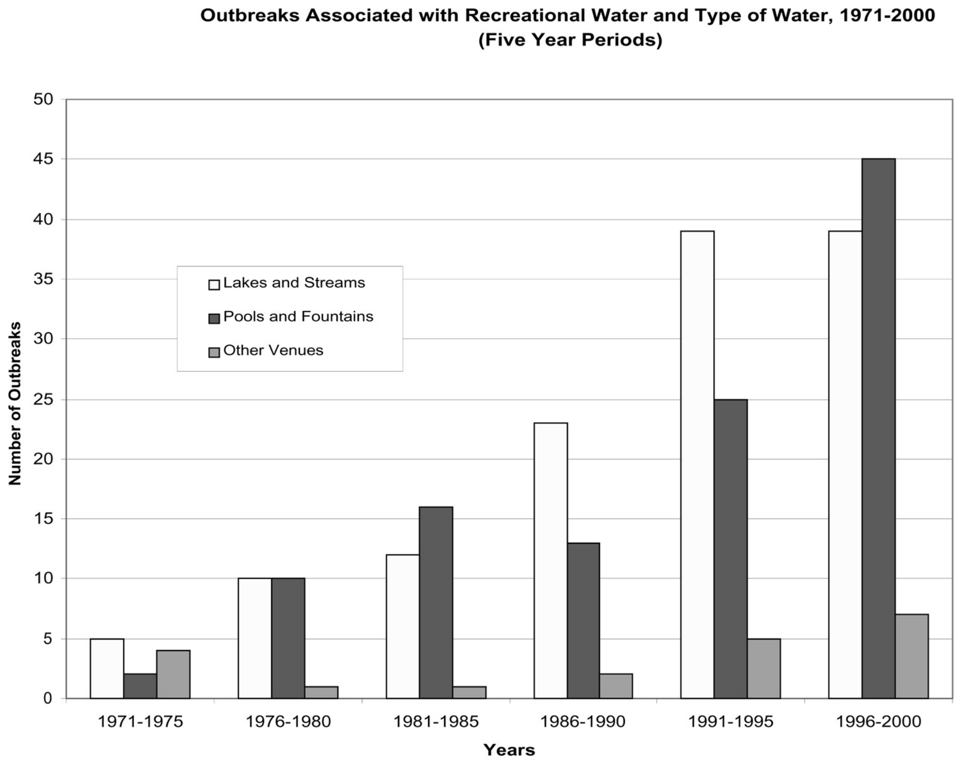


Figure 3.

these venues in 1999 and 1998, respectively. Before 1998, there were only two years when more than five outbreaks were reported in treated recreation water. The number of outbreaks reported during 1991 – 2000 in untreated water venues was significantly greater than the number reported during the previous ten-year period; however, the annual number varied greatly from as few as two outbreaks in 1997 to as many as thirteen in 1995.

Outbreak etiologies. A bacterial or protozoan etiology was identified in most outbreaks (75%) and cases of illness (79%) during 1971 – 2000 (Table I). A viral etiology was identified in only 7% of both outbreaks and illnesses. Exposures to high levels of pool chemicals (chlorine, bromine, sodium hydroxide) caused 2% of the outbreaks and less than 1% of the illnesses. An infectious etiologic agent was suspected but not identified in the remaining outbreaks and illnesses. All but two outbreaks of undetermined etiology resulted in acute gastroenteritis; in these two outbreaks, investigators suspected *Pseudomonas aeruginosa* as the cause of otitis externa and folliculitis.

Almost half (47%) of the protozoan outbreaks were associated with swimming or wading pools and water fountains; 35% of the outbreaks were associated with lakes or ponds. Outbreaks of bacterial etiology were also associated with swimming or wading pools and fountains (45%) and lakes and ponds (46%). In an outbreak that occurred at an interactive water fountain, both a bacterial and protozoan etiology (*Shigella* and *Cryptosporidium*) was identified. Of the 18 reported viral outbreaks, 56% were associated with lakes or ponds.

Two dermatitis outbreaks were associated with marine water. One outbreak was attributed to *Microcoleus lyngbyaceus* algae at an ocean beach in Hawaii (Moore et al. 1993). The second outbreak occurred at an ocean beach in Delaware where local snails were found to contain cercariae of *Austrobilharzia variglandis*, an avian schistosome that can cause cercarial dermatitis (CDC 1982).

In recent years, reports of outbreaks of undetermined etiology have decreased, and outbreaks of protozoan etiologies have increased. Only 11% of the outbreaks reported during 1991 – 2000 were of undetermined etiology; during 1986 – 90, 34% of the reported outbreaks were of unknown etiology. During 1991 – 2000, 50% of the reported outbreaks were caused by protozoa, but only 16% of the outbreaks reported during 1986 – 90 were caused by protozoa.

Seasonal distribution. The date of occurrence was available for 252 (97%) outbreaks reported during 1971 – 2000. More than 70% of the outbreaks occurred during June, July, and August when increased numbers of persons participate in water recreation. Persons have access to indoor pools, especially at resorts and motels during cold weather months, and some areas of the United States are warm enough for year-round water recreation. Some 17% of outbreaks occurred during the months of September through February. Almost all (90%) of the outbreaks of gastroenteritis occurred during the summer months. However, almost all (92%) of the outbreaks of dermatitis occurred during the months of September through February.

Severity of illness. The number of illnesses associated with outbreaks varied during the 30-year period. In 1981 only 14 illnesses were reported; 5,623 illnesses were reported in 1995. The largest outbreaks occurred most recently when an average of 137 and 83 illnesses per outbreak were reported during 1991 – 95 and 1996 – 2000, respectively (Figure 4).

Although information was not always reported about the severity of illness, at least 75 outbreaks (29%) resulted in one or more hospital admissions. Hospitalizations were caused by various etiologic agents. The most frequent admissions were for persons diagnosed with shigellosis (32%), cryptosporidiosis (24%), *E. coli* O157:H7 gastroenteritis (18%), and

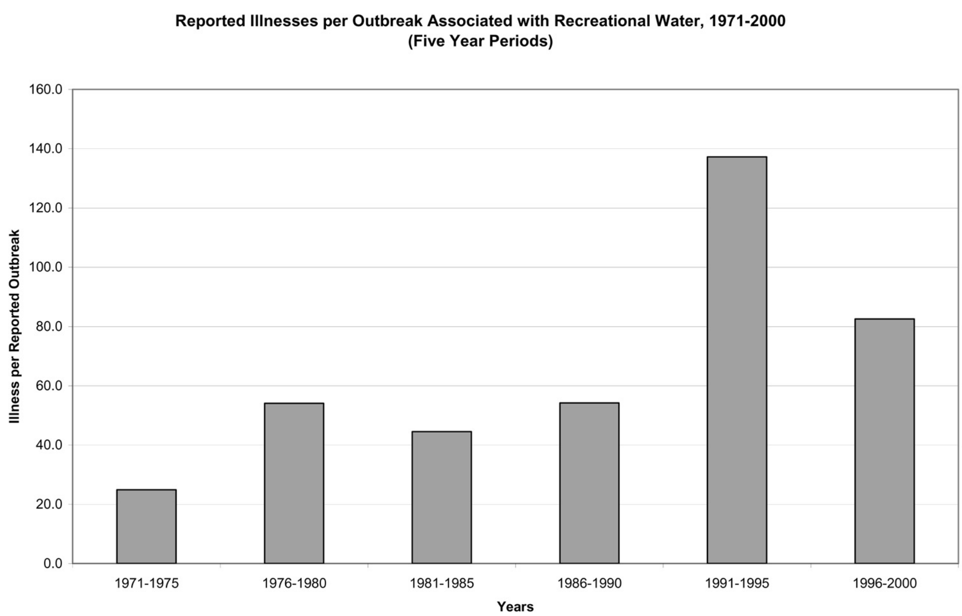


Figure 4.

leptospirosis (14%). In an additional four outbreaks, persons visited a hospital emergency room, primarily for medical treatment of shigellosis. Shigellosis is characterized by diarrhea accompanied by fever, nausea and sometimes vomiting, cramps, and tenesmus. In typical cases, the stools contain blood and mucus.

PAM, an infection of the central nervous system caused by free-living ameba *Naegleria fowleri*, usually occurs within 5 – 8 days after swimming and diving in warm fresh or brackish water. The majority of cases have been reported from states with more temperate climates like Florida and Texas, but cases have also been associated with lakes and ponds in Arkansas, North Carolina, New York, and Oklahoma and a thermal spring in California. The portal of entry is probably the nasal mucosa overlying the cribriform plate during underwater swimming and diving (Visvesvara and Stehr-Green 1990; CDC 1991). Infection is almost always fatal. Twenty-seven persons, mostly children and young adults 3 – 19 years old, died after becoming infected during the 30-year surveillance period. A single non-fatal case was reported; a nine-year-old child survived after being infected while bathing in a thermal spring.

A single death was associated with *E. coli* O157:H7, a pathogen that can elaborate potent cytotoxins and cause hemolytic-uremic syndrome. Diarrhea can be mild or very severe with stools that are virtually all blood.

Sources of contamination and deficiencies. In about half of the outbreaks reported during 1971 – 2000, information was available about sources of contamination and contributing factors such as a high density of bathers. Multiple sources of contamination and deficiencies including inadequate disinfection were reported in several outbreaks.

Inadequate attention to maintenance, operation, or treatment (e.g., disinfection or filtration) was frequently reported in treated-water outbreaks. Important sources of contamination for both treated and untreated waters included fecal accidents, ill bathers, and diaper-age children (Table IV). In several outbreaks, adults were reported rinsing diapers in the water. High bather density, heavy bather use, and bather crowding were often cited as the possible source of contamination or as a contributing factor. Overcrowding was a subjective conclusion based on investigators' comments. For example, investigators may have noted that the outbreak occurred during a time when the facility had a very large number of bathers due to a holiday, special event, or high temperatures

Table IV. Source of contamination and deficiencies, recreational water outbreaks, USA, 1971 – 2000.

	Percentage of outbreaks with listed contamination or deficiency [a]	
Source of contamination or deficiency	Treated water (%)	Untreated water (%)
Feces in water or ill bathers	36	31
Poor maintenance or operation; inadequate or malfunctioning filter or disinfection	52	–
Bather overloading or crowding	13	34
Diaper aged children	18	25
Seepage or overflow of sewage	2	21
Animals	2	18
Flooding, heavy rainfall	–	3

[a] Some outbreaks have multiple deficiencies; thus, totals are > 100%. One swimming pool outbreak is included where no treatment was provided.

(e.g., the hottest day of the season). Overcrowding was also noted without additional comments. Sources of contamination for untreated waters included sewage discharges, wildlife, and domestic animals.

Fecal accidents were identified or suspected in 39 outbreaks. In 15 (38%) of these outbreaks, *Cryptosporidium* was the etiologic agent. *E. coli* O15:H7 and *Shigella* each caused seven (18%) outbreaks where fecal accidents were reported. Norovirus and *Giardia* each caused three (8%) and two (5%) outbreaks, respectively. The remaining outbreaks (13%) were of undetermined etiology.

Cryptosporidium or *Shigella* caused most of the 25 outbreaks where diaper-age children were suspected sources of contamination. *Shigella* was identified as the etiologic agent in seven (28%) of these outbreaks, *Cryptosporidium* was identified in 6 (24%), and both agents were identified in one (4%) outbreak.

In the 28 outbreaks where large numbers of bathers were reported or overcrowding was noted, *Shigella* was the most frequently (32%) identified agent; no agent was identified in eleven (39%) outbreaks.

Water quality. Water samples collected during the investigation were found to be coliform-positive in 40 (85%) outbreaks where fecal accidents, diapered children, ill bathers, or bather crowding was reported and in 8 (73%) outbreaks where a sewage discharge was identified. Quantitative information about bacterial water quality was available for relatively few (38) outbreaks (Table V). Less than 500 organisms per 100 ml were detected in 59% of those outbreaks where water samples were analyzed for fecal coliforms, *E. coli*, or fecal streptococcus.

In 1986, EPA recommended microbiological water quality guidelines for fresh and marine recreational waters (EPA 1986; Dufour et al. 1984; Cabelli et al. 1983). The guideline for fresh waters is a monthly geometric mean (based on five equally spaced samples) of $\leqslant$ 33/100 ml for enterococci or $\leqslant$ 126/100 ml for *Escherichia coli*. For marine waters, the monthly geometric mean water quality indicator concentration should be $\leqslant$ 35/100 ml enterococci. State and local governments establish and enforce regulations for recreational water and may use different guidelines, including a single sample maximum value, to determine when to close beaches or post warning signs to alert potential bathers of poor water quality. The water quality of a recreational lake or pond was reported as meeting local bathing water quality standards in 12 (93%) of 13 outbreaks where this information was provided. In these 13 outbreaks, investigation reports provided no information about the limits that were applied. If

Table V. Number of outbreaks with water quality data obtained during outbreak investigations, 1971—2000.

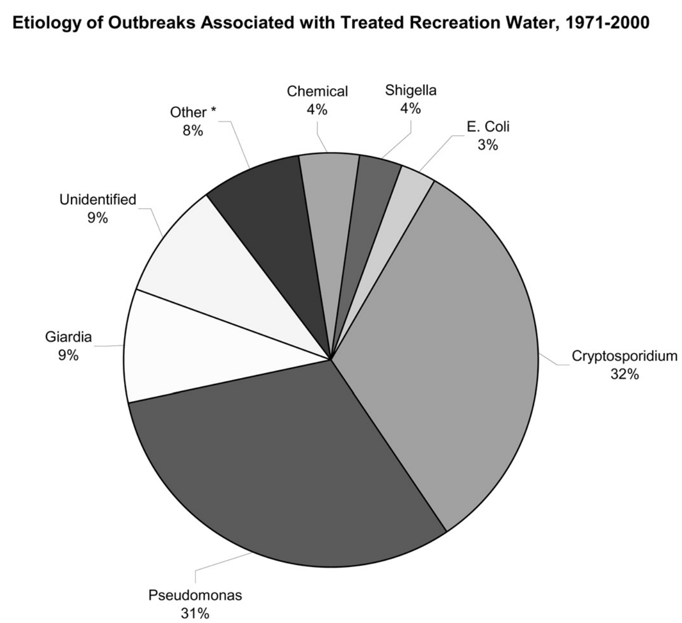
	Number of outbreaks and average number of coliforms/100 ml:		
Recreational water	$\leqslant$ 126/100 ml Fecal Coliform or *E. coli*	127 to 499/100 ml Fecal Coliform or *E. coli*	$\geqslant$ 500/100 ml Fecal Coliform or *E. coli*
Lakes, ponds	16[a]	7 [b]	12[c]
Rivers, streams, creek, or canal			2
Springs			1
All	16	7[a]	15

[a] Includes 2 outbreaks where samples were analyzed only for total coliforms (30 TC/100 ml and 30 TC/100 ml).
[b] Includes one outbreak where sample was analyzed for fecal streptococcus (380 FS/100ml) and one outbreak where sample was analyzed for total coliforms (208.5 TC/100ml). [c] Includes one outbreak where sample was analyzed for only for total coliforms (1400 TC/100ml).

the water samples that were collected during the outbreak investigations are assumed to be a measure of compliance with EPA guidelines, then 16 (42%) outbreaks occurred in recreational waters that met the EPA guidelines for fresh water (Table V). This includes water samples where only total coliforms were measured; (i.e., it was assumed that the coliforms were fecal coliforms). In an outbreak of *E. coli* O157:H7 in Wisconsin in 1999 (Lee et al. 2002), total and fecal coliforms were detected in water samples collected before and during the outbreak, but the levels detected did not exceed EPA guidelines for recreation in fresh waters.

Outbreaks associated with treated tecreational water

During 1971–2000, almost all (94%) treated-water outbreaks were associated with swimming and wading pools. Pool locations included community centers, parks and campgrounds, water theme parks, motels and hotels, country clubs, day-care centers, schools, and hospitals. *Cryptosporidium*, *Pseudomonas*, and *Giardia* were the three most frequently identified etiologies of outbreaks in treated recreational water (Figure 5). *Cryptosporidium* caused 32% of treated-water outbreaks and 80% of the cases of illness. *Pseudomonas* caused 31% of the outbreaks but only 10% of the illnesses. *Giardia* caused 9% of the outbreaks and 4% of the illnesses. An agent was not identified in 9% of the outbreaks and 4% of the cases of illness. *Cryptosporidium* was an important cause of outbreaks associated with swimming pools with and without wading pools, water slides, wave pools, and interactive water fountains.



* Other category includes adenovirus (1.8%), enterovirus (1.8%), *Campylobacter* (0.9%), hepatitis A (0.9%), norovirus (0.9%), *Salmonella* (0.9%), and both *Shigella* & *Cryptosporidium* (0.9%).

Figure 5.

Pseudomonas primarily caused outbreaks among users of swimming pools with and without hot tubs or whirlpool baths. *Giardia* was an important cause of outbreaks among children using wading pools.

A cryptosporidiosis outbreak in 1998 involved three community swimming pools in Wisconsin (Barwick et al. 2000). A child, later diagnosed with *Cryptosporidium*, had a fecal accident in each of the three pools on three successive days. Poor water quality and low chlorine levels were noted in two cryptosporidiosis outbreaks at an apartment complex and resort in Florida in 2000 (Lee et al. 2002). Fecal material was visible in the apartment pool. At the resort, there were complaints of ''cloudy'' water, and numerous diaper-aged children swam in the pool. In a third Florida outbreak in 2000, an infected child reportedly swam while ill (Lee et al. 2002). In a cryptosporidiosis outbreak that affected 700 members of a large private swim club in Ohio in 2000, oocysts were found in the water and inadequate operation, low chlorine residuals, high bather loads, and fecal accidents were reported (Lee et al. 2002). Another large outbreak of cryptosporidiosis was reported in Nebraska in 2000; 225 cases were associated with a cluster of four pools – outdoor adult and baby pools and indoor adult and baby pools (CDC 2002). Before the outbreak, several fecal accidents had occurred, and there was a two-day period when the baby pool was not chlorinated. In 1995, an outbreak at a water park in Georgia caused an estimated 5,449 cases of cryptosporidiosis after a probable fecal accident in the children's pool; several stool specimens were positive for both *Cryptosporidium* and *Giardia* (Levy et al. 1998). In 1996, an estimated 3,000 persons acquired cryptosporidiosis after being exposed to untreated water at a swimming pool and water from a jet-ski spray while watching a water show at a water park in California (Levy et al. 1998). As in the Georgia outbreak, some stool specimens were also found to be positive for *Giardia*.

Waterborne outbreaks of dermatitis (rash or folliculitis) caused by *Ps. aeruginosa* serotypes 0 – 11 have been reported since 1975 (McCausland and Cox 1975; Washburn et al. 1976). The rash has been described as intensely pruritic, progressing from a maculopapular to vesiculopustular eruption within hours to several days after exposure (CDC 1980). Extended and painful rashes associated with *Pseudomonas* outbreaks are unusual but have been reported (Lee et al. 2002). Most of these outbreaks have been associated with whirlpool baths and hot tubs, but swimming pool outbreaks have also been reported. Pseudomonads are well adapted to survival in whirlpools, hot tubs, and indoor pools because of the warm water temperatures. These waters are especially prone to contamination during periods of high use when it is difficult to maintain adequate disinfection levels. *Ps. aeruginosa* was identified as the etiologic agent in 36 outbreaks, 35 of which occurred in treated waters. In 16 outbreaks, ill persons reported using both a swimming pool and whirlpool, hot tub, or sauna. Also of interest are reports of conjunctivitis, otitis externa, and other symptoms reported in *Pseudomonas* outbreaks. In recent outbreaks in Alaska and Maine, persons reported nausea, headache, fever, fatigue and sore throat in addition to dermatitis (Lee et al. 2002). In another outbreak in Maine in 2000, 11 persons who were staying at a hotel during a sports tournament reported a rash accompanied by symptoms including ear infection, cough, headache and joint pain (Lee et al. 2002).

Other agents that caused outbreaks in treated water included *Shigella* (4%), chemicals (4%), and *E. coli* O157:H7 (3%). Three *Shigella* outbreaks occurred among children using wading pools or an interactive fountain and the fourth among water park visitors using a water slide. Three swimming pool-associated *E. coli* outbreaks were reported. One outbreak was associated with swimming in a poorly maintained and chlorinated indoor pool, and another occurred at a wading pool frequented by diaper-aged children. The third *E. coli* outbreak occurred at a water park where seven case-patients developed hemolytic uremic syndrome and one person died.

Outbreaks of chemical dermatitis were associated with incorrect dosing of chemicals to adjust the pH of swimming pool water, the addition of chemicals to remove excess chloramines, and high levels of disinfectants. Excess chlorine caused bronchial irritation in one outbreak and rash and conjunctivitis in another. Three cases of chemical keratitis resulted from exposure to bromine in a Vermont hotel swimming pool in February 2000 (Lee et al. 2002). Bromine levels were found to be greater than 5 mg/l.

Several outbreaks were associated with interactive fountains. In 1997, 369 persons became ill with cryptosporidiosis after playing in a sprinkler fountain at a Minnesota zoo (Barwick et al. 2000). The fountain was a popular place for diaper-age children to soak themselves during the heat of the summer. Water was sprayed through the air, drained through grates, collected, passed through a sand filter, chlorinated, and recirculated; however, it was not designed as an interactive fountain. In Massachusetts, nine persons became ill from *Shigella sonnei* in 1997 after play in a wading pool that included a sprinkler fountain. Recirculated water was disinfected with chlorine but not filtered, and many diaper-aged children were observed sitting in the wading pool (Barwick et al. 2000). An outbreak that occurred in August 1999 among 38 persons who visited a Florida beach park was attributed to both *S. sonnei* and *C. parvum* (Lee et al. 2002). The fountain's recirculation, filtration and disinfection systems were not approved by the health department and were inadequate or not completely operational at the time of its use.

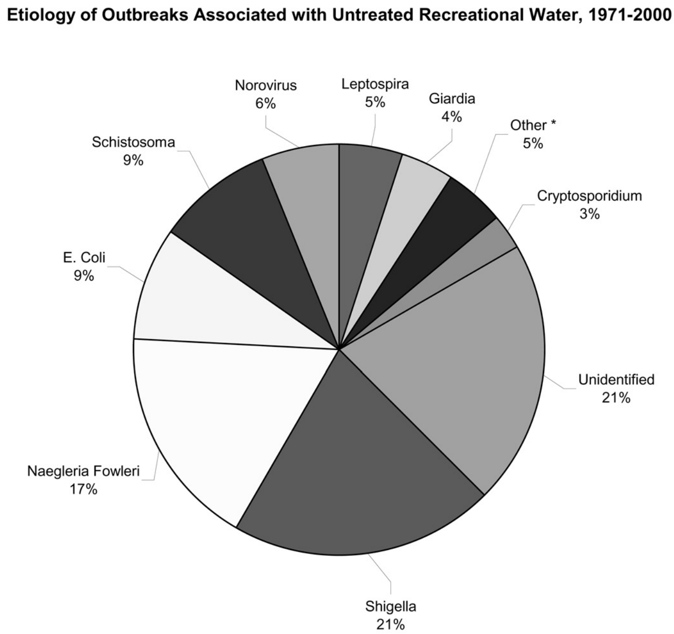
In 1995, an outbreak of *Salmonella* serotype Java was reported in Idaho among persons using a scuba dive pool that had been filled with fish (Levy et al. 1998). In 1999, an outbreak of *Campylobacter jejuni* was associated with a private pool in Florida that had ducks swimming in the pool but did not have continuous chlorine disinfection (Lee et al. 2002).

Two swimming-pool outbreaks of conjunctivitis and pharyngitis in 1980 and 1982 were caused by adenoviruses; the source of contamination was not identified in either outbreak (CDC 1982a, 1983). In 1987, two outbreaks of enterovirus-like illness and aseptic meningitis were reported in an inadequately chlorinated wading pool and a private pool that had been filled with water from a nearby creek (Levine and Craun 1990). In 1989, 20 persons in Louisiana contracted hepatitis after swimming in a campground pool; diaper-aged children were the likely source of contamination (Herwaldt et al. 1991). One norovirus outbreak in January 2000 was associated with swimming in a Wisconsin motel pool during a party where diapered infants were allowed in the water (Lee et al. 2002). Persons became ill within 48 hours after attending the event, and stool specimens tested positive for norovirus. An outbreak of suspected viral etiology occurred among 23 persons who attended three separate pool parties at a California apartment complex in June 1999 (Lee et al. 2002). Swimmers reported that the pool was overcrowded and toddlers were swimming in the pool.

Outbreaks associated with untreated recreational water

An etiologic agent was not identified in 21% of the outbreaks and 30% of the cases of illness associated with bathing activities in untreated water venues during 1971 – 2000 (Figure 6). Most of these outbreaks were associated with lakes and ponds (Table V). *Shigella* was the most frequently identified etiology of outbreaks (21%) and illnesses (29%) in untreated-water outbreaks. Other important agents included *N. fowleri* (17%), *E. coli* O157:H7 (9%), *Schistosoma* causing swimmer's itch (9%), norovirus (6%), *Leptospira* (5%), and *Giardia* (4%). *Cryptosporidium* caused only 3% of the outbreaks, all among swimmers in lakes or ponds. Causes of outbreaks among bathers in springs were due primarily to norovirus or *N. fowleri*.

Sixteen of the 28 single-case outbreaks of PAM were associated with swimming in lakes or ponds. Five PAM cases were associated with swimming in rivers and canals, and three cases



* Other category includes *Salmonella typhi* (1.4%), adenovirus (0.7%), enterovirus (0.7%), hepatitis A (0.7%), *Microcoleus* (0.7%), and *Pseudomonas* (0.7%).

Figure 6.

were associated with bathing in a thermal spring. PAM cases also occurred after facial immersion in a puddle and playing in mud holes and swimming in a waste water holding pond. In one outbreak, the infected person had fallen from a jet ski into the water.

In an *E. coli* O157:H7 outbreak of 36 laboratory-confirmed cases among visitors to a Washington state park in 1999, seven persons were hospitalized; *E.* coli O157:H7 was isolated from samples of lake water and sediment (Lee et al. 2002). *E. coli* O157:H7 also was implicated in a September 1999 outbreak among two young children who had been playing in ditch water in Florida (Lee et al. 2002); both clinical specimens and water samples were positive for *E. coli* O157:H7. A toddler with severe diarrhea reportedly swam in a Connecticut lake during a one-week period before an outbreak of *E. coli* O121:H19 in 2000 (Lee et al. 2002); lake water sampled after the outbreak was positive for total and fecal coliforms.

In a New Jersey park, at least 300 cases of shigellosis occurred in a 1994 outbreak among bathers in the swimming area of a reservoir where numerous fecal accidents had occurred and people were seen rinsing diapers (Kramer et al. 1996). During 1995–1996, swimming in a lake in Colorado contaminated with human feces caused 120 persons to become ill in two separate outbreaks; in a Pennsylvania outbreak where most cases occurred among children who were playing in the sand close to the water, soiled diapers were found near the implicated lake (Levy et al. 1998). *S. sonnei* was the cause of two outbreaks at freshwater lakes in Minnesota in 2000; in one outbreak, a large number of children were swimming, and a fecal accident was the likely source of contamination (Lee et al. 2002).

In August 2000, a cryptosporidiosis outbreak of 220 persons occurred at a Minnesota swimming beach where persons washed babies in the lake while changing diapers (Lee et al. 2002). Three persons in Indiana became ill with cryptosporidiosis in 1996 after heavy rains washed cattle feces into a lake (Levy et al. 1998). In Massachusetts, 18 persons were confirmed with *G. intestinalis* in a 1999 outbreak after swimming in a local pond that had high coliform levels (Lee et al. 2002). Two outbreaks of typhoid fever in 1971 and 1972 affected 11 children who swam and played in drainage pools and ditches contaminated with sewage (CDC 1973; Klein 1972).

An outbreak of conjunctivitis and pharyngitis attributed to enterovirus was reported among swimmers at a Wisconsin lake in 1983; the source of the contamination was not identified (CDC 1984). Adenovirus serotype 3 was isolated from clinical and water samples in a 1991 outbreak of 595 persons who reported conjunctivitis, pharyngitis, and fever after swimming in a pond in North Carolina (Moore et al. 1993). In Missouri in 1971, six persons contracted hepatitis A after swimming in a sewage-contaminated river (anon. 1971). In three norovirus outbreaks that occurred among bathers at lakes, fecal contamination from the bathers or sewage was identified as the source of contamination. In the four remaining norovirus outbreaks, a source of contamination was not identified. In one of two norovirus outbreaks in thermal springs, poor personal hygiene was the likely source of contamination.

The largest outbreak of leptospirosis ever reported in the United States occurred in Illinois during 1998 among competitors in a triathlon; 375 persons became ill after swimming in a lake; 28 were hospitalized (Barwick et al. 2000; CDC 1998). In 1991, an outbreak of leptospirosis was associated with swimming in a rural pond in Illinois; *Leptospira interrogans* was found in urine specimens from cases and in pond water (Moore et al. 1993). Swimming in waters contaminated by animal urine was the likely explanation for an outbreak of leptospirosis among 21 persons who participated in an adventure race in Guam in July 2000 (Lee et al. 2002). These persons reported various outdoor exposures including swimming in a river and a reservoir. *Leptospira* was confirmed via serology, and the epidemiologic investigation demonstrated that swimming in the reservoir, submerging one's head in the reservoir and swallowing water while swimming were significant risk factors for illness.

Discussion

The discussion includes a comparison of the recently reported 2001–2002 outbreak statistics (Yoder et al. 2004) and the 1971–2000 statistics. During 2001–2002 Yoder et al. reported outbreaks that included: 30 gastroenteritis outbreaks associated with recreation in lakes, pools, a river, a pool/hot spring, and a puddle; eight dermatitis outbreaks reported among persons who swam in a lake or used both a pool and spa; seven single-case outbreaks of PAM associated with lakes; one single-case outbreak of PAM associated with a river; two outbreaks of acute respiratory illness associated with exposure to an accidential release of chlorine gas at an indoor and outdoor pool; and two outbreaks of acute respiratory illness associated with exposure to an accumulation of chloramines at indoor pools. The seasonal distribution of these outbreaks was similar to that reported for outbreaks during 1971–2000.

Outbreaks associated with recreational water continued to increase during 2001–2000 when, an average of 25 outbreaks per year were reported. During 1991–1995 and 1996–2000, respectively, an annual average of 14 and 18 outbreaks were reported. This increase could reflect improved surveillance and reporting at the local and state level, a true increase in the number of outbreaks, or more likely, a combination of these factors. Although increased reporting of outbreaks occurred in both treated and untreated recreational water, a greater increase occurred in treated-water outbreaks where, on average, 14.5 outbreaks per year were reported during 2001 – 2002 compared to 5 and 9 outbreaks per year during 1991 – 1995 and 1996 – 2000, respectively. A relative modest increase occurred for outbreaks in untreated water where outbreaks increased from 8 to 9.5 per year during 1996 – 2000 to 2001 – 2002.

In recent years, cases of illness associated with these outbreaks have exceeded those reported in drinking water outbreaks. During 1995 – 2000, 13,131 illnesses were reported in recreational water outbreaks compared with 9,094 illnesses in drinking water outbreaks. During 2001 – 2002, this difference was even greater, as the number of illnesses reported in recreational water outbreaks was more than two-fold greater than illnesses in drinking water outbreaks.

Dose-response studies have shown that relatively few organisms of *Cryptosporidium, Giardia, Shigella* and *E. coli* O157:H7 are required to cause infection (Rendtorff 1954; DuPont et al. 1995, 1989; Moyer 1999; Benenson 1995). Thus, the unintentional ingestion of a single mouthful of contaminated water while swimming and bathing can cause illness, even in non-outbreak settings (Seyfried et al. 1986; Calderon et al. 1991). Swimming is essentially communal bathing, and pathogens can be introduced by the rinsing of soiled bodies and other bather activities. Most recreational outbreaks reported both during 1971 – 2000 and 2001 – 2002 occurred while swimming in lakes and swimming pools that were contaminated by fecal accidents and children in diapers and where bather overcrowding was suspected. However, in outbreaks during 2001 – 2002, bather overcrowding and diapered children were reported more frequently in treated water venues and less frequently in untreated water venues. High temperatures, special events, and holidays can result in bather overcrowding, and weather conditions (e.g., wind, rain, drought) can affect the water quality in pools and lakes. Outbreaks were also caused by contamination of untreated water by sewage discharges, watershed runoff from agricultural and residential areas, algal blooms, and various animal and avian species. Wild and domestic animals, as well as infected humans, can be sources of pathogens that can cause gastroenteritis, dermatitis, or other illnesses.

During both 1971 – 2000 and 2001 – 2002, relatively few (14 – 15%) outbreaks were of undetermined etiology. Outbreaks of undetermined etiology primarily occurred in untreated waters. Protozoan and bacterial agents caused almost three-fourths of the outbreaks reported during 1971 – 2000 and slightly more than half of the outbreaks reported during 2001 – 2002. During 2001 – 2002, norovirus was identified in 10% of the outbreaks compared to only 4% during 1971 – 2000. This may largely be due to improved laboratory capabilities and increased analyses. Outbreaks associated with chemical exposures were also more important during 2001 – 2002 (8%) than during 1971 – 2000 (2%). Outbreaks in 2001 and 2002 emphasize the importance of adequate operation, maintenance, and training; illnesses associated with exposure to chlorine gas or chloramines were characterized by respiratory symptoms, nausea, skin rash, and eye and throat irritation (Yoder et al. 2004). Chloramine exposures occurred at indoor pools, and chlorine gas exposures occurred at an indoor and an outdoor pool.

Outbreaks attributed to bacteria, such as *Shigella* and *E. coli* O157:H7, were associated primarily with swimming in fresh water (i.e., lakes, ponds, reservoirs). In contrast, most of the outbreaks caused by *Cryptosporidium* and *Giardia* were reported in chlorinated, filtered pool waters. Although *Cryptosporidium* and *Pseudomonas* were the principal causes of outbreaks in treated recreational water both in 2001 – 2002 (56%) and 1971 – 2000 (63%), other etiologies differed. During 2001 – 2002 compared to 1971 – 2000, there were increased percentages of treated-water outbreaks caused by norovirus (10 vs. < 1%) and *Shigella* (7 vs. 4%). No *Giardia* outbreaks were reported in treated water during 2001 – 2002, whereas 9% of the treated-water outbreaks during 1971 – 2000 were caused by *Giardia*. In untreated recreational waters, *N. fowleri, Cryptosporidium*, norovirus, and toxigenic *E. coli* increased in importance.

During 2001 – 2002 compared to 1971 – 2000, there were increased percentages of untreated-water outbreaks caused by *N. fowleri* (38 vs. 17%), *Cryptosporidium* (10 vs. 3%), norovirus (14 vs. 6%), and toxigenic *E. coli* (14 vs. 9%). No *Shigella* outbreaks were associated with treated recreational water during 2001—2002, whereas 21% of the treated-water outbreaks during 1971 – 2000 were caused by *Shigella*.

Cryptosporidium and *Giardia* are relatively resistant to disinfection by chlorine, and some pool filtration systems might not be effective in removing oocysts. Poor maintenance or operation and inadequate water treatment were identified as deficiencies that contributed to 52% of all outbreaks reported in treated water venues during 1971 – 2000. Similar statistics were reported during 2001—2002. Even pools with filters and disinfection practices capable of removing or killing these parasites may require hours or even a day to completely recirculate and disinfect the pool water once it becomes contaminated (e.g., by a fecal accident). Swimmers remain at risk until the contaminated water is recirculated through an effective water treatment process, and the water can become recontaminated if persons continue to swim. The risk for transmission of waterborne pathogens can also increase because of problems in the design of pools that result in areas with poor water circulation, mixing of water from different pools during filtration, and the depletion of residual disinfection levels (Carpenter et al. 1999; CDC 1976).

Although most bacterial outbreaks were associated with untreated water, outbreaks of bacterial etiology also occurred in treated water where residual disinfectant levels would be expected to prevent these outbreaks. Low chlorine levels found during an outbreak at a large water park emphasize the importance of frequent monitoring and maintaining adequate chlorination levels in large shallow pools, especially those used by young children. Since fecal contaminants and other organic material can rapidly consume the available chlorine, low chlorine residuals indicate possible water quality deterioration.

Although gastroenteritis is the most frequently reported illness in recreational water outbreaks, other illness have been reported and efforts to prevent these illnesses should not be forgotten. Outbreaks of *Pseudomonas* dermatitis are preventable if water is maintained at a pH of 7.2 – 7.8 with free, residual chlorine levels in the range of 2.0 – 5.0 mg/L (CDC 1981). A person's susceptibility and immersion time, along with the number of bathers per unit area, also influence the risk for infection (Highsmith and McNamara 1988). Close attention to bather overcrowding, as well as frequent disinfectant level monitoring and attention to maintaining adequate treatment, can help prevent these outbreaks.

Spring water was implicated in seven outbreaks during 1971 – 2000, five of which were associated with thermal springs. Springs and geothermal pools pose an increased risk to swimmers because their high levels of minerals and elevated temperatures may contribute to microbial growth and contamination. Improved education of patrons and staff about good hygiene practices is important, and in some instances, supplementary treatment or other measures may be needed to reduce risks.

Leptospira spp. can be found frequently in wild animal urine, and leptospirosis can be acquired through abrasions, inhalation of aerosols, or ingestion of water while swimming (Nelson et al. 1973; Lee et al. 2002). Swimmers should be educated about the potential risks of swimming in ponds and lakes that are not secured from entry by wild animals and that may be subject to contamination by wild or domestic animals.

The extent of the problem of cercarial dermatitis due to freshwater exposure is unknown, but it is likely that it occurs more frequently than what has been reported. Cercariae occur naturally in ecosystems that bring snails and birds closely together, and a number of the freshwater lakes in the United States have the potential to cause dermatitis among swimmers (Verbrugge et al. 2004). Persons should pay careful attention to where they swim, avoid shallow swimming areas known to be appropriate snail habitats in lakes associated with cercarial dermatitis, and report any incidents to their local health department to prevent further illnesses.

Inadequate pool operation and maintenance were often reported as contributing factors to treated-water outbreaks. Analysis of over 22,000 pool inspection records revealed that the majority of pool inspections had at least one pool code violation for water quality, recirculation system, or pool management and 8.3% of inspections resulted in immediate pool closure (CDC 2003). In partnership with a consortium of local and national pool associations, the CDC developed health communication materials for staff who work at treated water venues. This includes a caution to the public about health risks associated with swimming and wading pools, ways to reduce these risks, and a recreational water outbreak investigation tool kit for public health professionals (www.cdc.gov/healthyswimming). CDC has also developed and published technical information concerning laboratory diagnostics and a recreational water outbreak investigation toolkit that can be used by public health professionals.

Training of water park and pool managers, operators, and staff should include information about the transmission of waterborne illnesses and the critical role of treatment (i.e., disinfection, pH control, and filtration), operation/maintenance, and monitoring in preventing these illnesses. Health education activities should stress the potential for contamination by overcrowding, fecal accidents, and diaper-aged children. Good hygiene practices are required. Adequate toilet and diaper-changing facilities should be provided, and showers required before bathing. Although difficult to enforce, an important measure is preventing persons, especially young children from entering recreational waters if they are experiencing or convalescing from a diarrheal illness. The development of effective policies regarding fecal accidents and limiting the number of bathers in recreational water facilities are also needed, but additional research is needed, especially for water quality indicators and monitoring to assess bather contamination (CDC 2001). Contamination of pools and freshwater venues may require lengthy periods of closure or other ways to limit use before swimming can resume.

Because of the severe consequences associated with PAM, it is important to educate the public about the risks of PAM when swimming in certain locations. Since the amoebas associated with PAM are believed to enter through the nasal passage, limiting the amount of water forced into the nasal passages during jumping or diving by holding the nose or wearing nose plugs can reduce the risk of infection.

The effectiveness of health communication messages should be also assessed. For example, although cercarial dermatitis was a known problem at several of the lakes implicated in outbreaks during 1999 – 2000, signs posted by the health department regarding this problem were ignored by swimmers. Also, one gastroenteritis outbreak involved persons who swam in a lake that was clearly marked with signs indicating that the lake was unsafe for swimming. Additional microbial indicators and monitoring for fecal contamination may also be needed for freshwater recreational areas potentially contaminated by sewage. When one or more samples were collected during the outbreak investigations, it was surprising to find that current fecal indicators were present in relatively low numbers, and although the data are limited to only 13 outbreaks, investigators reported that the water quality had not exceeded state or local bathing water limits. In addition, limited water quality data are available for 26% of the outbreaks in fresh water. If it is assumed that water samples collected during the outbreak investigation reflect compliance samples, almost half of these outbreaks would have occurred in freshwaters that did not exceed EPA guidelines. It should be recognized that investigations were conducted after illnesses were reported, and the water quality of samples collected then might reflect improved water quality. However, these findings suggest the need for additional research on appropriate health indicators and monitoring frequency.

Current efforts by EPA under the Beaches Environmental Assessment, Closure, and Health (BEACH) program are aimed at reducing the risk for infection attributed to fresh and marine recreational water by strengthening water quality standards and monitoring frequency. Other activities include evaluating faster laboratory test methods, assessing sources of contamination, and increasing health- and methods-related research. To improve public access to information regarding both the quality of the water at their beaches and health risks associated with swimming in polluted water, EPA's Beach Watch (www.epa.gov/waterscience/beaches) provides online information regarding water quality, local protection programs, and other beach-related programs. Ongoing epidemiologic studies by EPA will provide information about the effectiveness of new, rapid (results in less than 2 hours) water quality indicator methods (www.epa.gov/nerlcwww/neearnerl.htm) and will be used to develop new EPA recreational water quality guidelines.

Conclusions

Although reporting is incomplete and the accuracy of case counts vary, surveillance activities have helped identify the types of recreational water, deficiencies and sources of contamination, and etiologic agents that are important causes of outbreaks in the United States. This information and recent increases in the number of reported outbreaks suggest the need to develop improved prevention and control to provide safe recreational waters. The prevention of illness from contaminated recreational waters requires a comprehensive approach that includes the control and remediation of environmental contamination, improved training of beach/pool managers and maintenance staff, and enhanced health education activities. To be effective, prevention and control programs depend on the close collaboration of local, state, and federal public health and regulatory agencies. It is also important that ways be developed to share surveillance, monitoring, and research information.

An important limitation of the outbreak surveillance data is the lack of information about sporadic waterborne illness. The outbreak statistics do not reflect the true incidence of waterborne outbreaks or illness, and additional regulations and control strategies may be necessary to prevent endemic waterborne illness. Recently initiated epidemiologic studies of endemic disease risks should help provide information in this regard.

Increased outbreak surveillance activities should also be considered to improve outbreak recognition and investigation, especially in localities where exposures may differ from the reported outbreaks in terms of recreational activities, water temperatures, contamination sources, or other local conditions. The evaluation and reporting of recreational-associated waterborne outbreak statistics from other countries is also encouraged.

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Current focus

Cryptosporidiosis: epidemiology and impact

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Abstract

Cryptosporidium was first recognized in humans in 1976 and came to prominence in the 1980s and 1990s as a cause of severe diarrheal illness in patients with AIDS. Its hardy, chlorine-resistant oocysts, tiny size, low infectious dose, fully infectious development when shed and zoonotic potential make it a threat in drinking and recreational water, contaminated food, day care centers, hospitals, and in persons with exposure to animals or unsanitary conditions, with potentially huge, long-term impact in malnourished children, as reviewed herein.

Keywords: Cryptosporidium; Protozoa; Diarrhea; Malnutrition; Parasite

1. History

Cryptosporidiosis, first recognized in the stomachs of autopsied mice by Tyzzer in 1907 [1] and first reported in humans in an immunocompetent child and in an immunosuppressed adult with diarrhea in 1976 [2,3], had only been anecdotally described in eight human patients (six with documented immunocompromise, including a child with congenital hypogammaglobulinemia and another with IgA deficiency) until AIDS patients with sometimes devastating, cholera-like diarrhea (and an outbreak among veterinary students with impressive calf diarrhea exposure) brought it to prominence [4–6]. Since then, cryptosporidiosis has become recognized as a highly chlorine-resistant pathogen for immunocompetent as well as immunocompromised humans. Most notorious is the huge waterborne outbreak affecting over 400,000 residents of South Milwaukee in 1993 [7]. In addition, cryptosporidiosis is increasingly appreciated as a cause of intestinal malabsorptive function not only in patients with AIDS [8,9], but also in malnourished children living in impoverished, developing areas [10–15].

As discussed in detail elsewhere, although over 20 different "species" of *Cryptosporidium* have been "named" in the literature, biologic, ribosomal RNA and genetic studies suggest that three larger, "gastric" (or cloacal) species (*C. muris*, *C. serpentis*, and *C. baileyi*, in rodents, reptiles and chickens, respectively) are distinct from the smaller, intestinal *C. parvum* (both the human genotype 1 and the bovine (and human genotype 2)) and its close "relatives" *C. felis*, *C. meleagridis* (seen in turkeys), *C. wrairi* (seen in guinea pigs) and possibly *C. saurophilum* (seen in skinks), and *C. nasorum* (seen in fish). To date, most human infections are with *C. parvum* (type 1 and some type 2). In addition, *C. meleagridis*, *C. felis*, and a dog genotype of *C. parvum* have been associated with human infections and/or diarrhea in adults with AIDS as well as in children in developing areas [16–20].

2. Cryptosporidiosis in immunocompromised and normal hosts

Cryptosporidiosis has now been reported from over 40 countries in six continents in both immunocompetent as well as immunocompromised patients around the world [21]. In a review of over 130,000 presumably immunocompetent patients with diarrhea in 43 studies in developing areas (Asia, Africa and Latin America) and in 35 studies in industrialized countries (in Europe, North America and Australia), Adal et al. [22] noted that 6.1% and 2.1% in developing and developed areas with diarrhea (vs. 1.5% and 0.15% in controls without diarrhea) had *Cryptosporidium* infections (see Table 1). Among HIV-positive patients with

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diarrhea, the percentages with *Cryptosporidium* infections rose to 24% and 14% (vs. 5% and 0 in HIV-infected controls without diarrhea) in over 1500 patients in nine studies in developing and in 13 studies in developed areas, respectively (Table 1) [22].

Serosurveys suggest that about 20% of individuals in the US experience cryptosporidial infections by young adulthood (numbers reach 58% in Oklahoma adolescents [23] and are higher, of course, for Peace Corps volunteers after working in developing areas [24]), while about 65% of children living in rural China become seropositive by 8–10 years of age and over 90% of children living in an urban shantytown in Northeast Brazil are seropositive by the end of their first year of life [10,25]. The consequences of these numerous infections (many of which may have important effects on absorption, nutrition and development, especially those in early childhood, with or without causing overt "diarrhea") are discussed in detail in Section 6.

3. Life cycle, biology and epidemiology

As shown in Table 2, critical to the epidemiology and spread of *Cryptosporidium* are five remarkable, distinguishing characteristics: (1) its impressively hardy oocysts that are highly resistant to chlorine and to acid; (2) its relatively small size; (3) its apparent low infectious dose; (4) its fully sporulated and infectious nature immediately upon shedding (unlike, for example, its "cousin", *Cyclospora*); and (5) its zoonotic potential (at least for strains other than *C. parvum*, genotype 1).

Table 1
Frequency of *Cryptosporidium* positivity in patients with diarrhea and controls with and without HIV in developing and developed countries [a]

	Cases (%)	Controls (%)
Developing countries		
HIV–	6.1% (1486/24,269)	1.5% (61/4146)
HIV+	24% (120/503)	5% (5/101)
Developed countries		
HIV–	2.1% (2232/107,329)	0.15% (3/1941)
HIV+	13.8% (148/1074)	0% (0/35)

[a] From a review of 52 studies in developing countries and 48 studies in developed countries [22].

3.1. Chlorine, iodine (and relative acid) resistance

A distinguishing characteristic of *Cryptosporidium* is its remarkable resistance to chlorine as used in drinking water treatment, swimming pools and even, for short periods, full strength household laundry bleach. A study of effects of fecal material on the chlorine concentration–time (Ct) calculation required to kill infectious *Cryptosporidium* oocysts for mice [26] confirms the previously published Ct values of 7200–9600 [27], i.e. >2 d (2880 min) at "normal" pool chlorine concentrations of 2 ppm. However, when fecal material was added, the Ct was increased over threefold; in fact, all oocysts remained infectious even after exposure of fecally contaminated material to 10 ppm chlorine for 48 h [26]. Consequently, *Cryptosporidium* is readily transmitted in fully chlorinated drinking water, as well as in public swimming pools [28]. Its relative acid resistance means that even though it does not multiply in foods, *Cryptosporidium* can also be transmitted in relatively acidic foods such as apple cider [29].

3.2. Relatively small size

As its 4–6-µm size is about one-third the size of amebic or *Giardia* cysts for which traditional filtration methods are effective, *Cryptosporidium* oocysts have proven to be a huge menace to the water treatment industry, requiring increasingly strict criteria for turbidity standards. The small size of cryptosporidial oocysts means that they are more difficult to filter (relative to oocysts of larger *Giardia* or *Cyclospora* parasites, for example), thus making *Cryptospo ridium* a particular challenge to effective water treatment methods. For example, the 1993 Milwaukee outbreak involved water with acceptable turbidity at that time (<0.5 NTU, nephilometry turbidity units), a standard that has subsequently been tightened. However, even the new, more stringent criteria may not be perfectly safe, as suggested by the outbreak of cryptosporidiosis in Clark Co., Nevada, despite full chlorination and "state-of-the-art" water treatment [30].

3.3. Low infectious dose

The infectious dose, initially reported to be a median of 132 oocysts for a single, type 2 (bovine) strain given to human volunteers [31], now appears to range from 9 to 1042 for three different bovine strains fed to humans [9,32], and

Table 2
Special characteristics of *Cryptosporidium* that are relevant to its epidemiology and transmission

	Characteristic	Epidemiologic significance
1	Hardy, chlorine (and acid)-resistant oocysts	Readily spread in fully chlorinated water or swimming pools and acidic foods (e.g. cider)
2	Relatively small size	Difficult to filter; menace to water industry
3	Low infectious dose	Easily acquired with high infection rates (e.g. Milwaukee water, DCC, hospitals, households)
4	Fully infectious when shed	Person-to-person spread (e.g. households, hospitals, day care centers)
5	Zoonotic potential	Animal contact (e.g. vet students; cattle, zoo related outbreaks)

even less is known about the infectious dose of the human strain that causes most outbreaks. Extrapolations from the Milwaukee outbreak suggest that the infectious dose may be as low as 1–10 oocysts for some individuals [10,33].

3.4. Fully infectious oocysts upon shedding

In addition to their remarkable chlorine resistance, small size and low infectious dose, the full development of *Cryptosporidium* oocysts by the time they are excreted to a promptly infectious stage means that it can be readily spread by direct person-to-person contact, and thus determines a key aspect of the epidemiology of *Cryptosporidium* infections. This is quite different, for example, for its sporozoan relative, *Cyclospora*, which, though also readily infectious with low oocyst doses, and also spread in foods (like raspberries) and water, is not initially infectious when shed, and thus does not appear to exhibit the same problem of secondary household spread or spread in institutions, at least to the extent that *Cryptosporidium* can. Consequently, taken with its low infectious dose described above, like other enteric pathogens with low infectious doses, *Cryptosporidium* can be readily spread by direct person-to-person contact in households [10], hospitals and extended care institutions [34,35], and day care centers [36,37]. Furthermore, especially difficult for control is the extended period of shedding often long after the overt symptomatic illness [36]. This may also lead to relapse or secondary spread, although the latter usually tapers off after an outbreak [38,39].

3.5. Zoonotic potential

While apparently not the case for genotype 1 *C. parvum*, genotype 2 *C. parvum* and other genotypes and *Cryptosporidium* species increasingly appear to have less stringent host species specificities. An impressive range of 152 different mammalian species have been reported to be infected with *C. parvum* or with a *C. parvum*-like organism [40]. This is again quite different from *Cyclospora*, which, despite a careful search in endemic Haiti, has not been found in animals [41]. As noted above, in addition to many human infections related to cattle exposure (as in veterinary students, farm and zoo exposures, and even cider from indirect cattle exposure), several reports now describe associations of *C. felis*, *C. meleagridis* and a dog genotype of *C. parvum* with human infections in adults with AIDS and in children in developing areas. One report describes the shedding of apparent *C. muris* oocysts by two asymptomatic Indonesian children [42]. Recently, the specificity of genotype 1 for humans has also been challenged by the report of its infecting a dugong [43].

Genetic studies of 39 outbreak isolates of *Cryptosporidium* (from nine different outbreaks in the US and Canada) suggest that most outbreaks in the US (six of eight) are with the human (genotype 1) parasite [44–46], which, when fed to calves or mice fails to infect, suggesting at least relative human host specificity [44]. In contrast, most sporadic cases of cryptosporidiosis reported in the UK are often with the bovine genotype [45], suggesting possible zoonotic sources. In an extensive study of over 1700 human fecal samples in cases in the UK, fully 61.5% were with genotype 2 (bovine) *C. parvum*, often with animal contact, while genotype 1 is seen (often with genotype 2 in mixed infections) in drinking water, swimming pool and travel-related cases [47]. Furthermore, epidemiologic studies in three of the four genotype 2 outbreaks in North America (one in Maine [29], one in British Columbia [48], one in Pennsylvania [44]) suggested zoonotic sources, and the fourth outbreak could well have been zoonotic, because it was associated with a sprinkler at a zoo [49].

Finally, new (as well as recognized) genotypes being found in wildlife suggest that sylvatic transmission of *C. parvum* genotype 2 and possibly novel genotypes is common in deer, chipmunks, mice, muskrats and occasional raccoons and skunks, and must be considered when strains are found in water sources or in association with outbreaks [50].

4. Means of transmission

As introduced above (and as shown in Fig. 1), the tiny, hardy, chlorine-resistant oocysts of *Cryptosporidium* can be transmitted in several different ways.

4.1. Drinking water

Probably most common is waterborne transmission, whether in fully chlorinated drinking water (that has been contaminated *usually* via contaminated surface water) or by sewage effluent, since sewage treatment often does not kill the parasite [27,40,51,52]. Fayer et al. have reviewed some 50 waterborne outbreaks reported from throughout the US, UK, Canada and New Zealand, and the documentation of widespread fecal contamination with oocysts in wastewater, activated sludge, ground and surface water, and treated drinking water [40]. Although questions remain about viability, species and sources of oocysts found in tap water, numerous outbreaks (including the huge Milwaukee outbreak) amply document the importance of waterborne transmission of *C. parvum* infections in humans.

4.2. Recreational water

In addition, 31 outbreaks [40] affecting over 10,000 people have associated cryptosporidiosis with exposure to recreational water, often despite its full chlorination, and often related to frequent fecal accidents by diapered infants, toddlers, or incontinent individuals.

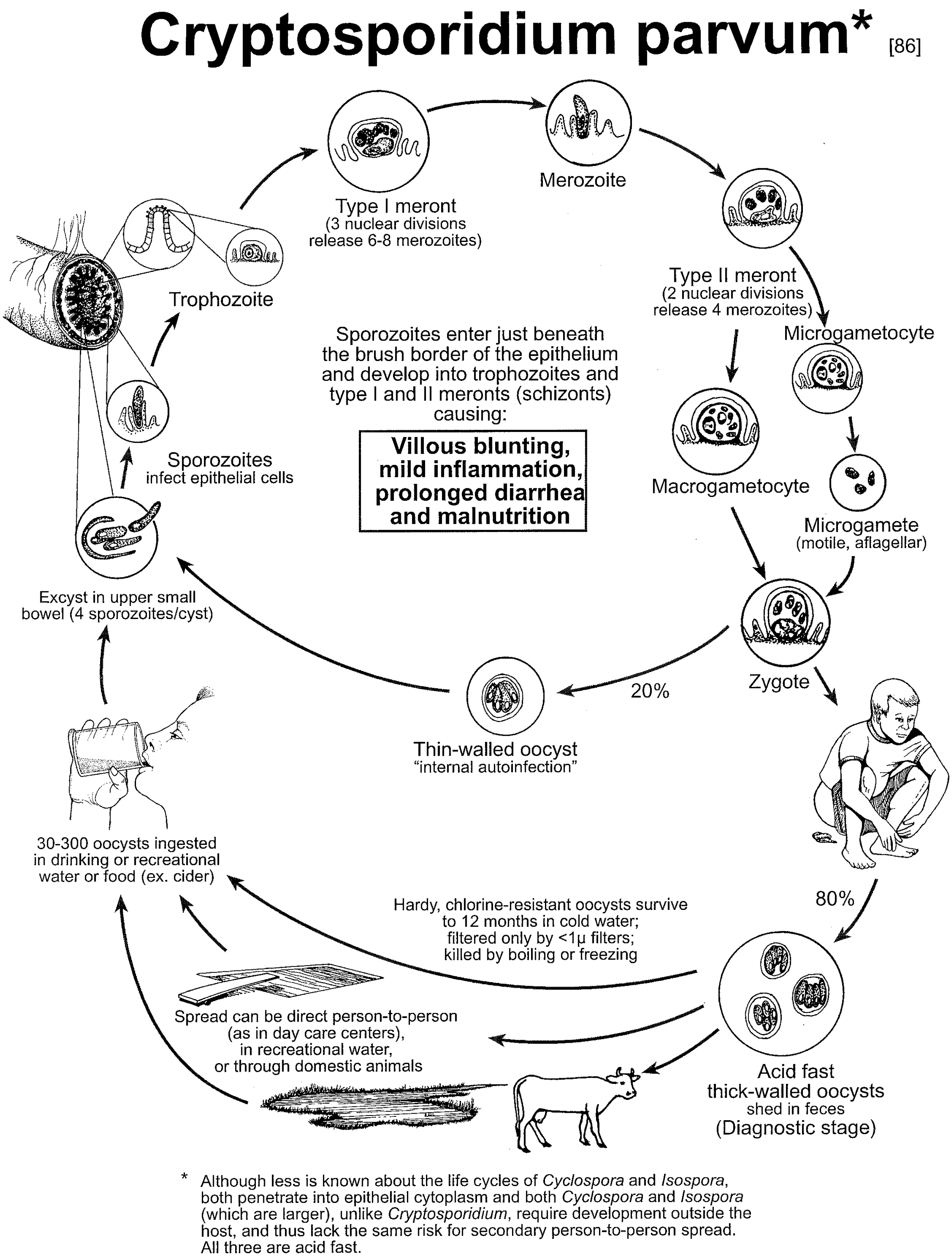

Fig. 1. Life cycle of cryptosporidia.

4.3. Food

Several documented food-associated outbreaks have implicated fresh pressed cider in Maine and New York [29,40], improperly pasteurized milk in the UK [53], chicken salad in Minnesota [54], uncooked green onions in Spokane, Washington [55], and an infected cook who cut fresh vegetables and fruit in a Washington, DC cafeteria [46].

4.4. Person-to-person

Person-to-person transmission occurred in households in 5.4% (of household contacts who developed symptomatic disease in the Milwaukee outbreak) to 19% (of family members of infected children in Fortaleza, Brazil developing disease or seroconversion) [38]. The nosocomial and day care center outbreaks noted above [34–36] are also likely related to direct person-to-person spread in institutional settings where sanitation is difficult. Association with anal sexual exposure also likely reflects person-to-person direct spread as well [56].

4.5. Animals-to-people

In addition to the links of bovine (genotype 2) cryptosporidiosis to cattle exposure on farms and through unpasteurized milk and apple cider, the outbreak of cryptosporidiosis among veterinary students in the early 1980s illustrates the potential for direct animal-to-person transmission of *C. parvum* [6]. The cases of human infection with cat, dog, and turkey genotypes mentioned above also implicate transmission from animals [10].

4.6. Other potential environmental sources

Environmental sources include well-documented common presence of *Cryptosporidium* oocysts in cattle, manure and farm watershed runoff [57,58]. Other potential environmental sources of cryptosporidial infections include Canada geese, Peking ducks, oysters, mussels and cockles [17,40,59,60], although care must be taken to distinguish similar acid fast *Haplosporidium* [61].

4.7. Flies, cockroaches and other potential vectors

Cockroaches, houseflies, dung beetles and microscopic rotifers in water have all been suggested as potential vectors of spread for *C. parvum* [40,62,63,64]. Finally, despite frequent pulmonary symptoms in immunocompromised patients with cryptosporidiosis and apparent respiratory acquisition of avian species among birds, airborne transmission to humans is not well documented.

5. Seasonality and other risk factors

Cryptosporidial infections are typically seen predominantly during the warm or wet season in tropical developing areas, when increased rainfall presumably results in greater spread of contaminated surface water used for drinking [10], an effect that is not seen with other enteric protozoa like microsporidia, for example [65]. Bern et al. [66] also found that *Cryptosporidium* infections were most common in the rainy season in Guatemala (while *Cyclospora* peaked in the warmer months, and tended to affect older children, and was more associated with diarrhea). Others, however, do not see a distinct association with the rainy season and see *Cyclospora* in cooler months (in Haiti) [67].

Another risk factor appears to be weaning, especially at a young age, suggesting that breast feeding provides some protection, whether by protective factors like immunoglobulin or simply by a safer source of primary sustenance in vulnerable young infants [68–71].

Other major risk factors include consumption of potentially contaminated water, lower household income and older age [72]. In addition, among homosexual and bisexual HIV-infected men, seropositivity was related to spa and sauna exposure and to anal sexual exposure, albeit not to CD-4 lymphocyte counts (while symptoms are highly correlated with low CD-4 counts) [73].

6. Impact and consequences

Without question, cryptosporidiosis constitutes a leading cause of persistent diarrhea in tropical, developing areas, both among children, in whom it often signals a period of increased diarrhea burden or nutrition shortfalls [14,15,74] as well as in patients with AIDS, in whom it can be devastating and often fatal [30,65,75].

In addition, however, the impact and consequences of cryptosporidiosis may well be far greater than generally appreciated, because of the lasting impairments in growth and development that may follow, especially with early childhood infections in impoverished areas, and possibly with malabsorption of key drugs such as antiretroviral or antituberculous drugs (Brantley, Silva, Lima, Guerrant, unpublished observations). Checkley et al. [11] reported impaired weight gain in the month following symptomatic or *even asymptomatic* cryptosporidial infections in children in Peru, where malnutrition did not appear to be a significant risk factor for infection. In a follow-up study of 185 children, those with cryptosporidial infection experienced several months of weight and height faltering, an effect that in young children (i.e. <6 months) or stunted children persisted (with about 1 cm growth shortfall) at 1 year after infection [12]; i.e. young and stunted children did not experience "catch-up" growth after their cryptosporidial infections.

Furthermore, we are now learning that the 4–8 dehydrating, malnourishing diarrheal illnesses that often occur each year in the critically formative first 2 years of life may have profound, lasting consequences for impaired fitness, growth, cognitive development and school performance several years later. Our initial studies in Northeast Brazil show reduced fitness 4–6 years later associated with early childhood diarrhea, and specifically with cryptosporidial infections in the first 2 years of life, *independent* of respiratory illnesses, anthropometry, anemia and intestinal helminths [76]. The fitness deficits that associate with the average diarrhea burdens in the first 2 years of life in these studies are comparable to that associated with a 17% decrement in work productivity in Zimbabwe sugar cane workers [76,77].

Furthermore, these early childhood diarrheal illnesses and intestinal helminthic infections in the first 2 years of life independently and additively associate with substantial long-term linear growth shortfalls that continue beyond 6 years of age (totaling an average of 8.2 cm (3 and 1/4 in.) growth shortfall at 7 years of age) [78]. We also find significant associations of early childhood diarrhea with long-term cognitive deficits (by standard "Test of Nonverbal Intelligence" or TONI) even when controlling for maternal education, breast-feeding duration and early helminthic infections [76,79]. Furthermore, WISC (Wechsler Intelligence Scale for Children; The Psychological Corp, San Antonio, TX) coding and digit span scores were lower in children with persistent diarrheal illnesses in their first 2 years of life, even when controlling for helminths and maternal education [79]. Finally, these effects are seen in a "best case" scenario in which we have documented substantial improvements in disease rates and in nutritional status over the several years in which we have conducted close, long-term surveillance of this population [78], effects that we have subsequently *not* found in other nearby shantytown communities that had not been under such intensive surveillance (Lima, Guerrant et al., unpublished observations).

These correlations of early childhood diarrhea with fitness, growth and cognitive deficits are also extending to school performance, with significant associations of diarrhea in the first 2 years of life with delayed age at starting school (late starters are also twofold more likely to have experienced cryptosporidial infections) and age for grade (the latter independent of height for age *Z* scores at 2 years of age) ([80]; R.L. Guerrant et al, trends Parasitol. 18 (2002) 191–193).

Consistent with these lasting effects of early childhood illnesses (or infections, without overt illness, with *Cryptosporidium*) are studies of the importance of early childhood years in human brain development [79,81–83]. The major brain growth and synapse formation occurs during the first 2 years of life in humans. If impaired at this critical formative stage, these synapses may never form or may be substantially delayed, thus potentially explaining why these early childhood infections may have such a lasting impact.

7. *Prevention of spread*

Special attention to care in the treatment of drinking water can prevent most cases of cryptosporidiosis. This is especially important for immunocompromised individuals in whom it can be devastating and life threatening. Probably the surest way to inactivate viable cryptosporidial oocysts is with heat (to 72 °C for 1 min or simply bring water to a boil) [84]. Water filters that limit to <1 μm and reverse osmosis filters are usually effective, but may fail [85]. As noted above, chlorine and iodine treatment is usually ineffective, and even the more rigorous water treatment standards (to <0.5 NTU) may not be fully protective [30].

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Policy Analysis

Regional Public Health Cost Estimates of Contaminated Coastal Waters: A Case Study of Gastroenteritis at Southern California Beaches

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Regional Public Health Cost Estimates of Contaminated Coastal Waters: A Case Study of Gastroenteritis at Southern California Beaches

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We present estimates of annual public health impacts, both illnesses and cost of illness, attributable to excess gastrointestinal illnesses caused by swimming in contaminated coastal waters at beaches in southern California. Beach-specific enterococci densities are used as inputs to two epidemiological dose−response models to predict the risk of gastrointestinal illness at 28 beaches spanning 160 km of coastline in Los Angeles and Orange Counties. We use attendance data along with the health cost of gastrointestinal illness to estimate the number of illnesses among swimmers and their likely economic impact. We estimate that between 627,800 and 1,479,200 excess gastrointestinal illnesses occur at beaches in Los Angeles and Orange Counties each year. Using a conservative health cost of gastroenteritis, this corresponds to an annual economic loss of $21 or $51 million depending upon the underlying epidemiological model used (in year 2000 dollars). Results demonstrate that improving coastal water quality could result in a reduction of gastrointestinal illnesses locally and a concurrent savings in expenditures on related health care costs.

Introduction

Each year between 150 million and nearly 400 million visits are made to California (CA) beaches generating billions of dollars in expenditures, by tourists and local swimmers, and nonmarket values enjoyed mostly by local area residents (*1*, *2*). Nonmarket benefits represent the value society places on resources, such as beaches, beyond what people have to pay to enjoy these resources (see Pendleton and Kildow (*1*) for a review of the nonmarket value of CA beaches). In an effort to protect the health of beach swimmers, the CA State Legislature passed Assembly Bill 411 (AB411) in 1997 with formal guidance and regulations for beach water quality which are formally codified as a state statute (*3*). AB411 requires monitoring of bathing waters for fecal indicator bacteria (FIB, including total coliform (TC), fecal coliform (FC), and enterococcci (ENT)) on at least a weekly basis during the dry season (1 April through 31 October) if the beach is visited by over 50,000 people annually or is located adjacent to a flowing storm drain. Beaches can be posted with health warnings if single-sample or geometric mean standards for TC, FC, and ENT exceed prescribed levels (see Supporting Information (SI) for standards).

Based on AB411 water quality criteria and their professional judgment, CA county health officials posted or closed beaches 3,985 days during 2004 (*4*). Sixty percent (2,408 beach-days) of these occurred at Los Angeles and Orange County (LAOC) beaches (*4*), and nearly all (93%) of the LAOC advisories and closures were caused by unknown sources of FIB. The number of beach closures and advisories in CA (and the country as a whole) rises each year as counties monitor more beaches (*4*). Needless to say, public awareness of coastal contamination issues is growing, and in some cases strongly influencing the development of programs to improve coastal water quality. For example, public pressure on the Orange County Sanitation District (OCSD) prevented them from reapplying for a waiver from the USEPA to release partially treated sewage to the coastal ocean. Instead, OCSD plans to implement a costly upgrade to their sewage treatment plant. New stormwater permits issued by CA Regional Water Boards require counties and municipalities to implement prevention and control programs to meet coastal water quality criteria. The cost of such mitigation measures is difficult to determine, yet cost has been used as an argument in court challenges to the permits (*4*). In 2004 elections, voters in the city of Los Angeles approved a measure to spend $500 million on stormwater mitigation (*5*).

To understand the potential public health benefits of cleaning up coastal waters, we need a better idea of the magnitude of health costs associated with illnesses that are due to coastal water contamination. Several previous studies address the potential economic impacts of swimming-related illnesses. Rabinovici et al. (*6*) and Hou et al. (*7*) focused on the economic and policy implications of varying beach closure and advisory policies at Lake Michigan and Huntington Beach, CA, respectively. Dwight et al. (*8*) estimated the per case medical costs associated with illnesses at two beaches in southern California and used this to make estimates of public health costs at two Orange County beaches. Our study is novel in that it provides the first regional estimates of the public health costs of coastal water quality impairment.

While many different illnesses are associated with swimming in contaminated marine waters, we focus our analysis on gastrointestinal illness (GI) because this is the most frequent adverse health outcome associated with exposure to FIB in coastal waters (*9*, *10*). We estimate daily excess GI based on attendance data, beach-specific water quality monitoring data, and two separate epidemiological models developed by Kay et al. (*11*) and Cabelli et al. (*12*) that model GI based on exposure to fecal streptococci and ENT, respectively. Finally, we provide estimates of the potential annual economic impact of GI associated with swimming at study beaches.

We conduct our analysis using data from 28 LAOC beaches during the year 2000. Together, these beaches span 160 km of coastline (Figure 1, Table S1). We limit our analysis to these beaches and the year 2000 in particular because we were able to obtain relatively complete daily and weekly attendance and water quality data for these beaches during

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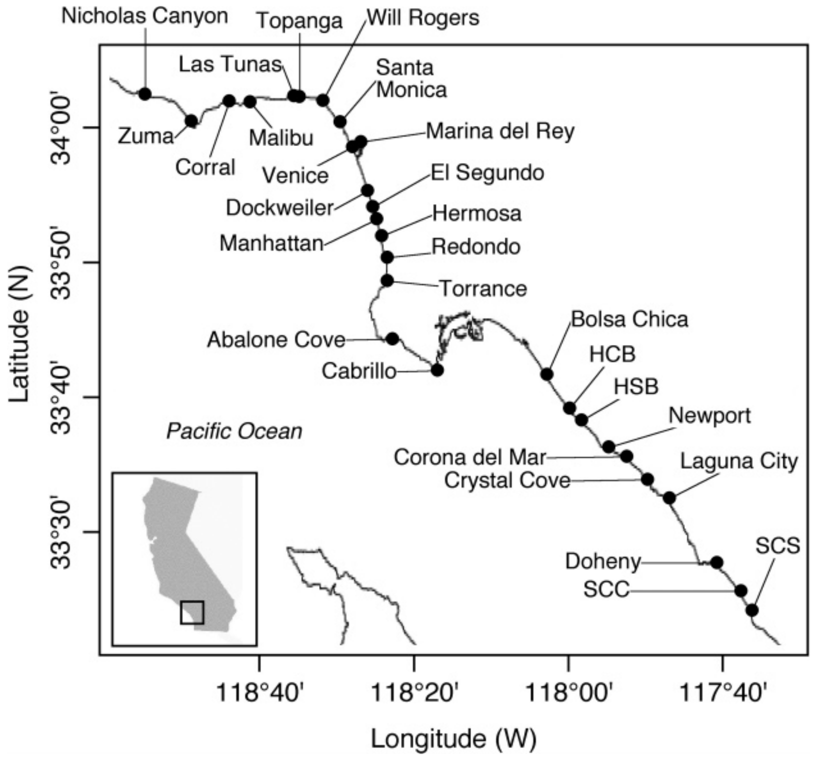

FIGURE 1. The 28 beaches considered in this study. HSB = Huntington State Beach, HCB= Huntington City Beach, SCC = San Clemente City Beach, and SCS = San Clemente State Beach.

this year. The 28 beaches represent a large, but incomplete, subset of the total beach shoreline in LAOC. Large stretches of relatively inaccessible beaches (e.g., portions of Laguna Beach, much of Malibu, and Broad Beach) were omitted from the analysis as were several large public beaches (e.g., Seal Beach and Long Beach) because of paucity of attendance and/or water quality data. The 1999−2000 and 2000−2001 winter rainy seasons were typical for southern CA (*13*), so 2000 was not particularly unique with respect to rainfall. A comparison of inter-annual water quality at a subset of beaches suggests that pollution levels in 2000 were moderate (data not shown). Thus, the estimates we provide can be viewed as typical for the region.

Methods

Number of Swimmers. Morton and Pendleton (*2*) compiled daily attendance data from lifeguards' records and beach management agencies. When data were missing, attendance was estimated using corresponding monthly median weekday or weekend values from previous years. (Table S1 shows the number of days in 2000 when data are available—for most beaches, this number approaches 366.) Because these data are based on actual counts, we do not need to factor in effects due to the issuance of advisories at a particular beach. Only a fraction of beach visitors enter the water. This fraction varies by month in southern CA from 9.56 to 43.62% (Table S2) (*14*). We applied the appropriate fraction to the attendance data to determine the number of individual swimmers exposed to coastal waters. Although research suggests the presence of FIB in sand in the study area (*15, 16*), we do not consider the potential health risk that may arise from sand exposure because it has not been evaluated.

Water Quality Data. ENT data were obtained from the local monitoring agencies and are publicly available. Local monitoring agencies sample coastal waters at ankle depth in the early morning in sterile containers. Samples are returned to the lab and analyzed for ENT using USEPA methods. When ENT values are reported as being below or above the detection limit of the ENT assay, we assume that ENT densities were equal to the detection limit.

During 2000, monitoring rarely occurred on a daily basis; ENT densities were measured 14−100% of the 366 days in 2000, depending on monitoring site (Table S1). For example, Zuma beach was monitored once per week during the study period, while Cabrillo beach was monitored daily. To estimate ENT densities on unsampled days, we used a Monte Carlo technique. Normalized cumulative frequency distributions of observed ENT densities at each monitoring site were constructed for the 1999−2000 wet season (Nov 1, 1999 through Mar 31, 2000), 2000 dry season (April 1, 2000 through Oct 31, 2000), and the 2000−2001 wet season (Nov 1, 2000 through Mar 31, 2001). ENT densities on unsampled days during 2000 were estimated by randomly sampling from the appropriate seasonal distribution. Because day-to-day ENT concentrations at marine beaches are weakly correlated and variable (*17*), we chose not to follow the estimation method of Turbow et al. (*18*) who assumed a linear relationship between day-to-day ENT densities at two CA beaches. Comparisons between the Monte Carlo method and a method that simply used the monthly arithmetic average ENT density indicated the two provided similar results (data not shown).

The beaches in our study area (Figure 1) are of variable sizes; each beach may include 1−7 monitoring sites (Table S1). If more than one monitoring site exists within the boundaries of a beach, the arithmetic mean of ENT at the sites was used as a single estimate for ENT concentrations within the beach (*19*). There is considerable evidence that ENT densities at a beach vary rapidly over as little as 10 minutes (*17, 20*). Therefore, even though we used up to 7 measurements or estimates to determine ENT at a beach on a given day, there is still uncertainty associated with our estimate because sampling is conducted at a single time each day.

Dose−Response. Of all the illnesses considered in the literature, GI is most commonly associated with exposure to polluted water (*10−12, 21−26*). To estimate the risk of GI

TABLE 1. Dose−Response Models for Predicting GI[a]

name	original model	model converted to excess risk
model C (*12*)	$1000(P - P_o) = 24.2 \log_{10}(\text{ENT}) - 5.1$	$(P - P_0) = (24.2 \log_{10}(\text{ENT}) - 5.1)/1000$
model K (*11*)	$X = \text{Ln}(P/(1 - P)) = 0.201\ (\text{FS} - 32)^{1/2} - 2.36$	$(P - P_0) = (e^X/(1 + e^X)) - P_0$

[a] ENT = enterococci, FS = fecal streptococci. Both ENT and FS are in units of CFU or MPN per 100 mL water. P is the risk of GI for swimmers, P_0 is the background risk of GI.

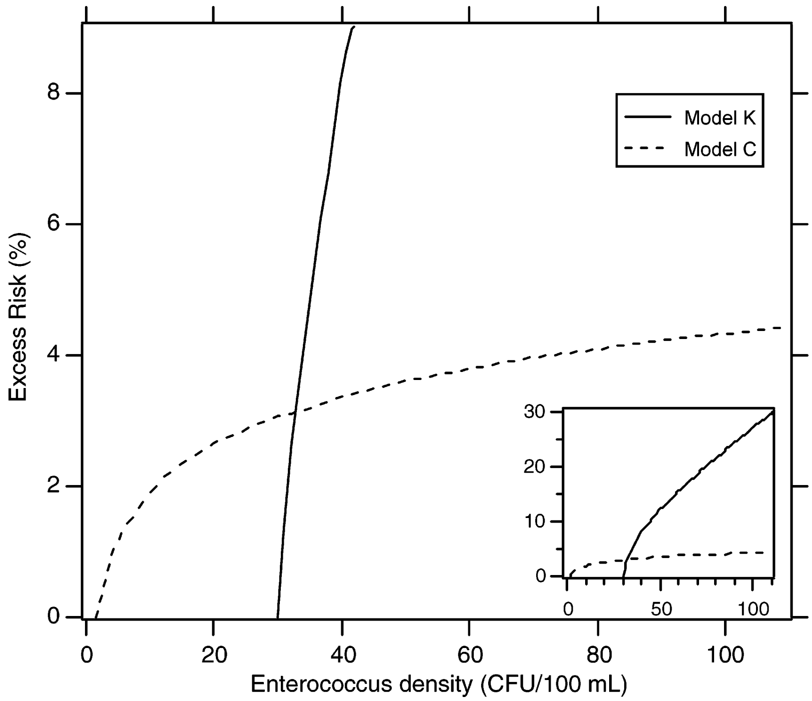

FIGURE 2. Dose−response relationships for the two epidemiological models. Excess risk of GI is shown as a function of ENT density. The inset more clearly shows the differences between the relationship for the randomized trial study (model K (*11*)) and the cohort study (model C (*12*)).

from swimming in contaminated marine waters in southern CA, we utilized two dose−response models (*11*, *12*) (Table 1) developed in epidemiology studies conducted elsewhere (in marine waters of the East U.S. coast and United Kingdom) (*18*, *27*). A local dose−response model for GI would be preferable, but does not exist. Haile et al. (*28*) conducted an epidemiology study at Los Angeles beaches and found that skin rash, eye and ear infections, significant respiratory disease, and GI were associated with swimming in waters with elevated FIB or near storm drains; however, they did not report dose−response models for illness and bacterial densities.

The two dose−response models (hereafter referred to as models C (*12*) and K (*11*)) are fundamentally different in that model C was derived from a prospective cohort study while model K was developed using a randomized trial study. Model C has been scrutinized in the literature (*20, 26, 29−31*). Among the criticisms are lack of ENT measurement precision and inappropriate pooling of data from marine and brackish waters. World Health Organization (WHO) experts (*10*) suggest that epidemiology studies that apply a randomized trial design, such as model K, offer a more precise dose−response relationship because they allow for better control over confounding variables and exposure (*26*). Thus, the WHO has embraced model K over cohort studies such as model C for assessing risk. We report GI estimates obtained from both models C and K in our study because they have both been applied in the literature (*8*, *18*), and form the basis for water quality criteria worldwide.

Models C and K were developed in waters suspected to be polluted with wastewater. The source of pollution at our study site during the dry season is largely unknown (*4*), although human viruses have been identified in LAOC coastal creeks and rivers (*32−36*) and an ENT source tracking study at one beach suggests sewage is a source (*37*). During the wet season, stormwater is a major source of FIB to coastal waters and Ahn et al. (*38*) detected human viruses in LAOC stormwater. Because we cannot confirm that all the ENT at our study site was from wastewater, there may be errors associated with the application of models C and K. In addition, there is evidence that dose−response relationships may be site specific (*30*). The results presented in our study should be interpreted in light of these limitations.

We converted incidence and odds, the dependent variables reported for model C and K, respectively, into risk of GI (P) (Table 1). P represents total risk of GI to the swimmer, and includes risk due to water exposure plus the background GI rate (P_0). Excess risk was calculated by subtracting the background risk from risk ($P - P_0$). While ENT is the independent variable for model C, model K requires fecal streptococci (FS), the larger bacterial group of which ENT are a subset, as the independent variable. We assumed that FS and ENT represent the same bacteria, following guidance from the WHO (*9*).

Models C and K provide different functional relationships between ENT and excess GI risk (Figure 2). Model C predicts relatively low, constant risks across moderate to high ENT densities relative to model K. At ENT less than 32 CFU/100 mL, model K predicts no excess risk; model C, however, does predict nonzero risks even at these low levels of contamination. The data range upon which each model was built varies considerably. Model C is based on measurements ranging from 1.2−711 CFU/100 mL and model K is based on measurements from 0−35 to 158 CFU/100 mL. We extrapolated models C and K when ENT densities were outside the epidemiology study data ranges. Given the lack of epidemiological data on illlness outside the ranges, extrapolation of the models represents a reasonable method of estimating excess GI.

Excess Illness Due To Swimming. The excess incidence of GI on day i at beach j ($GI_{i,j}$) is given by the following expression:

$$GI_{i,j} = A_{i,j}f_i(P_{i,j} - P_o) \quad (1)$$

$P_{i,j} - P_o$ is the excess risk of GI on day i at beach j as estimated from models C or K (Table 1), $A_{i,j}$ is the number of beach visitors, and f_i is the fraction of swimmers on day i (*14*). We assume P_0 is 0.06—the background risk for stomach pain as reported by Haile et al. (*28*) for beaches within Santa Monica Bay, CA. Daily values were summed across the year or season to estimate the number of excess GI per beach. Seasonal comparisons are useful in this region because of distinct differences between attendance and water quality between seasons. The wet season is defined as November through March and the dry season is defined as April through October. Note that the dry season corresponds to the season when state law mandates beach monitoring (*3*).

Public Health Costs of Coastal Water Pollution. GI can result in loss of time at work, a visit to the doctor, expenditures on medicine, and even significant nonmarket impacts that represent the "willingness-to-pay" of swimmers to avoid getting sick (sometimes referred to as psychic costs). Because there is a lack of information on the costs of waterborne GI, Rabinovici et al. (*6*) used the cost of a case of food-borne GI, $280 (year 2000 dollars) per illness from Mauskopf and French (*39*), as a proxy for the cost of water-borne GI for swimmers in the Great Lakes. The $280 per illness represents the willingness-to-pay to avoid GI and includes both market and nonmarket costs (*6*). Dwight et al. (*8*) conducted a cost of illness study for water-borne GI for two beaches in southern California (Huntington State Beach and Newport Beach) and determined the cost as $36.58 per illness in 2004 dollars based on lost work and medical costs. Discounting for inflation, this amount is equivalent to $33.35 in the year 2000 dollars. This value does not include lost recreational values or the willingness-to-pay to avoid getting sick from swimming. We use the more conservative estimate of Dwight et al. (*8*) to calculate the health costs of excess GI at LAOC beaches. However, we also provide more inclusive estimates of the cost of illness using Mauskopf and French's $280 willingness-to-pay value (*39*). Unless otherwise stated, all costs are reported in year 2000 dollars.

Results

Attendance and Swimmers. Beach attendance was higher during the dry season (from May through October) than in the wet season (November through April) (Figure 3). We estimate that the annual visitation to Los Angeles and Orange County (LAOC) beaches for the year 2000 approached 80 million visits.

Water Quality. Water quality (measured in terms of ENT concentration) varies widely across the beaches in the study. (Figure S1 shows the log-mean of ENT observations at each beach during the dry and wet seasons.) In general ENT densities are higher during the wet season compared to the dry. Water quality problems at a beach may exist chronically over the course of the year or may be confined to particularly wet days when precipitation washes bacteria into storm drains and into the sea. The most serious, acute water quality impairments can result in the issuance of a beach advisory or beach closure. According to CA state law, water quality

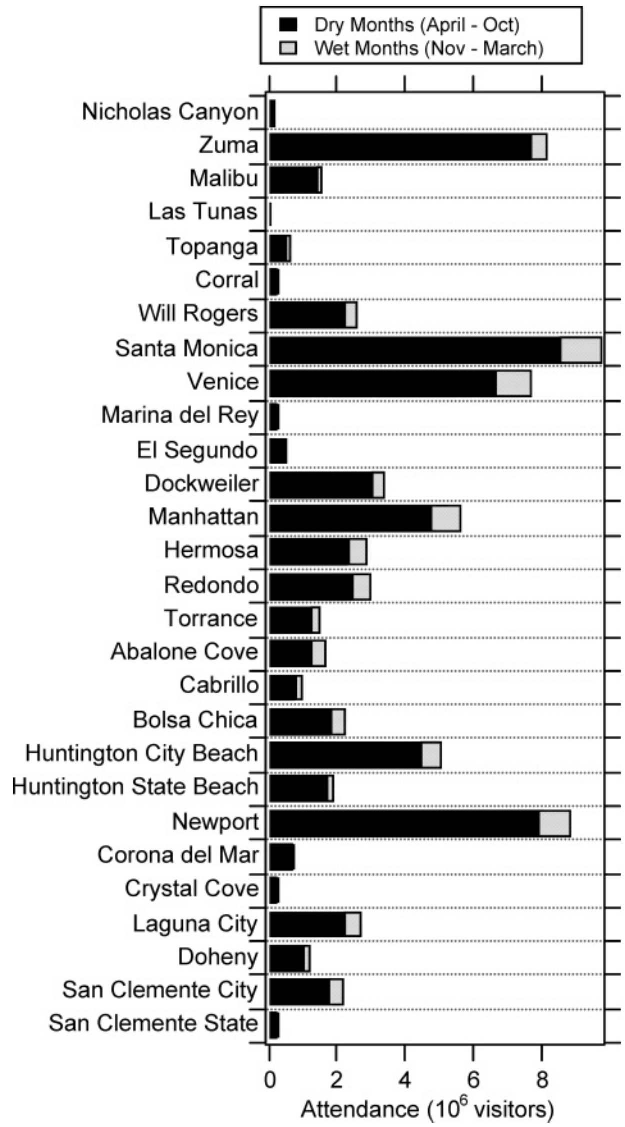

FIGURE 3. Beach attendance during wet and dry seasons 2000.

exceeds safe levels for swimming if a single beach water sample has a concentration of ENT greater than 104 CFU/100 mL. Figure 4 illustrates the percentage of the days for which daily estimated ENT concentrations were in excess of the state single sample standard. Exceedances during the wet months generally outnumber exceedances during the dry months. The exceptions are Corral, Bolsa Chica, and Crystal Cove, which are all relatively clean beaches, even in the wet season. Doheny, Malibu, Marina Del Rey, Cabrillo, and Las Tunas had the worst water quality with over 33% of the daily estimates in 2000 greater than 104 CFU/100 mL, while Newport, Hermosa, Abalone Cove, Manhattan, Torrance, and Bolsa Chica had the best water quality with less than 5% of daily estimates under the standard.

Estimates of Excess GI and Associated Public Health Costs due to Swimming. Figure 5 illustrates estimated annual excess GI at beaches based on models C and K; results are given for dry and wet months. Models C and K both indicate that Santa Monica, the beach with the highest attendance (Figure 3), has the highest excess GI of all beaches during wet and dry seasons. Both models predict that the three beaches with the lowest excess GI were San Clemente State, Nicholas Canyon, and Las Tunas, a direct result of these beaches being among the smallest and least visited in our study area (Figure 3).

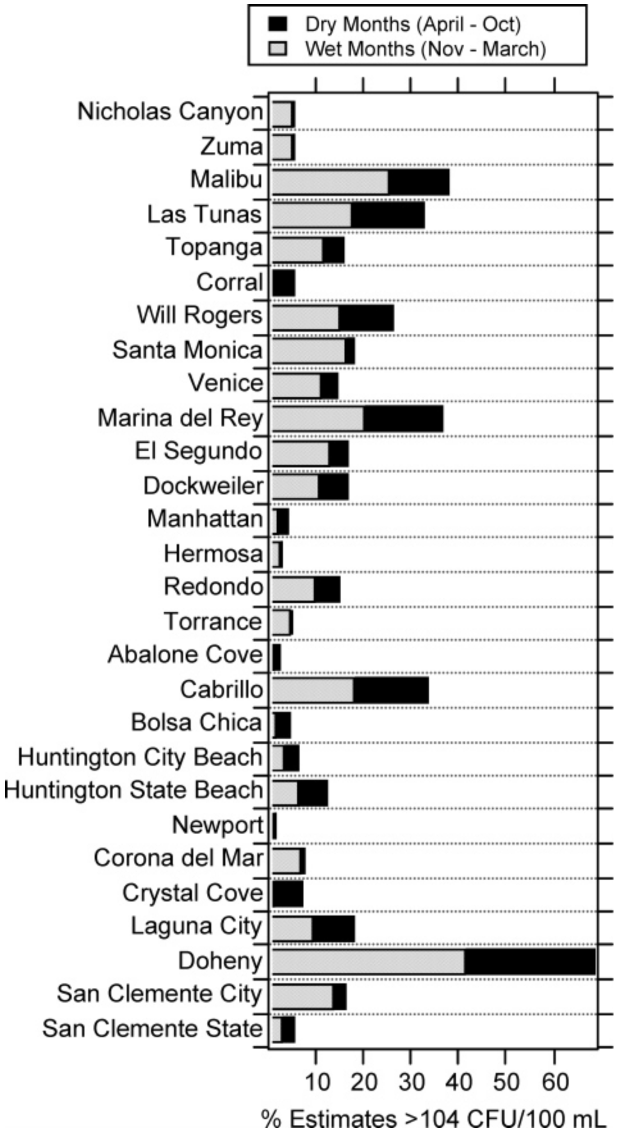

FIGURE 4. Percentage of days on which daily ENT estimates were greater than the CA Department of Health single-sample ENT standard of 104 CFU/100 mL.

There are marked seasonal differences between excess GI predictions. Although water quality is typically worse during the wet season compared to the dry (Figures 4 and S1), more excess GI are predicted for the dry season for most beaches. This result is driven by seasonal variation in attendance (Figure 3). The exceptions are model K predictions for Zuma that indicate 0 and 6647 excess GI during the dry and wet seasons, respectively. Zuma had no ENT densities greater than 32 CFU/100 mL during the dry season, hence the prediction of 0 excess GI.

Numerical predictions of excess GI for the entire year from model C and model K vary markedly between beaches. At 24 beaches, model K predicts between 18% and 700% greater excess GI than model C. The greatest difference in the estimated GI is at Doheny beach where models C and K predict 18,000 and 153,000 excess GI, respectively. At 4 beaches (Zuma, Hermosa, Torrance, and Newport), model K predicts between 1 and 90% lower incidence of GI than model C. These beaches are generally clean with ENT densities below the model K threshold of 32 CFU/100 mL for excess risk.

The public health burden of coastal contamination depends on both attendance and water quality. Figure 6 illustrates how excess GI, based on predictions from models C and K, varies as a function of water quality (percent of daily ENT estimates in exceedance of standard) and attendance. Red, yellow, and green symbols indicate beaches with increasing numbers of GI. If reduction of public health burden is a goal of local health care agencies, then beaches with a red symbol are candidates for immediate action. Nearly all beaches are categorized as high priority during the dry season based on model K (panels A and B). Model C indicates that dry weather mitigation measures at Venice, Zuma, Santa Monica, and Newport, some of the most visited beaches, would significantly reduce the public health burden (panel C), more so than wet weather mitigation measures (panel D).

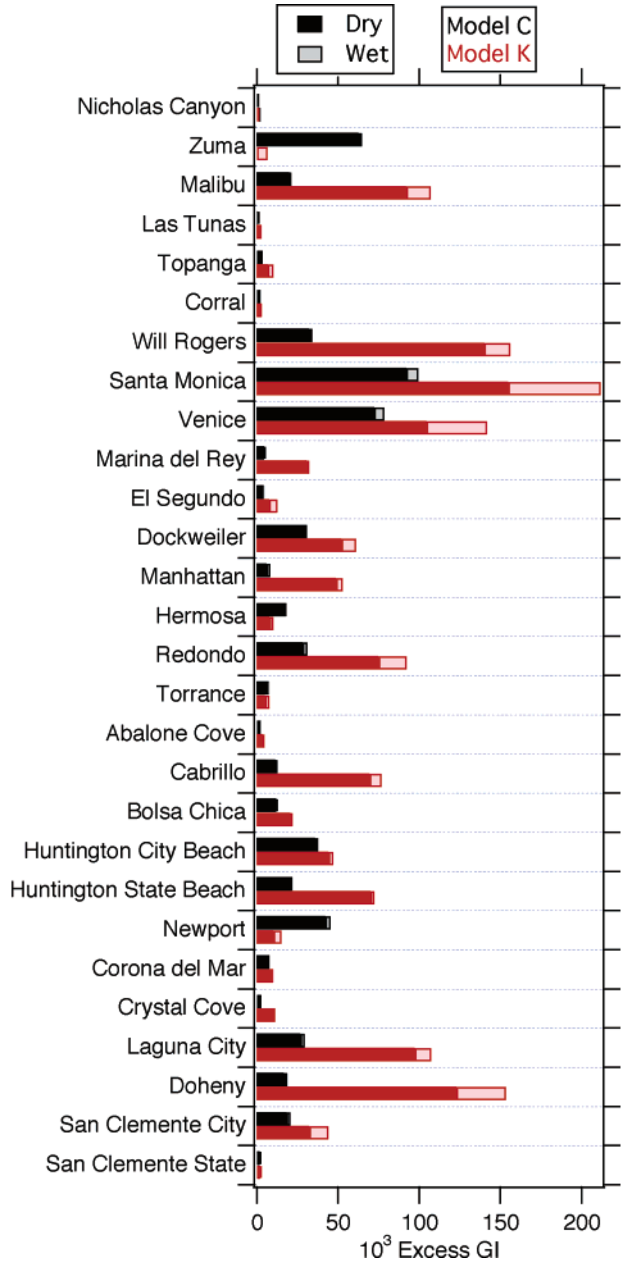

FIGURE 5. Excess GI by beach and season for models C and K.

Another way of prioritizing beach remediation is to examine the risk of GI relative to the USEPA guideline of 19 illnesses per 1000 swimmers (Figure S2). Model K indicates that at 19 and 15 of the 28 LAOC beaches during the wet and dry seasons, respectively, risk is greater than twice the EPA acceptable risk. Model C, on the other hand, indicates that only two beaches (Marina del Rey and Doheny) during the

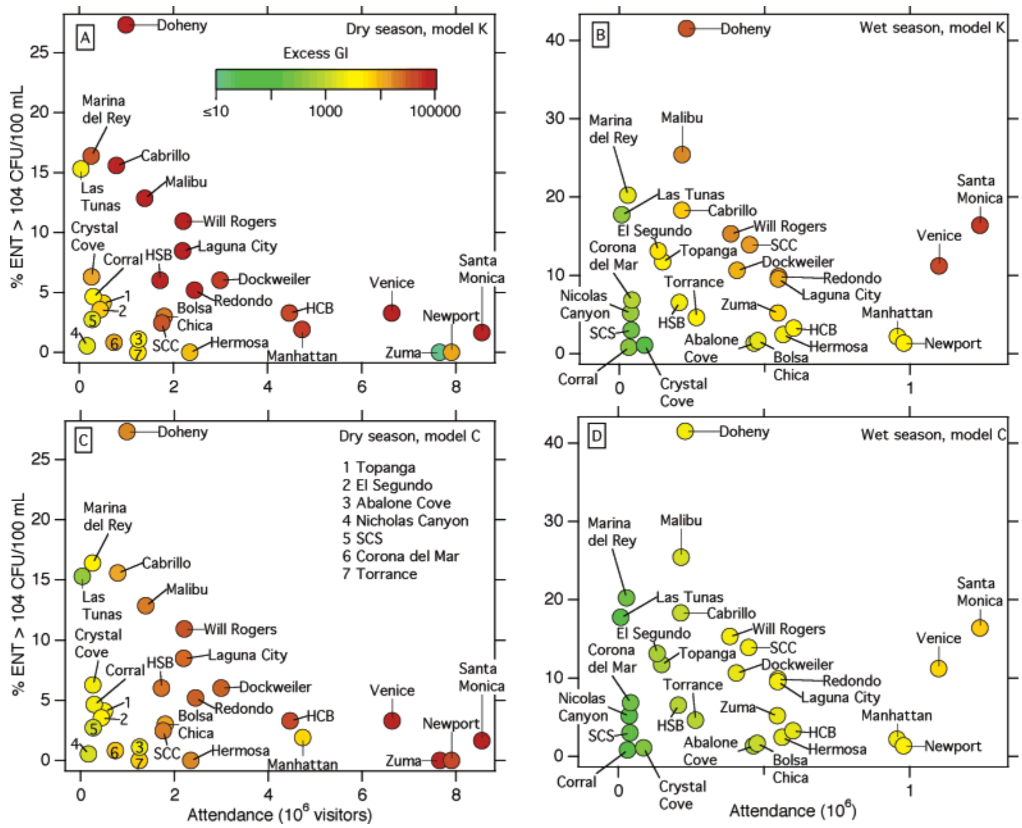

FIGURE 6. Excess GI at each beach as a function of % ENT in exceedance of the single sample standard and attendance. Results for the dry (panels A and C) and wet (panels B and D) seasons are shown for Models K (panels A and B) and C (panels C and D). Beaches are labeled; SCC is San Clemente City Beach, SCS is San Clemente State, HSB is Huntington State Beach, and HCB is Huntington City Beach. In panels A and C, numbers on symbols correspond to beaches, as indicated in the upper right corner of panel C. The color scale in panel A applies to all panels.

TABLE 2. Countywide Public Health Impacts and Costs for Wet and Dry Months (2000)

county/ region	season	GI cases		health costs	
		model C	model K	model C	model K
Los Angeles					
	dry	394,000	804,000	$13,100,000	$28,800,000
	wet	33,800	189,000	$1,130,000	$6,310,000
	total	427,800	993,000	$14,230,000	$35,110,000
Orange					
	dry	185,000	420,000	$6,180,000	$14,000,000
	wet	15,000	66,200	$500,000	$2,210,000
	total	200,000	486,200	$6,680,000	$16,210,000
region total					
	dry	579,000	1,224,000	$19,280,000	$40,800,000
	wet	48,800	255,200	$1,630,000	$8,520,000
	total	627,800	1,479,200	$20,910,000	$51,320,000

dry season, and six (Marina del Rey, Doheny, Santa Monica, Las Tunas, Will Rogers, and Malibu) in the wet season fall into this "high" risk category.

Public Health Costs of Coastal Water Pollution. Table 2 summarizes the number of excess GI and associated public health costs during wet and dry periods by county and season. Based on the conservative cost of illness given by Dwight et al. (*8*), the estimated health costs of GI based on models C and K is over $21 million and $50 million, respectively. If we follow Rabinovici et al. (*6*) and use $280 per GI, the estimated public health impacts are $176 million based on model C and $414 million based on model K. For both LA and OC beaches, county-wide costs obtained using model K yield higher results than those obtained from model C, a direct result of the difference in GI estimates (Figures 5 and 6). Health costs are greater in the dry season compared to the wet suggesting that money may be well spent on dry-weather diversions.

Discussion

A significant public health burden, in terms of both numbers of GI and the costs of GI, is likely to result from beach water quality contamination in southern CA. The corollary to this finding is that water quality improvements in the region would result in public health benefits. Specifically, we make three key findings: (1) removing fecal contamination from coastal water in LAOC beaches could result in the prevention of between 627,800 and 1,479,200 GI and a public health cost of between $21 and $51 million (depending upon the epidemiological model used) each year in the region using the most conservative cost estimates and as much as $176 million or $414 million if we use the larger estimate of health costs (*6*, *39*); (2) even beaches within the same region differ significantly in the degree to which swimming poses a public health impact; and (3) public health risks differ between seasons. Findings (2) and (3) are not surprising given spatio–temporal variation in water quality (*17*, *40*) and attendance within the study site.

A previous study by Turbow et al. (*18*) estimated 36,778 excess HCGI (highly credible GI) per year from swimming at Newport and Huntington State beaches (*8*). Our estimates for the same stretch of shoreline are higher (68,011 and 87,513 excess GI based on models C and K, respectively). Not only did we use a different measure of illness (GI vs. HCGI) we also used a Monte Carlo scheme to estimate ENT on unsampled days whereas Turbow et al. (*18*) used linear interpolation, and we used higher, empirically determined

(*14*) measures of the percent of beach goers that swim. Dwight et al. (*8*) used Turbow et al.'s (*18*) estimate to determine that the health costs of excess GI at the same beaches were $1.2 million. Our health cost estimates are higher ($2.3 and $2.9 million for models C and K, respectively), due to the higher incidence of ilness predicted by our models.

Beaches with chronic water quality problems are obvious candidates for immediate contamination mitigation. Many beaches in LAOC, however, are relatively clean and meet water quality standards on most days. Clean beaches with moderate to low levels of attendance do not represent a significant public health burden (Figure 6). Nevertheless, public health impacts are still substantial at heavily visited beaches (for instance those with over 6,000,000 visitors per year) even when water quality is good (e.g., Manhattan Beach) (Figure 6). Generally speaking, it will be more difficult to reduce contaminant levels at cleaner beaches. At beaches with high attendance and generally good water quality (like Newport Beach and Zuma), policy managers should continue dry weather source reduction efforts (e.g., education campaigns and watershed management), but should also recognize that the cost of eliminating all beach contamination may outweigh the marginal public health benefits of doing so.

Our estimates of the potential health benefits that might result from removing bacterial contamination from coastal water in LAOC beaches have limitations. First, we focus on a lower bound estimate of the health cost of GI that does not consider the amount a beach goer is willing to pay to avoid getting sick (estimates using higher, but less scientifically conservative estimates also are provided). Second, while we focus on the public health impacts from GI. Exposure to microbial pollution at beaches also increases the chance of suffering from various symptoms and illnesses (*28*, *41*). For instance, Haile et al. (*28*) and Fleisher et al. (*41*) document associations between water quality and respiratory illnesses, acute febrile illness, fever, diarrhea with blood, nausea, and vomiting, and earaches. Third, if the public believes swimming is associated with an increased risk of illness, they may be discouraged from going to the beach, resulting in a loss of beach-related expenditures to local businesses and recreational benefits to swimmers in addition to the loss in health benefits described here. Fourth, we consider GI occurring at a subset of LAOC beaches for which water quality and attendance data were available (Figure 1). Fifth, implicit in our analysis is the assumption that models C and K can be applied to LAOC beaches. Despite these limitations, the results reported here represent the best estimates possible in light of imperfect information. Future studies that establish dose−response relationships for the LAOC region or confirm incidence of swimming GI medically would improve estimates of public health burden and costs.

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Note Added after ASAP Publication

The Model C discussion in the Methods section published ASAP July 15, 2006 has been revised. The corrected version was published July 26, 2006.

Supporting Information Available

Tables S1 and S2, Figures S1 and S2, and the California state water quality standards. This material is available free of charge via the Internet at http://pubs.acs.org.

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The Health Effects of Swimming in Ocean Water Contaminated by Storm Drain Runoff

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Waters adjacent to the County of Los Angeles (CA) receive untreated runoff from a series of storm drains year round. Many other coastal areas face a similar situation. To our knowledge, there has not been a large-scale epidemiologic study of persons who swim in marine waters subject to such runoff. We report here results of a cohort study conducted to investigate this issue. Measures of exposure included distance from the storm drain, selected bacterial indicators (total and fecal coliforms, enterococci, and *Escherichia coli*), and a direct measure of enteric viruses. We found higher risks of a broad range of symptoms, including both upper respiratory and gastrointestinal, for subjects swimming (a) closer to storm drains, (b) in water with high levels of single bacterial indicators and a low ratio of total to fecal coliforms, and (c) in water where enteric viruses were detected. The strength and consistency of the associations we observed across various measures of exposure imply that there may be an increased risk of adverse health outcomes associated with swimming in ocean water that is contaminated with untreated urban runoff. (Epidemiology 1999;10:355–363)

Keywords: environmental epidemiology, gastrointestinal illness, ocean, recreational exposures, sewage, storm drains, waterborne illnesses, waterborne pathogens.

Runoff from a system of storm drains enters the Santa Monica Bay adjacent to Los Angeles County (CA). Even in the dry months of summer 10–25 million gallons of runoff (or non-storm water discharge) per day enter the bay from the storm drain system. Storm drain water is not subject to treatment and is discharged directly into the ocean. Total and fecal coliforms, as well as enterococci, are sometimes elevated in the surf zone adjacent to storm drain outlets; pathogenic human enteric viruses have also been isolated from storm drain effluents, even when levels of all commonly used indicators, including F2 male-specific bacteriophage, were low.[1]

Approximately 50–60 million persons visit Santa Monica Bay beaches annually. Concern about possible adverse health effects due to swimming in the bay has been raised by numerous interested parties.[2] Previous reports indicate that swimming in polluted water (for example, due to sewage) increases risks of numerous adverse health outcomes (Pruss[3] provides a recent review of this literature). To our knowledge, however, there has never been a large epidemiologic study of persons who swim in marine waters contaminated by heavy urban runoff.

These circumstances provided the motivation to study the possible health effects of swimming in the bay. We present here the main results from a large cohort study of people that addressed the issue of adverse health effects of swimming in ocean water subject to untreated urban runoff.

Methods

Design and Subjects

The exposures of interest were distance swimming from storm drains, levels of bacterial indicators (total coli-

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forms, fecal coliforms, enterococcus, *Escherichia coli*) for pathogens that potentially produce acute illness, and human enteric viruses. We studied three beaches located in Santa Monica Bay (CA) that exhibited a wide range of pathogen indicator counts and a high density of swimmers (Santa Monica, Will Rogers, and Surfrider).

Persons who immersed their heads in the ocean water were potential subjects for this study. There was no restriction based on age, sex, or race. We excluded anyone who swam at the study beaches or in heavily polluted areas (that is, Mothers' Beach in Marina del Rey or near the Santa Monica Pier) within 7 days before the study date, or between the date of the beach interview and the telephone follow-up interview. We excluded subjects who swam on multiple days, as one of our primary questions was whether risk of health outcomes was associated with levels of indicator organisms on the specific day a subject entered the water. We targeted persons bathing within 100 yards upcoast or downcoast of the storm drain and persons bathing greater than 400 yards beyond a storm drain.

For this study, 22,085 subjects were interviewed on the beach from June 25 to September 14, 1995, to ascertain eligibility and willingness to participate. We found that 17,253 of these subjects were eligible and able to participate (that is, had a telephone and were able to speak English or Spanish). Of these, 15,492 (90% of the eligible subjects) agreed to participate. They were interviewed about their age, residence, and swimming, particularly immersion of the head into ocean water. The interviewer noted distance from the storm drain (within the categories 0, 1–50, 51–100, or 400 yards), gender, and race of the subject. (Distances from each drain were marked with inconspicuous objects such as beach towels and umbrellas.)

Nine to 14 days after the beach interview, subjects were interviewed by telephone to ascertain the occurrence(s) of: fever, chills, eye discharge, earache, ear discharge, skin rash, infected cuts, nausea, vomiting, diarrhea, diarrhea with blood, stomach pain, coughing, coughing with phlegm, nasal congestion, and sore throat. For this study we defined *a priori* three groupings of symptoms indicative of gastrointestinal illness or respiratory disease. In particular, following Cabelli *et al*,[4] subjects were classified as having highly credible gastrointestinal illness 1 (HCGI 1) if they experienced at least one of the following: (1) vomiting, (2) diarrhea and fever, or (3) stomach pain and fever. We also classified subjects as having highly credible gastrointestinal illness 2 (HCGI 2) if they had vomiting and fever. Finally, we classified subjects as having significant respiratory disease (SRD) if they had one of the following: (1) fever and nasal congestion, (2) fever and sore throat, or (3) coughing with phlegm.

We were able to contact and interview 13,278 subjects (86% follow-up). Of those interviewed, 1,485 were found to be ineligible because they swam (and immersed their heads) at a study beach or in heavily polluted waters between the day of the beach interview and the telephone follow-up. We excluded 107 subjects because they did not confirm immersing their faces in ocean water, leaving 11,686 subjects. One subject had a missing value for age, which we imputed (as the median value among all subjects) for inclusion in the adjusted analyses (discussed below). For the bacteriological analyses, we excluded an additional 1,227 subjects who had missing values, leaving 10,459 subjects. In the virus analyses we included only the 3,554 subjects who swam within 50 yards of the drain on days when viruses were measured (as the samples were collected only at the storm drain).

Collection and Analysis of Samples for Bacterial Indicators

Samples were collected on days that subjects were interviewed on the beaches. Each day, ankle depth samples were collected from each location (0 yards, 100 yards upcoast and downcoast of the drain, and one sample at 400 yards). One duplicate sample per site was collected daily. Samples were collected in sterile 1 liter polypropylene bottles and transferred on ice to the microbiology laboratory. All samples were analyzed for total coliforms, fecal coliforms, enterococcus, and *E. coli*. Densities of total and fecal coliforms and enterococci were determined using the appropriate membrane filtration techniques in Ref 5. *E. coli* densities were determined by membrane filtration using Hach Method 10029 for m-ColiBlue24 Broth.

Collection and Analysis of Samples for Enteric Viruses

For looking at enteric viruses, we collected samples from the three storm drain sites on Fridays, Saturdays, and Sundays, using Method 9510 C g of Ref 5. Ambient pH, temperature, conductivity, and total dissolved solids were measured. Samples as large as 100 gallons chosen to minimize the impacts of seawater dilution were filtered through electropositive filters at ambient pH. Adsorption filters were eluted in the field with 1 liter of sterile 3% beef extract adjusted to pH 9.0 with sodium hydroxide. Field eluates were reconcentrated in the laboratory using an organic reflocculation procedure.[6] All final concentrates were detoxified before analysis.[7]

All samples were analyzed for infectious human enteric viruses in Buffalo green monkey kidney cells (BGMK) by the plaque assay technique. Ten percent of the final concentrate was tested in this manner to determine whether there were a quantifiable number of viruses present. The remaining concentrate volume was divided in half and analyzed using the liquid overlay technique known as the cytopathic effect (CPE) assay.[8] The CPE assay generally detects a greater number of viruses than the plaque assay, but it is not quantitative. Flasks that did not exhibit CPE were considered to be negative for detectable infectious virus. We further examined any flask exhibiting CPE by the plaque-forming unit method to confirm the presence of infectious viruses.

STATISTICAL ANALYSIS

Our analysis addressed two main questions. First, are there different risks of specific outcomes among subjects swimming 0, 1–50, 51–100, and 400 or more yards from a storm drain? If pathogens in the storm drain result in increased acute illnesses, one would expect higher risks among swimmers closer to the drain. Second, are risks of specific outcomes associated with levels of specific bacterial indicators or enteric viruses?

To address the second question, we estimated risks arising from exposure to levels within categories defined *a priori* by existing standards or expert consensus. Specifically, for total coliforms we defined categories using 1,000 and 10,000 colony-forming units (cfu) per 100 ml as cutpoints, which are based on the California Code of Regulations (S.7958 in Title 17).[9] For fecal coliforms we created categories using cutpoints of 200 and 400 cfu per 100 ml, which reflect criteria set by the State Water Resources Control Board.[10] For enterococcus we used cutpoints of 35 and 104 cfu per 100 ml of water, which were established by the U.S. Environmental Protection Agency.[11] Finally, categories for *E. coli* were selected in meetings with staff from the Santa Monica Bay Restoration Project (SMBRP), Heal the Bay, and the Los Angeles County Department of Health Services. These meetings resulted in initially selecting categories based on cutpoints of 35 and 70 cfu per 100 ml, and then subsequently adding categories using cutpoints of 160 and 320 cfu per 100 ml; the latter were added because it is believed that *E. coli* comprises about 80% of the fecal coliforms. Using these knowledge-based categories, however, assumes a homogeneous risk between cutpoints. This might not be a reasonable assumption because the adequacy of these cutpoints is unclear, and because a large percentage of the subjects were in a single (that is, the lowest) category. Therefore, we further explored the bacteriological relations using categories defined by deciles.

In addition to considering total and fecal coliforms separately, we investigated the potential effect of the ratio of total to fecal coliforms. Motivation for this arose from our expectation that the risk of adverse health outcomes might be higher when the ratio is smaller, indicating a relatively greater proportion of fecal contamination. We used categories of this ratio defined by a cutpoint of 5 (where 5 corresponds to there being 5 times as much total as fecal coliform in the water). The human enteric virus exposure was reported as a dichotomous (that is, virus detected *vs* not detected) measure.

We first calculated simple descriptive statistics giving the number of subjects with each adverse health outcome who swam (1) at the prespecified distances from the drain or (2) in water with the prespecified levels of pathogens. From these counts we estimated the crude risk associated with each exposure. We then used logistic regression to estimate the adjusted relative risks of each outcome. For each exposure/outcome combination, we fit a separate model. All models adjusted for the potential confounding of: age (three categories: 0–12 years, 13–25 years, >25 years); sex; beach; race (four categories: white, black, Latino/a, and Asian/multiethnic/other); California *vs* out-of-state resident; and concern about potential health hazards at the beach (four categories: not at all, somewhat, a little, and very).

Results

Table 1 presents results for each of the adverse health outcomes by distance swimming from the storm drain. Across all distances, risks ranged from about 0.001 (that is, 1 per 1,000) for diarrhea with blood to about 0.1 for runny nose. The risk of numerous outcomes was higher for people who swam at the drain (0 yards away), in comparison with those who swam 1–50, 51–100, or >400 yards from the drain. In particular, we observed increases in risk for fever, chills, ear discharge, coughing with phlegm, HCGI 2, and SRD. In addition, the risks for eye discharge, earache, sore throat, infected cut, and HCGI 1 were also slightly elevated. A handful of outcomes exhibited small increased risks among swimmers at 1–50 yards (skin rash) or at 51–100 yards (cough, cough with phlegm, runny nose, and sore throat). Adjusted estimates of relative risk (RR) comparing swimmers at 0, 1–50, or 51–100 yards from the drain with swimmers at least 400 yards away from the drain showed similar relations as the aforementioned patterns of risks (Table 1). Among the positive associations for swimmers at the drain, RRs ranged in magnitude from about 1.2 (eye discharge, sore throat, HCGI 1) to 2.3 (earache), with varying degrees of precision; most of these RRs ranged from 1.4 to 1.6.

In Table 2 we see that the risk of skin rash increased for the highest prespecified category of total coliforms (that is, >10,000 cfu). Furthermore, the adjusted RR comparing swimmers exposed at this level *vs* those exposed to levels ≤1,000 cfu was 2.6. Whereas the RR for diarrhea with blood also suggested a positive association, this result was based on a single adverse health event (as evinced by the wide 95% CIs). When looking at deciles, in relation to the lowest exposure level (that is, the lowest 10%), we observed increased risks of skin rash at all other levels (Figure 1). The adjusted RRs ranged from 1.6 to 6.2, with five of the nine RRs in the 2–3 range. In addition, there were increased risks of HCGI 2 for all deciles except one (the eighth); the corresponding adjusted RRs ranged from 1.4 to 4.7, with varying levels of precision (Figure 1).

When looking at fecal coliforms, we again observed among those in the highest category (that is, >400 cfu) an increased risk for skin rash (Table 3). There were also *slight* increased risks for infected cut, runny nose, and diarrhea with blood in the highest category, as well as for nausea, vomiting, coughing, sore throat, and HCGI 2 in the middle category (200–400 cfu). The adjusted RRs also indicated positive associations for these outcomes (Table 3). When we used deciles to categorize subjects, however, in comparison with the lowest decile, we only observed marginal increased risks for infection and skin rash (not shown). In our investigation of the ratio of

TABLE 1. Adverse Health Outcomes by Distance Swimming from Drain: Number Ill, Acute Risks, Adjusted Relative Risk (RR) Estimates and 95% Confidence Intervals (CI)

	Distance from Drain (in Yards)										
	>400 (N = 3030)*		51–100 (N = 3311)			1–50 (N = 4518)			0 (N = 827)		
Outcome	No. Ill	Risk	No. Ill	Risk	RR (95% CI)†	No. Ill	Risk	RR (95% CI)†	No. Ill	Risk	RR (95% CI)†
Fever	138	0.046	158	0.048	1.06 (0.84–1.34)	208	0.046	1.07 (0.85–1.33)	59	0.071	1.61 (1.16–2.24)
Chills	72	0.024	85	0.026	1.07 (0.77–1.47)	108	0.024	1.05 (0.77–1.42)	31	0.037	1.60 (1.03–2.50)
Eye discharge	61	0.020	59	0.018	0.88 (0.61–1.27)	73	0.016	0.77 (0.55–1.09)	19	0.023	1.15 (0.67–1.98)
Earache	116	0.038	116	0.035	0.89 (0.68–1.16)	136	0.030	0.81 (0.63–1.04)	38	0.046	1.34 (0.91–1.98)
Ear discharge	21	0.007	19	0.006	0.78 (0.42–1.46)	25	0.006	0.80 (0.45–1.44)	13	0.016	2.09 (1.01–4.33)
Skin rash	23	0.008	30	0.009	1.16 (0.67–2.01)	53	0.012	1.50 (0.91–2.46)	4	0.005	0.62 (0.21–1.83)
Infected cut	17	0.006	16	0.005	0.79 (0.40–1.58)	37	0.008	1.51 (0.84–2.69)	6	0.007	1.48 (0.57–3.87)
Nausea	133	0.044	115	0.035	0.77 (0.60–1.00)	143	0.032	0.75 (0.59–0.95)	40	0.048	1.13 (0.78–1.65)
Vomiting	57	0.019	58	0.018	0.97 (0.67–1.40)	63	0.014	0.76 (0.53–1.09)	25	0.030	1.40 (0.85–2.31)
Diarrhea	204	0.067	163	0.049	0.70 (0.56–0.86)	202	0.045	0.69 (0.56–0.84)	53	0.064	1.04 (0.75–1.44)
Diarrhea with blood	7	0.002	2	0.001	0.26 (0.05–1.26)	3	0.001	0.27 (0.07–1.06)	2	0.002	0.87 (0.15–4.57)
Stomach pain	206	0.068	194	0.059	0.85 (0.70–1.05)	271	0.060	0.93 (0.77–1.12)	61	0.074	1.11 (0.82–1.51)
Cough	209	0.069	263	0.079	1.18 (0.97–1.42)	296	0.066	0.98 (0.82–1.18)	55	0.067	1.01 (0.73–1.38)
Cough and phlegm	90	0.030	114	0.034	1.16 (0.88–1.54)	143	0.032	1.09 (0.83–1.43)	39	0.047	1.65 (1.11–2.46)
Runny nose	273	0.090	351	0.106	1.18 (1.00–1.40)	371	0.082	0.95 (0.80–1.12)	74	0.089	1.10 (0.84–1.46)
Sore throat	190	0.063	244	0.074	1.17 (0.96–1.43)	304	0.067	1.12 (0.93–1.35)	59	0.071	1.25 (0.92–1.71)
HCGI 1	102	0.034	96	0.029	0.88 (0.66–1.17)	121	0.027	0.84 (0.64–1.10)	35	0.042	1.21 (0.81–1.82)
HCGI 2	26	0.009	28	0.008	1.04 (0.61–1.79)	32	0.007	0.90 (0.53–1.53)	15	0.018	1.64 (0.84–3.21)
Significant respiratory disease	139	0.046	177	0.053	1.18 (0.94–1.49)	205	0.045	1.03 (0.82–1.23)	63	0.076	1.78 (1.29–2.45)

The total number of swimmers in each category is given in parentheses (N). HCGI1, highly credible gastrointestinal illness with vomiting, diarrhea and fever or stomach pain and fever. HCGT2, highly credible gastrointestinal illness with vomiting and fever only. Significant respiratory disease, fever and nasal congestion, fever and sore throat or coughing with phlegm.

* Referent category (RR = 1.0).

† Adjusted for age, sex, beach, race, California *vs* out-of-state resident, and concern about potential health hazards at the beach.

total to fecal coliforms, we observed a consistent pattern of higher risks for diarrhea and HCGI 2 as the ratio category became lower (not shown, but available in Ref 12). Because any effect of this lower ratio should be stronger when there was a higher degree of contamination, indicated by total coliform counts in excess of 1,000 or 5,000 cfu, we then restricted our analysis to subjects swimming in water above these levels. In the first case, increased risks with decreasing cutpoints were observed for nausea, diarrhea, and HCGI 2.[12] When we restricted our investigation to subjects in water in which the total coliforms exceeded 5,000 cfu, we observed

TABLE 2. Adverse Health Outcomes by Total Coliform Levels: Number Ill, Acute Risks, Adjusted Relative Risk (RR) Estimates and 95% Confidence Intervals (CI)

	Total Coliforms (cfu/100ml)							
	≤1,000 (N = 7,574)*		>1,000–10,000 (N = 1,988)			>10,000 (N = 757)		
Outcome	No. Ill	Risk	No. Ill	Risk	RR†	No. Ill	Risk	RR†
Fever	368	0.049	88	0.044	0.92 (0.72–1.17)	42	0.055	1.23 (0.87–1.73)
Chills	193	0.025	51	0.026	1.03 (0.75–1.42)	9	0.012	0.51 (0.26–1.01)
Eye discharge	151	0.020	21	0.011	0.46 (0.29–0.74)	15	0.020	0.81 (0.47–1.41)
Earache	270	0.036	66	0.033	0.96 (0.72–1.27)	21	0.028	0.86 (0.54–1.38)
Ear discharge	51	0.007	15	0.008	1.22 (0.67–2.23)	2	0.003	0.46 (0.11–1.93)
Skin rash	65	0.009	14	0.007	0.75 (0.41–1.36)	19	0.025	2.59 (1.49–4.53)
Infected cut	49	0.006	11	0.006	0.97 (0.49–1.91)	3	0.004	0.82 (0.25–2.72)
Nausea	292	0.039	69	0.035	0.94 (0.72–1.24)	18	0.024	0.71 (0.43–1.16)
Vomiting	137	0.018	34	0.017	0.90 (0.61–1.33)	9	0.012	0.64 (0.32–1.29)
Diarrhea	434	0.057	85	0.043	0.80 (0.63–1.03)	33	0.044	0.95 (0.65–1.39)
Diarrhea with blood	8	0.001	2	0.001	1.08 (0.22–5.35)	1	0.001	1.73 (0.19–15.88)
Stomach pain	487	0.064	125	0.063	1.05 (0.85–1.29)	29	0.038	0.69 (0.47–1.02)
Cough	546	0.072	133	0.067	0.90 (0.73–1.10)	51	0.067	0.94 (0.69–1.28)
Cough and phlegm	267	0.035	58	0.029	0.81 (0.60–1.09)	27	0.036	1.03 (0.68–1.57)
Runny nose	703	0.093	170	0.086	0.93 (0.78–1.12)	67	0.089	1.06 (0.81–1.40)
Sore throat	534	0.071	116	0.058	0.83 (0.67–1.03)	47	0.062	0.95 (0.69–1.30)
HCGI 1	242	0.032	54	0.027	0.84 (0.62–1.14)	17	0.022	0.74 (0.44–1.23)
HCGI 2	72	0.010	16	0.008	0.89 (0.51–1.55)	5	0.007	0.83 (0.32–2.12)
Significant respiratory disease	396	0.052	84	0.042	0.80 (0.62–1.02)	42	0.055	1.11 (0.79–1.55)

The total number of swimmers in each category is given in parentheses (N).

* Referent category (RR = 1.0).

† Adjusted for age, sex, beach, race, California *vs* out-of-state resident, and concern about potential health hazards at the beach.

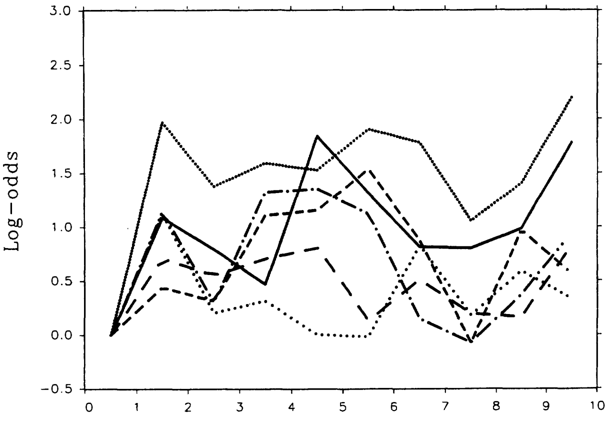

FIGURE 1. Log odds of adverse health outcomes by deciles of exposure for selected bacterial exposures. —, Total coliform and skin rash; - - - ·, total coliform and HCGI 2; · · ·, Enterococci and infected cut; — — —, *E coli* and eye discharge; ···, *E coli* and skin rash; · — ··, *E coli* and infected cut. HCGI 2 = highly credible gastrointestinal illness with vomiting and fever only.

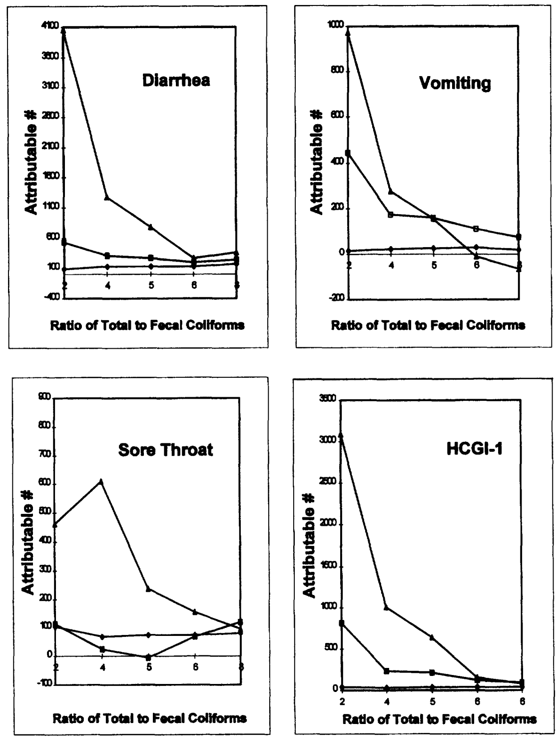

FIGURE 2. Selected attributable numbers/10,000 exposed subjects for total to fecal coliforms. ♦, All days; ■, >1000; ▲, > 5000. HCGI 1 = highly credible gastrointestinal illness with vomiting, diarrhea and fever or stomach pain and fever.

increased risks with eye discharge, ear discharge, skin rash, nausea, diarrhea, stomach pain, nasal congestion, HCGI 1, and HCGI 2.[12] There was a consistent pattern of stronger risk ratios as the cutpoint became lower (when the analyses were restricted to times when total coliforms exceeded 1,000 or 5,000 cfu), with the strongest effects generally observed with the cutpoint of 2, as illustrated in Figure 2 for diarrhea, vomiting, sore throat, and HCGI1.

Table 4 gives results for the relation among enterococci and the adverse health outcomes. Again, we observed an increased risk of skin rash among those in the highest category (that is, >104 cfu). In addition, comparing the highest to other categories of exposure, there

TABLE 3. Adverse Health Outcomes by Fecal Coliform Levels: Number Ill, Acute Risks, Adjusted Relative Risk (RR) Estimates and 95% Confidence Intervals (CI)

	Fecal Coliforms (cfu/100ml)							
	≤200 (N = 8,005)*		>200–400 (N = 768)			>400 (N = 1,636)		
Outcome	No. Ill	Risk	No. Ill	Risk	RR†	No. Ill	Risk	RR†
Fever	381	0.048	39	0.051	1.04 (0.74–1.46)	80	0.049	1.02 (0.80–1.32)
Chills	197	0.025	24	0.031	1.14 (0.74–1.76)	34	0.021	0.78 (0.54–1.14)
Eye discharge	149	0.019	11	0.014	0.70 (0.38–1.31)	30	0.018	0.97 (0.65–1.46)
Earache	275	0.034	26	0.04	0.93 (0.62–1.41)	57	0.035	1.00 (0.75–1.35)
Ear discharge	53	0.007	8	0.010	1.29 (0.60–2.73)	7	0.004	0.56 (0.25–1.24)
Skin rash	69	0.009	5	0.007	0.64 (0.26–1.60)	26	0.016	1.86 (1.17–2.95)
Infected cut	47	0.006	2	0.003	0.40 (0.10–1.65)	15	0.009	1.50 (0.83–2.74)
Nausea	289	0.036	38	0.049	1.29 (0.91–1.84)	57	0.035	0.93 (0.69–1.24)
Vomiting	133	0.017	18	0.023	1.33 (0.81–2.21)	31	0.019	1.07 (0.71–1.60)
Diarrhea	425	0.053	50	0.065	1.17 (0.86–1.60)	81	0.050	0.90 (0.70–1.15)
Diarrhea with blood	7	0.001	1	0.001	1.22 (0.15–10.01)	3	0.002	1.69 (0.42–6.75)
Stomach pain	495	0.062	51	0.066	1.04 (0.77–1.41)	103	0.063	0.98 (0.78–1.23)
Cough	551	0.069	70	0.091	1.34 (1.03–1.74)	117	0.072	1.06 (0.86–1.31)
Cough and phlegm	265	0.033	31	0.040	1.16 (0.79–1.70)	60	0.037	1.10 (0.82–1.47)
Runny nose	722	0.090	72	0.94	1.03 (0.79–1.33)	160	0.098	1.11 (0.93–1.34)
Sore throat	527	0.066	70	0.091	1.40 (1.07–1.82)	106	0.065	0.99 (0.80–1.24)
HCGI 1	239	0.030	28	0.036	1.18 (0.79–1.77)	50	0.031	0.99 (0.72–1.36)
HCGI 2	65	0.008	11	0.014	1.63 (0.85–3.12)	17	0.010	1.13 (0.65–1.95)
Significant respiratory disease	399	0.050	42	0.055	1.08 (0.77–1.50)	85	0.052	1.04 (0.81–1.33)

The total number of swimmers in each category is given in parentheses (N).
* Referent category (RR = 1.0).
† Adjusted for age, sex, beach, race, California *vs* out-of-state resident, and concern about potential health hazards at the beach.

TABLE 4. Adverse Health Outcomes by Enterococci Levels: Number Ill, Acute Risks, Adjusted Relative Risk (RR) Estimates and 95% Confidence Intervals (CI)

	Enterococci (cfu/100ml)								
	≤35 (N = 7,689)*		>35–104 (N = 1,863)			>104 (N = 857)			
Outcome	No. Ill	Risk	No. Ill	Risk	RR†	No. Ill	Risk	RR†
Fever	371	0.048	84	0.045	0.91 (0.71–1.16)	45	0.053	1.00 (0.72–1.40)
Chills	198	0.026	33	0.018	0.67 (0.46–0.97)	24	0.028	0.94 (0.60–1.48)
Eye discharge	149	0.019	25	0.013	0.69 (0.45–1.07)	16	0.019	1.01 (0.58–1.75)
Earache	270	0.035	57	0.031	0.82 (0.61–1.11)	31	0.036	0.88 (0.59–1.31)
Ear discharge	52	0.007	12	0.006	0.85 (0.45–1.62)	4	0.005	0.53 (0.19–1.51)
Skin rash	74	0.010	13	0.007	0.71 (0.39–1.30)	13	0.015	1.72 (0.89–3.31)
Infected cut	46	0.006	12	0.006	0.95 (0.49–1.82)	6	0.007	0.90 (0.37–2.18)
Nausea	271	0.035	72	0.039	1.07 (0.82–1.41)	41	0.048	1.19 (0.84–1.70)
Vomiting	130	0.017	34	0.018	1.13 (0.77–1.67)	18	0.021	1.20 (0.71–2.04)
Diarrhea	398	0.052	101	0.054	0.99 (0.78–1.25)	57	0.067	1.01 (0.75–1.36)
Diarrhea with blood	8	0.001	0	—	—	3	0.004	2.90 (0.66–12.68)
Stomach pain	464	0.060	126	0.068	1.09 (0.89–1.35)	59	0.069	0.97 (0.72–1.30)
Cough	554	0.072	121	0.065	0.91 (0.73–1.12)	63	0.074	1.00 (0.75–1.34)
Cough and phlegm	266	0.035	59	0.032	0.91 (0.68–1.22)	31	0.036	1.03 (0.69–1.54)
Runny nose	704	0.092	165	0.089	0.96 (0.80–1.15)	85	0.099	1.01 (0.79–1.30)
Sore throat	533	0.069	118	0.063	0.89 (0.72–1.10)	52	0.061	0.80 (0.59–1.09)
HCGI 1	230	0.030	51	0.027	0.92 (0.67–1.26)	36	0.042	1.31 (0.89–1.92)
HCGI 2	67	0.009	14	0.008	0.82 (0.46–1.48)	12	0.014	1.30 (0.67–2.51)
Significant respiratory disease	397	0.052	84	0.045	0.86 (0.67–1.11)	45	0.053	0.98 (0.70–1.37)

The total number of swimmers in each category is given in parentheses (N).
* Referent category (RR = 1.0).
† Adjusted for age, sex, beach, race, California *vs* out-of-state resident, and concern about potential health hazards at the beach.

were increased risks of nausea, vomiting, diarrhea with blood, HCGI 1, and HCGI 2. Our adjusted RRs suggested similar positive associations, except for diarrhea; although the risk increased from 0.05 to 0.07, the adjusted RR comparing the highest to lowest category was 1.0 (Table 4). When comparing the lowest to higher deciles, we observed increased risks in most categories for infected cut and skin rash (Figure 1). Other adverse health outcomes—infected cut, nausea, diarrhea, diarrhea with blood, HCGI 1, and HCGI 2—exhibited increased risks only in particular quantiles. In comparison with the lowest decile, the risk of each of these outcomes was higher in the 10th decile. For example, the risk for HCGI 2 was 0.007 in the first decile, but 0.015 in the 10th.

Table 5 presents results for *E. coli*. We once again found an increased risk of skin rash in the highest prespecified category (that is, >320 cfu). Furthermore, we observed slight increased risks in this highest category for eye discharge, earache, stomach pain, coughing with phlegm, runny nose, and HCGI 1 (Table 5). In our decile-based analysis, however, we only observed materially increased risks for eye discharge, skin rash, and infection (Figure 1).

Numerous adverse health outcomes exhibited higher risks among subjects swimming on days when samples were positive for viruses (Table 6). In particular, the risk of fever, eye discharge, vomiting, sore throat, HCGI 1, and HCGI 2, and to a lesser extent, chills, diarrhea, diarrhea with blood, cough, coughing with phlegm, and SRD were higher on days when viruses were detected. Our adjusted RR estimates showed similar relations, most ranging from 1.3 to 1.9 (Table 6). Additionally, adjusting for each bacterial indicator (one-at-a-time) also left these results essentially unchanged.[12] As expected, there was an association between presence of virus and fecal coliforms within 50 yards of the drain. The mean density of fecal coliforms when no virus was detected was 234.8 cfu (SD 542.5 cfu); whereas it was 2,233.8 (SD 2,634.1) when viruses were detected (N = 386). The median values were 47.8 and 452.6 cfu, respectively.

Discussion

We observed differences in risk for a number of outcomes when we compared subjects swimming at 0 yards *vs* 400+ yards. Most of the relative risks suggested an approximately 50% increase in risk. Furthermore, as evinced by both the risks and RRs, there is an apparent threshold of increased risk occurring primarily at the drain: no dose response is evinced with increasing closeness to the drain, but there is a jump in risk for many adverse health outcomes among those swimming at the drain. We also found that distance is a reasonably good surrogate for bacterial indicators, with higher levels observed closer to the drain.[12]

For bacterial indicators, we observed a relation among numerous higher exposures and adverse health outcomes. These increases were mostly restricted to the highest knowledge-based categories (no effect was observed below any existing standards). When looking at quantiles, we found higher risks of skin rash and infection at fairly low levels. In contrast with what one might expect, however, there was no clear dose-response pattern across increasing levels of bacteriological exposures.

TABLE 5. Adverse Health Outcomes by E. coli Levels: Number Ill, Acute Risks, Adjusted Relative Risk (RR) Estimates and 95% Confidence Intervals (CI)

	E. coli (cfu/100ml)													
	≤35 (N = 6,104)*		>35–75 (N = 1,620)			>75–160 (N = 1,145)			>160–320 (N = 518)			>320 (N = 991)		
Outcome	No. Ill	Risk	No. Ill	Risk	RR†	No. Ill	Risk	RR†	No. Ill	Risk	RR†	No. Ill	Risk	RR†
Fever	274	0.045	89	0.055	1.22 (0.95–1.56)	61	0.053	1.20 (0.90–1.60)	29	0.056	1.22 (0.81–1.84)	45	0.045	0.98 (0.70–1.37)
Chills	145	0.024	41	0.025	1.00 (0.70–1.44)	28	0.024	1.00 (0.66–1.52)	18	0.035	1.38 (0.82–2.33)	22	0.022	0.79 (0.49–1.26)
Eye discharge	116	0.019	30	0.019	0.99 (0.65–1.49)	14	0.012	0.65 (0.37–1.15)	6	0.012	0.61 (0.26–1.43)	23	0.023	1.36 (0.84–2.19)
Earache	214	0.035	45	0.028	0.75 (0.54–1.04)	33	0.029	0.78 (0.53–1.14)	18	0.035	0.91 (0.55–1.50)	47	0.047	1.25 (0.89–1.77)
Ear discharge	42	0.007	8	0.005	0.60 (0.28–1.28)	5	0.004	0.57 (0.22–1.46)	6	0.012	1.28 (0.52–3.15)	6	0.0066	0.67 (0.27–1.62)
Skin rash	57	0.009	15	0.009	1.01 (0.56–1.80)	7	0.006	0.66 (0.30–1.46)	6	0.012	1.21 (0.49–2.98)	15	0.015	2.04 (1.11–3.76)
Infected cut	42	0.007	7	0.004	0.53 (0.24–1.20)	3	0.003	0.33 (0.10–1.06)	3	0.006	0.66 (0.20–2.19)	9	0.009	1.02 (0.48–2.19)
Nausea	216	0.035	74	0.046	1.22 (0.93–1.61)	34	0.030	0.80 (0.55–1.16)	18	0.035	0.88 (0.53–1.46)	42	0.042	1.03 (0.73–1.47)
Vomiting	107	0.018	31	0.019	1.09 (0.72–1.64)	16	0.014	0.82 (0.48–1.40)	8	0.015	0.87 (0.41–1.85)	20	0.020	1.05 (0.63–1.74)
Diarrhea	310	0.051	101	0.062	1.14 (0.90–1.44)	63	0.055	1.00 (0.75–1.33)	25	0.048	0.80 (0.52–1.23)	56	0.057	0.91 (0.67–1.23)
Diarrhea with blood	5	0.001	3	0.002	2.06 (0.48–8.89)	1	0.001	1.03 (0.12–9.01)	2	0.004	3.98 (0.68–23.21)	0	—	—
Stomach pain	353	0.058	124	0.077	1.28 (1.03–1.59)	70	0.061	1.02 (0.78–1.33)	31	0.060	0.95 (0.64–1.40)	70	0.071	1.06 (0.80–1.40)
Cough	444	0.073	96	0.059	0.81 (0.64–1.02)	86	0.075	1.04 (0.82–1.33)	29	0.056	0.77 (0.51–1.14)	82	0.083	1.14 (0.88–1.48)
Cough and phlegm	226	0.037	41	0.025	0.66 (0.47–0.92)	34	0.030	0.78 (0.54–1.12)	11	0.021	0.53 (0.28–1.00)	43	0.043	1.12 (0.79–1.59)
Runny nose	566	0.093	136	0.084	0.87 (0.71–1.06)	105	0.092	0.96 (0.77–1.20)	38	0.073	0.76 (0.53–1.08)	108	0.109	1.12 (0.89–1.41)
Sore throat	417	0.068	99	0.061	0.86 (0.68–1.08)	82	0.072	1.02 (0.80–1.31)	29	0.056	0.78 (0.52–1.17)	75	0.076	1.04 (0.80–1.37)
HCGI 1	183	0.030	51	0.031	1.03 (0.75–1.42)	30	0.026	0.88 (0.59–1.30)	17	0.033	1.06 (0.63–1.80)	36	0.036	1.12 (0.76–1.64)
HCGI 2	48	0.008	21	0.013	1.55 (0.92–2.64)	8	0.007	0.85 (0.40–1.81)	6	0.012	1.25 (0.51–3.03)	10	0.010	1.04 (0.51–2.13)
Significant respiratory disease	319	0.052	71	0.044	0.82 (0.62–107)	58	0.051	0.96 (0.72–1.28)	21	0.041	0.74 (0.47–1.18)	56	0.057	1.03 (0.76–1.40)

The total number of swimmers in each category is given in parentheses (N).

* Referent category (RR = 1.0).

† Adjusted for age, sex, beach, race, California *vs* out-of-state resident, and concern about potential health hazards at the beach.

TABLE 6. Number Ill, Risks, and Adjusted Relative Risk (RR) Estimates of Adverse Health Outcomes by Virus

	Viruses				
	No (N = 3,168)*		Yes (N = 386)		
Outcome	No. Ill	Risk	No. Ill	Risk	RR (95% CI)†
Fever	126	0.040	23	0.060	1.56 (0.98–2.50)
Chills	65	0.021	10	0.026	1.25 (0.63–2.50)
Eye discharge	36	0.011	8	0.021	1.86 (0.85–4.09)
Earache	93	0.029	10	0.026	0.92 (0.47–1.80)
Ear discharge	15	0.005	0		
Skin rash	32	0.010	4	0.010	0.97 (0.34–2.82)
Infected cut	31	0.010	2	0.005	0.57 (0.13–2.40)
Nausea	101	0.032	12	0.031	0.93 (0.50–1.73)
Vomiting	44	0.014	10	0.026	1.86 (0.92–3.80)
Diarrhea	130	0.041	21	0.054	1.27 (0.78–2.07)
Diarrhea with blood	2	0.001	1	0.003	5.82 (0.45–75.72)
Stomach pain	191	0.060	23	0.060	0.92 (0.58–1.45)
Cough	181	0.057	28	0.073	1.22 (0.80–1.86)
Cough and phlegm	92	0.029	13	0.034	1.20 (0.66–2.18)
Runny nose	246	0.078	32	0.083	1.01 (0.68–1.49)
Sore throat	198	0.063	32	0.083	1.38 (0.93–2.06)
HCGI 1	72	0.023	15	0.039	1.69 (0.95–3.01)
HCGI 2	22	0.007	6	0.016	2.32 (0.91–5.88)
Significant respiratory disease	133	0.042	21	0.054	1.34 (0.83–2.18)

The total number of swimmers in each category is given in parentheses (N).
* Referent category (RR = 1.0).
† Adjusted for age, sex, beach, race, California *vs* out-of-state resident, and concern about potential health hazards at the beach.

When looking at the ratio of total to fecal coliforms using the entire dataset, no consistent pattern emerged.[12] This is not entirely surprising inasmuch as an analysis of all data points treats all ratios of similar numerical value equally. Thus, for example, even though a ratio of 5 when the total coliforms are very low may not increase risk, the same ratio may be associated with increased risks when the density of total coliforms is above 1,000 or 5,000 cfu. When the analysis was restricted to swimmers exposed to total coliform densities above 1,000 or 5,000 cfu, a consistent pattern emerged, with higher risks associated with low ratios.[12]

This is the first large-scale epidemiologic study that included measurements of viruses. A number of adverse health effects were reported more often on days when the samples were positive, suggesting assays for viruses may be informative for predicting risk. Norwalk-like viruses are a plausible cause of gastroenteritis.[4,13] Enteroviruses, the most common viruses in sewage effluent, can cause respiratory symptoms. Not only are viruses responsible for many of the symptoms associated with swimming in ocean water but also they die off at slower rates in sea water than do bacteria, and they can cause infection at a much lower dose.[14]

Our design substantially reduced the potential for confounding by restricting the study entirely to swimmers and making comparisons between groups of swimmers (for example, defined by distance from the drain) to estimate relative risks. Previous studies looking at the effects of exposure to polluted recreational water (for example, due to sewage outflows) have been criticized for comparing risks in swimmers with risks in nonswimmers.[4,14,15] In these earlier studies, background risks among subjects who swim *vs* those choosing not to swim may differ because there are many other (potentially noncontrollable) exposures/pathways that can produce the symptoms under investigation. By restricting the present study to swimmers, we have reduced potential differences between the background risks of exposed *vs* unexposed subjects (for example, swimmers choosing to swim at the drain *vs* those swimming at the same beach but farther away from the drain). Furthermore, we were able to adjust our relative risk estimates for a number of additional factors (listed above) that could confound the observed relations. Of course, this does not exclude the possibility that residual confounding in these factors, or other unknown factors, might have confounded the observed relations.

Nevertheless, any actual (that is, causal) effects may be higher than we observed in this study because both distance and pathogenic indicators are proxy measures of the true pathogenic agents. Also, recall that we excluded subjects who frequently entered the water at these beaches. If there is a dose-response relation such that higher cumulative exposures are associated with increased risk, then one may infer that persons who frequently enter the water and immerse their heads (for example, surfers) may have a higher risk of adverse health outcomes than the relatively infrequent swimmers included in this study.

In summary, we observed positive associations between adverse health effects and (1) distance from the drain, (2) bacterial indicators, and (3) presence of enteric viruses. Taken together, these results imply that there may be an increased risk of a broad range of adverse health effects associated with swimming in ocean water subject to urban runoff. Moreover, attributable numbers—that is, estimates of the number of new cases of an adverse health outcome that is attributable to the exposure of interest—reached well into the 100s per

10,000 exposed subjects for many of the positive associations observed here.[12] This finding implies that these risks might not be trivial when we consider the millions of persons who visit these beaches each year. Furthermore, the factors apparently contributing to the increased risk of adverse health outcomes observed here are not unique to Santa Monica Bay (similar levels of bacterial indicators are observed at many other beaches). Consequently, the prospect that untreated storm drain runoff poses a health risk to swimmers is probably relevant to many beaches subject to such runoff, including areas on the East, West, and Gulf coasts of North America, as well as numerous beaches on other continents.

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Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure

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Summary

The health effects of bathing in coastal waters is an area of scientific controversy. We conducted the first ever randomised "trial" of an environmental exposure to measure the health effects of this activity. The trial was spread over four summers in four UK resorts and 1216 adults took part. Detailed interviews were used to collect data on potential confounding factors and intensive water quality monitoring was used to provide more precise indices of exposure. 548 people were randomised to bathing, and the exposure included total immersion of the head. Crude rates of gastroenteritis were significantly higher in the exposed group (14·8 per 100) than the unexposed group (9·7 per 100; p=0·01).

Linear trend and multiple logistic regression techniques were used to establish relations between gastroenteritis and microbiological water quality. Of a range of microbiological indicators assayed only faecal streptococci concentration, measured at chest depth, showed a significant dose-response relation with gastroenteritis. Adverse health effects were identified when faecal streptococci concentrations exceeded 32 per 100 mL. This relation was independent of non-water-related predictors of gastroenteritis.

We do not suggest that faecal streptococci caused the excess of gastrointestinal symptoms in sea bathers but these microorganisms do seem to be a better indicator of water quality than the traditional coliform counts. Bathing water standards should be revised with these findings in mind.

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Introduction

There is a widespread belief that sea bathing may lead to illness[1] but evidence for such an association has proved elusive. No consistent relation between any single microbiological indicator of water quality and disease has emerged[2,3] from the many studies that have been undertaken.[4-15] Despite this, microbiological standards and recommendations have been drawn up for bathing waters in Europe and North America.[16-21]

Previous studies have suffered from three methodological flaws: (1) those who bathed and those who did not selected themselves and may have differed in respect to factors other than the quality of the bathing water to which they were exposed, (2) the microbiological quality of water was not assigned to each bather at the time and place of bathing, and (3) non-water-related risk factors were not measured or adequately controlled for. To overcome these problems, and following a suggestion of the World Health Organization (WHO),[22] we have conducted a randomised controlled exposure study. Groups of healthy adults were allocated at random to bathe or not to bathe at UK resorts that met the mandatory EC standards. The levels of indicator organisms to which each bather was exposed were precisely determined. Four such studies were done, and preliminary results, based on the first two, have been published.[23,24] In this paper we present the dose-response relation between gastroenteritis and exposure to sea water of varying microbial quality based on the results of the full four year programme.

Methods

Epidemiological

Four sites around the UK coast that had met EC mandatory bacteriological criteria for bathing waters[18] in the previous summer were selected by an independent committee. Adult volunteers (>18 years) were recruited in population centres close to the study sites in the 3 weeks before the study. Background rates in previous studies gave our study the power to detect an increase in illness rate of 5% above background at alpha=0·01 and beta=0·02. The acceptable water quality and minimum age of volunteers were both requirements defined in ethical clearance for the studies granted by the Royal College of Physicians Committee for Research on Healthy Volunteers. The studies took place at four separate locations on four successive bathing seasons between 1989–1992 (beaches A–D)

Volunteers attended for an extensive structured personal enrolment interview and medical examination not more than 2 days before the exposure day. The interview recorded age, sex, occupation, household size, general health, illness in previous 3 weeks, water contact activities, and other possible confounding factors associated with gastroenteritis. Those deemed unfit at the

Age grouped by 10 yr intervals
Sex
History of migraine headaches
History of stress or anxiety
Frequency of diarrhoea (often/sometimes/rarely/never)
Current use of prescription drugs
Illnesses in 4 weeks before study day lasting more than 24 h
Use of prescription drugs in 4 weeks before study day
Consumption of following foods in period from 3 days before to 7 days after trial day
Mayonnaise
Purchased sandwiches
Chicken
Eggs
Hamburgers
Hot dogs
Raw milk
Cold meat pies
Seafood
Illness in household within 3 weeks after trial day
Alcohol consumption within 7 day period after trial
Frequency of usual alchol consumption
Taking of laxatives within 4 weeks of trial day
Taking of other stomach remedies within 4 weeks of trial day
Additional bathing within 3 day before and 3 weeks after trial day*

*Included to control for possible confounding due to multiple exposures among bathers and exposure among non-bathers before or after trial day.

Table 1: **Non-exposure-related risk factors for gastroenteritis**

medical examination (eg, serious heart conditions, life-threatening illness, fear of water; n=16) and those who failed to attend on the trial day (n=183) were excluded from further analysis. 23 volunteers who failed to comply with their randomisation status were also excluded because no water quality data were available for "non-bathers" who bathed and the research team could not confirm that "bathers" who claimed to have stayed on the beach had not entered the water unobserved.

The remaining 1306 volunteers (table 2) were randomised into bathing and non-bathing groups using a computerised table of random numbers on the exposure day. Some recruitment was also undertaken on the trial day itself. Participants recruited on the trial day were given the initial trial day interviews, examined by a physician, and randomised to bather or non-bather. Volunteers were unaware of their group status until they reported to the beach. The bathing group (exposed) entered a defined and roped-off 20 m area of water for at least 10 min, completely immersing their heads at least three times. Their location and duration of exposure was closely monitored. The non-bathing (unexposed) group remained in a designated area on the adjacent beach. Before they left the beach, participants were interviewed about their current health and dietary habits for the days before the trial day.

Further extensive interviews inquiring about health, diet, water contact activity, and other possible non-water-related risk factors for gastroenteritis in the intervening period were done 1 week after the trial day. Volunteers were also given medical examinations. Interviewers were blind to exposure status. 3 weeks subsequent to the trial date participants each received a final questionnaire by post on which to record any further symptoms of illness and exposure to bathing water. These delay periods were chosen to accommodate the incubation periods of illness often attributed to bathing exposures such as viral gastroenteritis and cryptosporidiosis. The study was not designed to pick up longer incubation illness such as hepatitis A.

Site	Taking part		Excluded*		Final cohort†		Follow-up
	B	NB	B	NB	B	NB	
Site A	133	133	13	0	120	133	95·1%
Site B	138	166	37	2	101	164	87·2%
Site C	178	186	6	0	172	186	98·4%
Site D	178	194	23	9	155	185	91·4%
Total	627	679	79	11	548	668	93·1%

B=bather, NB=non-bather. *Excluded from further analysis due to missing indicator organism exposure data or incomplete follow-up. †Includes 104 participants who reported gastroenteritis symptoms on exposure day.

Table 2: **Study population**

Faecal streptococci exposure class (/100 mL)	No	Rate of gastroenteritis (per 100) (and 95% CI)
Unexposed	605	9·7 (7–12)
Exposed 0–34	307	11·1 (7–14)
Exposed 35–69	149	16·1 (10–22)
Exposed >70	51	33·3 (20–46)

p (trend) for all classes <0·01, p (trend) exposed classes only <0·01.

Table 3: **Gastroenteritis among non-bathers and bathers exposed to less than 34, 35–69, and greater than 70 faecal streptococci (per 100 mL) derived from samples taken at chest depth**

Bacteriological

The bathing area was sampled every half-hour over the exposure period every 20 m and at surf, mid (1 m depth, 30 cm below the surface) and chest (1·3–1·4 m depth, 30 cm below the surface) depths. The chest depth was the location at which immersion exposure took place. Samples were analysed for total and faecal coliforms, faecal streptococci, and *Pseudomonas aeruginosa.*[25] Total staphylococci[26,27] were counted at three of the study sites. All samples were analysed by standard methods and the results were first known to the researchers at 24–48 h post exposure.

Analytical

The success of randomisation was assessed by comparing the distribution of non-water-related risk factors for gastroenteritis among bathers and non-bathers (table 1). This was done by univariate χ^2 analysis. If randomisation was successful, the crude rates of illness for bathers and non-bathers would be adjusted for any of those extraneous risk factors. If randomisation was not successful for any non-water-related risk factor, the effect of this failure was assessed, for any association between illness at increasing indicator organism exposure, by multiple logistic regression.

The indicator organism concentrations to which individual bathers were exposed were assigned from results obtained from samples at three depths taken at the location of, and during, the exposure to sea water. Where this was not possible the closest sample results of the time of the individual exiting the water were assigned as the measure of exposure. All volunteers (and interviewers) were "blind" to the water quality attributed to each bather.

Statistical analyses examined dose-response relationships between exposure (as measured by microbiological water quality) and gastroenteritis, whilst controlling for the effects of confounding factors. Confounding factors included non-exposure-related variables likely to affect the incidence of gastroenteritis. Gastroenteritis was defined as any case of vomiting or diarrhoea plus any case of either indigestion or nausea accompanied by fever reported at either post-exposure interview. Participants were excluded from the analysis where they failed to attend for follow-up interviews or where precise details of the indicator organism density to which the bathers were exposed could not be determined.

Trends in gastroenteritis rate with indicator organism exposure measured at the three depths were examined by the Mantel-Haenszel χ^2 test for linear trend.[28] Exposure classes for total and faecal coliforms were defined, on the basis of existing standards, as 0–2400 and greater than 2400 total coliforms per 100 mL (dL) and 0–200 and greater than 200 faecal coliforms/dL and 0–34, 35–69, and greater than 70 faecal streptococci/dL. Rates of gastroenteritis amongst the unexposed were thus compared with rates amongst those exposed to water quality judged to comply with or to exceed current standards for total coliforms, faecal coliform, and faecal streptococci.

Since no quantitative standards exist for *Pseudomonas aeruginosa* and total staphylococci, median values were used to define exposure, the classes being 0 and greater than 0 *P aeruginosa*/dL and 0–172 and greater than 172 total staphylococci/dL. Trend analyses were repeated excluding the unexposed group. A trend was considered significant if the

Mantel Haenszel χ^2 for linear trend yielded a p value less than 0·05 with and without the inclusion of the unexposed group. This ensured that the trend statistic was not unduly influenced by an excessive difference between the gastroenteritis rate in the unexposed group and that in the lowest exposure class.[31,32] Trends were also assessed by study site and the differences in stratum-specific gastroenteritis rates between sites examined to justify combining the data.

Any significant trends were further analysed by quartiles and 20-unit intervals to define exposure categories. Multiple logistic regression analysis was then used to examine the details of significant dose relationships between indicator organism concentration and gastroenteritis whilst controlling for significant non-exposure-related risk, or confounding, factors found by univariate χ^2 analyses between the exposed and reference groups. Two independent analyses were conducted with the BMDP[33] (by JMF) and MULTLR[34] (by RLS) statistical packages. This provided an independent validation of the results of the BDMP models which are reported herein and were constructed with backwards selection procedures to control for confounding factors.

Results

Table 2 shows numbers of volunteers who came to the beach on the four trial days, the exclusions due to missing indicator organism data or incomplete follow-up, the final cohorts, and the percentages with complete follow-up information. The median ages of the volunteers before exclusions were bathers 31·8 years and non-bathers 32·4 years. After exclusions the medians were 31·7 years and 32·1, respectively. The sex balance was 47·4% males/52·6% females before exclusions and 45·6%/54·4% afterwards.

Each trial covered a 3 h period. Significant spatial and temporal variability was observed in indicator organism concentrations at each site throughout the 3 h period of sea water sampling during which bathers were exposed.

Crude rates of gastroenteritis were significantly higher in the exposed group (14·8%) than in the unexposed group (9·7%) (p=0·01). A check on non-water-related risk factors showed randomisation to have been successful in that no confounding was observed between non-water-related factors and the excess risk for bathers at increasing levels of indicator organism exposure.

34·3% of gastroenteritis cases developed within 7 days of the study day, the remaining 65·7% had onset dates up to 21 days. No difference in onset of symptoms was observed between the exposed and unexposed groups. Of the five indicator organisms analysed, only increases in faecal streptococci concentrations derived from samples taken at chest depth were associated with a trend towards increased rates of gastroenteritis when the unexposed (non-bather) group was included or excluded in the trend analysis (table 3). The exposure categories were then defined by the quartile points of the faecal streptococci distribution. This was done to remove any possible subjectivity in the selection of cut-points and to look for relations between faecal streptococci exposure and gastroenteritis at indicator organism density below 35/dL. Table 4 shows gastroenteritis rates for exposure categories defined by quartiles of faecal streptococci exposure at chest depth and provides data on bathers exposed to concentrations below 35/dL. A significant trend was observed with or without inclusion of the non-bather group (table 4).

Faecal streptococci exposure class (/100 mL)	No	Rate of gastroenteritis (per 100) (and 95% CI)
Unexposed	605	9·7 (7–12)
Exposed 0–13	159	10·7 (6–15)
Exposed 14–26	109	11·0 (5–17)
Exposed 27–49	121	14·0 (8–20)
Exposed 50–158	118	24·6 (17–32)

p (trend) for all classes <0·001, p (trend) exposed classes only <0·002.

Table 4: **Gastroenteritis among non-bathers and bathers exposed in quartile categories of faecal streptococci (per 100 mL) derived from samples taken at chest depth**

Better to delineate the faecal streptococci exposure at which bathers reported gastroenteritis significantly more than non-bathers, the data were split into 20 unit intervals of faecal streptococci exposure (table 5). The increased risk to the bathers did not start until exposure to 40 or more faecal streptococci/dL.

Faecal streptococci exposure class (/100 mL)	No	Rate of gastroenteritis (per 100) (and 95% CI)
Unexposed	605	9·7 (7–12)
Exposed 0–19	184	10·9 (6–15)
Exposed 20–39	161	10·6 (5–16)
Exposed 40–59	82	18·3* (10–26)
Exposed 60–79	57	28·1* (16–39)
Exposed 80+	23	30·4* (11–49)

p (trend) for all classes <0·001, p (trend) exposed classes only <0·001.
*Rates significant relative to unexposed (non-bather) group (p<0·05).

Table 5: **Gastroenteritis among unexposed individuals *vs* bathers exposed to 20 unit categories of faecal streptococci (per 100 mL) derived from samples taken at chest depth**

Trends in gastroenteritis rate with increasing faecal streptococci exposure were not significantly different between sites, indicating that the pooling of data from all four studies was justified. Furthermore, all volunteers at all sites were healthy adults who underwent medical examination and screening before exposure.

Logistic regression analysis (BMDP model) revealed no confounding of the relation between exposure to faecal streptococci and illness by the risk factors shown (table 6). Nor was there any significant interaction between faecal streptococci exposure and any of these non-water-related risk factors. Results with the MULTR model were similar. The lack of interaction between faecal streptococci exposure and the non-water-related risk factors shows that the non-water factors are independent predictors of illness.

The analyses presented thus far suggest that the risk of gastroenteritis does not increase until bathers are exposed to about 40 faecal streptococci/dL. To define the threshold more precisely, we did logistic regression modelling comparing ill with non-ill bathers as the

Variable	Likelihood ratio	p	OR (95% CI)
Faecal streptococci (/100 mL)	11·83	0·008	
0–39			1·00
40–59			1·91 (1·60–2·28)
60–79			2·90 (1·43–5·88)
80+			3·17* (1·12–8·97)
Non-water†	3·54	0·06	1·17 (0·98–2·99)
Age‡	3 66	0·056	0·81 (0·65–1·01)
Gastroenteritis in family member§	5·21	0·02	4·44 (1·34–14·64)
Gender¶	5·09	0·02	1·81 (1·08–3·04)

*p (trend)=0·009. †Composite variable of non-water-related risk factors (a), (b), (c), (g), and (h) (see figure legend). ‡Modelled continuously in intervals of 10 years. §Symptoms in family members preceding symptom in individual bathers. ¶Reference group males.

Table 6: **Logistic regression analysis of gastroenteritis amongst bathers only**

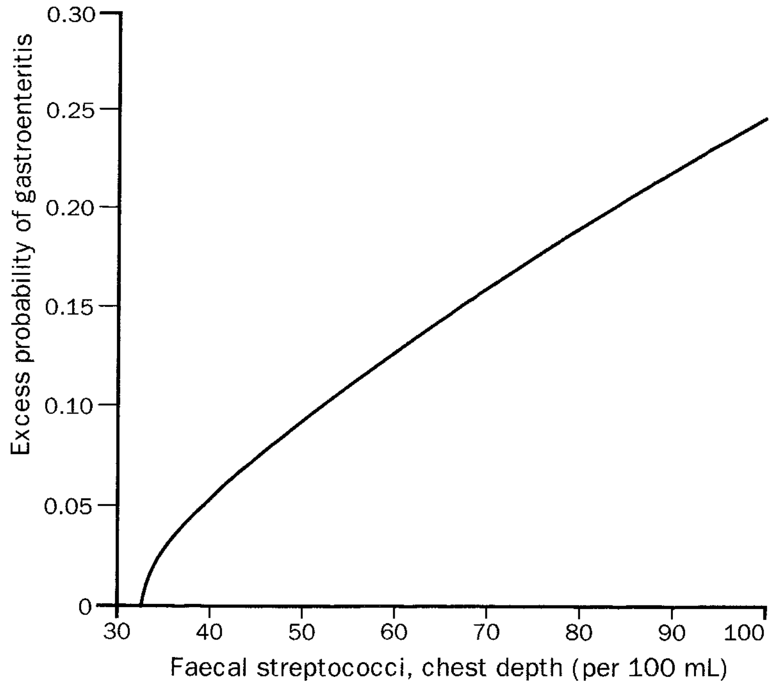

Figure: **Probability of gastroenteritis amongst bathers exposed to increasing faecal streptococci densities from samples taken at chest depth**

Model is adjusted for following significant non-exposure related risk factors identified between bathers reporting gastroenteritis and those not reporting gastroenteritis post exposure:
(a) Predisposition to diarrhoea (diarrhoea at least once per month *vs* less than twice per year) (site A). (b) Diarrhoea lasting over 24 h within 3 weeks before exposure (all sites). (c) Usual fatigue lasting over 23 h within 3 weeks before exposure (site B). (d) Gastroenteritis in family members preceding illness experienced by individual bathers (all sites)
(e) Gender (all sites). (f) Age (10 unit categories) (all sites).
(g) Hamburgers or take-out food consumed within 3 days before and 7 days post exposure (site B). (h) Purchased sandwiches consumed within 3 days before and 7 days post exposure (site A).
From equaton (see Results) a value of 32 per 100 mL on x axis for faecal streptococcal density is the approximate density at which predicted probability of gastroenteritis equals observed proportion of non-bathers reporting gastroenteritis (ie, y, the excess, is zero).

outcome variable with faecal streptococci density as a continuous variable. Since all previous models had indicated no excess risk among bathers exposed to 20–39 faecal streptococci/dL, the median faecal streptococci density to which bathers were exposed within this exposure grouping was chosen as the cut-point on which to conduct separate logistic regression analysis—ie, separate logistic models were computed for bathers exposed to less than 32 and more than 32 faecal streptococci/dL. Because the faecal streptococci density was a continuous variable here, a square-root transformation was necessary to ensure linearity.

The model for bathers exposed to less than 32 faecal streptococci/dL was not significant (likelihood ratio χ^2=0·67, p=0·41) but for bathers exposed to more than 32 faecal streptococci/dL there was a significant result (χ^2=6·33, p=0·02), as follows:

$$\text{Log}_n \text{ odds (of gastroenteritis)} = 0{\cdot}20102\sqrt{(\text{FS}-32)} - 2{\cdot}3561$$

This model was then used to calculate the probability of gastroenteritis symptoms with increased faecal streptococci exposure (figure 1). The model predicts a probability of illness of 0·0866 at 32 faecal streptococci/dL and 0·1039 at 33/dL. When compared with the observed rate of gastroenteritis symptoms among non-bathers (0·0975) the model does provide strong evidence that 33 faecal streptococci/100 mL is the threshold of increased risk.

Discussion

This is the first time that the methods of a randomised controlled clinical trial have been applied to an environmental exposure. The relation between faecal streptococci concentration measured at chest depth and gastroenteritis is robust. No other microbiological indicator at any sampling depth displayed a significant trend relating concentration to gastroenteritis rate. The biological basis of this observation is unknown and there is no suggestion that faecal streptococci was the aetiological agent involved. However, it would be logical to presume that whatever causes gastroenteritis co-partitions in sea water with faecal streptococci. A Norwalk-like virus is one possibility. The case definition of gastroenteritis used gave relative prominence to upper gastrointestinal symptoms which frequently predominate in outbreaks of viral gastroenteritis.[35] These viruses cannot be isolated from surface waters with current techniques.[36]

The use of organisms similar to faecal streptococci as indicators of risk of gastroenteritis has been proposed before.[6,20] Nevertheless the increase in rates of illness in those studies with increasing indicator concentration is lower than in our study. US Environmental Protection Agency (EPA) studies report lower rates of gastroenteritis, both in bathing and non-bathing groups, than we found.[5,6] We cannot explain this discrepancy though methodological criticisms have been levelled at EPA type studies.[13,30] The healthy volunteers in our study may dffer from the weekend family groups who participated in the EPA studies. Prospective cohort studies in the UK[14] confirm the generally higher rates of gastroenteritis observed in UK investigations than in EPA studies.[5]

Our findings are not applicable to younger bathers or special interest groups such as surfers, sailboarders, and divers; nor can they be extrapolated to freshwater recreation sites. They do show that existing EC standards[18] have very little public health significance to coastal bathing waters in temperate north-west Europe. Indeed coliforms seem to have little value as indicators of the risks of gastroenteritis from sewage pollution of coastal waters. Faecal streptococci concentrations are a better microbiological indicator of whether sea water is fit for bathing in and should replace coliform concentrations as the basis for official standards. Some movement in this direction is evident in proposed amendments to EC bathing water standards.[21] Information on faecal streptococci concentrations, resort-by-resort and day-by-day, may have an important role in helping people decide whether to bathe or not

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REVIEW ARTICLE

Critical processes affecting *Cryptosporidium* oocyst survival in the environment

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SUMMARY

Cryptosporidium are parasitic protozoans that cause gastrointestinal disease and represent a significant risk to public health. *Cryptosporidium* oocysts are prevalent in surface waters as a result of human, livestock and native animal faecal contamination. The resistance of oocysts to the concentrations of chlorine and monochloramine used to disinfect potable water increases the risk of waterborne transmission via drinking water. In addition to being resistant to commonly used disinfectants, it is thought that oocysts can persist in the environment and be readily mobilized by precipitation events. This paper will review the critical processes involved in the inactivation or removal of oocysts in the terrestrial and aquatic environments and consider how these processes will respond in the context of climate change.

Key words: *Cryptosporidium*, survival, environment, inactivation, processes, review, climate change.

INTRODUCTION

Protozoan parasites of the genus *Cryptosporidium* are ubiquitous and a significant enteropathogen of a wide range of vertebrates, including mammals, birds, reptiles, and fish (O'Donoghue, 1995; Sturdee *et al.* 1999; Fayer *et al.* 2000*a*; Sreter and Varga, 2000; Alvarez-Pellitero and Sitja-Bobadilla, 2002; Xiao *et al.* 2004). They are the cause of the gastro-intestinal disease cryptosporidiosis, which primarily involves watery diarrhoea in mammals and birds and gastritis in reptiles and fish (O'Donoghue, 1995). While the disease is normally self-limiting, persistent infections have been associated with severe, chronic disease, particularly in snakes or immuno-compromised mammals (Current *et al.* 1983). Recent research has identified drugs (e.g. nitrazoxanide) for the treatment of cryptosporidiosis, making this less of a concern in immuno-compromised human patients (Smith and Corcoran, 2004; Rossignol *et al.* 2006).

The infectious form is the oocyst, and a single oocyst is sufficient to produce infection and disease in an animal model (Pereira *et al.* 2002). In humans, the median infectious dose for some isolates of *C. parvum* has been reported to be as low as 12 oocysts or as high as 2066 oocysts (Messner *et al.* 2001). Transmission can occur via the faecal/oral route or by ingestion of contaminated food or water, the latter of which serves as an excellent vehicle for its transmission (Juranek, 1997). Following ingestion by the host of an infectious oocyst, exposure to stomach acid, bile salts and host metabolic temperature promotes destabilization of the oocyst wall, resulting in release of the sporozoites that can then infect the epithelial cells lining the luminal surfaces of the digestive and respiratory tract of the host (O'Donoghue, 1995; Chen *et al.* 2004). The life-cycle is complex, comprising asexual and sexual stages, with the sexual cycle resulting in the production of millions of thick walled, environmentally robust oocysts (Fayer *et al.* 2000*a*; Atwill *et al.* 2003), which are excreted with the faeces of the host and subjected to the rigors of the environment until rendered non-infectious or ingested by a susceptible host (Fayer *et al.* 1998*a*).

Cryptosporidium sparked enormous public health interest after the large human waterborne outbreak in Milwaukee in 1993 (MacKenzie *et al.* 1995). In the past decade, *Cryptosporidium* has been identified as the cause of numerous outbreaks of waterborne disease affecting hundreds of thousands of individuals (SoloGabriele and Neumeister, 1996; Smith and Rose, 1998; Causer *et al.* 2006). However, because diagnosis of cryptosporidiosis is frequently not considered by many clinicians outside of the context of immunodeficient patients and many laboratories do not routinely test stool specimens for *Cryptosporidium* unless specifically requested, there

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is speculation that many cases of gastroenteritis caused by this parasite go undiagnosed and therefore unreported (Tillett *et al*. 1998; Hunter *et al*. 2001).

Cross-infection studies lead to the proposal that *Cryptosporidium* are zoonotic, with transmission reported between animals and humans (Meisel *et al*. 1976; Anderson *et al*. 1982; Reese *et al*. 1982). More recent genetic studies have demonstrated considerable genetic diversity among isolates of the same species suggesting that some species are in fact species complexes and that some of these species, such as *C. hominis*, may be host specific (Monis and Thompson, 2003). However, *Cryptosporidium parvum* is truly zoonotic and small percentages of human infections may also be caused by species other than *C. parvum* or *Cryptosporidium hominis* (Caccio, 2005). The widespread distribution of *Cryptosporidium* amongst vertebrates highlights the potential for transmission between host species (Sturdee *et al*. 1999; Hunter and Thompson, 2005).

Oocysts in the terrestrial environment are often associated with faeces from domestic and wild animals (Power *et al*. 2003) and can be readily mobilized by precipitation events (Davies *et al*. 2004). Consequently, oocysts of various species of *Cryptosporidium* are frequently detected in rivers and lakes and have also been detected in groundwater and treated drinking water (Smith and Rose, 1990; LeChevallier *et al*. 1995). In addition to being extremely resistant to chemical disinfection, oocysts can survive for several months in the aquatic environment (Robertson *et al*. 1992; Johnson *et al*. 1997). Therefore, *Cryptosporidium* represents not only a threat to public health but also a challenge to suppliers of drinking water.

The aim of this paper is to review the critical processes involved in the inactivation or removal of oocysts from both the terrestrial and aquatic environments, identify those processes that warrant further attention and consider how these processes will be impacted by climate change. A comprehensive understanding of these environmental matrices may help in mitigating the threat that *Cryptosporidium* oocysts pose by providing a valuable framework for risk assessment of *Cryptosporidium* oocysts in both the terrestrial and aquatic environments.

DETERMINATION OF *CRYPTOSPORIDIUM* OOCYST INACTIVATION

Studies investigating oocyst inactivation via biotic and abiotic mechanisms have used a variety of methods including animal infectivity or surrogate *in vitro* assays to determine oocyst viability. Before discussing critical processes affecting oocyst viability, it is prudent that the different methodologies used for determination of oocyst viability and the validity of these methods be considered.

A number of studies have examined oocyst survival using techniques such as *in vitro* excystation or vital dye staining (Robertson *et al*. 1992; Chauret *et al*. 1998), but such methods are only indictors of viability and are known to overestimate infectivity (Black *et al*. 1996; Bukhari *et al*. 2000). It therefore will be noted in this review where only these methods have been used for the determination of oocyst inactivation rates for particular biotic/abiotic stresses. Animal bioassays are considered the 'gold standard' for the assessment of *Cryptosporidium* oocyst infectivity, and the neonatal mouse model has been used extensively in the assessment of oocyst inactivation for *Cryptosporidium parvum* (Bukhari *et al*. 2000; Rochelle *et al*. 2002). However, this model is limited in its application for the assessment of *C. hominis* as this species cannot infect mice. While *C. hominis* ('human genotype') can be cultured in gnotobiotic pigs (Widmer *et al*. 1999), and this model has been used to assess drug efficacy (Theodos *et al*. 1998), it has not been used for the study of environmental oocyst inactivation.

Significant progress in the measurement of oocyst infectivity was catalysed by the development of cell culture (CC) assays for both *C. hominis* oocysts (Hijjawi *et al*. 2001) and *C. parvum* oocysts (Current and Haynes, 1984; Upton *et al*. 1994; Di Giovanni *et al*. 1999; Hijjawi *et al*. 2001; Rochelle *et al*. 2002). Evaluation of the cell culture assays using the human ileocecal adenocarcinoma (HCT-8) cells have shown them to be equivalent to the gold standard neonatal mouse infectivity assay (Shin *et al*. 2001; Rochelle *et al*. 2002; Slifko *et al*. 2002). A range of methods, including reverse transcriptase PCR (Rochelle *et al*. 1997), immunofluorescence microscopy (Slifko *et al*. 1997), colorimetric *in situ* hybridization (Rochelle *et al*. 2002) and real-time PCR (Keegan *et al*. 2003) have been applied for the analysis of CC infection. The combination of high throughput rapid molecular methods with CC, used to measure oocyst inactivation by temperature and solar inactivation (King *et al*. 2005, and manuscript in preparation), promises to improve our ability to rapidly quantify environmental oocyst inactivation through biotic and abiotic mechanisms. However, it is reasonable to consider that differences in reported inactivation rates for oocysts challenged by a particular stress may result from actual differences in the sensitivities of different methodologies employed by particular investigators to quantify oocyst inactivation. During this review we will endeavour to identify dissimilar methodologies used by investigators where significant differences in inactivation rates are reported for the same environmental stress.

TEMPERATURE

The ability of *Cryptosporidium* oocysts to initiate infection has been linked to finite carbohydrate energy reserves in the form of amylopectin, which are consumed in direct response to ambient environmental temperatures (Fayer *et al.* 1998*b*). King *et al.* (2005), using ATP and CC-PCR assays, demonstrated that temperature inactivation at higher temperatures (up to 37 °C) is a function of increased metabolic activity. Temperature, therefore, is one of the most critical processes governing the fate of oocysts in the environment. While *Cryptosporidium* oocysts appear to be resilient to a wide range of temperatures (Chauret *et al.* 1998; Fayer *et al.* 1998*b*; Widmer *et al.* 1999; Freire-Santos *et al.* 1999, 2000*a*; Jenkins *et al.* 2000) increased holding temperatures correspond to decreased oocyst infectivity (Fayer *et al.* 1998*b*). At temperatures below 15 °C oocysts can maintain high levels of infectivity for periods of at least 24 weeks (Fayer *et al.* 1998*b*) with one report suggesting oocysts may remain infectious for periods longer than a year (Jenkins *et al.* 2002) (using 4′6-diaminidino-2-phenylindole (DAPI) and propidium iodide (PI) vital dyes). In contrast, at slightly increased environmental temperatures of 20 °C and 25 °C, inactivation is more rapid, leading to complete inactivation after 12 weeks and 8 weeks, respectively (King *et al.* 2005) (using CC-PCR). While Fayer *et al.* (1998*b*) (using neonatal BALB/c mice) described a longer survival time at these holding temperatures (24 weeks and 12 weeks for 20 °C and 25 °C respectively), they too described rapid inactivation rates as determined by reductions in mouse infectivity at these temperatures. At higher temperatures, King *et al.* (2005) described complete oocyst inactivation at 30 °C and 37 °C within 500 h and 72 h respectively. Therefore, *Cryptosporidium* oocysts, which have a finite energy supply, are highly susceptible to higher (>15 °C) environmental temperatures which they may encounter in the environment.

Oocysts are extremely susceptible to temperatures above 37 °C. Fayer (1994), using neonatal BALB/c mice, reported oocysts held at 64·2 °C for 5 min completely lost their ability to infect mice while Moriarty *et al.* (2005) reported that oocysts were rendered non-infective against monolayers of HCT-8 cells following treatments of 60 °C for 45 s and 75 °C for 20 s. Work in our laboratory (unpublished observations, using CC-PCR) has identified greater than 2 logs of inactivation at temperatures as low as 45 °C for holding times of only 20 min. These results suggest that oocysts are particularly susceptible to heat-shock. Oocysts may encounter such extreme temperatures, especially within manure, with bovine faecal material exhibiting temperature peaks over 40 °C and up to 70 °C if exposed to ambient temperatures of mid-20 to 30 °C under direct exposure to solar radiation (Li *et al.* 2005). Li *et al.* (2005) examined oocyst viability using a thermocycler to emulate the diurnal cycles found in bovine faeces and under such conditions they reported a complete loss of infectivity in the first diurnal cycle (using neonatal BALB/c mice). They determined that once climatic conditions generate internal faecal temperatures of greater than 40 °C, rapid inactivation occurs at rates of greater than 3 logs per day for *C. parvum* oocysts deposited in the faeces of beef and dairy cattle. Therefore, any substantial delays in time between the deposition of faeces containing *Cryptosporidium* and hydrological events such as rainfall, particularly under arid climatic conditions, will have the consequence that the initial load of infective oocysts may be significantly reduced by thermal inactivation.

The effects of low freezing temperatures also pose a serious challenge to oocyst survival. Fayer and Nerad (1996) reported that oocysts frozen at −10 °C for up to 168 h and then thawed out at room temperature were able to retain viability and infectivity to neonatal BALB/c mice. However, oocysts frozen at −20 °C for greater than 24 h and then thawed at room temperature did not infect mice. A similar study (using DAPI/PI vital dyes) by Robertson *et al.* (1992) found that after 21 h at −22 °C, 67% of oocysts were no longer viable. Kato *et al.* (2002) found that 99% of oocysts that were frozen at −10 °C in soils became inactivated within 50 days (using DAPI/PI vital dyes). Robertson and Gjerde (2004), using a vital dye assay, reported that oocysts did not persist in a Norwegian terrestrial environment over winter. They postulated that shear forces generated during the freeze-thaw cycles disintegrated the parasites. However, Fayer and Nerad (1996) predicted that for surface soil temperatures just below freezing and insulated by a cover of snow, oocysts may survive for weeks or months. The survival (measured using DAPI/PI vital dyes) of sentinel and control oocysts in field soil for 39 days (Jenkins *et al.* 1999*b*) adds weight to this prediction.

The predicted global temperature increases in the near future may have dramatic consequences for oocyst longevity in the environment, with small increases in temperatures above 15 °C increasing inactivation. However, warmer temperatures may also increase survival of oocysts in areas prone to soil subsurface freezing or lake ice covers, resulting in substantial numbers remaining infective after the winter period, where previously they may have been inactivated.

AMMONIA

Ammonia occurs naturally in the environment as a product of urea hydrolysis and of microbiological degradation of proteins and other nitrogen containing compounds (ammonification) (Jenkins *et al.* 1998). Other important sources of ammonia can include fertilizers, human and animal wastes, and by-products from industrial manufacturing processes (www.telwf.org/watertesting/ammonia.htm). Ammonia exists in 2 forms simultaneously, with the equilibrium between these forms governed largely by pH and temperature. These forms are NH_3 (un-ionized ammonia) and NH_4^+ (ionized ammonia or ammonium) and it is the NH_3 form that is particularly harmful to aquatic organisms (Arauzo and Valladolid, 2003). The formation of NH_3 is favoured at higher pHs but is also affected by increased temperature, so while the concentration of total ammonia may remain constant in a water body, the proportion of un-ionized ammonia fluctuates with temperature and pH. Significant formation of NH_3 can occur within a single day as water temperatures fluctuate (www.ec.gc.ca/substances/ese/eng/psap/final/ammonia.cfm).

Fayer *et al*. (1996) reported that oocysts suspended in water exposed to one atmosphere of pure ammonia at room temperature (21 °C to 23 °C) for 24 h were no longer infectious when fed to neonatal BALB/c mice, identifying ammonia as a useful disinfectant for oocysts. Jenkins *et al*. (1998) identified significant decreases in oocyst viability using DAPI/PI dye permeability and *in vitro* excystation assays. Based on their kinetic analysis, they predicted exposure to 0·06 M ammonia would inactivate 99·999% of freshly purified oocysts in 8·2 days at a temperature of 24 °C. The rate of inactivation for oocysts exposed to the same concentration at 4 °C was significantly less, with a hypothetical 55·1 days to reach 99·999% inactivation (Jenkins *et al*. 1999*a*). They concluded that environmentally relevant concentrations of free ammonia may significantly increase the inactivation of oocysts in ammonia-containing environments.

Significant concentrations of ammonia can be present in decomposing manure, especially manure storages (Muck and Steenhuis, 1982; Muck and Richards, 1983; Patni and Jui, 1991). Concentrations in cattle slurries have been measured at an initial concentration of 0·05 M, rising to 0·2 M after 3 weeks (Whitehead and Raistrick, 1993). According to ammonia induced oocyst inactivation data produced by Jenkins *et al*. (1998), exposures to such high concentrations would have significant effects on oocyst viability even at cool temperatures given longer exposure times (Ruxton, 1995). Therefore, storage of animal waste products may be regarded as an effective strategy to reduce oocyst numbers in livestock wastes before being spread onto the land (Hutchison *et al*. 2005).

While ammonia levels in manure storages may be high enough to substantially affect oocyst viability, Brookes *et al*. (2004) concluded that the impact of free ammonia on oocyst viability would be negligible in drinking water reservoirs. They calculated that levels of ammonium in the hypolimnion of lakes (often the highest concentration) are typically less than 1 mg/l, values considerably less than the lowest concentration tested by Jenkins *et al*. (1998), 0·007 M being equivalent to 1780 mg/l at a pH of 8 in an aquatic environment. Even for lakes undergoing eutrophication, ammonia levels according to these data would be too low to significantly affect oocyst viability. However, anthropogenic induced increases in ammonia levels in aquatic systems may have indirect effects on the survival of oocysts in aquatic systems through the disruption of benthic fauna and flora responsible for oocyst removal (see section on predation). Such fauna and flora may be significantly more susceptible to the lower ammonia levels that oocysts resist. Additionally, small increases in global temperature due to climatic change and the concomitant increase in water temperatures may raise ammonia levels in some water bodies. Such changes in the aquatic environment could possibly see an increase in the presence of oocysts due to slight increases in ammonia, to which some predatory organisms may be more susceptible.

Oocysts may reside in the soil for a considerable degree of time before mobilization by a precipitation event. Soils typically contain ammonia levels ranging between 1 and 5 ppm, which are not high enough to affect oocyst viability. However, freshly fertilized soils may contain levels as high as 3000 ppm (http://www.npi.gov.au/database/substance-info/profiles/8.html#env-whateffect), which is high enough to have an effect. It may be prudent to revisit the effect of ammonia on oocyst viability, but measuring inactivation using cell culture models instead of vital dye and *in vitro* excystation methods, which are too conservative for estimating reductions in oocyst infectivity. Any dramatic increases recorded in oocyst sensitivity to ammonia levels would have consequences for the predicted survival of oocysts, especially in manures, soils and possibly heavily polluted waters.

DESICCATION

While the robust nature of *Cryptosporidium* oocysts is well recognized and they are known to be resistant to many forms of environmental stress, desiccation is apparently an exception. Robertson *et al*. (1992) reported desiccation to be lethal with only 3% of oocysts viable as judged by DAPI/PI vital dye staining after being air-dried for 2 h at room temperature. Deng and Cliver, (1999), using PI vital dye staining, reported similar rates of survival with only 5% of oocysts remaining viable after 4 h of air drying at room temperature. This is in contrast to another coccidian, *Eimeria*, which has been reported to maintain viability under conditions of severe desiccation (Thomas *et al*. 1995).

Once oocysts are excreted into the terrestrial environment and released from faeces by precipitation or other physical processes, their survival above the soil milieu is greatly limited due to the process of desiccation. It is envisaged that this may vary greatly depending on the climatic setting, with increased rates of inactivation expected in more arid environments. However, once within the soil environment oocysts appear to be protected from desiccation as indicated by a majority of studies identifying soil moisture and soil water potential to have little effect on oocyst viability (see section on the physical terrestrial environment). Therefore, while those oocysts above the soil matrix may be extremely vulnerable to desiccation, those within it may be protected.

Since only vital dye assays have been used to measure oocyst inactivation from desiccation, it would be prudent to conduct further studies using the neonatal mouse or cell culture assays for more accurate measurements of inactivation. In addition, due to the limited number of studies undertaken it would be warranted to further investigate the process of desiccation by challenging oocysts to a variety of environmental conditions, with particular attention to synergistic interactions with temperature. In the light that climate change is predicted to increase the frequency and duration of droughts, it is possible that the process of desiccation may predominate in such areas as one of the critical processes inactivating oocysts.

THE PHYSICAL TERRESTRIAL ENVIRONMENT (SOIL MATRIX AND VEGETATION)

Once oocysts are shed in faeces, they may be released from the faecal matrix by the action of rainfall (Davies *et al*. 2004). After dispersal from the faecal matrix, inactivation may be dependent on the physical, chemical and biological properties of the soil environment (Ferguson *et al*. 2003). Jenkins *et al*. (2002) used DAPI/PI dye permeability and Davies *et al*. (2005) used fluorescent *in situ* hybridization (FISH) to estimate oocyst viability and found significant differences in oocyst survival in different soil types, identifying soil texture as important for survival. However, neither gave detailed explanations of how this parameter was able to influence oocyst viability, with the exception of Jenkins *et al*. (2002),who indicated that while unlikely, lower soil pH may contribute somewhat to this inactivation. Soil moisture in the ranges tested were not shown to be influential to oocyst survival (Jenkins *et al*. 2002; Kato *et al*. 2004; Davies *et al*. 2005), with the exception of a study by Nasser *et al*. (2003) that suggested oocysts in a loam soil can become susceptible to dehydration. Increased water potential (measured using osmotic potential as a surrogate for total water potential in soils, which can avoid problems such as heterogeneity in soil moisture distribution) has been identified as leading to oocyst population degradation (measured using PI vital dye and microscopy) (Walker *et al*. 2001). Therefore, it is possible that environmental soil moisture content may affect oocyst survival, but this requires further research effort for this to be conclusively determined. From the limited studies, biotic status appears to have little effect on oocyst inactivation within the soil environment (Davies *et al*. 2005). However, temperature (see above section) was identified (Jenkins *et al*. 2002; Davies *et al*. 2005) as the critical factor affecting oocyst survival within the soil profile, indicating that oocysts within the soil profile at 4 °C may remain infectious for very long periods (even years) regardless of soil texture.

Pathogen fate within the soil environment is not only a function of survival within the soil but also retention by soil particles (Zyman and Sorber, 1988). Soils have been shown to act as an effective pathogen filter, with a number of studies indicating that the majority of bacteria and/or viruses are removed in a relatively short distance (Cilimburg *et al*. 2000). Soil pore size may significantly affect the movement of protozoa through soil and protozoan cysts and oocysts are likely to be dependent on macropores for their transport and may be expected to show an even greater response to a lack of macropores in disturbed soils from precipitation events (Mawdsley *et al*. 1995; Davies *et al*. 2004). However, a study of the movement of *Cryptosporidium parvum* through 3 contrasting soil types identified distribution within the cores as similar in all 3 soil types, with the majority of oocysts in the top 2 cm of soil, and oocyst numbers decreasing with increasing depth (Mawdsley *et al*. 1996). Depending on soil saturation and soil type, this suggests the possibility for remobilization of those oocysts close to the soil surface with further precipitation events. Soils that consist predominantly of clay-sized particles can cake with wetting and drying, or freezing and thawing, and may pool water, establish water tables, and encourage runoff (Fuller and Warrick, 1985). However, Zyman and Sorber (1988) found that in soils with high clay content, adsorption plays an important role in virus removal. So it is possible that for less than saturating conditions, soils with higher clay contents may retain oocysts more readily under such conditions. Soil pH may also affect properties like adsorption (Mawdsley *et al*. 1996). However, Davies *et al*. (2005) identified little adsorption of oocysts in intact soils plots without vegetation.

Vegetation surfaces have been identified as very effective in reducing *Cryptosporidium* in surface runoff into drinking and irrigation water supplies (Tate *et al*. 2004; Trask *et al*. 2004). Grassland buffers of only 1·1 to 2·1 m in width have been shown to generate between 3 and 8 log retention of *Cryptosporidium* oocysts (Atwill *et al*. 2006), and in combination with soil type, vegetated buffered strips constructed on soils of lower bulk densities have been identified as most effective in retaining oocysts

(Atwill *et al.* 2002). Davies *et al.* (2004), using intact soil blocks, showed that runoff load was significantly affected by vegetation status, the slope of the soil, and event characteristics in terms of rainfall intensity and duration. Based on their observations, a significant risk existed for the dispersion of oocysts from recent animal faecal deposits and their transport into nearby surface waters on sloping land of 10° or more with little or no vegetation after a short burst of rainfall of significant intensity.

On the soil surface, high temperature, desiccation and ultraviolet radiation (see relevant individual sections) can be lethal to pathogens. Therefore, oocysts within the soil column are to a large extent protected from inactivation depending on soil temperature and to a lesser extent soil texture. Oocysts within the soil column are tied away from host ingestion until a precipitation event can mobilize them. Climate change models predict more intense precipitation events in the future for a number of geographical locations (Easterling *et al.* 2000). Such scenarios may increase saturation of soil profiles, mobilize infectious oocysts within the soil column more often, and in combination with urbanization and deforestation of the landscape, may significantly increase the risk that *Cryptosporidium* oocysts pose. Further attention to particular watersheds at risk of oocyst contamination may therefore be warranted, so as to better predict source water quality.

SOLAR INACTIVATION

It is well established that solar radiation is a genotoxic agent with short-wavelength UV radiation, UV-B (280 nm to 320 nm) and UV-A (320 nm–400 nm), the most biologically damaging and mutagenic component of the electromagnetic spectrum (Caldwell, 1971; Ravanat *et al.* 2001). Although short-wavelength UV radiation can disturb most macromolecules, including proteins, lipids and nucleic acids, studies in animal systems suggest that damage to the structure and function of DNA is the primary mechanism responsible for cell injury and loss of viability by UV radiation (Friedberg *et al.* 1995; Malloy *et al.* 1997). UV exposure has been identified as being detrimental to a wide range of organisms including bacteria (Slieman and Nicholson, 2000; Whitman *et al.* 2004), fungi (Hughes *et al.* 2003), plants (Deckmyn and Impens, 1999; Ries *et al.* 2000; Hollosy, 2002) and animals (Misra *et al.* 2005). As well as impacting organisms on the terrestrial environment, surface irradiances are high enough to cause injurious effects in aquatic organisms even in coastal waters characterized by strong attenuation of UV radiation (Piazena and Hader, 1994). Therefore, oocysts in both terrestrial and aquatic environments are targets for solar inactivation.

While the efficacy of UV-C on *Cryptosporidium parvum* oocyst infectivity has been well documented during the last 10 years (for a review see Rochelle *et al.* (2005)), the effects of solar radiation on *Cryptosporidium* are little known. The effect of solar inactivation of *Cryptosporidium* has been limited to one study carried out in marine waters which identified a 90% reduction in viability (measured using excystation) after a 3-day exposure period (Johnson *et al.* 1997). Recent work has investigated the inactivation of *C. parvum* oocysts incubated in tap water and a range of environmental waters exposed to solar radiation over consecutive winter and summer periods (CC-PCR) (King *et al.*, manuscript in preparation). These experiments, conducted on days with varying levels of solar insolation, identified rapid inactivation of *Cryptosporidium* oocysts in tap water, with up to 90% inactivation occurring within the first hour on the highest UV index days.

Results from these tap water inactivation experiments indicate that *C. parvum* oocysts are particularly susceptible to inactivation via solar insolation, indicating the potential for solar insolation to play a significant role in inactivating oocysts in the terrestrial environment. *Cryptosporidium* oocysts present on the soil surface may be exposed to the microbicidal effects of solar radiation and become quickly inactivated. While it is assumed that the majority of oocysts will be protected in the bulk of the soil matrix, including those in the top few centimetres of the bulk soil (Mawdsley *et al.* 1996; McGechan, 2002), precipitation events may re-mobilize oocysts and deposit them on top of the soil matrix, exposing previously protected oocysts to inactivation via solar radiation. This cycle may be repeated multiple times depending on the frequency of precipitation and presence of vegetation buffer zones which reduce oocyst runoff, therefore effecting significant reductions in the infectivity of the total oocyst load of the terrestrial environment.

Outdoor tank experiments have also identified rapid oocyst inactivation in environmental waters of varying water quality with up to 2 log inactivation recorded on a winter's day and up to 3 log inactivation recorded on a summer's day for *C. parvum* (King *et al.*, manuscript in preparation). Dissolved Organic Carbon (DOC) content of the environmental waters was identified as significantly affecting oocyst induced solar inactivation, with increased DOC levels rapidly reducing oocyst inactivation. It is well known that in freshwater environments, the penetration of UV is dependent on the concentration and type of DOC as it is highly absorptive in the ultraviolet spectrum and determines the extinction co-efficient of UV light in a particular water body (Morris *et al.* 1995; Jerome and Bukata, 1998; Hutchison *et al.* 2005). Therefore, waters high in DOC can provide a natural shield to harmful solar radiation (Jerome and Bukata, 1998). However, while solar inactivation of *Cryptosporidium* oocysts may be negligible in waters of high DOC content at depth, it may still play an important contributing factor to oocyst inactivation when oocysts mobilized by precipitation events are carried into such water bodies by warm water inflows. Inflows are controlled by their density relative to that of the lake, such that warm inflows will flow over the surface of the lake as a buoyant surface flow and cold, dense inflows will sink beneath the lake water where they will flow along the lake bed towards the deepest point (Brookes *et al*. 2004). With >90% oocyst inactivation occurring in just a few hours in the top 5 cm of environmental waters high in DOC (King *et al*., manuscript in preparation), oocysts present in warm water inflows into water bodies with strong UV light attenuation may still be strongly inactivated by UV light as they would be in the top few cm of the water column. Oocysts in water bodies with low DOC levels may, on the other hand, be quite vulnerable to solar inactivation at significant depths because UV can penetrate to a depth of 46 m in fresh water bodies (Brookes *et al*. 2004). However, those oocysts carried into water bodies by cold water inflows may escape the damaging effects of solar radiation if water bodies are high in DOC content.

Long-pass filter experiments have identified UV-B as the most germicidal wavelength (King *et al*. 2006). However, a pronounced but lesser effect on oocyst infectivity from UV-A (<380 nm) was also identified by the end of each experimental period. This is consistent with other findings that UV-A light also exhibits cytotoxic and mutagenic effects, however, to a smaller extent than UV-B (Ravanat *et al*. 2001). While UV-A may be less cytotoxic, it may have greater ecological significance, especially where oocysts are found in water bodies where lower wavelengths are more rapidly attenuated in the water column.

While it has been recently demonstrated that solar UV can substantially affect *Cryptosporidium* oocyst infectivity in environmental waters of varying water quality, suggesting that it may be a major factor driving oocyst inactivation in both terrestrial and aquatic environments, there is an enormous lack of data on solar inactivation rates in different environments. Previously, models for determining oocyst fate have incorporated solar radiation inactivation rates of other surrogate organisms (Brookes *et al*. 2004). However, data produced by King *et al*. (manuscript in preparation) suggests a greater degree of susceptibility to solar radiation than previously thought, warranting further effort to study the impact that solar radiation has on oocyst survival. For some environments, this may be the critical process determining oocyst inactivation.

Drastic stratospheric ozone depletion over the Antarctic and Artic, as well as moderate decreases in total ozone column over high and mid-latitude waters, have been reported (Hader *et al*. 1998). Changes in the spectral composition exceeding those experienced during the evolution of exposed organisms may pose significant stress for diverse aquatic ecosystems (IASC, 1995). Any anthropogenic increases in UV-B radiation from atmospheric ozone destruction may not just affect exposed oocysts in the terrestrial environment or upper water column of low DOC water bodies, but may affect oocysts deeper in the water column in higher DOC water bodies due increased solar UV photolytic degradation of dissolved organic carbon (Naganuma *et al*. 1996), resulting in a significant effect on oocyst survival in environments susceptible to such changes.

BIOTIC ANTAGONISM

It has been proposed that natural biological antagonism has a pronounced influence in determining the environmental stability of *Cryptosporidium* oocysts (Chauret *et al*. 1998). Yet to date, little work has been published on the predation of *Cryptosporidium* oocysts in either the terrestrial (Huamanchay *et al*. 2004) or aquatic environments (Fayer *et al*. 2000*b*; Stott *et al*. 2001, 2003), while no work has identified bacterial antagonism of oocysts. Rotifers, ciliates, amoebae, gastrotrichs and platyhelminths have previously been reported as capable of ingesting oocysts (Fayer *et al*. 2000*b*; Harvey, 2004; Huamanchay *et al*. 2004; Stott *et al*. 2001, 2003). However, there is minimal information on the effect that predation has on either the removal of oocysts from the environment, or on oocyst infectivity.

Fayer *et al*. (2000*b*) noted that rotifers excreted oocysts in boluses. King *et al*. (2005) also identified oocyst clumping in a number of raw water experiments, which was absent in the sterilized water controls, and concluded this to be a result of predation. It is therefore possible that oocyst predation and then excretion of oocysts in boluses or clumps may hasten the settling of oocysts in water bodies, removing them more quickly to the sediment (Brookes *et al*. 2004) (see Hydrological Parameters section). Brookes *et al*. (2004) noted that the feeding experiments reported in the literature exposed predatory organisms to prey densities greater than 10^4 oocysts/ml. This density is far greater than the oocyst density in the environment, which in a water reservoir can typically be 0 to 100 oocysts per 100 litres, leading to their suggestion that this represents an extremely low prey density for grazing (Brookes *et al*. 2004). However, of those organisms shown to ingest oocysts, many are phagotrophic size-selective filter feeders, therefore their prey range may include a plethora of particles in the same size range as *Cryptosporidium* oocysts. Prey and predatory densities may therefore be high enough to effectively ingest large numbers of oocysts finding their way into the aquatic environment. The effects of predation in the natural terrestrial environment are unknown, with the limited oocyst microcosm studies in the soil environment not yet identifying any effect of microbial activity on oocyst survival (Davies *et al.* 2005).

Caenorhabditis elegans has been shown to ingest oocysts and excrete both intact oocysts and empty oocysts (Huamanchay *et al.* 2004). King *et al.* (2005) observed variation in oocyst FITC staining which they concluded could be a result of partial digestion of the oocyst wall due to predation of oocysts in raw water samples. Harvey (2004) found that DAPI- and FITC-stained oocysts were being degraded through the gut of a number of predatory organisms in feeding experiments. While empty oocysts can be safely assumed to be no longer infectious, it is not known if intact excreted oocysts are still infectious, or if these oocysts are as environmentally robust as they were before ingestion. It is also not known whether ingestion of oocysts by any of these organisms may act to protect oocysts from other biotic or abiotic stresses, potentially enhancing oocyst survival in the environment before ingestion by a susceptible host.

A number of RNA viruses have been identified as infecting protozoan parasites including *Leishmania* (Patterson, 1993), *Giardia* (Wang and Wang, 1986; Tai *et al.* 1996), *Trichomonas*, *Eimeria* and *Babesia* (Wang and Wang, 1991). Viral-like double-stranded RNAs and virus-like particles have also recently been identified in *Cryptosporidium* sporozoites (Khramtsov and Upton, 1998, 2000). However, it is unknown whether the presence of the virus affects sporozoite infectivity, survival, or fitness. It is therefore interesting to speculate that oocysts containing infected sporozoites may be more susceptible to environmental degradation through synergistic interactions with other stresses. For example, increased metabolic temperatures may result in increased viral replication within the sporozoite, increasing metabolic demand, therefore reducing the longevity of the oocyst in the environment.

Invertebrates, such as dung beetles which feed on dung, can rapidly degrade manure pats and reduce the activity of other organisms within the pat such as flies, fungi and nematodes (Fincher, 1975; Beesley, 1982; Biggane and Gormally, 1994). Such activity may also reduce the survival of *Cryptosporidium* oocysts in faeces either directly by feeding activity and ingestion and inactivation of oocysts (Mathison and Ditrich, 1999) or through the breakdown of the dung pat and exposure to abiotic stresses such as solar radiation and desiccation. Considering the enormous oocyst load in animal faeces, the activity of dung beetles may significantly impact on the survival of oocysts in the terrestrial environment, and the study of this warrants further effort. However, invertebrates have also been implicated in the spread and dissemination of *Cryptosporidium* oocysts (Graczyk *et al.* 2000, 2005; Follet-Dumoulin *et al.* 2001; Szostakowska *et al.* 2004). The feeding mechanisms, breeding habits and indiscriminate travel between filth and food make some groups of insects, such as non-biting flies and cockroaches, efficient vectors of protozoan parasites of concern to human health (Graczyk *et al.* 2005).

Many potential predators of *Cryptosporidium* oocysts may be found in animal faeces, the soil or aquatic environments. Invertebrate organisms may pose both negative and positive stresses on oocyst survival. Further experiments designed to measure oocyst removal or attenuation from the environment due to such biotic processes are needed if we are able to effectively model the fate of *Cryptosporidium* oocysts in the environment. Until we know which organisms are responsible for removal, attenuation, dissemination or reduction in general fitness of oocysts, it is not possible to predict the effect that climate change may have on these organisms and therefore the fate of oocysts in the environment.

HYDROLOGICAL PARAMETERS

Once oocysts escape the terrestrial environment and enter the aquatic ecosystem, water can serve as an excellent vehicle for their transmission and subsequent contact with and ingestion by hosts. However, there are a number of important processes controlling the transport and distribution of pathogens within water bodies. These include dispersion, dilution, horizontal and vertical transport. The settling of pathogen particles and their partition into the sediment (hydrodynamic processes) is discussed in detail by Brookes *et al.* (2004).

Horizontal transport is predominantly driven by inflows and basin-scale circulation patterns including wind-driven currents and internal waves. The riverine inflow is considered to be a major source of pathogens to water bodies. Inflows are controlled by their density relative to that of the lake, such that warm inflows flow over the surface of the lake and cold dense inflows sink beneath the lake water. As already discussed, this can impact enormously on the solar radiation exposure oocysts receive. The inflow will entrain water from the lake, increasing its volume and diluting the concentration of oocysts. The speed at which an inflow travels through the lake, its entrainment of lake water and resulting dilution of its characteristics and its insertion depth are all of critical importance in determining the hydrodynamic distribution of oocysts (Brookes *et al.* 2004).

The vertical distribution of pathogens can be affected by the settling rate of the pathogen, which in turn is affected by its size and density (Reynolds, 1984). Aggregation of pathogens to particulate material or integration into an organic matrix will influence settling. Medema *et al.* (1998) identified individual oocysts with a settling velocity in water of 0·03 m/day, and when attached to particles from biological effluent the rate increased to 2·5 m/day. Hawkins *et al*. (2000) estimated sedimentation rates of oocysts of 5–10 m/day. Therefore, while the settling of individual oocysts is extremely slow, when attached to other particles this can increase their settling velocity by 2 orders of magnitude (Brookes *et al*. 2004). Therefore, the size of particles with which *Cryptosporidium* associates is a major factor influencing its transport in a water body. However, a study by Dai and Boll (2003) determined that oocysts do not attach to natural soil particles and would travel freely in water. This is supported by the negative surface charge of oocysts at neutral pH, suggesting that they would not readily adsorb to particles (Ongerth and Pecoraro, 1996). However, this conflicts with the high settling velocities recorded by Hawkins *et al*. (2000) and Medema *et al*. (1998), suggesting that in some situations oocysts must associate with larger particles. Brookes *et al*. (2004) suggested that the aggregation of oocysts to particles in water will be primarily controlled by turbulence, therefore if aggregation is to occur it is much more likely in inflowing rivers than within lakes and reservoirs due to the higher rates of turbulence in riverine systems. Vertical transport may also be affected by internal waves, which may generate significant vertical movements in the order of tens of metres, resulting in the vertical advection of pathogens and particles (Deen and Antenucci, 2000; Brookes *et al*. 2004).

While sedimentation of oocysts may remove them from host ingestion, it may only be temporarily. Since oocysts can remain viable for lengthy periods of time within the sediment, especially if cold and dark, any re-suspension and subsequent redistribution will be important in estimating the risk such oocysts still pose. Turbulence generated by underflow events and internal waves can result in sediment re-suspension of particulate material (Michallet and Ivey, 1999). If the turbulent zone of benthic boundary layers coincides with the zone of substantial sediment accretion, then large amounts of suspended oocysts may occur in this region. Climate change predictions forecast more intense precipitation events, this may in turn result in increased disturbance of sediments and re-suspension of infectious oocysts; however it may also result in increased settling of oocysts due to increased turbulence combined with increased organic matter in waters in which oocysts may become enmeshed.

SALINITY AND ACCUMULATION IN FILTER FEEDING SHELLFISH

Large quantities of oocysts find their way to the ocean from precipitation events or through the discharge of treated and untreated waste products, resulting in contamination of marine waters. Any survival of oocysts for significant periods at environmental temperatures provides potential for exposure to humans and marine animals. Significant reductions in oocyst viability have been identified in seawater trials using DAPI/PI vital dyes (Robertson *et al*. 1992), with concentrations of 20 ppt and higher demonstrated to have a significant effect on *Cryptosporidium* infectivity (Fayer *et al*. 1998*a*) (neonatal BALB/c mice). Salinity, time and salinity-time interactions have been described as important factors affecting infection intensity (Freire-Santos *et al*. 1999) (neonatal CD-1 mice). Fayer *et al*. (1998*a*) also identified a strong synergistic interaction of salinity and temperature, with oocysts held at 20 °C infectious at salinities of 0 and 10 ppt for 12 weeks, 20 ppt for 4 weeks, and 30 ppt for 2 weeks. While these findings demonstrate that salinity can have a pronounced effect on oocyst viability, they also suggest that oocysts could survive in marine waters long enough to be removed by filter feeders or infect marine animals. This is supported by the identification of *Cryptosporidium* species in marine mammal species (Fayer *et al*. 2004; Appelbee *et al*. 2005; Hughes-Hanks *et al*. 2005) fish (Sitja-Bobadilla *et al*. 2005) and the detection and recovery of infectious oocysts in filter feeding shellfish worldwide (Fayer *et al*. 1998*a*, 2002; Freire-Santos *et al*. 2001, 2000*b*; Gomez-Couso *et al*. 2003, 2004; Giangaspero *et al*. 2005; MacRae *et al*. 2005). Because shellfish are able to filter large volumes of water and concentrate oocysts, this poses a threat not only to human health but to marine wildlife that may feed on these shellfish as well. With increased global temperatures predicted and subsequent estimates of large rises in sea levels due to melting of the Artic and Antarctic ice sheets (Overpeck *et al*. 2006), any large decreases in salinity or ocean freshening (Wadhams and Munk, 2004) may result in increased survival of oocysts. Any lengthening of the period of exposure for marine wildlife to oocysts may have detrimental consequences for marine ecosystems due to increased parasitism.

CONCLUSIONS

While *Cryptosporidium* oocysts are considered to be environmentally robust, they are sensitive to a number of abiotic and biotic processes that they may encounter in either the terrestrial or aquatic environment. While a number of these processes (e.g. temperature) have been well quantified by researchers, other processes affecting oocyst viability (e.g. solar radiation/biotic antagonism) need much more attention. Importantly, it is largely unknown what synergetic processes occur between these different stresses and how they affect oocyst survival and/or viability in the environment. When further studies are undertaken, attention must be paid to the methodology used to measure oocyst inactivation. Using either the neonatal mouse assay or cell culture assays for measuring oocyst viability (instead of vital dye or excystation) after being challenged by these stresses will help provide accurate data for estimating *Cryptosporidium* risks in different environments.

While much progress has been made in the disinfection of oocysts in treated water supplies using artificial UV-C (Clancy *et al.* 2004; Johnson *et al.* 2005; Hijnen *et al.* 2006), it is important to realize that the vast amount of potable water used for consumption by the world's populus will not be disinfected using such processes and *Cryptosporidium* oocysts will continue to pose a threat to many communities, as well as impacting wildlife and domestic animals. Climate change and climate warming have been predicted to increase pathogen development and survival rates, disease transmission and host susceptibility. However, while the severity and frequency of diseases are predicted to increase for many host-parasite systems, a subset of pathogens might decline, releasing hosts from disease (Harvell *et al.* 2002). Our analysis of the critical processes involved in the inactivation and removal of oocysts from the environment leads us to predict that while some regions of the world will experience increasing incidences of cryptosporidiosis, other areas will see a decline in the disease. Further attention to those critical processes affecting oocyst survival in particular environments will help us to determine which areas may become more susceptible to outbreaks of cryptosporidiosis. Finally it has not escaped our attention that the processes discussed in this review and how they may respond to climate change will also have important implications for other coccidians with an infectious oocyst stage. Any substantial changes in the levels of host parasitism by coccidian parasites will have important ramifications for the ecology of those particular systems.

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LEPTOSPIROSIS AFTER RECREATIONAL EXPOSURE TO WATER IN THE YAEYAMA ISLANDS, JAPAN

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Abstract. Leptospirosis is a global zoonotic disease with a variety of clinical manifestations. We report an outbreak of leptospirosis in the Yaeyama Islands, Japan, in the summer of 1999 associated with heavy rainfall. Fourteen people were diagnosed with leptospirosis and required hospitalization. All cases were found to have exposure to contaminated soil or water. A history of recreational activities involving water sports was more frequent (71%) than occupational risk factors related to agriculture or construction (29%). Fever was the primary symptom in all cases, followed by chills (93%), headache (86%), myalgias (57%) and conjunctival suffusion (57%). All cases were successfully treated with antimicrobial therapy except one patient who improved spontaneously. Jarisch-Herxheimer reactions were seen in six cases (43%). The increasing incidence of leptospirosis related to recreational sports is an important public health problem in resort areas. A high-index of suspicion, early treatment, and prevention are crucial in this latently endemic area.

INTRODUCTION

The Yaeyama Islands consist of two major islands, Ishigaki and Iriomote, and a number of smaller islets located in the most southern part of Japan. Due to its location at a latitude of 24 degrees, the climate of the Yaeyama Islands is subtropical. As in other subtropical resort locations, such as Hawaii and Florida, marine sports are major tourist attractions in this area.

Leptospirosis has a worldwide distribution. The incidence is higher in the tropics than in temperate regions.[1] In both developing and developed countries, leptospirosis is an important public health problem related to poor housing conditions. The disease is seasonal, with peak incidence occurring in summer or fall in temperate regions.[2] Extensive flooding and seasonal rainfall are significant risk factors for exposure to water contaminated with leptospires. A report from Brazil described a relationship between rainfall and human leptospirosis.[3] Leptospirosis was formerly considered to be primarily an occupational disease associated with agriculture, mining, livestock farming, and military maneuvers.[1,4]

Although leptospirosis related to occupational exposure has decreased, reports of recreational exposure involving water-sports including swimming, canoeing, or rafting have been increasing conspicuously.[5–8] Travelers returning from locations where leptospirosis is endemic are at risk. The incubation period of the disease is usually 5–14 days but may last up to 1 month.[9] Therefore, the relationship between symptoms and the water exposure may not always be apparent. In this report, we describe 14 cases of leptospirosis requiring hospitalization in a 2-month period, with the majority acquiring the disease after recreational exposure to water.

MATERIALS AND METHODS

The medical records of all patients with a laboratory diagnosis of leptospirosis during summer 1999 at the Yaeyama Hospital, Okinawa, Japan, were retrospectively reviewed. In some cases, patients were also interviewed during hospitalization.

In this institution, all patients suspected to have leptospirosis on the basis of history and symptoms were studied by two methods: culture isolation and serological diagnosis. Blood, urine, and cerebrospinal fluid were inoculated into Korthof media (Denka Seiken Co. Ltd., Tokyo, Japan), followed by subculturing 300 μL into 5 mL of Stuart media. Repeated weekly subculturing was continued at least 4 weeks until positive growth was visualized by dark-field microscopy. Otherwise, the results were regarded as negative. Serotyping of isolates was performed by cross-agglutination absorption.

As for serological diagnosis, microcapsule agglutination test and microagglutination tests were performed on paired acute and convalescent sera.

A positive laboratory diagnosis of leptospirosis required one of the following two criteria: 1) culture isolation or 2) serological diagnosis by greater than 4-fold elevation in paired sera, or a titer of greater than 1/80 in a single serum. Laboratory studies were performed by the microbiology section at the Okinawa Prefectural Institute of Health and Environment.

The following clinical information was collected; exposure history of contaminated water or soil, injury on extremities, resident or nonresident, occupation, clinical symptoms, and clinical data from all hospitalized 14 cases.

Descriptive weather data including the amount of rainfall during summer 1999 in the Yaeyama Islands were retrieved from the Ishigakijima Local Meteorological Observatory. The timing of the onset of symptoms of leptospirosis and heavy rainfall were compared.

RESULTS

Fourteen cases met criteria for a laboratory diagnosis of leptospirosis. 11 cases occurred in the Iriomote Island and 3 cases in the Ishigaki Island. Most patients were males (86%). The average age was 35 years. All patients reported exposure to contaminated water or soil, and 4 cases (29%) had an open

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wound on hands or feet. Nine patients (64%) were nonresidents of the Yaeyama Islands. Ten of 14 (71%) patients reported recreational exposures, including kayaking or canoeing.

In terms of clinical findings, fever (100%), chills (93%), and headache (86%) were the most common symptoms. Myalgia, arthralgia, and conjunctival suffusion were seen more than half of cases. Jarisch-Herxheimer reactions (JHR) characterized by rigors followed by hypotension were seen in 6 cases (43%) after ampicillin administration. Urinary protein was seen in 50%.

Leptospires were isolated in 9 cases (2 cases from the Ishigaki Island, 7 cases from the Iriomote Island). Serovar identity was determined either by serotyping of isolates or by serology and the majority of cases were due to serovars hebdomadis or grippotyphosa (Table 1).

Detailed clinical information is provided in Table 2. The incubation period was defined as the period from the day of exposure to contaminated water or soil to the day of onset of symptoms. Incubation periods were determined in only 4 cases, ranging from 6 to 14 days (Table 2). The other cases were exposed to water or soil on a regular basis, making it difficult to estimate the incubation period. There were no cases of icteric leptospirosis (Weil disease). One case had a typical biphasic clinical course accompanied by aseptic meningitis. All cases recovered without long-term complications.

Cases were clustered from July to September in 1999, after unusually heavy rainfall (Figure 1). During summer 1999, peaks of rainfall alternated with dry weather. According to the the Ishigakijima Local Meteorological Observatory, only September 1999 showed a significant increase in precipitation of 150–200% greater than average. Most of this excess precipitation occurred over a relatively short period of a few days.

DISCUSSION

The epidemiology of human leptospirosis reflects the ecologic relationship between humans and chronically infected mammalian reservoir hosts.[10] Three epidemiologic patterns of leptospirosis were originally defined by Faine.[11] The first pattern is a "farming type" that occurs in temperate regions, due to direct contact with animals through exposure to a limited number of serovars that infect cattle and pigs.[12] The second pattern is an "urban type" that comprises rodent-borne infection in the urban environment.[13,14] The third pattern is a "tropical type" that occurs in humid, wet areas, from exposure to a larger number of serovars infecting a diversity of reservoir animals including rodents and farm animals. The outbreak in the Yaeyama Islands corresponds typically to this "tropical type." Eleven out of our 14 cases were from the Iriomote Island.

Transmission frequently occurs via skin abrasion or exposed mucous membranes. Haake and others reported in their case report of leptospirosis that leech bites, skin abrasions, and maceration might have served as risk factors for infection.[6] All cases in the outbreak presented here demonstrated water-soil exposure, and 4 cases (29%) were confirmed with some injury on extremities. Many of our cases were tour guides or water-sports instructors with frequent exposure to white water. Three cases reported swimming in a river and were presumed to be exposed through immersion in contaminated water. In our study, skin maceration, conjunctivae, and skin injury were possible portals of entry. In mice, immunity to leptospirosis is exclusively humoral.[15] Immunity is strongly restricted to the homologous serovar or closely related serovars.[16–18]

Nine cases (64%) of leptospirosis occurred in nonresidents of the Yaeyama Islands. This finding may suggest that nonresidents are more susceptible to leptospirosis in the Yaeyama Islands, due to a lack of immunity. A report from the Okinawa Prefectural Institute of Health and Environment showed that 29% of the population in Iriomote Island had positive antibodies for serovars hebdomadis and 13% for serovars grippotyphosa.[19] Leptospirosis can be prevented by serovar-specific vaccination or prophylactic antibiotic therapy. Of note, from 1977, serovar-specific vaccination as protection against serovars pyrogenes, autumnalis, and hebdomadis had succeeded in the Izena Island, Okinawa, Japan.[20] As Bharti and others mentioned, several problems confront the development of vaccines to prevent human leptospirosis.[1] Further study of the role of immunization for prevention of leptospirosis in water-sports instructers in endemic areas is warranted.

Human infections of leptospirosis may be acquired through two types of exposures: occupational and recreational.[2] In the previous report from this area, the main occupational exposures were secondary to agriculture or construction.[21] By contrast, the current report from the Yaeyama Islands found that recreational exposure predominated. Recent case reports and review articles describe recreational exposure including ecotourism as epidemiologic risk factors.[2,5–10,22,23] Young male patients were predominantly affected in this outbreak, a predilection that seems to be related to recreational exposure. The leading demographic characteristics of this outbreak were tour guides or instructors of water-sports, such as kayaking or canoeing. They were exposed to contaminated turbulent river water daily while paddling. Their activity as the

TABLE 1
Identified serovars of leptospirosis

Serovar(s)	Ishigaki: Serologically diagnosed	Ishigaki: Isolated microbiologically	Iriomote: Serologically diagnosed	Iriomote: Isolated microbiologically	Total
Hebdomadis	0	0	3	4	7
Grippotyphosa	0	2	1	2	5
Kremastos	1	0	0	0	1
Pyrogenes	0	0	0	1	1
Total	1	2	4	7	14

TABLE 2
Detailed clinical information of 14 cases of leptospirosis

Summary of leptospirosis (all cases)							
Case	Occupation	Water/soil exposure	Native vs. nonresident	Serovar(s)	Clinical course	JHR	IP (days)
61 yr M	Agriculture	Rice farming	Nonresident	Pyrogenes	Thrombocytopenia, renal failure	+	NA
22 yr M	Agriculture	Rice farming	Nonresident	Hebdomadis	Improved without antibiotics	−	NA
26 yr M	Tour guide	River	Nonresident	Hebdomadis	Thrombocytopenia	+	NA
49 yr M	Tour guide	River	Nonresident	Hebdomadis	Conjunctival hemorrhage, calf muscle pain	+	NA
13 yr M	Junion high student	Swimming	Native	Grippotyphosa	Vomiting, diarrhea, concerned HUS	−	6
28 yr M	Tour guide	River	Nonresident	Grippotyphosa	Headache without meningismus	+	NA
54 yr M	Tour guide	River	Native	Hebdomadis	Arthralgia, myalgia	−	NA
22 yr M	Agriculture	Swimming	Nonresident	Hebdomadis	General joint pain	−	NA
22 yr M	Tour guide	River	Nonresident	Hebdomadis	Doxycycline, switched to ampicillin	−	NA
48 yr M	Tour guide	River	Nonresident	Hebdomadis	Headache and lower back pain	−	NA
31 yr M	Doctor	Canoeing	Nonresident	Grippotyphosa	Biphasic clinical course with aseptic meningitis	−	10
30 yr M	Laborer	River	Native	Kremastos	Severe JHR	+	NA
25 yr F	Office worker	Swimming	Native	Grippotyphosa	Well clinical course	−	14
60 yr F	Agriculture	Rice farming	Native	Grippotyphosa	General fatigue, joint pain	+	7

HUS, hemolytic uremic syndrome; JHR, Jarisch-Herixheimer reaction; IP, incubational period; NA, not assessed.

cause of infection related to river water in the Yaeyama Islands represents an overlap between occupation and recreation.

The clinical spectrum of leptospirosis is broad, ranging from asymptomatic illness to the classic syndrome of Weil disease. The great majority of infections caused by leptospirosis are subclinical, thus, patients probably will not seek medical attention.[2] In Japan, including the Yaeyama Islands, leptospirosis only became a reportable disease in 2003 through a revision of the infectious diseases reporting regulations. We have only described hospitalized cases; it is likely that larger numbers of people were affected who did not require hospitalization.

The biodiversity of leptospirosis in the environment is affected by geography, climate, biotic interactions, and anthropogenic activities.[1] Leptospiral diversity is limited in islands such as Barbados, where only four pathogenic serovars infectious to people have been identified.[24,25] On the other side, in tropical regions with a rich diversity of animal reservoir species such as in the Amazon basin or rural areas in Southeast Asia, leptospires are also highly diverse. A report described 11 serovars in Okinawa Prefecture includes the Yaeyama Island.[26] The result showed 9 cases of serovars kremastos, 5 cases of canicola, 3 cases each of hebdomadis, pyrogenes, rachmati, 2 cases each of autumnalis and javanica, one case each of australis, castellonis, icterohemorrhagiae, and pomona. The result on Table 1 shows four serovars. It is interesting to observe that serovars grippotyphosa was found on two separate islands, Ishigaki and Iriomote. Multiple serovars are also found in the mainland of Japan because the climate and reservoir animals are diverse.

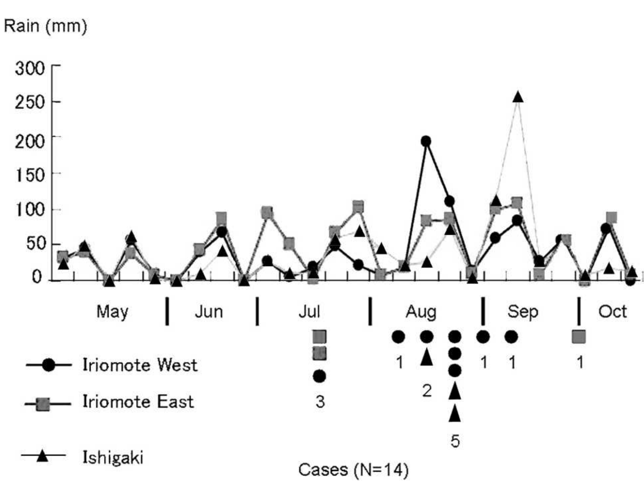

FIGURE 1. Relationship between rainfall and leptospirosis. Cases of leptospirosis were clustered from July to September 1999, several days (latent period) after unusually heavy rainfall. According to the Ishigakijima Local Meteorological Observatory, most of this excess precipitation occurred over a relatively short period of a few days.

For prevention, travelers and tour instructors should know how to minimize exposure to contaminated soil and water. Protective clothing is recommended to prevent skin injury.[6] Walking with bare feet should be avoided. Swimming in fresh water should be abandoned in endemic areas.

Chemoprophylaxis with doxycycline is likely to be useful for adventure travelers who visit endemic areas.[1,27] In a study of U.S. army soldiers in Panama, doxycycline, 200 mg weekly, was found to reduce the attack rate of symptomatic leptospirosis.[27] A study suggested that the use of postexposure chemoprophylaxis may be useful for rural residents of an area of high endemicity with flood-associated outbreaks of leptospirosis.[28] In the Yaeyama area, chemoprophylaxis should be considered, especially for high-risk subjects to prevent serious complications. Administrative interventions including public information and education also play an important role.

Human exposure to leptospires is not limited by occupation but results more often from widespread environmental contamination, particularly during rainy seasons. Large outbreaks of leptospirosis are most likely to occur following floods, hurricanes or other disasters. Outbreaks of leptospirosis associated with water sports have demonstrated the ability of pathogenic leptospira species to survive in water for extended periods.[9] Survival of pathogenic leptospires in the environment is dependent of several factors, including pH, temperature, and the presence of inhibitory compounds. In soil saturated with rainwater, leptospires have been found to survive for at least 3 weeks.[29] Outbreaks of leptospirosis followed extensive flooding.[30–32] Cases of leptospirsis in Puerto

Rico increased in 1966 after a hurricane,[33] and a report of urban epidemic in Brazil described peaks of leptospirosis after excessive rainfall.[3] Other authors found an association between leptospirosis and seasonal rainfall.[3,33–36] As Figure 1 shows, most of the cases of leptospirosis in this outbreak occurred after heavy rainfall. The role of rainfall in outbreak of leptospirosis is thought to occur as follows: Leptospires are shed by reservoir hosts and accumulate in moist soil during drier periods. The spirochete requires a warm, moist climate of 25°C and water and soil pH level of 7.0–8.0 for optimal survival outside the host.[37] When precipitation from a heavy rainstorm exceeds the capacity of the soil to absorb the moisture, leptospires are gathered from contaminated soil into rivers. The meteorological conditions during the outbreak described in this report meets with these environmental conditions. The caveat is that this relationship between rainfall and outbreaks of leptospirosis might represent the exception rather than the rule. Further investigation is warranted for public health of residents and visitors to the Yaeyama Islands.

CONCLUSION

Increasing cases of leptospirosis due to recreational sports affects public health in a resort area. Our study suggested the relationship of large volume of rain precipitation and the outbreak of leptospirosis. Understanding the relationship between the epidemiology and rainfall in a subtropical area is crucial to prevention of leptospirosis outbreaks. Further observational study is warranted to confirm these conclusions.

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Multitiered Approach Using Quantitative PCR To Track Sources of Fecal Pollution Affecting Santa Monica Bay, California

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The ubiquity of fecal indicator bacteria such as *Escherichia coli* and *Enterococcus* spp. in urban environments makes tracking of fecal contamination extremely challenging. A multitiered approach was used to assess sources of fecal pollution in Ballona Creek, an urban watershed that drains to the Santa Monica Bay (SMB) near Los Angeles, Calif. A mass-based design at six main-stem sites and four major tributaries over a 6-h period was used (i) to assess the flux of *Enterococcus* spp. and *E. coli* by using culture-based methods (tier 1); (ii) to assess levels of *Enterococcus* spp. by using quantitative PCR and to detect and/or quantify additional markers of human fecal contamination, including a human-specific *Bacteroides* sp. marker and enterovirus, using quantitative reverse transcriptase PCR (tier 2); and (iii) to assess the specific types of enterovirus genomes found via sequence analysis (tier 3). Sources of fecal indicator bacteria were ubiquitous, and concentrations were high, throughout Ballona Creek, with no single tributary dominating fecal inputs. The flux of *Enterococcus* spp. and *E. coli* averaged 10^9 to 10^{10} cells h^{-1} and was as high at the head of the watershed as at the mouth prior to discharge into the SMB. In addition, a signal for the human-specific *Bacteroides* marker was consistently detected: 86% of the samples taken over the extent during the study period tested positive. Enteroviruses were quantifiable in 14 of 36 samples (39%), with the highest concentrations at the site furthest upstream (Cochran). These results indicated the power of using multiple approaches to assess and quantify fecal contamination in freshwater conduits to high-use, high-priority recreational swimming areas.

The Santa Monica Bay (SMB), California, is home to some of the most popular beaches in the world. It is located adjacent to metropolitan Los Angeles, and more than 50 million beachgoers visit SMB shorelines every year—more than those visiting all other beaches in California combined (38). However, there are serious concerns about beach water quality because of continued exceedances of water quality thresholds based on fecal indicator bacteria such as total coliforms, fecal coliforms, or *Escherichia coli* and *Enterococcus* spp., particularly in areas impacted by urban runoff. Thirteen percent of the shoreline mile-days in the SMB exceeded water quality thresholds between 1995 and 2000, with over 50% of these exceedances located near storm drains (37). The public health risk associated with urban runoff has been directly demonstrated through epidemiology studies. Haile et al. (19) demonstrated that swimmers near storm drain discharges in the SMB had a higher likelihood of respiratory and/or gastrointestinal symptoms than swimmers more than 400 m from a storm drain.

Despite the impairment of water quality and risks to human health, identification and elimination of the sources of bacteria responsible for the beach warnings remain elusive. The difficulty in identifying and eliminating the sources of bacteria results from three important factors. First, the traditional indicators of fecal pollution on the basis of which the water quality thresholds were developed are not specific to humans. These fecal indicator bacteria can be shed from any warm-blooded organism, including wild and domesticated animals (12). Therefore, source tracking turns into a challenging scenario when these diffuse and frequently intermittent or episodic fecal releases occur. The second difficulty in identifying and eliminating sources of fecal indicator bacteria is their ubiquity in urban environments. Finally, unlike many human pathogens of concern, fecal indicator bacteria may survive and even grow in the environment (see, e.g., references 24, 39, and 44).

Viruses are one tool that could prove useful in source-tracking studies, because they include many pathogens of concern, and they are generally species specific. Viruses are known to cause a significant portion of waterborne disease, mostly from ingestion of sewage-contaminated water and seafood (10). Until recently, however, virus detection and quantification have relied on cell culture-based approaches that are much too slow to be effective source-tracking tools. Recently developed molecular techniques, such as quantitative reverse transcriptase PCR (QRT-PCR), can detect and quantify viral genetic material directly from water samples. Tests conducted previously in Southern California (11, 22, 32, 41, 42), Florida (17, 36), and Europe (35) using conventional RT-PCR or PCR have detected genetic material from human-specific viruses, including enterovirus, hepatitis A virus, rotavirus, and adenovirus, in urban runoff discharges or seawater samples.

A different approach would be to use alternative bacterial indicators for source tracking that might be much more abundant in urban discharges. For example, *Bacteroides* spp. make up approximately one-third of the human fecal microflora,

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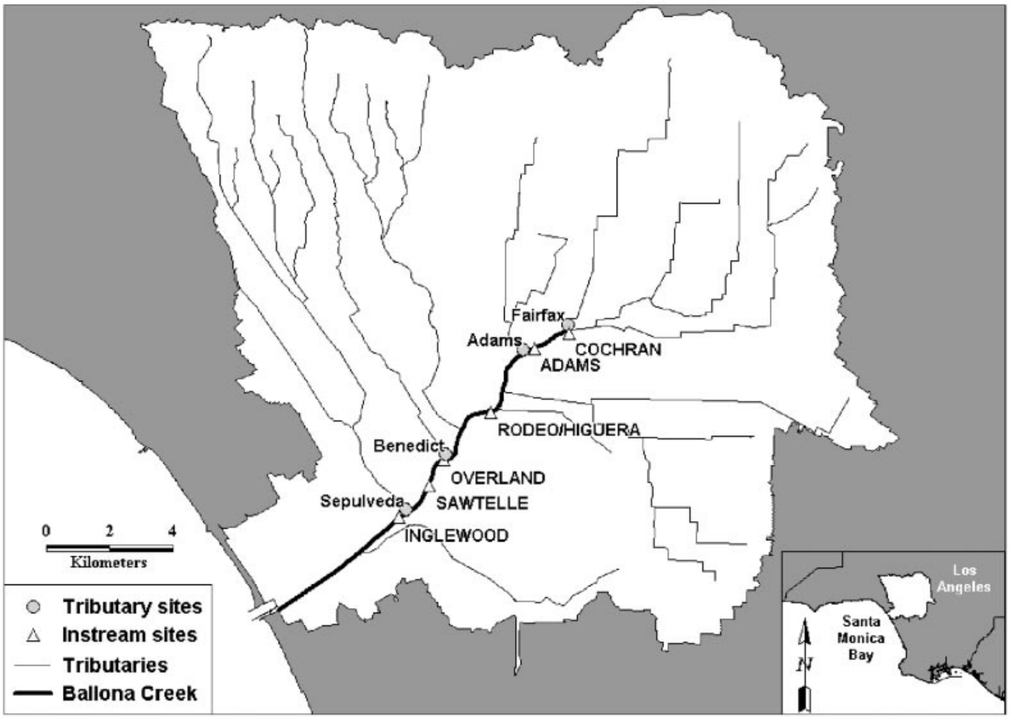

FIG. 1. Map of the Ballona Creek watershed in Los Angeles, Calif. Tributary and main-stem sampling sites for the water quality study are indicated. (Inset) Santa Monica Bay, in Southern California.

considerably outnumbering fecal coliforms, *E. coli*, and *Enterococcus* spp. *Bacteroides* spp. are obligate anaerobes, so there is little concern over persistence or regrowth in the environment. More importantly, human-specific *Bacteroides* markers have been developed, increasing the value of this potential indicator (2, 3, 7).

Both viruses and alternative bacterial indicators such as *Bacteroides* spp. have been shown to be potentially useful source-tracking tools. Griffith et al. (18) and Noble et al. (33) concluded that genetics-based methods, such as PCR, consistently provided the best information in efforts to conduct source tracking on mixed-source samples. To date, however, no single method has all of the traits necessary to be the consummate source-tracking tool. Therefore, a multitiered, multiindicator approach has been recommended by some investigators (4, 40). By using multiple tools, investigators can utilize the strengths of each to ascertain inputs and track fates that will ultimately lead to successful management solutions.

The objective of this study was to identify the contributions and quantify the loading of fecal contamination affecting the SMB by using a multitiered approach. The first tier included traditional measurements of fecal indicator bacteria. The second tier included molecular assays developed and conducted for *Enterococcus* spp., a human-specific *Bacteroides* marker, and enterovirus. These methods rely on conventional PCR, quantitative PCR (QPCR), or QRT-PCR, which have not previously been applied in conjunction with one another for source-tracking studies in urban watersheds. The third tier involved sequencing of the enterovirus from the field samples with the greatest concentrations so as to determine the likely type of enterovirus amplified in the assay. The multitiered approach was applied using a mass-based design to quantify inputs and flux through an urban watershed to the beach.

MATERIALS AND METHODS

Study site. This study quantified inputs of flow, bacterial concentrations, and virus genomic equivalents and then tracked them through an urban watershed over time. This mass-based design was applied in the watershed of Ballona Creek, the largest tributary to the SMB. Ballona Creek is over 85% developed and currently has the largest inputs of fecal indicator bacteria to the SMB (Fig. 1).

Sample collection and filtration. Samples were collected at six main-stem sites and four major tributaries to Ballona Creek. The six main-stem sites extended from Cochran Ave. (where the system daylights from the underground storm drainage system) to Inglewood Ave. (located at the head of tide just prior to discharge into the SMB) (Table 1). The four tributaries represented the four

TABLE 1. Sampling sites along the main stem and major tributaries of Ballona Creek

Site	Description	GPS coordinates (NAD 83 datum)[a]
Cochran Ave.	Main stem/ tributary	34°02.662″N, 118°21.237″W
Fairfax drain	Tributary	34°02.298″N, 118°22.136″W
Adams Ave.	Main stem	34°02.009″N, 118°22.494″W
Adams drain	Tributary	34°02.009″N, 118°22.494″W
Rodeo/Higuera	Main stem	34°01.305″N, 118°22.693″W
Benedict Box Channel	Tributary	34°00.925″N, 118°23.432″W
Overland Ave.	Main stem	33°00.429″N, 118°23.771″W
Sawtelle Ave.	Main stem	33°59.816″N, 118°24.164″W
Sepulveda channel	Tributary	33°59.512″N, 118°24.693″W
Inglewood Ave.	Main stem	33°59.394″N, 118°24.696″W

[a] GPS, global positioning system; NAD 83 datum, North American datum of 1983.

largest hydrodynamic inputs to the system and were located in reaches between each of the main-stem sampling sites. Flow was calculated as the product of the flow rate and the wetted cross-sectional area (43). Doppler area-velocity sensors (Teledyne ISCO, Los Angeles, CA) were used to measure flow rate. Pressure transducers that measure stage, along with verified as-built cross sections, were used to estimate the wetted cross-sectional area. One-minute instantaneous flow was logged electronically during the entire 6-h sampling period. Both the area-velocity sensors and the pressure transducers were calibrated prior to sampling.

One-hour composite water samples were collected immediately downstream of flow measurement devices at each site (Table 1, GPS coordinates) between 8:00 and 14:00 on 26 August 2004. The 6-h sampling period corresponds to the approximate hydrodynamic travel time from Cochran Ave. to Inglewood Ave. (1). The hourly 4-liter composite samples at each site were created after combining 10 individual 400-ml grab samples collected every 6 min into a single container. In total, 60 composite samples were collected at Ballona Creek as a result of sampling 6 h at 10 different sites.

After collection, samples were placed on ice and transported immediately to the University of Southern California for processing. For each composite sample, 100 ml of water was devoted to indicator bacteria analysis, and 200 to 600 ml of the sample volume was vacuum filtered through replicate 47-mm-diameter, 0.4-μm-pore-size polycarbonate filters (Poretics, Inc., Livermore, CA) using a filter funnel and receiver (Millipore, Inc., Bedford, MA) for *Enterococcus* sp. analyses by QPCR or *Bacteroides* sp. analysis by conventional PCR, as suggested by Haugland et al. (20). In addition, replicate filtrations were also conducted using 47-mm-diameter, 0.45-μm-pore-size type HA (Millipore, Inc., Bedford, MA) mixed cellulose ester filters for enterovirus analysis as suggested by Fuhrman et al. (11). For each filter, the volume filtered was the maximum filterable within ca. 10 min of the start of filtration. The total volume filtered was dependent on the location and turbidity of each individual sample, and the filter volumes were carefully recorded to the nearest 1 ml. All filters were placed in microcentrifuge tubes and stored at −80°C for further analysis.

Analysis of indicator bacteria. Concentrations of *E. coli* and *Enterococcus* spp. were measured by defined substrate technology using kits supplied by IDEXX Laboratories, Inc. (Westbrook, ME) according to the manufacturer's instructions. Briefly, 10-fold and 100-fold dilutions of the water samples were made with deionized water containing the appropriate media and sodium thiosulfate, mixed to dissolve, dispensed into trays (Quanti-Tray/2000), and heat sealed. *E. coli* was measured using the Colilert-18 reagents, while *Enterococcus* spp. were measured using Enterolert reagents. Samples were incubated overnight according to the manufacturer's instructions and inspected for positive wells. Conversion of positive wells from these tests to a most probable number was done following Hurley and Roscoe (21).

Extraction of DNA. The polycarbonate filters were processed for DNA extraction using the UltraClean fecal DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's alternative protocol for maximum yield. Eluted DNA extracts were stored at −20°C until use.

The concentration of the extracted DNA was measured using the Quant-iT Picogreen double-stranded DNA reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Standard curves were generated in duplicate using Lambda DNA standards between 2.5 ng/ml and 600 ng/ml and a negative control (0 ng/ml). Fluorometric measurements were made using a Bio-Rad VersaFluor fluorometer.

Bacterial analyses using QPCR. The total *Enterococcus* sp. primers and probe are described by Ludwig and Schleifer (28) and were constructed using the 23S rRNA gene regions around the target site of a well-established *Enterococcus* group-specific primer (ENC854R). Primer ECST748F targets *Enterococcus* spp., lactococci, and several clostridia. The target site of probe GPL813TQ is present in the 23S rRNA genes from a variety of representatives of gram-positive bacteria with low G+C DNA contents (28).

The master mix contained 1× *Taq* buffer, 4 mM $MgCl_2$, 3 mM deoxynucleoside triphosphates (dNTPs), 2.5 U Ex *Taq* R polymerase (R PCR kit for quantitative PCR; TaKaRa Mirus Bio, Madison, WI), 1 μM ENC854R, 1 μM ECST748F, 0.1 μM GPL813 TQ Cy3 Probe (synthesized by MWG Biotech, High Point, NC), and nuclease-free water, yielding a final volume of 20 μl, to which 5 μl of sample (either DNA extract from an environmental sample, ranging from 1 to 76 ng genomic DNA, or 5 μl of lysed cell suspension or genomic equivalents) was added for a final volume of 25 μl. The samples were run under the following optimized assay conditions for PCR: 1 cycle consisting of an initial hold at 95°C for 2 min and 45 cycles of denaturation at 94°C for 15 s and annealing/extension at 60°C for 30 s (the optics were turned on during the annealing step). The Cepheid Smart Cycler II was set with the following specific parameters for this assay. The Dye Set was set for FCTC25. The cycle threshold analysis mode was set for growth curve (linear) analyses, with a manual threshold typically set at 5 to 15 fluorescence units. The background subtract level was set at a minimum of 12 and a maximum of 40. The BoxCar averaging feature was set at zero. The assay was previously optimized for Taq, Mg^{2+}, and dNTP concentrations as well as all cycling parameters (data not shown). For quality control, *Enterococcus faecalis* ATCC 29212 and *Enterococcus faecium* ATCC 35667 combined were used as our calibration strains for the total-*Enterococcus* primer and probe set. Control bacterial preparations were prepared by boiling bacteria for 5 min, centrifuging for 2 min at 12,000 × *g* at 4°C, and storing immediately on ice. *E. faecalis* and *E. faecium* cells were enumerated using SYBR Green I epifluorescence microscopy (30). Serial dilutions of the standards were made in diethyl pyrocarbonate-treated sterile water, and four-point standard curves were run in duplicate in concert with the unknown samples on the Smart Cycler II instrument. Total-*Enterococcus* primers were tested with all 19 validly described species of the genus *Enterococcus* and demonstrated amplification of rRNA genes of all strains, with varying efficiencies (28).

Analysis of *Bacteroides* spp. using conventional PCR. Amplification of the human-specific *Bacteroides*/*Prevotella* marker generally followed the procedure of Bernhard and Field (2), with PCR primers that amplify partial 16S rRNA from the human feces-specific group (BAC708R and HS183F). A range of extracted DNA quantities (representing 1 to 70 ng per assay, with most samples in the range of 5 to 20 ng) was tested to avoid problems with inhibition of the PCR. DNA was amplified with the *Bacteroides-Provotella*-specific primers described by Bernhard and Field (2). The PCR mixture was 1× *Taq* polymerase buffer, 1μm each primer, 200 μm dNTPs, 1.25 U *Taq* polymerase, 640 ng μl^{-1} BSA, and 1.5 mM $MgCl_2$. The PCR conditions were specifically optimized for this study and differed from those in the original publication: 2 min at 95°C, then 25 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, followed by a 5-min extension at 72°C. Then 1 μl of each PCR product was reamplified using the conditions given above for another 25 cycles. PCR was performed on a 3000MX thermal cycler (Stratagene). PCR products were visualized in a 2% agarose gel stained with 1× SYBR Gold (Molecular Probes, Eugene, OR) and compared to a 100-bp DNA ladder (Promega). Positive results yielded 525-bp amplicons. The positive control was a human fecal sample extracted with a QIAamp DNA stool kit (QIAGEN, Valencia, CA). Negative controls contained water instead of sample. All samples were initially run with 5 μl of extracted material. After the initial analyses, all negative samples (14 of 60) were spiked with 0.1 ng of positive-control DNA and reanalyzed to determine possible inhibition. Three of the 14 negative samples (two from Benedict Box Channel and one from Adams) were determined to be inhibited. Inhibited samples were reanalyzed using 2 μl of sample, but all three remained negative.

Enterovirus analyses. Samples, along with negative controls, were extracted using a modified RNeasy plant minikit (QIAGEN Plant and Fungi RNA isolation protocol). The RLT homogenization buffer supplied was supplemented with polyvinyl pyrrolidone 40 (PVP-40) at a final concentration of 2%. Prior to the extraction, fresh β-mercaptoethanol (Sigma Chemical Co.) was added to the extraction buffer in the exact concentration recommended by the manufacturer. Filters (type HA) were manually homogenized with a pipette tip, and 700 μl of the RLT/filter slurry was applied to a QiaShredder column (QIAGEN) and spun at maximum speed, ≥8,000 × *g*, for 2 min to aid in viral lysis as well as to separate filter particles from the filtrate (11). The filtrate was then carefully removed without disturbing the pelleted material and was placed into a new 1.5-ml tube. The volume of solution in each tube was estimated by pipetting, and 0.4 volume of 5 M potassium acetate (pH 6.5) was added. Tubes were mixed by inversion and incubated on ice for 15 min. The mixture was then spun (12,000 × *g*) at 4°C for 15 to 30 min and the supernatant transferred to a new 1.5-ml microcentrifuge tube. Subsequently, the Plant and Fungi RNA isolation protocol was followed starting at step 5. RNA was eluted with 50 μl of the supplied RNase-free water. A one-step TaqMan QRT-PCR was performed on the extracted RNA, with final reaction volumes of 25 μl, using a QIAGEN One-Step RT-PCR kit. Five microliters of extracted RNA was added to 20 μl of master mix containing 1× RT buffer, 6 mM $MgCl_2$, 500 nM dNTPs, 700 nM EV1 reverse primer (5′-TGTCA CCATA AGCAGCCA-3′), 700 nM EV1 forward primer (5′-CCCTGAATGCG GCTAAT-3′), 30 μg bovine serum albumin, 20 U of recombinant RNasin (Promega Corp.), 1.5% PVP-25 (29) (Sigma Chemical Co.), 100 genomic equivalent units of a competitive internal positive control (CIPC) developed in-house (16) by following the general approach of Kleiboeker (25), plus 300 nM CIPC probe 5′-Cy5-TGTGCTGCAAGGCGATTAAGTTGGGT–BHQ-2–3′, and 300 nM EV-BHQ probe 5′–6-carboxyfluorescein–ACGGACACCCAAAGTAGTCGGT TC–BHQ-1–3′, and 1 μl of enzyme mix (containing both reverse transcriptase and DNA polymerase). The probe and primers were synthesized by MWG Biotech, Inc. The Cepheid Smart Cycler II was programmed as follows: a 1-h reverse transcription step at 50°C followed by a 15-min hold at 95°C for DNA polymerase activation, then 45 cycles of 94°C for 15 s (denaturation) and 60°C for 1 min (annealing and extension, with optics on).

QRT-PCR results were available 3 h after the start of analysis, making the total RNA extraction, QRT-PCR preparation, and analysis time less than 5 h. After the first analyses, those samples that appeared to have inhibition of the QRT-PCR (as indicated by the CIPC) had RNA sample volumes reduced in half and were rerun. No inhibition was observed at this lower RNA concentration. Standard curves were generated using a synthetic enterovirus transcript that was quantified using fluorometric analysis, and sample genome concentrations were interpolated from the standard curve using the manufacturer's curve-fitting software. Quantitative results are reported per liter sample volume.

Sequencing of enterovirus QRT-PCR positives. QRT-PCR-positive samples from the enterovirus analyses were sequenced to assess the specificity and fidelity of our enterovirus QRT-PCR and to elucidate the identities of the enterovirus genomes being amplified by the assay. Following the initial enteroviral analysis of the Ballona Creek samples, RNA samples identified as positive for enterovirus genomes were again amplified using the QRT-PCR protocol. However, after numerous repetitions of freezing and thawing of the extracted RNAs for various analyses, only Cochran Ave. samples from 9:00, 10:00, and 11:00 contained amplifiable enterovirus genetic material (11). The 144-bp enterovirus QRT-PCR product was distinguished from the 126-bp internal positive control product using a 10% polyacrylamide–1× Tris-borate-EDTA gel. The gel was stained with ethidium bromide and visualized with a 254-nm UV light. The larger 144-bp enterovirus QRT-PCR products were excised, homogenized in 50 μl of 1× QIAGEN One-Step RT-PCR buffer using a microcentrifuge pestle, and incubated overnight at 37°C with shaking. The enterovirus products were purified (Wizard SV gel and PCR Clean Up System; Promega), cloned into the 3.9-kilobase pCR 2.1 TOPOVector (TOPO TA cloning kit; Invitrogen, Carlsbad, CA), transformed into TOP10 chemically competent *E. coli*, and plated on Luria-Bertani agar plates containing 100 μg/ml of ampicillin. Bacterial clones were screened using QPCR, and positive colonies from each of the three sites were selected and grown individually in 3 ml of 2× medium overnight at 37°C. Plasmid DNA was isolated (PerfectPrep plasmid minikit; Eppendorf, Westbury, CT), and the DNA concentration was calculated using yeast extract-tryptone Ribogreen (Molecular Probes). Plasmid DNA was sequenced bidirectionally by MWG Biotech, and the chromatograms were inspected using Sequencher, version 4.2 (Gene Codes Corp., Ann Arbor, MI). The sequences were aligned to sequences in the NCBI GenBank using BLAST and are available by searching nucleotide accession numbers DQ196482 through DQ196487.

Calculations and statistical analyses. Data analysis comprised four steps. First, the hydrologic budget was evaluated to determine if the majority of the flow was sampled. This evaluation was conducted by comparing the volumetric inputs from each of the tributaries to the volumetric discharges along the main stem of Ballona Creek. The second step was to examine temporal and spatial trends in the flux of fecal indicator bacteria. The flux of indicator bacterial cells per hour was calculated by multiplying the concentration of the indicator bacteria (per deciliter [100 ml]) by the flow rate (in deciliters per hour). The mean hourly flux (temporal analysis) was calculated by averaging the flux of indicator bacteria at all main-stem locations for each hourly interval ($n = 6$). The mean flux at each site (spatial analysis) was calculated by averaging the flux at each main-stem or tributary site for all hourly samples ($n = 6$). Statistical analysis of the differences in bacterial flux between hourly time periods or, alternatively, between main-stem sites was conducted using analysis of variance (46). The third data analysis step was to examine spatial and temporal patterns in the frequency of *Bacteroides* detection. The *Bacteroides* method used in this study was a presence/absence end point. This examination was conducted by tabulating the locations and time periods of *Bacteroides* detection to detect patterns moving downstream, adjacent to tributaries, or over time. The fourth data analysis step was to examine the spatial and temporal extent of enterovirus concentrations. Unlike that of *Bacteroides*, the presence of enterovirus was quantified, so the magnitude of enterovirus concentrations was tabulated among the different locations across the different time periods. As in the *Bacteroides* data analysis, patterns moving downstream, adjacent to tributaries, or over time were examined.

RESULTS

The total volume discharged from Ballona Creek during the 6-h sampling period was 13,390 m^3 (Fig. 2). Of this volume, 97% was attributed to monitored inputs from Cochran, Fairfax, Adams, Benedict, and Sepulveda tributaries. The largest volume was contributed at Cochran Ave., where the creek emerges into daylight from beneath downtown Los Angeles. Flow remained relatively stable over the study period at all sites, with little variation or pattern in discharge. For example, the coefficient of variation for flow at the most-downstream site, Inglewood Ave., was less than 8%, approaching the resolution of our flow-monitoring devices.

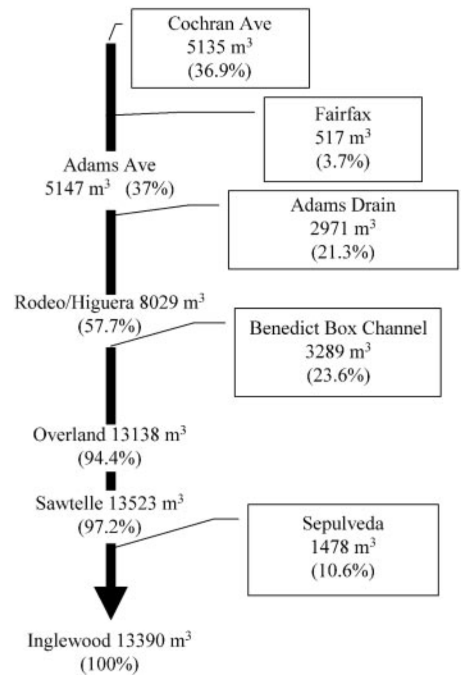

FIG. 2. Schematic diagram depicting additive flow in the main channel of Ballona Creek and the percentage contributed by each tributary sampled.

There was no observed spatial trend in the flux of fecal indicator bacteria in Ballona Creek during this study (Fig. 3). The average flux of *E. coli* ranged from 1.1×10^{10} to 5.3×10^{10} cells h^{-1} at the six main-stem sites. The average flux of *Enterococcus* spp. ranged from 6.6×10^8 to 1.4×10^9 cells h^{-1} at the six main-stem sites. In both cases, there was no discernible increase in bacterial flux as one moved downstream; no two main-stem sites were significantly different from one another in the flux of either *E. coli* or *Enterococcus* spp. ($P > 0.05$ by analysis of variance).

A temporal trend in the flux of fecal indicator bacteria in Ballona Creek was observed during this study (Fig. 4). The average flux of *Enterococcus* spp. was highest at 9:00 (2.9×10^{10} cells h^{-1}) and decreased monotonically throughout the study period. The lowest flux was measured at 14:00 (3.0×10^9 cells h^{-1}). Similar patterns were observed for *E. coli* (data not shown). In contrast to the results obtained by the culture-based methods, the QPCR results for *Enterococcus* spp. did not decrease over time. The flux of *Enterococcus* spp. ranged from 2.7×10^{10} to 4.7×10^{10} cells h^{-1}, and the 9:00 and 14:00 samples were nearly equivalent (Fig. 4).

The relative patterns of *Enterococcus* contributions from the tributaries were similar at all time periods (Fig. 5). Benedict tributary always had the greatest flux of fecal indicator bacteria, followed by Sepulveda, Fairfax, and Adams tributaries. A

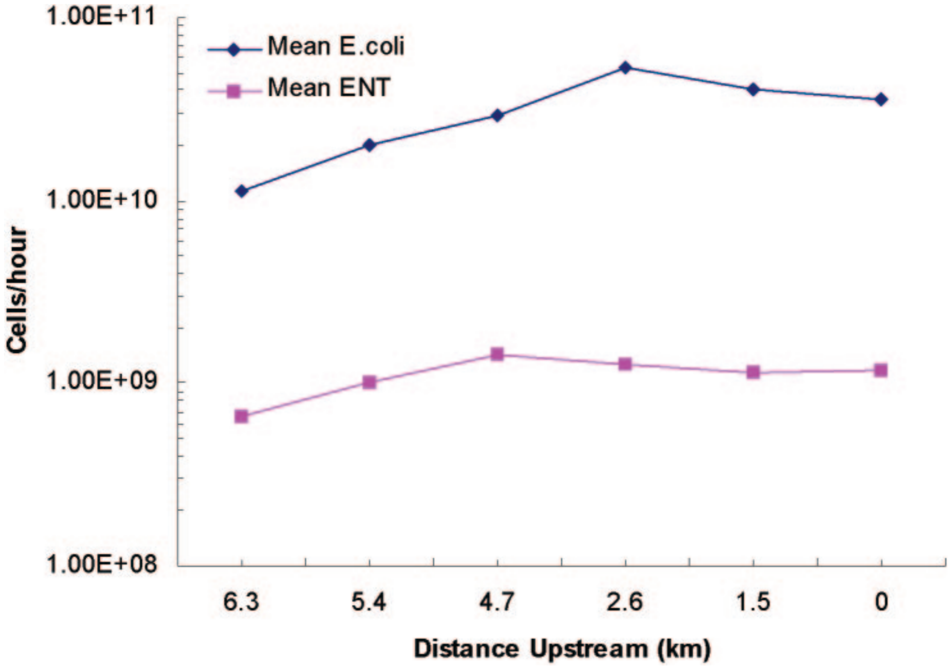

FIG. 3. Mean flux of *E. coli* and *Enterococcus* cells (expressed as cells per hour) at main-channel sampling sites of Ballona Creek (26 August 2004).

similar pattern was also observed for *E. coli*. The flux of *Enterococcus* spp. from Benedict tributary ranged from 4.1×10^9 to 1.4×10^{10} cells h^{-1} throughout the sampling period while the flux of *Enterococcus* spp. from Adams tributary ranged from 3.7×10^5 to 4.4×10^6 cells h^{-1}. On average, Benedict tributary contributed 81% of the *Enterococcus* spp. loading from all four tributaries.

The hourly flux of *Enterococcus* spp. (determined by culture-based methods) from each of the four main tributaries approximated the load being passed down Ballona Creek (Fig. 5). Regardless of the hour, the flux from each of the tributaries was within a factor of 10^1 compared to its nearest downstream site on the main stem of Ballona Creek. The only exception was the Adams tributary, for which the flux was as much as 4

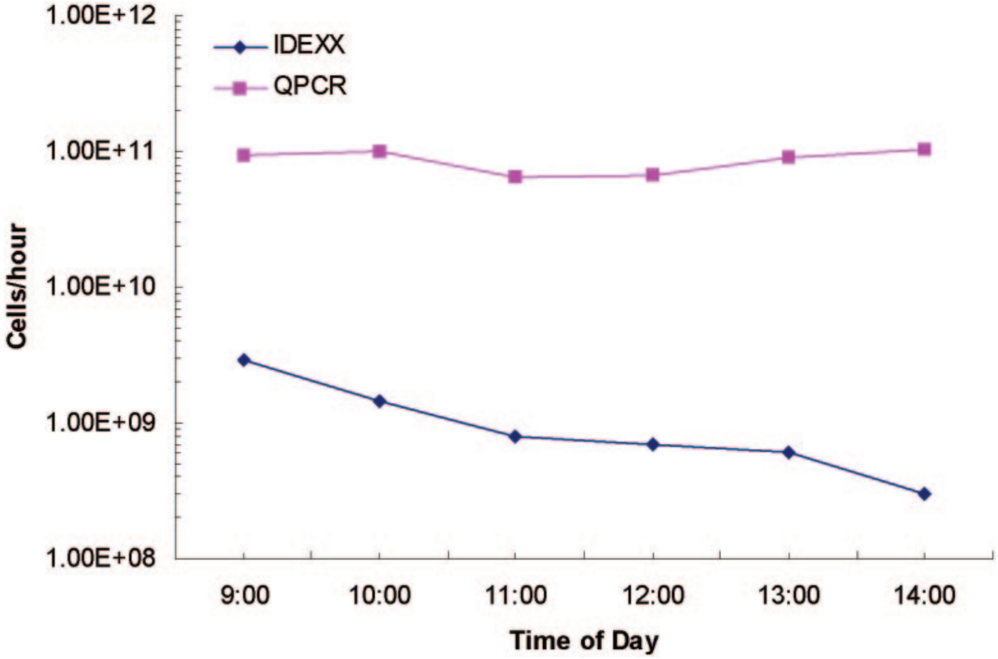

FIG. 4. Mean hourly flux of *Enterococcus* spp. (expressed as cells per hour) along the main channel of Ballona Creek, measured by using either an IDEXX chromogenic substrate (Enterolert) or QPCR methods on 26 August 2004.

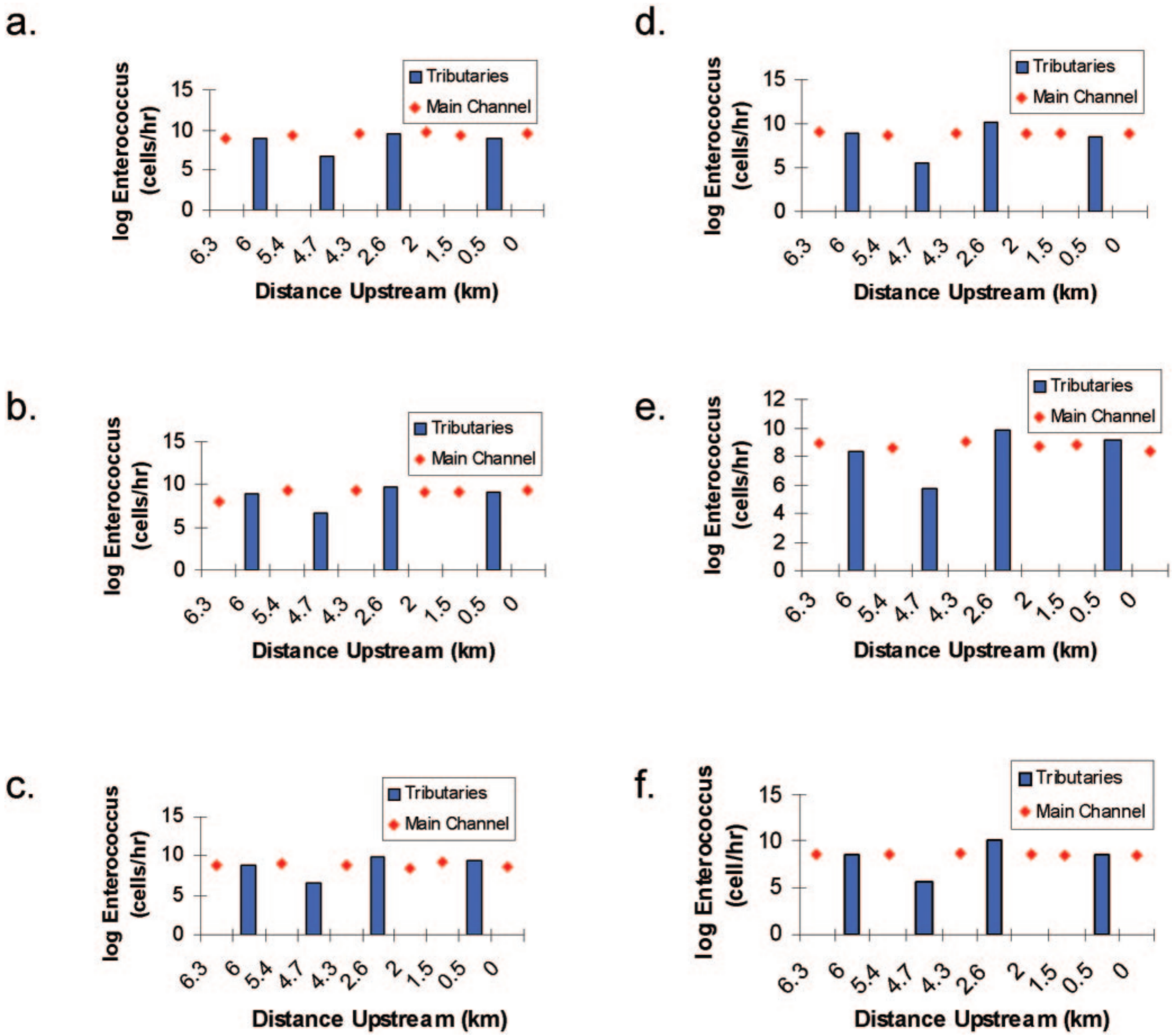

FIG. 5. Loading of *Enterococcus* spp. (expressed as cells per hour) in the main channel and tributaries of Ballona Creek at 9:00 (a), 10:00 (b), 11:00 (c), 12:00 (d), 13:00 (e), and 14:00 (f) on 26 August 2004.

orders of magnitude less than that for the nearest downstream site. The main stem showed virtually no response to any of these tributary inputs, including that of Adams. The flux of *Enterococcus* spp. remained virtually unchanged from upstream to downstream of each of the tributary inputs (Fig. 3 and 5).

The human-specific *Bacteroides* marker was positively detected throughout the main stem of Ballona Creek; its presence in 31 of the 36 main-stem samples tested (86%) was confirmed (Table 2). A positive signal was observed at all sites during the early morning hours, but during periods of heightened UV radiation (midday and late afternoon), a decrease in the number of positive results for the human-specific *Bacteroides* marker was observed. For example, only three of six samples at 14:00 were positive for the human-specific *Bacteroides* marker and exceeded the water quality threshold for *E. coli*.

Enteroviruses were detected and quantified in 14 of the 36 main-stem samples tested (39%) (Table 2). Moreover, spatial and temporal patterns in enterovirus concentrations were also evident in the Ballona Creek system. Main-channel locations in the upper reaches of the study area were more likely to be positive for enteroviruses than downstream sites. The most consistently positive site was Cochran Ave., where 89% of the samples contained measurable levels of enterovirus. In addition, some of the highest concentrations of enterovirus were measured at Cochran during four of the six time periods (concentrations ranging from 1,336 to 3,255 enterovirus genomes per liter). A general pattern in enterovirus detection was observed during the course of the day. Enterovirus was detected during the early morning and at midday at upstream sites but was detected most frequently late in the day at the downstream sites. The 12:00 sampling interval had the most frequent de-

TABLE 2. Number of enterovirus genomes per liter along the main stem of Ballona Creek in Los Angeles, CA

Distance upstream from Santa Monica Bay (km) (name of site)	No. of enterovirus genomes/liter and presence or absence of the human-specific *Bacteroides* marker[a] at the following time of day:					
	9:00	10:00	11:00	12:00	13:00	14:00
6.3 (Cochran)	3,255*	1,391*	1,714*	1,440*	1,336*	*
5.4 (Adams)	*	630*	200[b]	290*	*	*
4.7 (Rodeo/Higuera)	*	96*	*	1,641*	579	
2.6 (Overland)	*	*	*	926*	*	*
1.5 (Sawtelle)	*	*	*	61*	*	384
0 (Inglewood)	*	*	*	*	*	*

[a] Asterisks indicate presence.
[b] PCR inhibited for human-specific *Bacteroides* marker.

tection of enterovirus, with the highest concentrations observed, at the middle sites in the watershed. In nearly all of the tributary samples, no enterovirus was detected (data not shown); only Adams Drain tributary had any detectable enterovirus.

The highest sequence homology observed for the three enterovirus-positive Cochran Ave. samples that were sequenced (>95%) was with human coxsackievirus A22 (GenBank accession no. AF499643), human coxsackievirus A19 (accession no. AF499641), and human enterovirus 90 (accession no. AY773285). The 144-bp QRT-PCR product corresponded to nucleotides 453 to 596 of human coxsackievirus A22, nucleotides 457 to 600 of human enterovirus 90, and nucleotides 454 to 597 of human coxsackievirus A19.

DISCUSSION

The Ballona Creek watershed is a system impacted by fecal pollution. The flux of fecal indicator bacteria was as high at the head of the watershed as it was at the mouth of the creek, where it discharges into the SMB. Although we focused on the flux of these fecal indicator bacteria, it should be noted that 92% of all samples collected from Ballona Creek in this study exceeded the water quality thresholds established by the State of California (CA State Assembly Bill 411). The presence of human enterovirus and of human-specific markers of *Bacteroides* spp. further characterizes the fecal inputs and should increase an environmental manager's awareness of the human health risks associated with these discharges.

Our study is not the first to examine the presence of viruses in urban runoff entering shorelines in the SMB and other Southern California urban watersheds. For example, Gold and colleagues (13, 14) found viruses in repeated samples from multiple storm drains to the SMB by using both cell culture and RT-PCR techniques. Haile et al. (19) detected human-specific viruses in all three storm drains tested in their epidemiological study of the SMB. Noble and Fuhrman (32) found human enteric virus genomes in the near-shore marine waters of the SMB. Jiang et al. (22) found human adenovirus in samples collected at 12 sites between Malibu and the Mexican border, and Fuhrman et al. (11) previously found human enterovirus genomes in Ballona Creek.

The multitiered approach used in this study can assist watershed managers in determining sources and efficiently abating the most significant inputs of fecal indicator bacteria. If managers relied solely on the patterns in fecal indicator bacteria from Ballona Creek, then the only option would be to treat the entire 37-m^3 s^{-1} discharge furthest downstream at Inglewood Ave., because the flux of fecal indicator bacteria was similar at all sources. The use of multiple tools, however, allows managers to prioritize the most important sources. In this case, the presence of human enterovirus was greatest at the Cochran Ave. site, where the system daylights from the underground storm drain system beneath Los Angeles and the discharge volume is one-third of the volume at Inglewood Ave. Previous studies of Southern California storm drains have detected a human-pathogenic virus signal (22, 23, 31, 32). Since Cochran Ave. had the most frequent occurrence and highest concentrations of enterovirus, plus a consistent co-occurrence of the human-specific *Bacteroides* marker, this source would appear to be the most likely candidate for future management actions. The sequencing results that confirmed the presence of several potential risks to human health (human coxsackievirus and enterovirus) should provide the reassurance most managers would need before planning future management steps.

The lack of correlation between bacterial indicator levels and levels of human-pathogenic viruses has been observed in previous studies (8, 9) and demonstrates the value of the multitiered approach used here for source identification. For example, analysis of wild shellfish from the Atlantic coast of France indicated no significant correlation between fecal coliforms and enteroviruses or hepatitis A virus (26, 27), and viruses have sometimes been found in oysters without coliform contamination (15, 45). Noble and Fuhrman (32) detected enterovirus in 35% of the 50 shoreline samples they examined over a 5-year period, and no significant statistical relationship to any of the standard bacterial indicators was found. Virus and fecal indicator bacteria were measured in dry-weather urban runoff in drains along 300 km of shoreline from Santa Barbara to San Diego, CA (31). Although 40% of the storm drains contained detectable enterovirus, there was no correlation with concentrations of fecal indicator bacteria. It is also possible that differential rates of degradation of viruses and bacteria can explain much of the discordant relationship between viral pathogens and indicator bacteria (see, e.g., reference 34).

The use of QPCR to measure fecal indicator bacteria presents unique opportunities and challenges. An advantage of using QPCR for measuring fecal indicator bacteria is speed; the method potentially provides measurements in less than 3 h (18). However, culture-based methods quantify only viable bacteria, while QPCR measures the DNA from both cultivable and noncultivable microbes. This was most apparent in the temporal trends from Ballona Creek. Levels of *Enterococcus* spp. determined using culture-based methods generally decreased as the day progressed, most likely as a result of photoinactivation of cells by sunlight (5, 6, 34). Ballona Creek is a 40-m-wide concrete-lined channel, concentrating solar energy into the shallow creek in the channel invert. The QPCR results, however, remained steady, indicating that the bacterial DNA was still intact and detectable, even though the *Enterococcus* spp. were not viable.

Overall, the use of multiple approaches provided convincing evidence of the extent and types of microbial contamination in this urban watershed. We believe such studies should provide invaluable information for researchers and managers trying to balance regulatory burdens and public safety.

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Characterizing fecal contamination in stormwater runoff in coastal North Carolina, USA

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ABSTRACT

Microbial contaminants in stormwater runoff have the potential to negatively impact public health. Stormwater runoff to coastal waters is increasing in amount and rate of discharge due to loss of vegetated landscape and increasing coastal development. However, the extent and nature of microbial contamination of stormwater runoff in North Carolina (NC) has not been previously characterized. The aim of this study was to measure a range of fecal indicator bacteria (FIB) and molecular markers at three coastal sites. *E. coli* and *Enterococcus* sp. were measured in addition to molecular markers including *Bacteroides* Human-Specific Marker (HS) and fecal *Bacteroides* spp. Levels of FIB in stormwater far exceeded recreational water quality guidelines, frequently by several orders of magnitude. High concentrations of fecal *Bacteroides* spp. and the presence of HS indicated the presence of human fecal contamination in the stormwater runoff, but only during specific storms. Examinations of levels of fecal contamination in stormwater over multiple seasons and a range of storm conditions will allow managers to consider appropriate design of effective mitigation strategies necessary to maintain and restore coastal water quality.

1. Introduction

Stormwater runoff, one of the most common forms of non-point source (NPS) pollution, has been identified as a potential threat to human and ecosystem health due to the high levels of chemical and biological contaminants it contains that have been directly linked to disease outbreaks (Curriero et al., 2001; Gaffield et al., 2003), toxic effects in aquatic life (Bay et al., 2003; Heaney et al., 1999), and dramatic negative impacts on water quality (Ahn et al., 2005; Makepeace et al., 1995). As precipitation washes over land, it picks up and transports a variety of chemicals, pesticides, metals, petroleum products, sediment, and human and animal fecal wastes. Knowledge of the composition of the resultant runoff, as well as its delivery pathways and distribution in the environment, is crucial in managing the overall risk associated with stormwater runoff.

Discharge of stormwater runoff onto recreational beaches in the US is particularly problematic in terms of public health, as it is the largest known cause of beach closures and advisories in the US (Dorfman, 2006). Many of these advisories (75% in 2005) are in response to elevated fecal indicator bacteria (FIB) levels that exceed USEPA recommended beach water quality standards (Dorfman, 2006; USEPA, 1986). Furthermore, US recreational waters serve as a known route of exposure to human pathogens, with 95 documented recreational water-associated outbreaks occurring from 1996 to 2000 (Arnone and Walling, 2007). Evidence from epidemiological studies of recreational water-associated health effects

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suggests a causal dose-related relationship between gastrointestinal symptoms and FIB counts and strong relationships between urban runoff and illness (Pruss, 1998). Therefore, mitigating stormwater runoff to decrease loading rates of FIB and viral, bacterial, and protozoan pathogens to recreational beaches is a direct way to improve beach water quality and protect public health.

In some locations, such as the coast of California, the microbial contaminants in stormwater and their associated health risks have been studied (Dwight et al., 2004; Haile et al., 1999). However, in coastal NC, the microbial contaminants in stormwater and their impacts on recreational beaches have not yet been assessed. North Carolina ranks sixth of the fifty US states in tourism (by person-trip volume, NC Department of Commerce, 2008), with more than 6.5 million coastal tourists annually (Dorfman, 2006). Stormwater runoff in coastal NC is also receiving greater attention from the public as coastal development, number of septic tanks, and percentage of impervious surface area increase along with tourism and visitation of beaches. In Carteret County, located in coastal NC, 68% of housing units utilize septic tank systems for wastewater disposal, according to 1990 census data, which is considerably higher than the statewide average of 50% (NC National Estuarine Research Reserve, 2004). A study on southeastern NC coastal watersheds showed the percentage of watershed impervious surface is the most important anthropogenic factor associated with FIB concentrations in estuarine creeks (Mallin et al., 2000). Furthermore, the shellfish-harvesting industry is a prominent economic and cultural resource in NC which is impacted by poor microbiological water quality. These factors provide numerous opportunities for stormwater runoff to affect the coast of NC, increasing the need for research to identify and quantify the microbial contaminants therein.

To date, most published studies of microbial contaminants in stormwater runoff are limited by reliance on quantification of single types of FIB only and by low numbers of storms characterized. In this study of NC stormwater runoff, a sampling scheme was implemented to assess microbial contaminant concentrations in both baseflow and stormwater runoff samples by measuring several types of FIB and alternate molecular markers of human fecal contamination over the course of many storm events. Concentrations of FIB, including total coliforms, *E. coli*, and *Enterococcus* spp., and the *Bacteroides* Human-Specific Marker (HS) and fecal *Bacteroides* spp., were examined in an attempt to quantify levels of fecal contamination and determine the likelihood for the presence of human fecal contamination in stormwater runoff.

2. Materials and methods

2.1. Sample site selection

The study was conducted at three sites in coastal Carteret County, NC: two impacted by stormwater outfalls (pipes emptying to the beach, also called storm drains) and one impacted by a culvert/ditch system that funnels runoff to an estuarine marina. The two stormwater outfall sites are Bogue Inlet Pier (BIP), located in Emerald Isle, NC, and Triple S Pier (TSP), located in Atlantic Beach, NC (Fig. 1). Both are located at fishing piers frequented by fishermen and collect water from parking lots and residential areas which is discharged at the dune lines of popular recreational beaches. Stormwater outfall sites were selected based on historical data demonstrating poor microbial water quality of recreational beach waters after rainfall (R. T. Noble, unpublished data), anecdotal reports of failing health of surfers at recreational beaches receiving stormwater runoff (J. D. Siekmann, personal communication), and a need to initiate an examination of stormwater runoff in NC due to findings in other locations (Jeong et al., 2005; Noble et al., 2003). The estuarine site, Town Creek Marina (TCM), is located on Turner Street in Beaufort, NC (Fig. 1), where the majority of runoff from the nearby

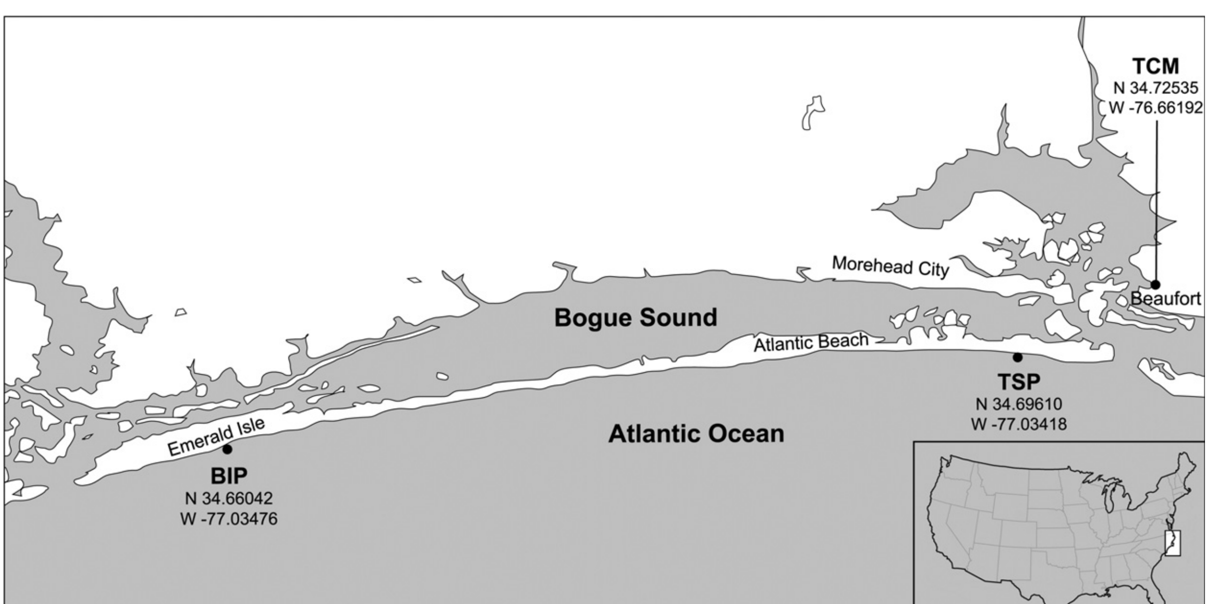

Fig. 1 – Map of sample sites.

watershed flows into a popular marina in close proximity to high priority shellfish-harvesting waters and sound-side swimming areas. TCM was selected based on results from a pilot study which demonstrated high levels of FIB at the site after rainfall (Parker, 2004).

2.2. Water sample collection

The sampling scheme was designed to collect stormwater runoff from a range of storm events. Storm sampling was initiated at any point after a sustained period of moderate to heavy rainfall produced precipitation of at least ~0.5 cm until ~1 h after the storm ended. This ensured that flow was visible from the stormwater outfalls. Rainfall was monitored in real-time using online data from weather stations in close proximity to each sampling site to make this determination. Weather stations used for each sampling site were: BIP – Oceanside of Emerald Isle Crystal Shores Station (ID# KNCEMERA1) from Weather Underground; TSP – Atlantic Beach Water Plant Station (ID# COOP 310356) from the National Climatic Data Center; TCM – Beaufort Michael J Smith Field Station (ID# WBAN 93765) from the National Climatic Data Center. Grab samples were collected using a triple acid-rinsed bucket (5% HCl) which was rinsed twice with water from the sample site before filling 4 L sample bottles prepared in the same way. At BIP, samples were taken directly from the pipe or, under conditions of very heavy rainfall, at the interface of flow from the pipe and the pool of accumulated water (storm, $n = 21$, baseflow $n = 4$). At TSP, the pipe rises ~1 m from ground level, and samples were taken as water flowed out at this height (storm $n = 14$, baseflow $n = 4$). At TCM, samples were taken ̃0.3 m below the surface of the water (storm, $n = 14$, baseflow, $n = 7$). Baseflow samples from TSP and BIP were collected after a period of at least five days without rain, and were taken from the wavewash area of the Atlantic Ocean, as pipe discharge was absent. Samples were stored on ice and analyzed in the laboratory within 2 h of collection.

2.3. Time series sampling

During several rainfall events, time series experiments were conducted where multiple samples were collected over durations of 2–3 h. Two of the time series studies were started immediately prior to the peak rainfall rate for the given storm (BIP 24 Oct 2005 and TSP 21 Nov 2005), while one was initiated during the peak rainfall rate (BIP 7 Oct 2005). Samples for the first BIP time series (BIP 7 Oct 2005) were collected over 2.1 h, beginning after 14 h (5.26 cm) of rainfall, ca. 1.5 h after the peak rainfall rate for the storm. Samples for the second BIP time series (BIP 24 Oct 2005) were collected over 1.3 h, beginning after 5 h (1.30 cm) rainfall, ca. 2.5 h before the peak rainfall rate for the storm. Samples for the TSP time series (TSP 21 Nov 2005) were collected over 2.7 h, beginning after 5 h (0.53 cm) rainfall, ca. 4.5 h before the peak rainfall rate for the storm.

2.4. Fecal indicator bacteria measurements

Concentrations of FIB used as monitoring standards for both freshwater (TC and EC) and marine waters (ENT) were enumerated, since the stormwater samples were essentially freshwater, yet were contaminating marine recreational waters. Colilert-18® was used for enumeration of total coliforms and *E. coli* and Enterolert™ was used for *Enterococcus* spp. (IDEXX Laboratories, Westbrook, ME). Since concentrations of FIB were typically high, duplicate samples were diluted to 10 ml, 1 ml, or 0.1 ml per 100 ml with deionized water. The reagents were added to each sample, dissolved by shaking 10 times, poured into a Quanti®-Tray/2000 tray, and incubated overnight at 35 °C for Colilert-18® and 41 °C for Enterolert™ as required by manufacturer's instructions. Trays were counted according to manufacturer's instructions and most probable number (MPN) per 100 ml of sample was determined using the manufacturer's MPN tables. For each set of duplicate MPN values (per dilution), average MPN values were calculated using the dilution set that yielded usable counts, with preference to the least diluted sample set if more than one set was usable.

2.5. Molecular analyses of stormwater samples

For molecular analyses, duplicate 100 ml samples were vacuum filtered through 0.4 μm polycarbonate (PC) filters (GE Osmonics, Minnetonka, MN). The filters were placed into sterile, DNase/RNase-free microcentrifuge tubes and stored at −80 °C. DNA extractions were performed on the filters using the UltraClean™ Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA) following the protocol for maximum yield, with extracts stored at −20 °C.

Quantitative PCR (QPCR) was performed on extracted DNA using the fecal *Bacteroides* spp. primer-probe set (Converse et al., 2009). The DNA template in each reaction ranged from 1 to 50 ng, determined fluorometrically with PicoGreen (Invitrogen Carlsbad, CA) using a Turner TBS-380 Fluorometer. QPCR was conducted on a SmartCycler® II (Cepheid, Sunnyvale, CA) using TaKaRa Ex Taq version 2.1 (Mirus Bio, Madison, WI), with each 25 μl reaction containing the following: 300 $\mu mol\ l^{-1}$ dNTPs, 4 $mmol\ l^{-1}$ $MgCl_2$, 0.05 U Taq polymerase, 1× Taq buffer, and 5 μl DNA template. Reaction conditions were as follows: 95 °C for 2 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 45 s. Quantification was conducted using *Bacteroides thetaiotaomicron* cells (Converse et al., 2009) enumerated via epifluorescence microscopy (Noble and Fuhrman, 1998). Quantified cells were used to establish a 4-log standard curve, with reactions run in duplicate. QPCR amplification efficiencies of >90% and R^2 values of >0.95 were documented for all standard curves.

Conventional PCR was performed on the DNA extracts using the human-specific *Bacteroides/Prevotella* marker, referred to in this manuscript as "HS" (Bernhard and Field, 2000) using primers targeting a segment of the 16S rRNA gene from the human feces-specific group (HS183F and BAC708R). PCR master mix was composed of 1.25 U Hot Master polymerase (Eppendorf, Westbury, NY), 1× Taq Polymerase self-adjusting magnesium buffer (Eppendorf, Westbury, NY), 1 $\mu mol\ l^{-1}$ each primer, 200 $\mu mol\ l^{-1}$ dNTPs, and 640 ng μl^{-1} bovine serum albumin. PCR was performed on a Genius thermal cycler (Techne, Burlington, NJ) using the following cycling parameters: 2 min at 94 °C, then 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 68 °C for 30 s, followed by 5 min at 68 °C.

PCR products were visualized in a 1.2% agarose gel stained with 1 μg ml^{-1} ethidium bromide and compared to a 100-bp DNA ladder (Promega, Madison, WI).

2.6. Statistical analyses

Normality tests on non-transformed data indicate that the FIB data are not normally distributed. Therefore, FIB measurements were log_{10} transformed prior to all statistical analyses. Normality tests were conducted for the datasets to select the appropriate statistical analyses. Independent sample *t*-tests examined significant differences (alpha (α) = 0.05, two-tailed) between FIB concentrations for storm and baseflow samples and for seasonal comparisons. Levene's test for equality of variances was used to determine whether equal variances were or were not assumed (α = 0.05, two-tailed). Seasonal differences for FIB concentrations were determined using the one-way ANOVA with the post-hoc comparison Bonferroni (e.g. Salkind, 2004). A significant relationship was determined with respect to an alpha (α) of 0.05 (two-tailed). The percent of samples positive for the molecular markers was calculated by dividing the number of samples with positive detection of target by the total number of samples and multiplying by 100.

Ongoing research is being conducted on fecal *Bacteroides* spp. QPCR data to establish relationships among fecal contamination types. Preliminary work (Coulliette and Noble, 2008) suggests an "action threshold" of 5000 cells per 100 ml (as determined by QPCR), i.e. water samples exhibiting concentrations of fecal *Bacteroides* spp. above this concentration should be further examined for the potential presence of human fecal contamination using other "toolbox" approaches such as those presented previously (Noble et al., 2006). Furthermore, ratios of *Enterococcus* spp. and *E. coli* and the fecal *Bacteroides* spp. QPCR numbers have been suggested to be predictive of source (Converse et al., 2009). Some ratios from this work are presented here to examine these ideas. The HS marker, developed by Bernhard and Field (2000), has been utilized for a range of source tracking studies, including Noble et al. (2006). Recently, Ahmed et al. (2009) conducted an evaluation study on *Bacteroides*-based PCR markers and found the HS marker, reported for use here, was able to discriminate between human and animal feces 99% of the time.

3. Results

3.1. Fecal indicator bacteria measurements

Coastal areas of eastern NC are subject to extensive microbial contamination due to stormwater runoff. Concentrations of FIB in runoff samples collected during storm events reached as high as 2.39×10^6, 1.20×10^5, and 1.00×10^5 MPN per 100 ml for total coliforms, *E. coli* and *Enterococcus* spp., respectively. Even the mean concentrations of FIB across storms were at least one order of magnitude above existing single-sample standards for recreational waters (Table 1). All three sites appeared to be impacted by stormwater runoff contributing high concentrations of FIB to receiving waters, though concentrations of FIB varied widely from sample to sample and season to season. Precipitation amounts prior to collection of storm samples ranged from 0.2 to 11.4 cm, with a median value of 1.3 cm. There was no correlation of rainfall to FIB concentrations, but the sample size was relatively small.

Baseflow FIB concentrations ranged from 79–2.18×10^4, 10–8.31×10^2, and 10–4.78×10^2 for total coliforms, *E. coli*, and *Enterococcus* spp., respectively. Concentrations of FIB in baseflow samples were significantly lower than concentrations in stormwater samples (Table 1, $p < 0.001$). However, compared to historical data examined from the NCDENR Recreational Water Quality Section, some baseflow concentrations of FIB were higher than expected.

Concentrations of FIB exceeded the single-sample marine recreational water quality standard of 104 MPN per 100 ml *Enterococcus* spp. in almost all stormwater samples (Table 1), often by an order of magnitude. Stormwater samples from the outfall sites were more consistent in the degree of contamination and showed the most marked differences between stormflow and baseflow (Table 1). TCM had FIB concentrations that were more variable from sample to sample (Table 1). The total coliform:*E. coli* ratio was analyzed for each sample at all sites. When total coliform concentrations exceed 1000 CFU or MPN per 100 ml and the total coliforms:*E. coli* ratio is less than 5, stormwater runoff may be predictive of gastrointestinal illness and the presence of "fresh" human sewage is indicated (Haile et al., 1999). In all stormwater samples, this ratio

Table 1 – Average, standard deviation, and median values for total coliforms, *E. coli*, and *Enterococcus* spp. concentrations in MPN per 100 ml for all stormwater samples and baseflow samples collected from Bogue Inlet Pier (BIP), Triple S Pier (TSP) and Town Creek Marina (TCM).

	Total coliforms			*E. coli*			*Enterococcus*		
	Average	Standard deviation	Median	Average	Standard deviation	Median	Average	Standard deviation	Median
Stormwater samples									
BIP	1.80E5	5.20E5	2.93E4	7.21E3	2.60E4	5.70E2	7.92E3	2.15E4	3.12E3
TSP	1.65E5	1.41E5	1.65E5	4.92E3	1.14E4	1.64E3	4.36E3	5.89E3	2.23E3
TCM	2.91E5	6.26E5	1.10E5	5.17E3	7.32E3	1.15E3	1.15E4	2.01E4	2.44E3
Baseflow samples									
BIP	7.82E2	7.09E2	6.41E2	5.70E1	2.2E1	5.8E1	1.5E1	1.4E1	1.0E1
TSP	4.88E2	4.78E2	4.23E2	7.50E1	7.5E1	6.2E1	8.0	1.0	8.0
TCM	8.46E3	6.66E3	6.52E3	3.95E2	2.46E2	3.66E2	1.29E2	1.62E2	7.5E1

exceeded 5.0. Linear regressions and correlations of log-transformed *Enterococcus* versus *E. coli* were also examined. Stormwater runoff from TSP and TCM demonstrated strong, significant correlations between *Enterococcus* and *E. coli* ($r^2 = 0.85$ and 0.92, respectively, Table 3). The presence of HS and the high concentrations of fecal *Bacteroides* spp. indicate a strong likelihood for the presence of human fecal contamination at BIP and TCM. The overall relationship between the presence of HS and fecal *Bacteroides* spp. was significant ($r^2 = 0.50$, $p = 0.001$, $n = 39$), yet weak.

3.2. *Bogue Inlet Pier, Town of Emerald Isle, NC*

Overall mean FIB storm sample concentrations at BIP were high and were similar for *E. coli* (7212 MPN per 100 ml) and *Enterococcus* spp. (7915 MPN per 100 ml, $n = 21$, Table 1). Concentrations of total coliforms, *E. coli*, and *Enterococcus* spp. in stormwater samples were statistically higher than baseflow concentrations (Fig. 2a, $p < 0.001$). None of the baseflow samples exceeded the single-sample recreational water quality standard for *Enterococcus* spp. *E. coli* concentrations tended to be higher in the spring and summer, while *Enterococcus* spp. concentrations were highest from spring to fall (Fig. 3a). The relationship between *Enterococcus* spp. and *E. coli* in BIP storm samples was weak and insignificant ($r^2 = 0.15$). The HS marker was found in 1 of 15 samples (7%), indicating an ephemeral human fecal contamination signal during a single intense storm event (Table 2). This sample also contained BT (1148 cells/100 ml), but it did not exceed 5000 cells per 100 ml. Fecal *Bacteroides* spp. were detected in 82% of samples, but the concentrations were generally low, with only 2 of 17 storm samples having concentrations greater than 5000 cells per 100 ml (Table 2).

3.3. *Triple S Pier, Town of Atlantic Beach, NC*

Overall mean FIB concentrations at TSP were similar for *E. coli* (4920 MPN per 100 ml) and *Enterococcus* spp. (4358 MPN per 100 ml, $n = 14$, Table 1). Storm total coliforms, *E. coli*, and *Enterococcus* spp. concentrations were statistically higher than baseflow concentrations ($p < 0.05$, Fig. 2b). Baseflow samples never exceeded the single-sample limit for recreational guidelines. Seasonal trends showed that *E. coli* concentrations were highest in the summer and fall, while *Enterococcus* spp. concentrations were highest in the fall (Fig. 3b). Storm sample *Enterococcus* spp. and *E. coli* concentrations had a significant, positive, and strong correlation ($r^2 = 0.85$), a departure from the weak relationship observed at BIP. The *Bacteroides* HS Marker was found in 0 of 13 samples, and fecal *Bacteroides* spp. were detected in 64% of samples, generally at very low concentrations. The quantity of fecal *Bacteroides* spp. cells in one sample was greater than 30,000 per 100 ml, however HS was not detected in this sample. These results indicate a low likelihood of human fecal contamination in TSP storm samples for the periods studied.

3.4. *Town Creek Marina, Town of Beaufort, NC*

Overall mean FIB concentrations at TCM were much higher for *Enterococcus* spp. (11,486 MPN per 100 ml) than *E. coli* (5174 MPN per 100 ml, $n = 14$, Table 1). Although mean stormwater concentrations were generally higher for total coliforms, *E. coli*, and *Enterococcus* spp. than in baseflow samples (Table 1, Fig. 2c), only *Enterococcus* spp. concentrations were significantly different ($p < 0.05$). Seasonal trends showed that *E. coli*

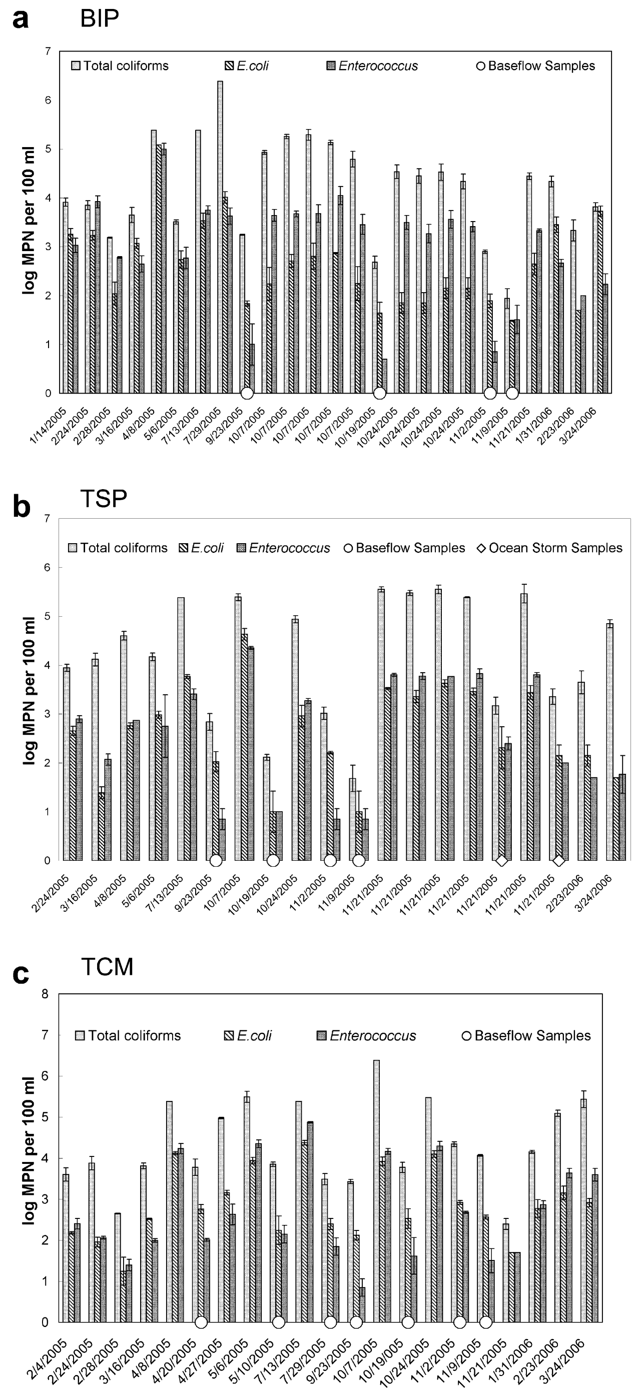

Fig. 2 – Log-transformed fecal indicator bacteria concentrations reported in MPN per 100 ml for samples collected from a) Bogue Inlet Pier, b) Triple S Pier, and c) Town Creek Marina. On 6 May 2005 a sewage spill contributed 12,000 gallons of sewage to Town Creek Marina. Error bars are ± one standard deviation.

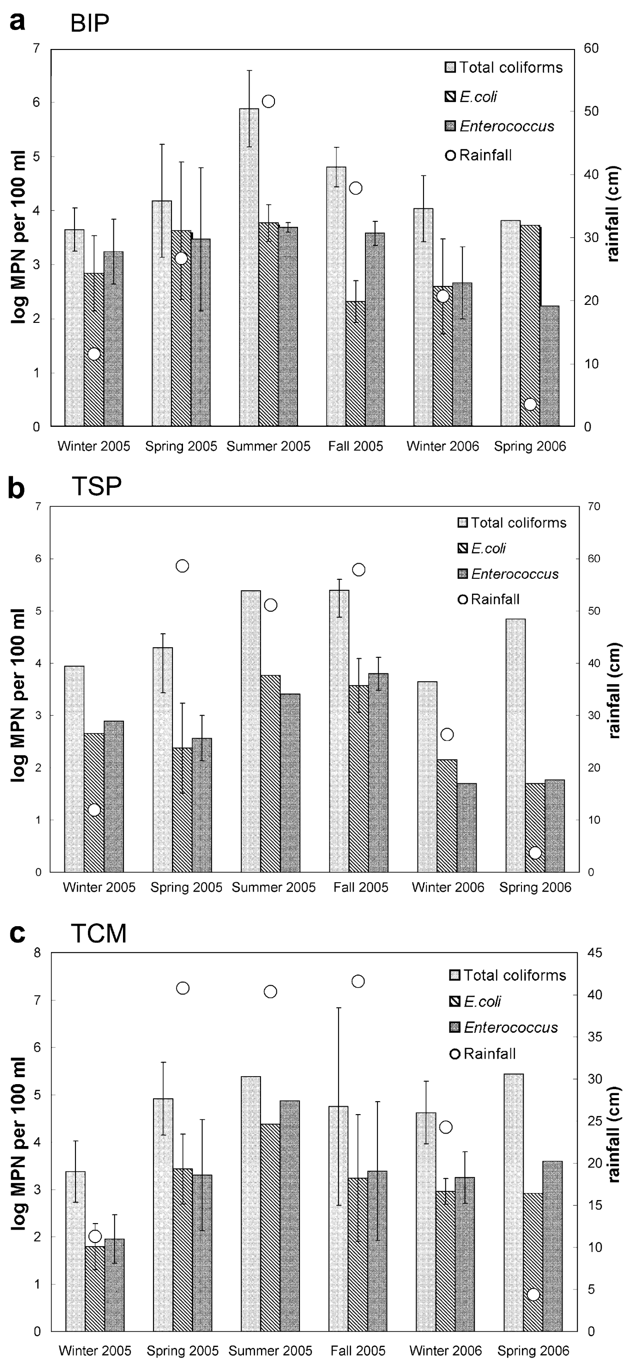

Fig. 3 – Log-transformed fecal indicator bacteria concentrations reported as seasonal averages in MPN per 100 ml for samples collected from a) Bogue Inlet Pier, b) Triple S Pier, and c) Town Creek Marina. Total rainfall (cm) is on the secondary y-axis. Error bars are ± one standard deviation.

and *Enterococcus* spp. were highest in the summer (Fig. 3c). The correlation between *Enterococcus* spp. and *E. coli* concentrations in storm samples was strong, ($r^2 = 0.91$). *Bacteroides* HS marker was found in 4 of 13 samples (31%, Table 2). Fecal *Bacteroides* spp. were detected and quantified in 11 of 11 samples, and 8 of the 11 samples exceeded concentrations of 5000 cells per 100 ml (Table 2). All of the TCM samples that were positive for HS also had BT concentrations greater than 5000 cells per 100 ml (Table 2.) These findings suggest that human fecal contamination was present during the period studied.

Table 2 – Percent of samples testing positive by PCR for *Bacteroides* HS Marker (HS) and *Bacteroides thetaiotaomicron* (BT) from samples collected at Bogue Inlet Pier (BIP), Triple S Pier (TSP) and Town Creek Marina (TCM).

Sample Site	Percent positive (# samples tested)	Percent HS positive with >5000 BT cells/100 ml	Percent positive (# samples tested)	Percent samples with >5000 cells/100 ml
	HS	HS	BT	BT
BIP	6.7 (15)	0	82 (17)	12
TSP	0.0 (13)	0	64 (11)	9
TCM	44 (9)	100	100 (11)	73

3.5. *Time series*

Time series experiments were conducted to see if FIB and molecular marker concentrations would change over short time scales. Observed FIB concentrations indicated the magnitude of the storm response during the time series events was similar to that typically seen for other storm events of similar-size. Concentrations of FIB were high and varied little over time, with variability generally not exceeding an order of magnitude (data not shown). There were no clear patterns of either increasing or decreasing concentrations according to the progression of the storm.

Concentrations of FIB were notably similar in both BIP time series (Fig. 4a and b), and the total storm rainfall amounts were similar, although storm hydrographs are not available. Ocean samples taken during the TSP time series exceeded recreational guidelines for *Enterococcus* spp. concentrations (Fig. 4c), though the magnitude of exceedance was much less than for the samples taken directly from the stormwater outfall.

Table 3 – Linear regression of log-transformed *E. coli* and *Enterococcus* spp. concentrations for all three sampling sites, for all samples (baseflow and storm) and for storm samples only.

	Regression	R^2	intercept *p*-value	x *p*-Value	n
All samples	$y = 0.9678x + 0.1511$	0.5887	0.610	1.42E-13	64
All storm	$y = 0.722x + 1.1101$	0.546	4.46E-4	1.34E-9	49
BIP storm	$y = 0.3094x + 2.4573$	0.1502	8.92E-5	0.0826	21
BIP all	$y = 0.7037x + 1.0976$	0.3307	0.0714	0.00264	25
TSP storm	$y = 0.8968x + 0.4302$	0.8524	0.228	2.49E-6	14
TSP all	$y = 1.0718x - 0.2517$	0.8105	0.509	3.58E-7	18
TCM storm	$y = 1.0951x - 0.1325$	0.916	0.669	8.22E-8	14
TCM all	$y = 1.2331x - 0.7921$	0.8092	0.066	2.91E-8	21

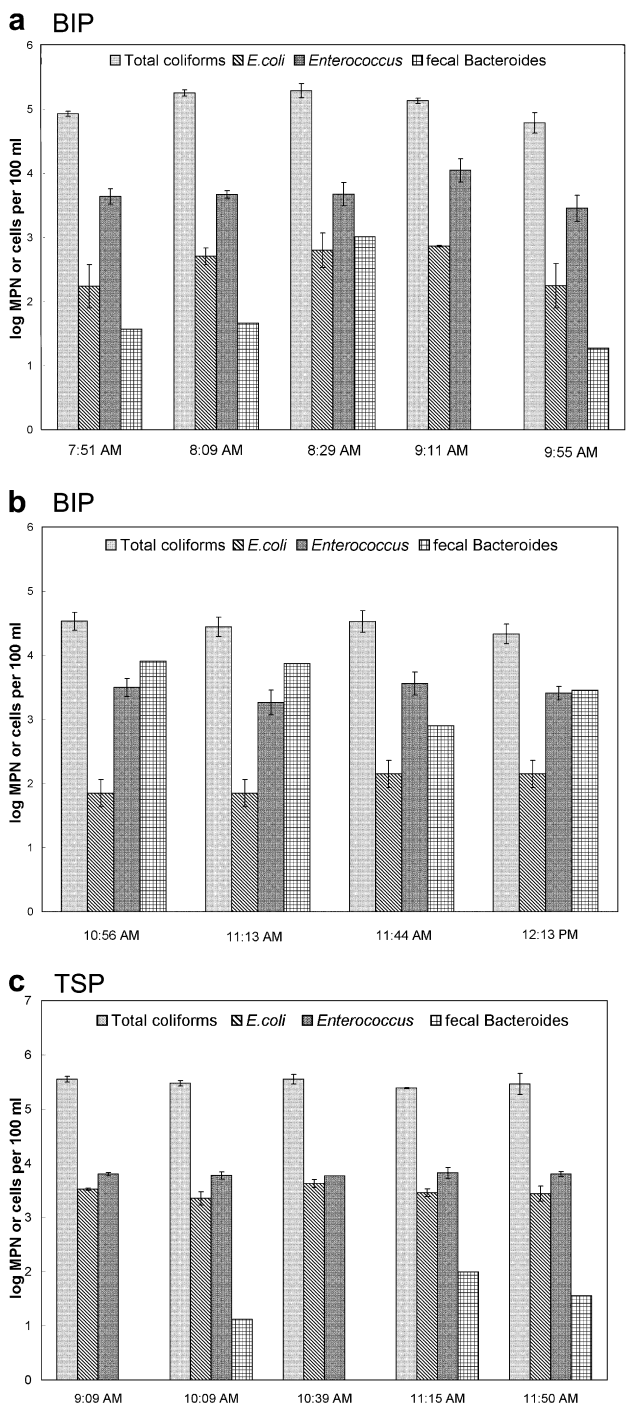

Fig. 4 – Samples collected during time series sampling. Error bars are ± one standard deviation. Samples shown were collected at: a) Bogue Inlet Pier on 7 October 2005; b) Bogue Inlet Pier on 24 October 2005; c) Triple S Pier on 21 November 2005.

4. Discussion

Stormwater runoff is a public health concern in coastal NC. Recreating on beaches with stormwater outfalls during and after storm periods could pose a significant public health risk. Concentrations of all three types of FIB in stormwater samples far exceeded recreational water quality standards, frequently by one or two orders of magnitude. This was true regardless of the amount of precipitation occurring prior to storm sampling. Overall, 88% of stormwater samples exceeded the single-sample threshold for designated recreational waters of 104 MPN per 100 ml for *Enterococcus* sp. (USEPA, 1986). All sites were impacted by both *E. coli* and *Enterococcus* spp. The higher levels of *E. coli* and *Enterococcus* spp. in summer and fall corresponded to warmer temperatures, greater amounts of rainfall, and increased human use of the watersheds draining to the sample sites. Positive confirmation of human fecal contamination in a subset of the stormwater samples tested is particularly problematic because of greater potential for the presence of human pathogens, increasing the risk of a waterborne disease outbreak.

Based on historical and baseflow data collected during this study, it appears to be safe to recreate at beaches close to stormwater outfalls during non-storm periods. FIB concentrations generally do not exceed water quality guidelines in dry weather, probably due to lack of runoff. Similar differences between stormwater and baseflow FIB concentrations have been found in other studies (Brownell et al., 2007; Krometis et al., 2007; Noble et al., 2003). NC runoff is dominated by rain events, and stormwater runoff flow is greatly reduced or absent during dry weather. In this study, flow from the stormwater outfalls at BIP and TSP was only observed during and directly after storm events. This differs from stormwater outlets in areas like California, which can discharge millions of gallons of urban runoff even in dry weather (Haile et al., 1999; Stein and Ackerman, 2007). The possible exception to this is TCM, where baseflow samples exceeded guidelines on occasion. TCM collects non-point source runoff from inland areas. Flow is always present at the sample site and may collect dry weather runoff, possibly contributing human contamination in the form of septic system leachate, bird and dog feces, and agricultural runoff to the area.

From a management standpoint, there is more interest in mitigating stormwater contamination at BIP and TSP, which are located on recreational beaches where humans are more likely to come into direct contact with stormwater. Stormwater outfalls at these sites discharge directly onto the beach, creating large standing pools of contaminated water that children are often observed playing in, as well as streams of water which flow downhill into the ocean. Total loading amounts are of great concern for these sites, as large volumes of water flow from the pipe continuously during and immediately after a storm. Although flow measuring equipment was not in place during this study, similar ongoing studies in Dare County, NC are equipped for flow measurements, and estimated total flows have ranged from 3.8×10^6 to 1.2×10^7 L for 2–5 cm storms of roughly 12 h duration in watersheds of similar-size to those studied here ($n = 10$, Converse, 2009). In addition, the loading of FIB during these storms has consistently ranged from 10^{11} to 10^{12} FIB per storm (Converse, 2009). For very large storms of long duration at BIP and TSP, the standing water and visible plume can last for days (J.K. Parker and R.T. Noble, unpublished data). Results indicate that the two stormwater outfall sites have a low likelihood of being heavily impacted by human fecal contamination, possibly indicating a lower public health risk. However, the risk of swimming in contaminated stormwater runoff has been demonstrated (Haile et al., 1999), and, as a major component of NPS runoff, it is of great concern for recreational water quality. Based on the magnitude of contamination observed during this study, even if human fecal contamination is not prominent, one could speculate that other types of contamination (e.g. bird or dog) could contain zoonotic pathogens which pose a health risk to those using the waters for recreation. Regardless of the associated public health risk, for compliance with USEPA recommended standards stormwater requires mitigation to reduce concentrations of FIB contributed to receiving waters.

While not discharging directly to the beach, stormwater contamination at TCM is of concern because it is proximal to high priority (Type SA) shellfish-harvesting waters that are valuable to the NC economy and society (North Carolina Division of Shellfish Sanitation, Map 29, Area E5 and E6). Though used for swimming less often than the beach sites, TCM is located in a marina frequented by recreational boaters and the site of sailing camps for children. Based on the relatively strong relationship between *Enterococcus* spp. and *E. coli* at TCM and the number of samples concurrently containing HS and high concentrations of fecal *Bacteroides* spp., it can be speculated that this site was contaminated by human fecal material, at least during the course of this study. While these noted markers on their own might cause one to only suggest the possible presence of human fecal contamination, their simultaneous presence lends strength to the likelihood that human fecal contamination is present. The main source of human fecal contamination is probably stormwater runoff, but it is also possible that illicit dumping of boat vessel waste within the marina or leakage from septic tanks in the developments surrounding the marina contribute to the human fecal contamination signal during storms (during poor weather many boaters who live aboard are forced to keep the boat within the marina). Further investigations are necessary to determine the source of contamination at this site and to determine whether the contamination is chronic or episodic. However, this example shows that measuring both ENT and EC, and then analyzing the correlation between the two datasets, might be an informative and fairly simple addition to current FIB testing schemes used by recreational water quality monitoring agencies. This practice is currently conducted in the State of California, where they use the total coliform to fecal coliform (or EC) ratio as one means of posting or closing a beach (Noble et al., 2003).

The time series analysis of stormwater outfall FIB concentrations was intended to provide insight as to how FIB concentrations vary over short time scales during a storm event. In this study, sampling was conducted for a short duration (2–3 h). The concentrations of FIB remained high and varied only slightly over the sampling time. Another short time scale study of stormwater FIB found similar results (Dorner et al., 2007). Results are consistent with a study of urban surface runoff drainage outfalls which concluded there is usually enough pollution available for wash-off to occur throughout the duration of normal storm events (Deletic, 1998). Also, observed concentrations of FIB from the two different BIP time series presented here, conducted during two different storms, showed extremely similar FIB concentrations.

It is notable that ocean samples collected during the TSP time series storm event exceeded recreational water quality guidelines. None of the baseflow samples at TSP exceeded guidelines, so this is evidence that the ephemeral stream created by the stormwater runoff directly impacts the ocean waters nearby. Dorner et al. (2007) found that trends in stormwater FIB concentrations are more easily observed over longer time spans. Also, it has been demonstrated in Florida, though it differs from NC in having distinct wet/dry seasons, that FIB concentrations increase with proximity to the shoreline, especially during the time subsequent to high tide when wash-off from contaminated beach areas occurs (Shibata et al., 2004). It would be important to repeat this set of time series experiments for the entire duration of a storm, with concomitant measurements of flow, to thoroughly examine the process of first flush. Future studies to determine the duration of stormwater impact and to assess the area of influence of stormwater plumes are necessary to better understand the potential for adverse public health outcomes.

5. Conclusions

This study has shown that stormwater in NC contains high levels of FIB and includes human fecal contamination at times. These findings demonstrate the need for further characterization of possible health risks. Storm events contribute fecal matter to receiving waters, likely from a range of sources. These sources need to be identified to help with risk assessment and mitigation efforts. This study also demonstrates the successful use of molecular markers to confirm the presence of human fecal contamination.

Total FIB loading to receiving waters also needs to be determined for storm events. This would require instrumentation at the stormwater outfalls to accurately measure flow rates and volumes so that total microbial contaminant loading can be estimated. Loading rates and fate and transport may also be affected by variability of partitioning of FIB and pathogens during the course of a storm, and this effect should be more thoroughly examined (Krometis et al., 2007). Epidemiological research should be conducted along the NC coast to ascertain actual human health risks from contact with stormwater runoff. It has been shown that rapid molecular methods (notably QPCR for *Enterococcus* spp.) can be used to predict swimming-associated health effects (Wade et al., 2006), so further application of molecular methods in conjunction with collection of epidemiological data in NC is recommended.

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July 23, 2012

Mr. Sam Unger, Executive Officer, and Members of the Board
California Regional Water Quality Control Board, Los Angeles Region
320 West 4th Street, Suite 200
Los Angeles, CA 90013

Dear Mr. Unger and Members of the Board:

At the request of the Natural Resources Defense Council, I wish to submit the following comments regarding tentative Order No. R4-2012-XXXX; NPDES Permit No. CAS004001; Waste Discharge Requirements for Municipal Separate Storm Sewer System (MS4) Discharges. In formulating my comments I applied the experience of my 35 years of work in the stormwater management field and 11 additional years of engineering practice. During this period I have performed research, taught, and offered consulting services on all aspects of the subject, including investigating the sources of pollutants and other causes of aquatic ecological damage, impacts on organisms in waters receiving urban stormwater drainage, and the full range of methods of avoiding or reducing these impacts. Attachment A to this letter presents a more complete description of my background and experience. My full *curriculum vitae* are available upon request.

Synopsis of Comments

In this letter I express and provide documentation for my opinions that:

- The permit's key management criterion at paragraph 6.c.i(2)(b), page 70, properly requires retaining on-site (i.e., not discharging to a surface receiving water) runoff from a designated rainfall event. That standard that has been demonstrated to be feasible in California.

- I base my opinion of the criterion's feasibility on three analyses that I have performed in recent years, for Ventura County, the San Francisco Bay Area, and four climate regions nationally (including north San Diego County). Average annual precipitation for California sites covered in these studies varies from slightly under 10 to approximately 20 inches, essentially bracketing the range for the developed portion of Los Angeles County. Soil conditions examined in these investigations also represent the range of Los Angeles County soil types.

- I concluded from these analyses using the low-impact development (LID) practice of infiltrating bioretention alone would meet the permit's criterion in residential land use cases throughout the developed portion of Los Angeles County on all but the most restrictive soils. In fact, this management strategy could save all pre-development groundwater recharge and avoid any increase in pollutant mass loadings to receiving waters.

- With more intense urban land uses, like large retail commercial and infill redevelopment cases, I have shown that the criterion would be achieved in most of the County, possibly excluding higher rainfall, foothill areas. There, adding a special roof runoff management LID practice (e.g., water harvesting for some beneficial use) would achieve the standard for these more developed land uses.

- On the most restrictive soils present in the County, roof runoff harvesting in the most impervious developments or broad landscape dispersal in less intensive land uses would capture, on average, an estimated 37 to 66 percent of the total runoff produced. The pollutant loading reduction could be further increased by treating non-retained runoff with a vegetative practice. Relative to the retention volume required by the Los Angeles County's permit, more than 50 percent of the requirement can be met with any land use considered on this restrictive soil by using only roof runoff management strategies.

- In my quantitative analyses of surface runoff reduction through bioretention, I took no credit for evaporation or transpiration loss to the atmosphere, attributing the decrease only to infiltration. However, I performed an evaporation assessment showing Los Angeles has a much greater excess of evaporation over precipitation in the highest rainfall months than elsewhere in the nation where runoff retention standards are in force or under consideration. Thus, I believe atmospheric loss would add substantially to the surface runoff reduction via infiltration. This finding gives me further confidence in the feasibility of the permit's retention criterion.

- This letter presents data showing that installing an underdrain in a bioretention unit cuts the hydrologic advantage by roughly one-third to one-half; adding a liner diminishes that advantage by around two-thirds. Therefore, these design features should only be incorporated for a good reason. Without such a reason, bioretention cells and other soil- and vegetation-based LID practices should be built without an underdrain or liner for maximum infiltration opportunity and minimum surface discharge. I therefore disagree with the categorical provision in Appendix H of the permit stating, "Biofiltration BMPs are designed and constructed with an underdrain."

- The letter also presents data showing LID options are generally less costly than traditional drainage system designs (e.g., using curb and gutter collectors and pipe conveyances). These financial advantages add to the feasibility of achieving the permit's criterion.

- I agree with the permit's questioning the technical feasibility of infiltration where seasonal high groundwater table is within 5 ft of the surface but disagree with extending the concern to a separation of as much as 10 ft. In my experience 5 ft is adequate to ensure no groundwater intrusion. If the concern is with groundwater quality in rapidly infiltrating soils, a geotextile material can be placed in the bed to limit the infiltration rate to a safe value, e.g., 2 inches/hour.

I present my rationale for these opinions after a general discussion of municipal separate storm sewer systems and their problems.

The Nature of Stormwater Runoff and Other Urban Runoff Conveyed by Municipal Separate Storm Sewer Systems

When precipitation falls to earth, it can evaporate, infiltrate into soil, or generate surface runoff. Runoff typically first forms in a thin, broadly distributed sheet ("sheet flow") as rain drops strike the ground and coalesce. In a natural landscape, if they even form at all, sheet flows are often quickly attenuated as water travels slowly over the soil and through vegetation, infiltrating and percolating downward to groundwater or being drawn into plant tissues. In such a landscape sheet flows that persist may eventually concentrate into a more confined stream in a natural draw and flow, seasonally or permanently, to a receiving water, such as a creek, river, lake, estuary, or ocean.

This hydrologic picture changes when development occurs. Vegetation is removed and impervious surfaces (e.g., roofs, pavement) overlay some or even most of the soil, not admitting water for infiltration. Construction activity typically results in the removal of topsoils, and their associated water storage capacity, and compaction of the remaining soils, tending to reduce the perviousness of even revegetated areas. With this hardening, sheet flows are more rapid, greatly cutting the time for evaporation to occur. This reduced infiltration and evaporation results in a higher proportion of surface runoff than seen in a natural landscape. Sheet flows are typically collected close to their point of formation into a structural conveyance (e.g., pipe, channel) engineered to carry them to the receiving water as quickly as possible, to prevent accumulations that would interfere with the human activities on the landscape. A typical example is the "curb-and-gutter" system employed on urban roadways. The road is designed to pass sheet flows to a curb at the side, which guides them via periodically placed inlets to a subsurface pipe.

Not only is urban runoff proportionately greater than runoff from natural lands, but it also carries substances, present not at all or in smaller quantities in non-urban flows, that are detrimental to aquatic life and human consumers; i.e., pollutants. These contaminants are varied and numerous and occur in the categories of bacteria, some disease-causing; metals, oils, and organic chemicals, many toxic to living organisms; nutrients, which can cause excessive algal and aquatic plant growths deleterious to ecosystems and human uses of water; and sediments, which have a number of direct negative effects in receiving waters and also transport pollutants in the other categories. These pollutants are deposited by humans in their outdoor activities of driving, fertilizing plants, raising animals, killing pests, doing construction, manufacturing products, and many others. Water contacting these deposits entrains the solids and dissolves the soluble contaminants and conveys them along to receiving waters.

An important point about urban stormwater runoff arises from the dual burden of more flow and more pollutants that it imposes on the environment as compared to natural land runoff. The higher flow quantities, even if they would be uncontaminated, erode stream channels, increasing sediment pollutants and destroying aquatic habitats, and of course also contribute to downstream flooding. Pollutants occur at elevated concentrations (mass per unit volume) in urban stormwater, which can cause acute negative effects (e.g., toxic reactions). The combination of greater volumes and higher concentrations in this runoff impose the burden on the receiving water of increased cumulative mass loadings, loading being the multiplication product of volume times concentration. Even if acute effects do not occur, longer term chronic ones can as a result of cumulative mass of contaminants. Many pollutants tend to accumulate in the bottom muds and sands of water bodies. The solids deposited there and the dissolved pollutants reacting with and attaching to the bed materials harm organisms that dwell or feed there. They can also be resuspended or redissolved into the water column and impact the pelagic life.

A municipal separate storm sewer system is a network of pipes, channels, and the entrance points to them, operated by one or more municipalities, that collect and convey surface runoff from at or near the point of formation to a receiving water. The word “separate” denotes that these systems are designed for flows generated directly and immediately by precipitation, to be conveyed separately from waste streams produced after water is used and processed by humans, for example, as household water or supply for an industrial process. While separation of these flows is the intention, sanitary sewage can leak or overflow into storm sewers. Although not a factor in Los Angeles County, combined sanitary and storm sewers exist in many places in the nation. In these cases stormwater receives treatment at the municipal sewage treatment plant, until the stormwater volume exceeds its capacity, after which the combined flow bypasses the plant and discharges to a receiving water without treatment. In a separate storm sewer the flow always discharges to the receiving water with no treatment. In functioning as they do, MS4s convert what was originally dispersed flow to a “point source” of water pollution.

Flow can enter municipal drainage systems during dry weather too. The leading example is excess irrigation runoff, but vehicle and pavement washing and other sources also contribute. Not only do these flows entrain and dissolve pollutants in the same way as stormwater, but they also add contaminants directly associated with the operation, like oil residue on vehicles and detergents in cleaning products. Dry-weather runoff diversions that have been put in place successfully, though they handle only a limited quantity of runoff short of the amounts generally accompanying rainfall.

In recent years the classic MS4 as described above has begun to change to some degree. Recognizing the benefits of vegetation and soil in a natural landscape, scientists and engineers are attempting to replace the highly structural elements with features offering vegetation and soil contact, with the attendant opportunities to infiltrate and evaporate precipitation instead of allowing it to become surface runoff. These practices are usually termed “low impact development” (LID) techniques. They are many and diverse and can be decentralized through the landscape to manage runoff before it concentrates much or at all, when it is easier to manage. There are even opportunities to apply these methods in an already built, dense urban area, especially when it redevelops to a different form.

Feasibility of the Permit's Quantitative Management Criterion

The Criterion and Los Angeles County's Physiographic Setting

The permit's fundamental quantitative management criterion[1] properly requires retaining on-site (i.e., not discharging to a surface receiving water) runoff from a designated rainfall event, a standard that has been demonstrated to be feasible in California. Over the last five years I have analyzed the feasibility and benefits of this criterion in three locations in the state. Climatological and soil conditions in these locations bracket such circumstances found in Los Angeles County and thus constitute a feasibility demonstration valid in the County.

Average annual precipitation for the Los Angeles area is highly variable and terrain-dependent, ranging from approximately twelve inches at the ocean to about twice that in the foothills. At the Civic Center in downtown Los Angeles the average annual rainfall is 14.77 inches.[2] Regarding soils, Los Angeles County's Hydrology Manual[3] observes that valley soils are alluvial and grade from coarse sand and gravel near San Gabriel Mountain canyon mouths to silty clay and clay in the lower valleys and coastal plain. Residual soils in other mountainous and hilly areas are shallow and generally less pervious than those of the San Gabriel Mountains. Water infiltration potential thus ranges widely in the County, from high in the alluvium to restricted in the clays.

Scope of Previous Analyses

Ventura County

My first analysis concerned Ventura County, reported in Attachment B. The analysis was performed to evaluate proposed permit terms to limit effective impervious area (EIA) of certain types of new development and redevelopment projects to 5 percent of total development project area. EIA is defined as hardened surface hydrologically connected via sheet flow or a discrete hardened conveyance to a drainage system or receiving water body. The study modified this requirement to 3 percent, as a way to test both the feasibility of meeting the higher, 5 percent standard in the draft permit and a more environmentally protective 3 percent EIA.

The study's objective was to compare the water quantity and quality management benefits of LID methods in comparison to conventional stormwater management best management practices (BMPs) and to no stormwater management at all. In this investigation and all of the subsequent ones, including my analysis for this letter, I define LID practices as those that *retain* runoff and thus avoid discharge on the surface. Accordingly, vegetative methods that still produce a surface

[1] Retain on-site the Stormwater Quality Design Volume (SWQDv) defined as the runoff from: (a) The 0.75-inch, 24-hour rain event, or (b) The 85th percentile, 24-hour rain event, as determined from the Los Angeles County 85th percentile precipitation isohyetal map, whichever is greater, with exceptions as provided in Parts VI.D.6.c.ii and VI.D.6.c.v.

[2] http://www.wrh.noaa.gov/lox/climate/climate_intro.php

[3] Los Angeles County Department of Public Works. 2006. *Hydrology Manual*. Water Resources Division, Alhambra, CA.

effluent (e.g., by including an underdrain collecting percolating water and ultimately discharging it on the surface) are not included in my definition. The letter revisits this subject later under the heading Bioretention with Underdrains.

Average annual precipitation in the City of Ventura is 14.71 inches, rising to as much as 21.32 inches elsewhere.[1] Calculations in this analysis used a 0.75-inch quantity as the best estimate of the 85th percentile, 24-hour rainfall, the design event. The most prominent soils in developed and developing areas of Ventura County are loams, sandy loams, loamy sands, and silty clay loams.[2] The analysis involved six land use case studies, three residential developments, a restaurant, a relatively small office park, and a large retail commercial development.

The study evaluated the hydrologic and water quality benefits of draining all runoff produced by storms up to the design event to pervious area, to reduce the quantity discharging from the site and improve the quality of any remnant runoff. This pervious land could be in the form of bioretention (also known as a rain garden), a vegetated cell providing runoff storage, or via land dispersion on a parcel over which water travels as a sheet flow (often called a filter strip). While these LID practices offer the opportunity for runoff both to infiltrate the soil and evaporate or transpire to the atmosphere, the quantitative analysis conservatively assessed only infiltration as a runoff-reduction mechanism (please see the section later in this letter titled Evaporation Considerations for a discussion of the substantial role that this mechanism probably plays).

The investigation also examined how special efforts at roof water management harvesting could contribute to storm water management for case study sites where infiltration capacity, available space, or both appeared to be limited. Roof runoff can be subtracted from the surface runoff by harvesting and storing it for uses such as irrigation and toilet flushing or distributed over and/or in the soil in a dispersion trench (e.g., French drain). Water storage capacity in such a trench effectuates infiltration, even at a slow rate, and also increases the opportunity for evaporation. The study assumed the former mode for the large retail commercial development and the latter for less intense land uses needing special roof runoff management. The Ventura County analysis further included an assessment of the benefits of conventional stormwater management methods, used alone without any LID practices or applied just to effective impervious area.

San Francisco Bay Area

I followed up with analyses of a four-county region of the San Francisco Bay Area, the initial report for which is in Attachment C. I obtained rainfall records from a number of sites in the region, finding that the mean annual range is from 13.73 to 24.30 inches, with quantities close to

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[1] Ventura County Watershed Protection District (http://www.vcwatershed.org/fws/specialmedia.htm). The City of Ventura is considered to be representative of most of the developed and developing areas in Ventura County. However, there is some variation around the county, with the maximum precipitation registered at Ojai (annual average 21.32 inches). Ojai is about 15 miles inland and lies at elevation 745 ft at the foot of the Topatopa Mountains, the orographic effect of which influences its meteorology. Ojai's higher rainfall was taken into account in the calculations, and the report notes the few instances where it affected the conclusions.
[2] Cabrillo Port Liquefied Natural Gas Deepwater Port Draft EIS/EIR (Oct. 2004) (http://www.cabrilloport.ene.com/files/eiseir/4.05%20%20-Agriculture%20and%20Soils.pdf).

either 14 or 20 inches predominating. The report details how I estimated 85 percentile, 24-hour event quantities of 0.77 and 0.82 inch for the 14 and 20-inch Bay Area rainfall zones, respectively. Loam soils are common formations in the portion of the Bay Area covered by this report, those areas with Hydrologic Soil Groups (HSGs) A, B, and C.[1] However, a minority but still substantial fraction of the Bay Area has group D soils (39.3, 68.0, 18.3, and 50.1 percent of the mapped areas of Alameda, Contra Costa, San Mateo, and Santa Clara Counties, respectively). Whereas A and B soils generally effectively infiltrate water, and C soils can be amended with organic compost according standard techniques to do so, D soils are usually not amenable to infiltration. Accordingly, I prepared a supplementary analysis covering those soils in the 14-inch rainfall region, included here as Attachment D.

The initial analysis covered six urban land use types (four residential, a restaurant, and an office park). The supplementary analysis added to those case studies a large retail commercial development and an infill redevelopment. These studies evaluated the hydrologic and water quality benefits of soil- and vegetation-based and roof runoff management LID techniques, as well as conventional stormwater best management practices (BMPs) in a fashion similar to the Ventura County analysis.

National Study, Including North San Diego County

Finally, I extended the analysis nationally, in this case assessing the feasibility of four potential regulatory standards in addition to the 85 percentile, 24-hour event. Attachment E presents the resulting report. This study assessed five urban land use types (three residential, one retail commercial, and one infill redevelopment), each placed in four climate regions in the continental United States on two regionally common soil types. One of the regions was the Southwest, represented by San Marcos in north San Diego County. Its average annual precipitation, 9.68 inches, is based on the NOAA Hourly Precipitation Data Rainfall Event Statistics[2] for the nearest station with a long-term record, San Diego International Airport. The 85th percentile, 24-hour rainfall quantity was taken as 0.76 inch, determined as described in the report. Soils were characterized by a web soil survey for an 8267.5-acre area of interest centered in San Marcos. The leading HSG is D (58 percent of the area), followed by group C (26 percent) and group B (14 percent). Soil textures include sandy loam (19 percent), coarse sandy loam (17 percent), silt loam (15 percent), very fine sandy loam (14 percent), loamy fine sand (12 percent), loam (7 percent), and clay (5 percent). The leading drainage classification is well drained (51 percent of the area), followed by moderately well drained (34 percent). It was accordingly decided to perform the analysis based on characteristics consistent with both D and C soils for this climate region.

These analyses concerned only LID-type practices. Infiltrating bioretention was applied as an initial strategy in the analysis of each case. When the initial strategy could not fully retain post-

[1] http://gis.ca.gov/catalog/BrowseCatalog.epl?id=108, http://websoilsurvey.nrcs.usda.gov/applWebSoilSurvey.aspx
[2] National Climatic Data Center, Hourly Precipitation Data Rainfall Event Statistics (http://cdo.ncdc.noaa.gov/cgi-bin/HPD/HPDStats.pl).

development runoff, additional methods were applied, involving roof runoff harvesting in the most impervious development cases and roof water dispersion in those with substantial pervious area.

Basis of Estimates of Runoff Reduction Through LID in Previous Analyses

All of the previous analyses assumed that runoff discharged from the site on the surface would be reduced only by infiltration while draining into or over a vegetated LID practice on HSG A, B, or C (organically amended as necessary) soils, taking no credit for evapotranspiration. In the Ventura County and San Francisco Bay Area analyses, the chief basis for infiltration estimation was an assessment of infiltration capacity and benefits performed earlier for Los Angeles' San Fernando Valley by Chralowicz et al. (2001).[1] This study posited providing 0.1-0.5 acre for infiltration basins to serve each 5 acres of contributing drainage area. At 2-3 ft deep, it was estimated that such basins could infiltrate 0.90-1.87 acre-ft/year of runoff in San Fernando Valley conditions. Soils there are generally various loam textures with infiltration rates in the approximate range of 0.5-2.0 inches/hour. Similar soils predominate in Ventura County and are an important component of the Bay Area soil matrix, excepting the HSG D soil areas where zero infiltration was assumed. Information from the Chralowicz et al. study was used to estimate how much of each case study site's annual runoff would be infiltratable, and if the pervious portion would provide sufficient area for infiltration.

In the national study, bioretention cell surface areas were calculated using an equation in the City of Santa Barbara's Storm Water BMP Guidance Manual[2], runoff volume based on the design standard, a design infiltration rate based on saturated hydraulic conductivity, and other characteristics. HSG C soils, such as found in north San Diego County, either have conductivities in excess of 0.5 inch/hour, the rate often regarded in the stormwater management field as the minimum for the use of infiltration practices (e.g., Geosyntec Consultants 2008)[2] or, in my experience, can be and have been successfully organically amended to produce such a rate and infiltrate accumulated water within 72 hours, and usually less time. Bioretention sizing was accordingly based on a 0.5-inch/hour infiltration rate for such soils. The D soils also found in north San Diego County were regarded as not amendable to reach 0.5 inch/hour conductivity to host facilities designed specifically for infiltration.

The national study also incorporated a groundwater mounding analysis intended to avoid water table rise that would compromise the infiltration facility or any other infrastructure. The analysis was based on limiting the yearly rate of infiltration, as volume of runoff per unit infiltrating surface area, to hold water table rise to no more than 1 ft/year. As another conservative feature of the analysis, the yearly rate was capped at a maximum value based on the results of Chralowicz et al. (2001) from the San Fernando Valley.

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[1] Chralowicz, D., T. Goff, M. Mascali, and E. Taylor. 2001. Infiltration of Urban Stormwater Runoff to Recharge Groundwater Used for Drinking Water: A Study of the San Fernando Valley, California. Master of Environmental Science and Management Report, University of California at Santa Barbara, Santa Barbara, CA.

[2] Geosyntec Consultants. 2008. Post-Construction BMP Technical Guidance Manual. City of Santa Barbara, CA.

Comparison of Previous Study Locations to Los Angeles County

Overall, among the three previous sets of analyses, average annual precipitation for California sites varies from slightly under 10 to approximately 20 inches, essentially bracketing the range for the developed portion of Los Angeles County (roughly 12 inches to about twice that quantity). The 85^{th} percentile, 24-hour rainfall amounts are very similar for these sites, as well as for Los Angeles County. All four Hydrologic Soil Groups and the range of soil types present in the County were represented in these analyses. Results of an infiltration study based on soil conditions in Los Angeles' San Fernando Valley were important components of all previous analyses. Therefore, it is my strong opinion that their results apply in Los Angeles County and can be used to assess the feasibility of the permit's quantitative management criterion.

<u>Summary of Results of Previous Analyses</u>

Organization of the Summary

Previous studies conducted for areas with approximately 14 inches average annual precipitation on infiltrating soils (HSG A and B, as well as C with compost soil amendment) have the broadest relevance to Los Angeles County; i.e., the analyses for Ventura County and one Bay Area rainfall region, which are summarized first. Analysis of the 20-inch Bay Area rainfall region, along with observations for Ojai in Ventura County, provides insights applicable to the wetter portions of developed Los Angeles County. The C soil assessment in north San Diego County shows the relatively high retention potential present in a relatively low rainfall area. Results for these higher and lower rainfall cases are summarized next. More restrictive soils are represented by the HSG D soil analyses performed for the Bay Area and north San Diego County, the final topic in the summary.

Ventura County and San Francisco Bay Results for Predominant Los Angeles County Rainfall and Infiltratable Soils

These analyses demonstrated that, on infiltrating soils, all land use cases except the large retail development have sufficient pervious area to infiltrate *all* runoff produced in the average year, even that from the allowable EIA. This retention would save all pre-development groundwater recharge, whereas as much as 65 percent of that recharge would be lost with no stormwater BMPs or with conventional techniques having hardened beds (e.g., box filters). Full retention, of course, would also capture and prevent the discharge of all pollutants.

The large commercial development would have pervious area sufficient to infiltrate about 26 percent of the annual runoff, falling well short of performance with the less intense land uses. Nevertheless, it still would have the capacity to limit EIA to 3 percent and thus exceed the draft permit term under consideration at the time of the report. Harvesting its roof runoff would raise infiltration to 45 percent of the total remaining production. The combination of infiltration to recharge groundwater and harvesting for some beneficial use would reduce the loss of water supply available as a potential resource from 86 percent of the incident rainfall with no BMPs or with hard-bottom conventional ones to just 37 percent. With this LID management scheme plus conventional treatment of EIA runoff, I estimated that annual pollutant mass loadings to receiving waters would fall by at least 89 percent for total suspended solids (TSS) and the metals copper and zinc and by 83 percent for total phosphorus, compared to a case with no BMPs. Conventional BMPs would provide far less reduction in pollutant loadings; for example, as little as zero reduction of copper with the poorest treatment and no more than 55 percent phosphorus decrease with the best treatment for that pollutant.

Effects of Higher and Lower Rainfall

Repeating the analyses for the Bay Area 20-inch annual rainfall region yielded no change in the conclusions regarding infiltration capability, recharge, and pollutant loadings. That analysis did not consider a large retail commercial case, but that land use was represented in the Ventura County study. Assuming Ojai average annual rainfall of 21.32 inches would slightly reduce the infiltration estimate from 26 near the coast to 18 percent of average annual production with infiltrative BMPs only, and from 45 to 32 percent when roof harvesting is added. With the higher rainfall, the multi-family residential development could no longer infiltrate all runoff but could retain it all if the deficit were made up by special management of roof runoff (e.g., by harvesting or broad land dispersion).

With the lower rainfall in the San Diego area, not only the residential cases but also the large retail commercial and redevelopment scenarios would achieve full runoff retention with bioretention on the C soil. Full retention would save all pre-development groundwater recharge and attenuate all pollutant loading.

The national study did not include a climate region having average annual precipitation in the area of 20 ± 5 inches to serve in assessing the wetter portions of developed Los Angeles County. The closest case was from the South Central U.S., represented by Round Rock, TX, an Austin suburb. Average annual precipitation there is 32.67 inches, and the 85th percentile, 24-hour event quantity is 1.19 inch, both substantially higher than anywhere in developed areas of Los Angeles County. The analysis determined that, on HSG C soil with compost amendment as needed, use of bioretention alone would meet the 85th percentile, 24-hour standard in the residential land use cases. Adding roof water management in the large retail commercial and infill redevelopment cases would achieve that standard. Hence, I strongly believe that the Los Angeles permit's quantitative management criterion can be met with a wide range of land uses, anywhere A, B, and C soils occur, in any Los Angeles County climate regime. The criterion can probably be achieved with bioretention alone in most of the County, and roof water management can meet it with the more intensely developed land uses in foothill areas with higher rainfall.

Results with Low Infiltration Capabilities

The Bay Area analysis for HSG D soils in the 14-inch/year rainfall region assumed roof runoff harvesting for the multi-family residential, office park, restaurant, large retail commercial and redevelopment cases; harvesting supplemented by dispersion of roof runoff for three single-family residential scenarios of different scales; and treatment of remaining runoff by conventional extended-detention basins (EDBs). This management strategy was estimated to decrease the average annual runoff by 40 to 79 percent, compared to no BMPs, depending on the land use case. I estimated that the average annual mass loadings of TSS, metals, and phosphorus would drop by 61 to 92 percent, depending on land use and pollutant. Conventional EDB treatment alone would reduce runoff quantity by only 8 to 23 percent and pollutant loadings by 44 to 78 percent.

With the San Diego area D soil, a similar management plan would reduce average annual runoff quantity by 37 to 66 percent from the amount with no BMPs, depending on land use. The pollutant loading reduction would be at least equal, and could be increased to an estimated 67 to 85 percent by treating non-retained runoff with a vegetative BMP. Relative to the retention volume required by an 85th percentile, 24-hour event standard, more than 50 percent of the requirement can be met with any land use considered on this D soil by using only rooftop dispersion or harvest and reuse of rooftop water (100 percent for the redevelopment case).

Evaporation Considerations

As I pointed out above, I estimated surface runoff reduction in bioretention cells and filter strips only as a function of infiltration and assumed no loss as vapor to the atmosphere through evaporation from free water surfaces or transpiration from plants. If evapotranspiration (ET) is substantial, though, it would reinforce my conclusion that the permit's criterion of retaining runoff produced by events up to and including the 85th percentile, 24-hour storm can be met, and in fact exceeded, on all developed land uses in most of Los Angeles County. To examine the extent of the additional insurance that ET would provide, I prepared a report (Attachment F) comparing recorded evaporation and rainfall records in California with other locations in the nation that have adopted or considered stormwater runoff retention standards.

I based the analysis primarily on the excess of evaporation over precipitation in the three and six months of highest rainfall in the respective locations. I learned that Los Angeles and other Southern California locations exhibit a substantial excess of evaporation over precipitation in the six highest months of precipitation, almost twice the excess in the next highest location (Atlanta). Among the cities assessed elsewhere in the nation, only Philadelphia has any excess in the three highest rainfall months; and the southern California cities' excess is about two to four times as large as Philadelphia's in these months. Therefore, even though southern California's wet season coincides with its period of lowest evaporation, its generally warm, sunny winters give it an advantage over other locations in the nation that have adopted runoff retentive LID measures.

To gauge how this demonstrated evaporation potential would affect surface runoff I considered green roof research performed at Pennsylvania State University, located in central PA, a location for which I have evaporation data. The researchers found over 50 percent of annual stormwater volume to be retained and not discharged, even with as little as 20 mm (under 1 inch) of storage capacity, and peak discharge rate attenuation to no more than the pre-development level for the 2-, 25-, and 100-year frequency events. Central PA has a deficit of evaporation relative to precipitation in both the three and six months of highest precipitation. With the far more favorable position of Los Angeles in this regard, it can be expected that runoff retention afforded by green roofs would be well over 50 percent. This advantage would also accrue to other LID BMP types, such as bioretention, and gives me further confidence in the feasibility of the permit's retention criterion.

Bioretention with Underdrains

Bioretention facilities have been built and studied with and without impermeable liners and/or underdrains, either entirely eliminating (if lined) or reducing (if unlined but underdrained) infiltration. Obviously, this design feature would be expected to have a major influence on performance. Table 1 below presents a comparison in surface runoff volume reduction with and without underdrains. Installing an underdrain but leaving the facility unlined appears to cut the hydrologic advantage by roughly one-third to one-half, while adding a liner diminishes that advantage by around two-thirds. Therefore, these design features should only be incorporated for a good reason (e.g., high groundwater table; very restricted infiltration rate that cannot be sufficiently increased by soil amendment; buried contaminants in the soil below, which could be mobilized by concentrated infiltration). Without such a reason, bioretention cells and other soil- and vegetation-based LID practices should be built without these features for maximum infiltration opportunity and minimum surface discharge. I therefore disagree with the provision in Appendix H of the permit stating, "Biofiltration BMPs are designed and constructed with an underdrain."

While unlined, free-draining systems are preferable, lined and underdrained units still can provide a benefit where some good reason exists for incorporating one or both of these features. The 20-29 percent of the inflow lost from the lined facility reported in the table could only have departed via evapotranspiration, pointing out again the substantial role that loss to the atmosphere can play in surface runoff reduction.

Davis et al. (2009) summarized water quality performance registered by several bioretention studies in the eastern United States from New Hampshire to North Carolina. As shown in Table 2, the results were highly variable, most likely as a consequence of the several design variables listed earlier, differing behavior among geo-climatological regions, or both. The results overall do not provide good indices of effectiveness and signify that relative certainty is quite poor. They do, though, indicate the strong potential of bioretention to eliminate the discharge of almost all pollutant mass in the best applications and to meet or at least approach achievement of water quality standards at the point of release.

Table 1. Surface Runoff Volume Reduction Achieved by Bioretention Systems With and Without Underdrains (adapted from NRC [2009] and references cited)[1]

Design	Location	Volume Reduction (%)	Reference
Unlined, no underdrain	Connecticut	99	Dietz and Clausen (2006)
Unlined, no underdrain	Pennsylvania	86	Ermilio and Traver (2006)
Unlined, no underdrain	Florida	98	Rushton (2002)
Unlined, no underdrain	Australia	73	Lloyd et al. (2002)
Unlined, with underdrain	Ontario	40	Van Seters et al. (2002)
Unlined, with underdrain	North Carolina	40-60	Smith and Hunt (2007)
Unlined, with underdrain	North Carolina	52-56	Hunt et al. (2008)
Unlined, with underdrain	Maryland	52-65	Davis (2008)
Lined, with underdrain	North Carolina	20-29	Sharkey (2006)

Table 2. Summary of Water Quality Performance of Eastern United States Bioretention Cells (after Davis et al. 2009[2], excluding laboratory and pilot tests)

Pollutant	Effluent Concentration Range	Mass Loading Reduction Range (%)
Total suspended solids	13-20 mg/L	54-99
Total nitrogen	0.80-4.38 mg/L	32-97
Total phosphorus	58-560 μg/L	negative 240-79
Total zinc	17-48 μg/L	54-99

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[1] Davis, A.P. 2008. Field Performance of Bioretention: Hydrology Impacts. *Journal of Hydrologic Engineering* 13(2):90-95.

Dietz, M., and J. Clausen. 2006. Saturation to Improve Pollutant Retention in a Rain Garden. *Environmental Science and Technology* 40(4):1335-1340.

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Low-Impact Development Stormwater Management Costs

The U.S. Environmental Protection Agency (USEPA, 2007)[1] assembled a series of LID case studies, including costs. In general, the investigation concluded that:

> ... applying LID techniques can reduce project costs and improve environmental performance. In most cases, LID practices were shown to be both fiscally and environmentally beneficial to communities. In a few cases, LID project costs were higher than those for conventional stormwater management practices. However, in the vast majority of cases, significant savings were realized due to reduced costs for site grading and preparation, stormwater infrastructure, site paving, and landscaping. Total capital cost savings ranged from 15 to 80 percent when LID methods were used, with a few exceptions in which LID project costs were higher than conventional stormwater management costs.

Among examples covered by the USEPA report, the 2nd Avenue NW SEA Streets bioretention project in Seattle, WA saved $217,255 of the expected $868,803 cost (25 percent) of upgrading the street's previous "informal" drainage system to a conventional street curb-and-gutter configuration. Two parking lot rain garden retrofits in Bellingham, WA saved 76 and 80 percent of the costs of the conventional stormwater management alternative of underground vaults. A design study for a Pierce County, WA subdivision using an integrated range of LID techniques estimated 20 percent savings compared to managing stormwater conventionally. On the other hand, in a design study for another subdivision in the same county maximizing LID opportunities, including home roof water collection, capital costs were estimated as about twice as high as for a conventional system. These costs were expected to be offset somewhat by operating savings over time. The residential roof downspout disconnection program conducted by Portland, OR has cost the city $8.5 million thus far in materials and incentive payments but is expected to save $250 million in construction costs for piping to store an extra 1 billion gallons per year to prevent combined sewer overflows.

The City of Seattle[2] reported costs in comparison to traditional methods for two types of bioretention systems built in the City's Natural Drainage System program: (1) SEA Streets, a series of bioretention cells on relatively flat streets, and (2) Cascades, stepped bioretention cells divided by weirs placed on streets sloping at approximately 4-6 percent. All costs were for a residential area with 35 percent impervious land cover. The SEA Street cost per block (330 ft) on a local, low-traffic street was $325,000, compared to $425,000 for the same street built with a traditional curb and gutter and piped drainage system. In a 15-block network the per-block cost fell to $280,000. The Cascade system for a higher-traffic collector street was $285,000 per block, considerably below the $520,400 cost of a traditional system on a collector.

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[1] U.S. Environmental Protection Agency (USEPA). 2007b. Reducing Stormwater Costs through Low Impact Development (LID) Strategies and Practices. USEPA, Nonpoint Source Control Branch, Washington, DC.

[2] http://www.seattle.gov/util/groups/public/@spu/@usm/documents/webcontent/spu02_019986.pdf

Groundwater Separation

I agree with the permit provision at c.ii.(2)(b) on page 70 to question technical feasibility where seasonal high groundwater table is within 5 ft of the surface but not, everything else being equal, when it is more than 5 ft below the bed of the infiltration facility. In my experience such a separation is adequate to ensure no groundwater intrusion. If the concern is with groundwater quality in rapidly infiltrating soils, a geotextile material can be placed in the bed to limit the infiltration rate to a safe value, e.g., 2 inches/hour.

I would be pleased to discuss my comment with you and invite you contact me if you wish to do so.

Sincerely,

Richard R. Horner

Richard R. Horner, Ph.D.

ATTACHMENT A

RICHARD R. HORNER, PH.D.

BACKGROUND AND EXPERIENCE

I have 35 years of experience in the urban stormwater management field and 11 additional years of engineering practice. During this period I have performed research, taught, and offered consulting services on all aspects of the subject, including investigating the sources of pollutants and other causes of aquatic ecological damage, impacts on organisms in waters receiving urban stormwater drainage, and the full range of methods of avoiding or reducing these impacts.

I received a Ph.D. in Civil and Environmental Engineering from the University of Washington in 1978, following two Mechanical Engineering degrees from the University of Pennsylvania. Although my degrees are all in engineering, I have had substantial course work and practical experience in aquatic biology and chemistry. For 12 years beginning in 1981, I was a full-time research professor in the University of Washington's Department of Civil and Environmental Engineering. From 1993 until 2011, I served half time in that position and had adjunct appointments in two additional departments (Landscape Architecture and the College of the Environment's Center for Urban Horticulture). I spent the remainder of my time in private consulting through a sole proprietorship. My appointment became emeritus in late 2011, but I continue university research and teaching at a reduced level while maintaining my consulting practice.

I have conducted numerous research investigations and consulting projects involving all aspects of stormwater management. Serving as a principal or co-principal investigator on more than 40 research studies, my work has produced three books, approximately 30 papers in the peer-reviewed literature, and over 20 reviewed papers in conference proceedings. I have also authored or co-authored more than 80 scientific or technical reports. In addition to graduate and undergraduate teaching, I have taught many continuing education short courses to professionals in practice. My consulting clients include federal, state, and local government agencies; citizens' environmental groups; and private firms that work for these entities, primarily on the West Coast of the United States and Canada but in some instances elsewhere in the nation.

Over an 18-year period I spent a major share of my time as the principal investigator on two extended research projects concerning the ecological responses of freshwater resources to urban conditions and the urbanization process. I led an interdisciplinary team for 11 years in studying the effects of human activities on freshwater wetlands of the Puget Sound lowlands. This work led to a comprehensive set of management guidelines to reduce negative effects and a published book detailing the study and its results. The second effort, extending 10 years, involved an analogous investigation of human effects on Puget Sound's salmon spawning and rearing streams. These two research programs had broad sponsorship, including the U.S. Environmental Protection Agency, the Washington Department of Ecology, and a number of local governments.

I have helped to develop stormwater management programs in Washington State, California, and British Columbia and studied such programs around the nation. I was one of four principal participants in a U.S. Environmental Protection Agency-sponsored assessment of 32 state, regional, and local programs spread among 14 states in arid, semi-arid, and humid areas of the West and Southwest, as well as the Midwest, Northeast, and Southeast. This evaluation led to the 1997 publication of "Institutional Aspects of Urban Runoff Management: A Guide for Program Development and Implementation" (subtitled "A Comprehensive Review of the Institutional Framework of Successful Urban Runoff Management Programs").

My background includes 18 years of work in California, where I have been a federal court-appointed overseer of stormwater program development and implementation at the city and county level and for two Caltrans districts. I was directly involved in the process of developing the 13 volumes of Los Angeles County's Stormwater Program Implementation Manual, working under the terms of a settlement agreement in federal court as the plaintiffs' technical representative. My role was to provide quality-control review of multiple drafts of each volume and contribute to bringing the program and all of its elements to an adequate level. I have also evaluated the stormwater programs in San Diego, Orange, Riverside, San Bernardino, Ventura, Santa Barbara, San Luis Obispo, and Monterey Counties, as well as a regional program for the San Francisco Bay Area. My clients in these cases include Natural Resources Defense Council, Santa Monica Baykeeper, Orange County Coastkeeper, Ventura Coastkeeper, Santa Barbara Channelkeeper, Russian Riverkeeper, and San Diego Coastkeeper. At the recommendation of the latter organization, I have been a consultant on stormwater issues to the City of San Diego, the San Diego Unified Port District, and the San Diego County Regional Airport Authority.

For the last six years I have been a member of Salmon-Safe's assessment team. Salmon-Safe is an organization based in Portland, Oregon that certifies academic and professional campuses and other developed lands for maintaining practices supportive of salmon protection and recovery. We have assessed numerous parcels in Oregon and Washington and extended certification to those whose practices met our criteria or conditions imposed to achieve certification.

I was a member of the National Academy of Sciences-National Research Council (NAS-NRC) committee on Reducing Stormwater Discharge Contributions to Water Pollution. NAS-NRC committees bring together experts to address broad national issues and give unbiased advice to the federal government. The present panel was the first ever to be appointed on the subject of stormwater. Its broad goals were to understand better the links between stormwater discharges and impacts on water resources, to assess the state of the science of stormwater management, and to apply the findings to make policy recommendations to the U.S. Environmental Protection Agency relative to municipal, industrial, and construction stormwater permitting. The committee issued its final report in October 2008.

ATTACHMENT B

INVESTIGATION OF THE FEASIBILITY AND BENEFITS OF LOW-IMPACT SITE DESIGN PRACTICES ("LID") FOR VENTURA COUNTY

Richard R. Horner[†]

ABSTRACT

The Clean Water Act NPDES permit that regulates municipal separate storm sewer systems (MS4s) in Ventura County, California will be reissued in 2007. The draft permit includes provisions for requiring the use of low impact development practices (LID) for certain kinds of development and redevelopment projects. Using six representative development project case studies, the author investigated the practicability and relative benefits of the permit's LID requirements. The results showed that (1) LID site design and source control techniques are more effective than conventional best management practices (BMPs) in reducing runoff rates; (2) Effective Impervious Area (EIA) can practicably be capped at three percent, a standard more protective than that proposed in the draft permit; and (3) in five out of six case studies, LID methods would reduce site runoff volume and pollutant loading to zero in typical rainfall scenarios.

[†] Richard R. Horner, Ph.D., Research Associate Professor, University of Washington Departments of Civil and Environmental Engineering and Landscape Architecture; Adjunct Associate Professor, University of Washington Center for Urban Horticulture

INTRODUCTION

The Assessment in Relation to Municipal Permit Conditions

This purpose of this study is to investigate the relative water quality and water reuse benefits of three levels of storm water treatment best management practices (BMPs): (1) basic "treat-and-release" BMPs (e.g., drain inlet filters, CDS units), (2) commonly used BMPs that expose runoff to soils and vegetation (extended-detention basins and biofiltration swales and filter strips), and (3) low-impact development (LID) practices. The factors considered in the investigation are runoff volume, pollutant loading, and the availability of water for infiltration or other reuse. In order to assess the differential impact of storm water reduction approaches on these factors, this study examines six case studies typical of development covered by the Ventura County Municipal Separate Storm Sewer System Permit.

Low-impact development methods reduce storm runoff and its contaminants by decreasing their generation at sources, infiltrating into the soil or evaporating storm flows before they can enter surface receiving waters, and treating flow remaining on the surface through contact with vegetation and soil, or a combination of these strategies. Soil-based LID practices often use soil enhancements such as compost, and thus improve upon the performance of more traditional basins and biofilters. For the study's purposes, verification of the practicability and utility of LID practices was based on a modified version of the Planning and Land Development Program (Part 4, section E) in the Draft Ventura County Municipal Separate Storm Sewer

System Permit ("Draft Permit"). The Draft Permit requires that Effective Impervious Area (EIA) of certain types of new development and redevelopment projects be limited to five percent of total development project area. EIA is defined as hardened surface hydrologically connected via sheet flow or a discrete hardened conveyance to a drainage system or receiving water body. (Draft Permit p. 50) The study modified this requirement to three percent, as a way to test both the feasibility of meeting the higher, five percent standard in the draft permit and because the lower, three percent EIA is essential to protect the Ventura County aquatic environment (see Attachment A).

The Draft Permit further requires minimizing the overall percentage of impervious surfaces in new development and redevelopment projects to support storm water infiltration. The Draft Permit also directs an integrated approach to minimizing and mitigating storm water pollution, using a suite of strategies including source control, LID, and treatment control BMPs. (Draft Permit p. 50) It is noted in this section of the document that impervious surfaces can be rendered "ineffective" if runoff is dispersed through properly designed vegetated swales. In testing the practicability of the draft permit's requirements and a three percent EIA standard, this study broadened this approach to encompass not only vegetated swales (channels for conveyance at some depth and velocity) but also vegetated filter strips (surfaces for conveyance in thin sheet flow) and bioretention areas (shallow basins with a range of vegetation types in which runoff infiltrates through soil either to groundwater or a subdrain for eventual surface discharge). The Draft Permit's stipulation of "properly designed" facilities was interpreted to entail, among other requirements, either determination that existing site soils can support runoff reduction through infiltration or that soils will be amended using accepted LID techniques to attain this objective. Finally, the study further broadened implementation options to include water harvesting (collection and storage for use in, for example, irrigation or gray water systems), roof downspout infiltration trenches, and porous pavements.

The Draft permit was interpreted to require management of EIA, other impervious area (what might be termed Not-Connected Impervious Area, NCIA), and pervious areas as follows:

- Runoff from EIA is subject to treatment control and the Draft Permit's Hydromodification Mitigation Control requirements before discharge.

- NCIA must be drained onto a properly designed vegetated surface or its runoff managed by one of the other options discussed in the preceding paragraph. To the extent NCIA runoff is not eliminated prior to discharge from the site in one of these ways, it is subject to treatment control and the Draft Permit's Hydromodification Mitigation Control requirements before discharge.

- Runoff from pervious areas is subject to treatment control and the Draft Permit's Hydromodification Mitigation Control requirements before discharge. This provision applies to pervious areas that both do and do not receive drainage from NCIA.

Where treatment control BMPs are required to manage runoff from the site, the Draft Permit's Volumetric or Hydrodynamic (Flow Based) Treatment Control design bases were assumed to apply. The former basis applies to storage-type BMPs, like ponds, and requires capturing and treating either the runoff volume from the 85th percentile 24-hour rainfall event for the location, the volume of annual runoff to achieve 80 percent or more volume treatment, or the volume of runoff produced from a 0.75 inch storm event. The calculations in this analysis used the 0.75-inch quantity. The Hydrodynamic basis applies to flow-through BMPs, like swales, and requires treating the runoff flow rate produced from a rain event equal to at least 0.2 inches per hour intensity (or one of two other approximately equivalent options).

Scope of the Assessment

With respect to each of the six development case studies, three assessments were undertaken: a baseline scenario incorporating no storm water management controls; a second scenario employing conventional BMPs; and a third development scenario employing LID storm water management strategies.

To establish a baseline for each case study, annual storm water runoff volumes were estimated, as well as concentrations and mass loadings of four pollutants: (1) total suspended solids (TSS), (2) total recoverable copper (TCu), (3) total recoverable zinc (TZn), and (4) total phosphorus (TP). These baseline estimates were based on the anticipated land use and cover with no storm water management efforts.

Two sets of calculations were then conducted using the parameters defined for the six case studies.

The first group of calculations estimated the extent to which basic BMPs reduce runoff volumes and pollutant concentrations and loadings, and what impact, if any, such BMPs have on recharge rates or water retention on-site.

The second group of calculations estimated the extent to which commonly used soil-based BMPs and LID site design strategies ameliorate runoff volumes and pollutant concentrations and loadings, and the effect such techniques have on recharge rates. When evaluating LID strategies, it was presumed that EIA would be limited to three percent and runoff from EIA, NCIA, and pervious areas would be managed as indicated above. The assessment of basins, biofiltration, and low-impact design practices analyzed the expected infiltration capacity of the case study sites. It also considered related LID techniques and practices, such as source reduction strategies, that could work in concert with infiltration to serve the goals of: (1) preventing increase in annual runoff volume from the pre- to the post-developed state, (2) preventing increase in annual pollutant mass loadings between the two development states, and (3) avoiding exceedances of California Toxics Rule (CTR) acute saltwater criteria for copper and zinc.

The results of this analysis show that:

- Developments implementing no post-construction BMPs result in storm water runoff volume and pollutant loading that are substantially increased, and recharge rates that are substantially decreased, compared to pre-development conditions.

- Developments implementing basic post-construction treatment BMPs achieve reduced pollutant loading compared to developments with no BMPs, but storm water runoff volume and recharge rates are similar to developments with no BMPs.

- Developments implementing traditional basins and biofilters, and even more so low-impact post-construction BMPs, achieve significant reduction of pollutant loading and runoff volume as well as greatly enhanced recharge rates compared to both developments with no BMPs and developments with basic treatment BMPs.

- Typical development categories, ranging from single family residential to large commercial, can feasibly implement low-impact post-construction BMPs designed in compliance with the draft permit’s requirements, as modified to include a lower, three percent EIA requirement.

This report covers the methods employed in the investigation, data sources, and references for both. It then presents the results, discusses their consequences, draws conclusions, and makes recommendations relative to the feasibility of utilizing low-impact development practices in Ventura County developments.

CASE STUDIES

Six case studies were selected to represent a range of urban development types considered to be representative of coastal Southern California, including Ventura County. These case studies involved: a multi-family residential complex (MFR), a relatively small-scale (23 homes) single-family residential development (Sm-SFR), a restaurant (REST), an office building (OFF), a relatively large (1000 homes) single-family residential development (Lg-SFR) and a sizeable commercial retail installation (COMM).[1]

Parking spaces were estimated to be 176 sq ft in area, which corresponds to 8 ft width by 22 ft length dimensions. Code requirements vary by jurisdiction, with the tendency now to drop below the traditional 200 sq ft average. About 180 sq ft is common, but various standards for full- and compact-car spaces, and for the mix of the two, can raise or lower the average.[2] The 176 sq ft size is considered to be a reasonable value for conventional practice.

Roadways and walkways assume a wide variety of patterns. Exclusive of the two SFR cases, simple, square parking lots with roadways around the four sides and square buildings with walkways also around the four sides were assumed. Roadways and walkways were taken to be 20 ft and 6 ft wide, respectively.

Single-family residences were assumed each to have a driveway 20 ft wide and 30 ft long. It was further assumed that each would have a sidewalk along the front of the lot, which was calculated to be 5749 sq ft in area. Assuming a square lot, the front dimension would be 76 ft. A 40-ft walkway was included within the property. Sidewalks and walkways were taken to be 4 ft wide.

Exclusive of the COMM case, the total area for all of these impervious features was subtracted from the total site area to estimate the pervious area, which was assumed to have conventional landscaping cover (grass, small herbaceous decorative plants, bushes, and a few trees). For the COMM scenario, the hypothetical total impervious cover was enlarged by 10 percent to represent the landscaping, on the belief that a typical retail commercial establishment would typically be mostly impervious.

Table 1 (page 5) summarizes the characteristics of the six case studies. The table also provides the recorded or estimated areas in each land use and cover type.

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[1] Building permit records from the City of San Marcos in San Diego County provided data on total site areas for the first four case studies, including numbers of buildings, building footprint areas (including porch and garage for Sm-SFR), and numbers of parking spaces associated with the development projects. While the building permit records made no reference to features such as roadways, walkways, and landscaping normally associated with development projects, these features were taken into account in the case studies using assumptions described herein. Larger developments were not represented in the sampling of building permits from the San Marcos database. To take larger development projects into account in the subsequent analysis, the two larger scale case studies were hypothesized. The Lg-SFR scenario scaled up all land use estimates from the Sm-SFR case in the ratio of 1000:23. The hypothetical COMM scenario consisted of a building with a 2-acre footprint and 500 parking spaces. As with the smaller-scale cases, these hypothetical developments were assumed to have roadways, walkways, and landscaping, as described herein.

[2] J. Gibbons, *Parking Lots*, NONPOINT EDUCATION FOR MUNICIPAL OFFICERS, Technical Paper No. 5 (1999) (http://nemo.uconn.edu/tools/publications/tech_papers/tech_paper_5.pdf).

Table 1. Case Study Characteristics and Land Use and Land Cover Areas

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
No. buildings	11	23	1	1	1000	1
Total area (ft^2)	476,982	132,227	33,669	92,612	5,749,000	226,529
Roof area (ft^2)	184,338	34,949	3,220	7,500	1,519,522	87,120
No. parking spaces	438	-	33	37	-	500
Parking area (ft^2)	77,088	-	5808	6512	-	88,000
Access road area (ft^2)	22,212	-	6097	6456	-	23,732
Walkway area (ft^2)	33,960	10,656	1362	2078	463,289	7,084
Driveway area (ft^2)	-	13,800	-	-	600,000	-
Landscape area (ft^2)	159,384	72,822	17,182	70,066	3,166,190	20,594

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential;
REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; COMM—retail commercial

METHODS OF ANALYSIS

Annual Storm Water Runoff Volumes

Annual surface runoff volumes produced were estimated for both pre- and post-development conditions for each case study site. Runoff volume was computed as the product of annual precipitation, contributing drainage area, and a runoff coefficient (ratio of runoff produced to rainfall received). For impervious areas the following equation was used:

$$\mathbf{C = (0.009)\,\mathit{I} + 0.05}$$

where *I* is the impervious percentage. This equation was derived by Schueler (1987) from Nationwide Urban Runoff Program data (U.S. Environmental Protection Agency 1983). With ***I*** = 100 percent for fully impervious surfaces, C is 0.95.

The basis for pervious area runoff coefficients was the Natural Resource Conservation Service's (NRCS) Urban Hydrology for Small Watersheds (NRCS 1986, as revised from the original 1975 edition). This model estimates storm event runoff as a function of precipitation and a variable representing land cover and soil, termed the curve number (CN). Larger events are forecast to produce a greater amount of runoff in relation to amount of rainfall because they more fully saturate the soil. Therefore, use of the model to estimate annual runoff requires selecting some event or group of events to represent the year. A 0.75-inch rainfall event was used in the analysis here for the relative comparison between pre- and post-development and applied to deriving a runoff coefficient for annual estimates, recognizing that smaller storms would produce less and larger storms more runoff.

To select CN for the pre-development case, an analysis performed in the area of the Cedar Fire in San Diego County was used in which CN was determined before and after the 2003 fire.[3] In the San Diego analysis, CN = 83 was estimated for the pre-existing land cover, which was generally chaparral, a vegetative cover also typical of Ventura County. As indicated below, soils are also similar in Ventura and San Diego Counties, making the parameter selection reasonable for use in both locations. For post-development landscaping, CN = 86 was selected based on tabulated data in NRCS (1986) and professional judgment.

Pre- and post-development runoff quantities were computed with these CN values and the 0.75-inch rainfall, and then divided by the rainfall to obtain runoff coefficients. The results were 0.07

[3] American Forests, *San Diego Urban Ecosystem Analysis After the Cedar Fire* (Feb. 3, 2006) (http://www.ufei.org/files/pubs/SanDiegoUrbanEcosystemAnalysis-PostCedarFire.pdf).

and 0.12, respectively. Finally, total annual runoff volumes were estimated based on an average annual precipitation in the City of Ventura of 14.71 inches.[4]

Storm Water Runoff Pollutant Discharges

Annual pollutant mass discharges were estimated as the product of annual runoff volumes produced by the various land use and cover types and pollutant concentrations typical of those areas. Again, the 0.75-inch precipitation event was used as a basis for volumes. Storm water pollutant data have typically been measured and reported for general land use types (e.g., single-family residential, commercial). However, an investigation of low-impact development practices of the type this study sought to conduct demands data on specific land coverages. The literature offers few data on this basis. Those available and used herein were assembled by a consultant to the City of Seattle for a project in which the author participated. They appear in Attachment B (Herrera Environmental Consultants, Inc. undated).

Pollutant concentrations expected to occur typically in the mixed runoff from the several land use and cover types making up a development were estimated by mass balance; i.e., the concentrations from the different areas of the sites were combined in proportion to their contribution to the total runoff.

The Effect of Conventional Treatment BMPs on Runoff Volume, Pollutant Discharges, and Recharge Rates

The first question in analyzing how BMPs reduce runoff volumes and pollutant discharges was, What BMPs are being employed in Ventura County developments under the permit now in force? This permit is open-ended and provides regulated entities with a large number of choices and few fixed requirements. These options presumably include manufactured BMPs, such as drain inlet inserts (DIIs) and continuous deflective separation (CDS) units. Developments may also select such non-proprietary devices as extended-detention basins (EDBs) and biofiltration swales and filter strips. EDBs hold water for two to three days for solids settlement before releasing whatever does not infiltrate or evaporate. Biofiltration treats runoff through various processes mediated by vegetation and soil. In a swale, runoff flows at some depth in a channel, whereas a filter strip is a broad surface over which water sheet flows. Each of these BMP types was applied to each case study, although it is not clear that these BMPs, in actuality, have been implemented consistently within Ventura County to date.

The principal basis for the analysis of BMP performance was the California Department of Transportation's (CalTrans, 2004) BMP Retrofit Pilot Program, performed in San Diego and Los Angeles Counties. One important result of the program was that BMPs with a natural surface infiltrate and evaporate (probably, mostly infiltrate) a substantial amount of runoff, even if conditions do not appear to be favorable for an infiltration basin. On average, the EDBs, swales, and filter strips lost 40, 50 and 30 percent, respectively, of the entering flow before the discharge point. DIIs and CDS units do not contact runoff with a natural surface, and therefore do not reduce runoff volume.

The CalTrans program further determined that BMP effluent concentrations were usually a function of the influent concentrations, and equations were developed for the functional

[4] Ventura County Watershed Protection District (http://www.vcwatershed.org/fws/specialmedia.htm). The City of Ventura is considered to be representative of most of the developed and developing areas in Ventura County. However, there is some variation around the county, with the maximum precipitation registered at Ojai (annual average 21.32 inches). Ojai is about 15 miles inland and lies at elevation 745 ft at the foot of the Topatopa Mountains, the orographic effect of which influences its meteorology. Ojai's higher rainfall was taken into account in the calculations, and the report notes the few instances where it affected the conclusions.

relationships in these cases. BMPs generally reduced influent concentrations proportionately more when they were high. In relatively few situations influent concentrations were constant at an "irreducible minimum" level regardless of inflow concentrations.

In analyzing the effects of BMPs on the case study runoff, the first step was to reduce the runoff volumes estimated with no BMPs by the fractions observed to be lost in the pilot study. The next task was estimating the effluent concentrations from the relationships in the CalTrans report. The final step was calculating discharge pollutant loadings as the product of the reduced volumes and predicted effluent concentrations. As before, typical pollutant concentrations in the mixed runoff were established by mass balance.

Estimating Infiltration Capacity of the Case Study Sites

Infiltrating sufficient runoff to maintain pre-development hydrologic characteristics and prevent pollutant transport is the most effective way to protect surface receiving waters. Successfully applying infiltration requires soils and hydrogeological conditions that will pass water sufficiently rapidly to avoid overly-lengthy ponding, while not allowing percolating water to reach ground-water before the soil column captures pollutants.

The study assumed that infiltration would occur in surface facilities and not in below-ground trenches. The use of trenches is certainly possible, and was judged to be an approved BMP by CalTrans after the pilot study. However, the intent of this investigation was to determine the ability of pervious areas to manage the site runoff. This was accomplished by determining the infiltration capability of the pervious areas in their original condition for each development case study, and further assessing the pervious areas' infiltration capabilities if soils were modified according to low impact development practices.

The chief basis for this aspect of the work was an assessment of infiltration capacity and benefits for Los Angeles' San Fernando Valley (Chralowicz et al. 2001). The Chralowicz study posited providing 0.1-0.5 acre for infiltration basins to serve each 5 acres of contributing drainage area. At 2-3 ft deep, it was estimated that such basins could infiltrate 0.90-1.87 acre-ft/year of runoff in San Fernando Valley conditions. Soils there are generally various loam textures with infiltration rates of approximately 0.5-2.0 inches/hour. The most prominent soils in Ventura County, at least relatively near the coast, are loams, sandy loams, loamy sands, and silty clay loams, thus making the conclusions of the San Fernando Valley study applicable for these purposes.[5] This information was used to estimate how much of each case study site's annual runoff would be infiltratable, and if the pervious portion would provide sufficient area for infiltration. For instance, if sufficient area were available, the infiltration configuration would not have to be in basin form but could be shallower and larger in surface area. This study's analyses assumed the use of bioretention areas rather than traditional infiltration basins.

Volume and Pollutant Source Reduction Strategies

As mentioned above, the essence of low-impact development is reducing runoff problems before they can develop, at their sources, or exploiting the infiltration and treatment abilities of soils and vegetation. If a site's existing infiltration and treatment capabilities are inadequate to preserve pre-development hydrology and prevent runoff from causing or contributing to violations of water quality standards, then LID-based source reduction strategies can be implemented, infiltration and treatment capabilities can be upgraded, or both.

[5] Cabrillo Port Liquefied Natural Gas Deepwater Port Draft EIS/EIR (Oct. 2004) (http://www.cabrilloport.ene.com/files/eiseir/4.05%20%20-Agriculture%20and%20Soils.pdf).

Source reduction can be accomplished through various LID techniques. Soil can be upgraded to store runoff until it can infiltrate, evaporate, or transpire from plants through compost addition. Soil amendment, as this practice is known, is a standard LID technique.

Upgraded soils are used in bioretention cells that hold runoff and effect its transfer to the subsurface zone. This standard LID tool can be used where sufficient space is available. This study analyzed whether the six development case study sites would have sufficient space to effectively reduce runoff using bioretention cells, assuming the soils and vegetation could be amended and enhanced where necessary.

Conventional pavements can be converted to porous asphalt or concrete or replaced with concrete or plastic unit pavers or grid systems. For such approaches to be most effective, the soils must be capable of infiltrating the runoff passing through, and may require renovation.

Source reduction can be enhanced by the LID practice of water harvesting, in which water from impervious surfaces is captured and stored for reuse in irrigation or gray water systems. For example, runoff from roofs and parking lots can be harvested, with the former being somewhat easier because of the possibility of avoiding pumping to use the water and fewer pollutants. Harvesting is a standard technique for Leadership in Energy and Environmental Design (LEED) buildings.[6] Many successful systems of this type are in operation, such as the Natural Resources Defense Council offices (Santa Monica, CA), the King County Administration Building (Seattle, WA), and two buildings on the Portland State University campus (Portland, OR). This investigation examined how water harvesting could contribute to storm water management for case study sites where infiltration capacity, available space, or both appeared to be limited.

RESULTS OF THE ANALYSIS

1. "Base Case" Analysis: Development without Storm Water Controls

Comparison of Pre- and Post-Development Runoff Volumes

Table 2 (page 9) presents a comparison between the estimated runoff volumes generated by the respective case study sites in the pre- and post-development conditions, assuming implementation of no storm water controls on the developed sites. On sites dominated by impervious land cover, most of the infiltration that would recharge groundwater in the undeveloped state is expected to be lost to surface runoff after development. This greatly increased surface flow would raise peak flow rates and volumes in receiving water courses, raise flooding risk, and transport pollutants. Only the office building, the plan for which retained substantial pervious area, would lose less than half of the site's pre-development recharge.

[6] New Buildings Institute, Inc., *Advanced Buildings* (2005) (http://www.poweryourdesign.com/LEEDGuide.pdf).

Table 2. Pre- and Post-Development without BMPs: Distribution of Surface Runoff Versus Recharge to Groundwater

Annual Volume (acre-ft)	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
Precipitation[b]	13.4	3.72	0.95	2.60	162	6.37
Pre-development runoff[c]	0.94	0.26	0.07	0.18	11	0.45
Pre-development recharge[d]	12.5	3.46	0.88	2.42	150	5.92
Post-development impervious runoff[c]	8.48	1.59	0.44	0.60	69	5.50
Post-development pervious runoff[c]	0.54	0.25	0.06	0.24	11	0.07
Post-development total runoff[c]	9.02	1.83	0.50	0.84	80	5.57
Post-development recharge[d]	4.39	1.88	0.45	1.76	82	0.80
Post-development recharge loss (% of pre-development recharge)	8.08 (65%)	1.57 (46%)	0.43 (49%)	0.66 (27%)	68 (45%)	5.12 (86%)

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential;
COMM—retail commercial
[b] Volume of precipitation on total project area
[c] Quantity of water discharged from the site on the surface
[d] Quantity of water infiltrating the soil; the difference between precipitation and runoff

Pollutant Concentrations and Loadings

Table 3 presents the pollutant concentrations from the literature and loadings calculated as described for the various land use and cover types represented by the case studies. Landscaped areas are expected to release the highest TSS concentration, although relatively low TSS mass loading because of the low runoff coefficient. The highest copper concentrations and loadings are expected from parking lots. Roofs, especially commercial roofs, top the list for both zinc concentrations and loadings. Landscaping would issue by far the highest phosphorus, although access roads and driveways would contribute the highest mass loadings.

Table 3. Pollutant Concentrations and Loadings for Case Study Land Use and Cover Types

Land Use	**Concentrations**				**Loadings**			
	TSS (mg/L)	TCu (mg/L)	TZn (mg/L)	TP (mg/L)	Lbs. TSS/ acre-year	Lbs. TCu/ acre-year	Lbs. TZn/ acre-year	Lbs. TP/ acre-year
Residential roof	25	0.013	0.159	0.11	79	0.041	0.503	0.348
Commercial roof	18	0.014	0.281	0.14	57	0.044	0.889	0.443
Access road/driveway	120	0.022	0.118	0.66	380	0.070	0.373	2.088
Parking	75	0.036	0.097	0.14	237	0.114	0.307	0.443
Walkway	25	0.013	0.059	0.11	79	0.041	0.187	0.348
Landscaping	213	0.013	0.059	2.04	85	0.005	0.024	0.815

The CTR acute criteria for copper and zinc are 0.0048 mg/L and 0.090 mg/L, respectively. Table 3 shows that all developed land uses are expected to discharge copper above the criterion, based on the mass balance calculations using concentrations from Table 3. Any surface release from the case study sites would violate the criterion at the point of discharge, although dilution by the receiving water would lower the concentration below the criterion at some point. Even if copper mass loadings are reduced by BMPs, any surface discharge would exceed the criterion initially, but it would be easier to dilute below that level. In contrast, runoff from some land covers would not violate the acute zinc criterion. Because of this difference, the evaluation considered whether or not the zinc criterion would be exceeded in each analysis, whereas there was no point in this analysis for copper. There are no equivalent water quality criteria for TSS and TP; hence, their concentrations were not further analyzed in the different scenarios.

Table 4 shows the overall loadings, as well as zinc concentrations, expected to be delivered from the case study developments should they not be fitted with any BMPs. As Table 4 shows, all cases are forecast to exceed the 0.090 mg/L acute zinc criterion, and the retail commercial development does so by a wide margin. Because of its size, the large residential development dominates the mass loading emissions.

Table 4. Case Study Pollutant Concentration and Loading Estimates without BMPs

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
TZn (mg/L)	0.127	0.123	0.128	0.133	0.123	0.175
Lbs. TSS/year	1321	345	125	242	15016	853
Lbs. TCu/year	0.46	0.074	0.032	0.045	3.21	0.37
Lbs. TZn/year	3.09	0.607	0.174	0.301	26.4	2.64
Lbs. TP/year	6.58	2.39	0.72	1.78	104	3.36

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; COMM—retail commercial

2. "Conventional BMP" Analysis: Effect of Basic Treatment BMPs

Effect of Basic Treatment BMPs on Post-Development Runoff Volumes

The current permit allows regulated parties to select from a range of BMPs in order to treat or infiltrate a given quantity of annual rainfall. The range includes drain inlet inserts, CDS units, and other manufactured BMPs, detention vaults, and sand filters, all of which isolate runoff from the soil; as well as basins and biofiltration BMPs built in soil and generally having vegetation. Treatment BMPs that do not permit any runoff contact with soils discharge as much storm water runoff as equivalent sites with no BMPs, and hence yield zero savings in recharge. As mentioned above, the CalTrans (2004) study found that BMPs with a natural surface can reduce runoff by substantial margins (30-50 percent for extended-detention basins and biofiltration).

With such a wide range of BMPs in use, runoff reduction ranging from 0 to 50 percent, and a lack of clearly ascertainable requirements, it is not possible to make a single estimate of how much recharge savings are afforded by maximal implementation of the current permit. We made the following assumptions regarding implementation of BMPs. Assuming natural-surface BMPs perform at the average of the three types tested by CalTrans (2004), i.e., 40 percent runoff reduction, the estimate can be bounded as shown in Table 5 (page 11). The table demonstrates that allowing free choice of BMPs without regard to their ability to direct water into the ground forfeits substantial groundwater recharge benefits when hardened-surface BMPs are selected. Use of soil-based conventional BMPs could cut recharge losses from half or even more of the full potential to about one-quarter to one-third or less, except with the highly impervious commercial development. This analysis shows the wisdom of draining impervious to pervious surfaces, even if those surfaces are not prepared in any special way. But as subsequent analyses showed, soil amendment can gain considerably greater benefits.

Table 5. Pre- and Post-Development with Conventional BMPs: Distribution of Surface Runoff Versus Recharge to Groundwater

Annual Volume (acre-ft)	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
Precipitation[b]	13.4	3.72	0.95	2.60	162	6.37
Pre-development runoff[c]	0.94	0.26	0.07	0.18	11	0.45
Pre-development recharge	12.5	3.46	0.88	2.42	150	5.92
Post-development impervious runoff[c, d]	5.09-8.48	0.95-1.59	0.26-0.44	0.36-0.60	41-69	3.30-5.50
Post-development pervious runoff[c, d]	0.32-0.54	0.15-0.25	0.04-0.06	0.14-0.24	6.6-11	0.04-0.07
Post-development total runoff[c, d]	5.41-9.02	1.10-1.83	0.30-0.50	0.50-0.84	48-80	3.34-5.57
Post-development recharge[d, e]	4.39-7.99	1.88-2.62	0.45-0.65	1.76-2.10	82-114	0.80-3.03
Post-development recharge loss (% of pre-development recharge) [d, e]	4.51-8.08 (36-65%)	0.84-1.57 (24-46%)	0.23-0.43 (26-49%)	0.32-0.66 (13-27%)	36-68 (24-45%)	2.89-5.12 (49-86%)

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; COMM—retail commercial. Ranges represent 40 percent runoff volume reduction, with full site coverage by BMPs having a natural surface, to no reduction, with BMPs isolating runoff from soil.
[b] Volume of precipitation on total project area
[c] Quantity of water discharged from the site on the surface
[d] Ranging from the quantity with hardened bed BMPs to the quantity with soil-based BMPs
[e] Quantity of water infiltrating the soil; the difference between precipitation and runoff

Effect of Basic Treatment BMPs on Pollutant Discharges

Table 6 (page 12) presents estimates of zinc effluent concentrations and mass loadings of the various pollutants discharged from four types of conventional treatment BMPs. The manufactured CDS BMPs in this table, which do not expose runoff to soil or vegetation, are not expected to drop any of the concentrations sufficiently to meet the acute zinc criterion at the discharge point. The loading reduction results show the CDS units always performing below 50 percent reduction for all pollutants analyzed, and most often in the vicinity of 20 percent, with zero copper reduction.

When treated with swales or filter strips, effluents from each development case study site are expected to fall below the CTR acute zinc criterion. All but the large commercial site would meet the criterion with EDB treatment. These natural-surface BMPs, if fully implemented and well maintained, are predicted to prevent the majority of the pollutant masses generated on most of the development sites from reaching a receiving water. Only total phosphorus reduction falls below 50 percent for two case studies. Otherwise, mass loading reductions range from about 60 to above 80 percent for the EDB, swale, and filter strip. This data indicates that draining impervious to pervious surfaces, even if those surfaces are not prepared in any special way, pays water quality as well as hydrologic dividends.

Table 6. Pollutant Concentration and Loading Reduction Estimates with Conventional BMPs

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
Effluent Concentrations:						
CDS TZn (mg/L)[a]	0.095	0.095	0.098	0.102	0.095	0.131
EDB TZn (mg/L)[a]	0.085	0.086	0.084	0.084	0.086	0.098
Swale TZn (mg/L)	0.055	0.054	0.055	0.056	0.054	0.068
Filter strip TZn (mg/L)	0.039	0.039	0.039	0.041	0.039	0.048
Loading Reductions:						
CDS TSS loading reduction	15.7%	19.9%	22.0%	24.0%	19.9%	16.9%
CDS TCu loading reduction	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
CDS TZn loading reduction	22.7%	22.4%	22.9%	23.1%	22.4%	25.1%
CDS TP loading reduction	30.6%	41.5%	40.7%	45.9%	41.5%	20.3%
EDB TSS loading reduction	68.1%	73.7%	79.0%	81.1%	73.7%	71.7%
EDB TCu loading reduction	61.9%	55.7%	66.2%	63.0%	55.7%	66.8%
EDB TZn loading reduction	59.7%	59.6%	60.4%	61.9%	59.6%	66.6%
EDB TP loading reduction	61.9%	69.7%	69.1%	72.9%	69.7%	54.5%
Swale TSS loading reduction	68.8%	71.1%	73.1%	73.9%	71.1%	69.4%
Swale TCu loading reduction	72.5%	68.5%	78.2%	73.3%	68.5%	75.8%
Swale TZn loading reduction	78.4%	78.1%	84.3%	78.8%	78.1%	80.7%
Swale TP loading reduction	66.3%	70.7%	67.2%	76.2%	70.7%	55.0%
Filter strip TSS loading reduction	69.9%	75.4%	80.6%	82.6%	75.4%	72.3%
Filter strip TCu loading reduction	74.4%	69.1%	78.2%	75.4%	69.1%	78.7%
Filter strip TZn loading reduction	78.3%	77.9%	78.4%	78.7%	77.9%	80.9%
Filter strip TP loading reduction	48.4%	53.1%	63.7%	59.8%	53.1%	34.6%

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; COMM—retail commercial; CDS— continuous deflective separation unit; EDB—extended-detention basin

3. LID Analysis: Development According to Modified Draft Permit Provisions

(a) Hydrologic Analysis

The LID analysis was first performed according to the Draft Permit provisions under the Planning and Land Development Program (Part 4, section E). In this analysis, however, EIA was limited to three instead of five percent, under the reasoning presented in Attachment A. All runoff from NCIA was assumed to drain to vegetated surfaces, as provided in the Draft Permit.

One goal of this exercise was to identify methods that reduce runoff production in the first place. It was hypothesized that implementation of source reduction techniques could allow all of the case study sites to infiltrate substantial proportions of the developed site runoff, advancing the hydromodification mitigation objective of the Draft Permit. When runoff is dispersed into the soil instead of being rapidly collected and conveyed away, it recharges groundwater, supplementing a resource that maintains dry season stream flow and wetlands. An increased water balance can be tapped by humans for potable, irrigation, and process water supply. Additionally, runoff volume reduction would commensurately decrease pollutant mass loadings.

Accordingly, the analysis considered the practicability of more than one scenario by which the draft permit's terms could be met, as modified to reflect three percent EIA. In one option, all roof runoff is harvested and stored for some beneficial use. A second option disperses runoff into the soil via roof downspout infiltration trenches. The former option is probably best suited to cases like the large commercial and office buildings, while distribution in the soil would fit best with residences and relatively small commercial developments. The analysis was repeated with the assumptions of harvesting OFF and COMM roof runoff for some beneficial use and dispersing roof runoff from the remaining four cases in roof downspout infiltration systems.

Expected Infiltration Capacities of the Case Study Sites

The first inquiry on this subject sought to determine how much of the total annual runoff each property is expected to infiltrate. This assessment tested the feasibility of draining all but three percent of impervious area to pervious land on the sites. Based on the findings of Chralowicz et al. (2001), it was assumed that an infiltration zone of 0.1-0.5 acres in area and 2-3 ft deep would serve a drainage catchment area in the size range 0-5 acres and infiltrate 0.9-1.9 acre-ft/year. The conclusions of Chralowicz et al. (2001) were extrapolated to conservatively assume that 0.5 acre would be required to serve each additional five acres of catchment, and would infiltrate an incremental 1.4 acre-ft/year (the midpoint of the 0.9-1.9 acre-ft/year range). According to these assumptions, the following schedule of estimates applies:

Pervious Area Available for Infiltration	Catchment Served acres	Infiltration Capacity
0.5 acres	0-5 acres	1.4 acre-ft/year
1.0 acres	5-10 acres	2.8 acre-ft/year
1.5 acres	10-15 acres	4.2 acre-ft/year
(Etc.)	...	...

As a formula, infiltration capacity ≈ 2.8 x available pervious area. To apply the formula conservatively, the available area was reduced to the next lower 0.5-acre increment before multiplying by 2.8.

As shown in Table 7, five of the six sites have adequate or greater capacity to infiltrate the full annual runoff volume from NCIA and pervious areas where EIA is limited to three percent of the total site area (four at the higher Ojai rainfall). Indeed, five of the six development types have sufficient pervious area to infiltrate *all* runoff, including runoff from EIA areas. With the most representative rainfall, only the large commercial development, with little available pervious area, falls short of the needed capacity to infiltrate all rainfall, but it still has the capacity to meet the terms of the draft permit, as modified for this analysis. These results are based on infiltrating in the native soils with no soil amendment. For any development project at which infiltration-oriented BMPs are considered, it is important that infiltration potential be carefully assessed using site-specific soils and hydrogeologic data. In the event such an investigation reveals a marginal condition (e.g., hydraulic conductivity, spacing to groundwater) for infiltration basins, soils could be enhanced to produce bioretention zones to assist infiltration. Notably, the four case studies with far greater than necessary infiltration capacity would offer substantial flexibility in designing infiltration, allowing ponding at less than 2-3 ft depth.

Table 7. Infiltration and Runoff Volume With 3 Percent EIA and All NCIA Draining to Pervious Areas

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
EIA runoff (acre-ft/year)	0.38	0.11	0.03	0.07	4.6	0.18
NCIA + pervious area runoff (acre-ft/year)	**8.63**	**1.73**	**0.47**	**0.76**	**75.0**	**5.39**
Total runoff (acre-ft/year)	9.01	1.84	0.50	0.83	79.6	5.57
Pervious area available for infiltration (acres)	3.66	1.67	0.39	1.61	72.7	0.47
Estimated infiltration capacity (acre-ft/year)[b]	**9.8**	**4.2**	**1.4**	**4.2**	**203**	**1.4**
Infiltration capacity [c]	> 100%[d]	> 100%	> 100%	> 100%	> 100%	~26% [d]

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; COMM—retail commercial;
[b] Based on Chralowicz et al. (2001) according to the schedule described above
[c] Compare runoff production from NCIA + pervious area (**row 3**) with estimated infiltration capacity (**row 6**)
[d] At Ojai rainfall levels, capacity would be ~78 percent at the MFR site and ~18 percent at the COMM site.

As Table 7 shows, five of the six case study sites have the capacity to infiltrate *all* runoff produced onsite by draining impervious surfaces to pervious areas. Even runoff from the area assumed to be EIA could be infiltrated in most cases based on the amount of pervious area available in typical development projects. By showing that it is possible under normal site conditions and using native soils to retain *all* runoff in typical developments, these results demonstrate that a three percent EIA requirement, which would not demand that all runoff be retained, is feasible and practicable.

Additional Source Reduction Capabilities of the Case Study Sites: Water Harvesting Example

Infiltration is one of a wide variety of LID-based source reduction techniques. Where site conditions such as soil quality or available area limit a site's infiltration capacity, other source LID measures can enhance a site's runoff retention capability. For example, soil amendment, which improves infiltration, is a standard LID technique. Water harvesting is another. Such practices can also be used where infiltration capacity is adequate, but the developer desires greater flexibility for land use on-site. Table 8 shows the added implementation flexibility created by subtracting roof runoff by harvesting it or efficiently directing it into the soil through downspout dispersion systems, further demonstrating the feasibility of meeting the draft permit's proposed requirements, as modified to include a three percent EIA standard.

Table 8. Infiltration and Runoff Volume Reduction Analysis Including Roof Runoff Harvesting or Disposal in Infiltration Trenches (Assuming 3 Percent EIA and All NCIA Draining to Pervious Areas)

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
EIA runoff (acre-ft/year)	0.38	0.11	0.03	0.07	4.6	0.18
Roof runoff (acre-ft/year)	4.92	0.93	0.09	0.20	41	2.33
Other NCIA + pervious area runoff (acre-ft/year)	**3.71**	**0.79**	**0.39**	**0.56**	**35**	**3.06**
Total runoff (acre-ft/year)	9.01	1.84	0.50	0.83	79.6	5.57
Pervious area available for infiltration (acres)	3.66	1.67	0.39	1.61	72.7	0.47
Estimated infiltration capacity (acre-ft/year)[b]	**9.8**	**4.2**	**1.4**	**4.2**	**203**	**1.4**
Infiltration capacity [c]	> 100%	> 100%	> 100%	> 100%	> 100%	~45% [d]

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; COMM—retail commercial;
[b] Based on Chralowicz et al. (2001) according to the schedule described above
[c] Comparison of runoff production from NCIA + pervious area (**row 3**) with estimated infiltration capacity (**row 6**)
[d] If the higher rainfall at Ojai is assumed, capacity would be ~32 percent of the amount needed for the COMM case.

Effect of Full LID Approach on Recharge

Table 9 (page 15) shows the recharge benefits of preventing roofs from generating runoff and infiltrating as much as possible of the runoff from the remainder of the case study sites. The data show that LID methods offer significant benefits relative to the baseline (no storm water controls) in all cases. These benefits are particularly impressive in developments with relatively high site imperviousness, such as in the MFR and COMM cases. In the latter case the full LID approach (excluding the common and effective practice of soil amendment) would cut loss of the potential water resource represented by recharge and harvesting from 86 to 37 percent.

Table 9. Comparison of Water Captured Annually (in acre-ft) from Development Sites for Beneficial Use With a Full LID Approach Compared to Development With No BMPs

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
Pre-development recharge[b] (acre-ft)	12.5	3.46	0.88	2.42	150	5.92
No BMPs:						
post-development recharge [b] (acre-ft)	4.39	1.88	0.45	1.76	82	0.80
post-development runoff (acre-ft)	8.08	1.57	0.43	0.66	68	5.12
post-development % recharge lost	65%	46%	49%	27%	45%	86%
Full LID approach:						
post-development runoff capture (acre-ft)[c]	12.5	3.46	0.88	2.42	150	3.73
post-development runoff (acre-ft)	0	0	0	0	0	2.19
post-development % recharge lost	0%	0%	0%	0%	0%	37%

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; COMM—retail commercial
[b] Quantity of water infiltrating the soil; the difference between precipitation and runoff
[c] Water either entirely infiltrated in BMPs and recharged to groundwater or partially harvested from roofs and partially infiltrated in BMPs. For the first five case studies, EIA was not distinguished from the remainder of the development, because these sites have the potential to capture all runoff.

(b) Water Quality Analysis

As outlined above, it was assumed that EIA discharges, as well as runoff from all pervious surfaces, are subject to treatment control. For purposes of the analysis, treatment control was assumed to be provided by conventional sand filtration. This choice is appropriate for study purposes for two reasons. First, sand filters can be installed below grade, and land above can be put to other uses. Under the Draft Permit's approach, pervious area should be reserved for receiving NCIA drainage, and using sand filters would not draw land away from that service or other site uses. A second reason for the choice is that sand filter performance data equivalent to the data used in analyzing other conventional BMPs are available from the CalTrans (2004) work. Sand filters may or may not expose water to soil, depending on whether or not they have a hard bed. This analysis assumed a hard bed, meaning that no infiltration would occur and thus there would be no additional recharge in sand filters. Performance would be even better than shown in the analytical results if sand filters were built in earth.

Pollutant Discharge Reduction Through LID Techniques

The preceding analyses demonstrated that each of the six case studies could feasibly comply with the draft permit's requirements, as modified to include a more protective three percent EIA standard. Moreover, for five of the six case studies, *all* storm water discharges could be eliminated at least under most meteorological conditions by dispersing runoff from impervious surfaces to pervious areas. Therefore, pollutant additions to receiving waters would also be eliminated. This demonstrates not only that a lower EIA (three percent) is a feasible and practicable approach to maintaining the natural hydrology of land being developed, as discussed above, but that a lower EIA is a feasible and practicable way to eliminate the discharge of pollutants that could cause or contribute to violations of water quality standards.

While the high proportion of impervious area present on the large commercial site relative to pervious area would not allow eliminating all discharge, harvesting roof water and draining NCIA to properly-prepared pervious area would substantially decrease the volume discharged. Deployment of treatment control BMPs (e.g. sand filter treatment) could cut contaminant discharges from pollutants in the remaining volume of runoff to low levels.

Table 10 presents the pollutant reductions from the untreated case achievable through the complete LID approach described above in comparison to conventional treatments (from Table 6). Assuming EIA still discharges through sand filters, pollutant loadings from the untreated condition are expected to decrease by more than 96 percent for all but the COMM case. In that challenging case loadings would still fall by at least 89 percent for TSS and the metals and by 83 percent for total phosphorus, assuming City of Ventura rainfall levels, and slightly less assuming the higher Ojai rainfall levels. Thus, the Draft Permit's basic premise of disconnecting most impervious area, supplemented by specially managing roof water, is shown by both water quality and hydrologic results to be feasible and to afford broad and significant environmental benefits.

Table 10. Pollutant Loading Reduction Estimates With a Full LID Approach Relative to Conventional BMPs

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	COMM[a]
Conventional TSS loading reduction[b]	15.7-69.9%	19.9-75.4%	22.0-80.6%	24.0-82.6%	19.9-75.4%	16.9-72.3%
Conventional TCu loading reduction[b]	0.0-74.4%	0.0-69.1%	0.0-78.2%	0.0-75.4%	0.0-69.1%	0.0-78.7%
Conventional TZn loading reduction[b]	22.7-78.4%	22.4-78.1%	22.9-84.3%	23.1-78.8%	22.4-78.1%	25.1-80.9%
Conventional TP loading reduction[b]	30.6-66.3%	41.5-70.7%	40.7-69.1%	45.9-76.2%	41.5-70.7%	20.3-55.0%
LID TSS loading reduction[c]	99.4%	99.3%	99.5%	99.4%	99.3%	89.0% [d]
LID TCu loading reduction[c]	98.1%	96.7%	98.0%	96.2%	96.7%	90.6% [d]
LID TZn loading reduction[c]	99.1%	98.8%	98.9%	98.3%	98.8%	94.8% [d]
LID TP loading reduction[c]	98.1%	98.6%	98.8%	98.7%	98.6%	83.1%[d]

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; COMM—retail commercial; CDS— continuous deflective separation unit; EDB—extended-detention basin; NCIA—not connected impervious area; EIA—effective (connected) impervious area
[b] Range from Table 6 represented by treatment by CDS unit, EDB, biofiltration swale, or biofiltration strip
[c] Based on directing roof runoff to downspout infiltration trenches (MFR, Sm-SFR, REST, and Lg-SFR) or harvesting it (OFF and COMM), draining other NCIA to pervious areas, and treating EIA with sand filters
[d] If the higher rainfall at Ojai is assumed, reduction estimates for TSS, TCu, TZn, and TP would be 84.0, 86.3, 92.5, and 75.5 percent, respectively.

SUMMARY AND CONCLUSIONS

This paper demonstrated that common Ventura County area residential and commercial development types subject to the Municipal NPDES Permit are likely, without storm water management, to reduce groundwater recharge from the predevelopment state by approximately half in most cases to a much higher fraction with a large ratio of impervious to pervious area. With no treatment, runoff from these developments is expected to exceed CTR acute copper and zinc criteria at the point of discharge and to deliver large pollutant mass loadings to receiving waters.

Conventional soil-based BMP solutions that promote and are component parts of low-impact development approaches, by contrast, regain about 30-50 percent of the recharge lost in development without storm water management, although commercially-manufactured filtration and hydrodynamic BMPs for storm water management give no benefits in this area. It is expected the soil-based BMPs generally would release effluent that meets the acute zinc criterion at the point of discharge, although it would still exceed the copper limit. Excepting phosphorus, it was found that these BMPs would capture and prevent the movement to receiving waters of the majority of the pollutant loadings considered in the analysis.

It was found that a three percent Effective Impervious Area standard can be met in typical developments, and that by draining all site runoff to pervious areas, runoff can be eliminated entirely in most development types. This result was reached assuming the use of native soils. Soil enhancement (typically, with compost) can further advance infiltration. Draining impervious surfaces onto the loam soils typical of Ventura County, in connection with limiting directly connected impervious area to three percent of the site total area, should eliminate storm runoff from some development types and greatly reduce it from more highly impervious types. Adding roof runoff elimination to the LID approach (by harvesting or directing it to downspout infiltration trenches) should eliminate runoff from all but mostly impervious developments. Even in the development scenario involving the highest relative proportion of impervious surface, losses of rainfall capture for beneficial uses could be reduced from more than 85 to less than 40 percent, and pollutant mass loadings would fall by 83-95 percent from the untreated scenario when draining to pervious areas was supplemented with water harvesting. These results demonstrate the basic soundness of the Draft Permit's concept to limit directly connected impervious area and drain the remainder over pervious surfaces.

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ATTACHMENT A

JUSTIFICATION OF PROPOSED EFFECTIVE IMPERVIOUS AREA LIMITATION

Summary

The literature shows that adverse impacts to the physical habitat and biological integrity of receiving waters occur as a result of the conversion of natural areas to impervious cover. These effects are observed at the lowest levels of impervious cover in associated catchments (two to three percent) and are pronounced by the point that impervious cover reaches five percent. To protect biological productivity, physical habitat, and other beneficial uses, effective impervious area should be capped at no more than three percent.

I. Impacts to physical habitat of California receiving waters observed at three percent impervious cover

Stein *et al*.[7] note that while studies from parts of the country with climates more humid than California's indicate that physical degradation of stream channels can initially be detected when watershed impervious cover approaches 10%, biological effects, which may be more difficult to detect, may occur at lower levels (CWP 2003).[8] Recent studies from both northern and southern California indicate that intermittent and ephemeral streams in California are more susceptible to the effects of hydromodification than streams from other regions of the US, with stream degradation being recognized when the associated catchment's impervious cover is as little as 3-5% (Coleman *et al*. 2005).[9] Furthermore, supplemental landscape irrigation in semi-arid regions, like California, can substantially increase the frequency of erosive flows (AQUA TERRA Consultants 2004).[10]

Coleman, *et al*.[3] report that the ephemeral/intermittent streams in southern California (northwestern Los Angeles County through southern Ventura County to central Orange County) appear to be more sensitive to changes in percent impervious cover than streams in other areas. Stream channel response can be represented using an *enlargement curve*, which relates the percent of impervious cover to a change in cross-sectional area. The data for southern California streams forms a relationship very similar in shape to the enlargement curves developed for other North American streams. However, the curve for southern California streams is above the general curve for streams in other climates. This suggests that a specific enlargement ratio is produced at a lower value of impervious surface area in southern California than in other parts of North America. Specifically, the estimated threshold of response is approximately 2-3% impervious cover, as compared to 7-10% for other portions of the U.S. It is important to note that this conclusion applies specifically to streams with a catchment drainage area less than 5 square miles.

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7 Stein, E.D., S. Zaleski, (2005) *Managing Runoff to Protect Natural Streams: The Latest Developments on Investigation and Management of Hydromodification in California*. (Proceedings of a Special Technical Workshop Co-sponsored by California Stormwater Quality Association (CASQA), Stormwater Monitoring Coalition (SMC), University of Southern California Sea Grant (USC Sea Grant), Technical Report #475).

8 Center for Watershed Protection (CWP), (2003) *Impacts of Impervious Cover on Aquatic Systems*. Ellicott City, MD.

9 Coleman, D., C. MacRae, and E.D. Stein, (2005) *Effect of Increases in Peak Flows and Imperviousness on the Morphology of Southern California Streams.* Southern California Coastal Water Research Project Technical Report #450, Westminster, CA.

10 AQUA TERRA Consultants, (2004) *Urbanization and Channel Stability Assessment in the Arroyo Simi Watershed of Ventura County CA*. FINAL REPORT. Prepared for Ventura County Watershed Protection Division, Ventura CA.

This study concludes that disconnecting impervious areas from the drainage network and adjacent impervious areas is a key approach to protecting channel stability. Utilizing this strategy can make it practical to keep the effective impervious cover (*i.e*. the amount hydrologically connected to the stream) equal to or less than the identified threshold of 2-3%.

II. Impacts to biological integrity of receiving waters observed with any conversion from natural to impervious surface

Two separate studies conducted by Horner *et al*.[11,12] in the Puget Sound region (Washington State), Montgomery County, Maryland, and Austin, Texas built a database totaling more than 650 reaches on low-order streams in watersheds ranging from no urbanization and relatively little human influence (the reference state, representing "best attainable" conditions) to highly urban (>60 percent total impervious area, "TIA"). Biological health was assessed according to the benthic index of biotic integrity (B-IBI) and, in Puget Sound, the ratio of young-of-the-year coho salmon (*Oncorhynchus kisutch*), a relatively stress-intolerant fish, to cutthroat trout (*Oncorhynchus clarki*), a more stress-tolerant species. The following discussion summarizes the results and conclusions of these two studies.

There is no single cause for the decline of water resource conditions in urbanizing watersheds. Instead, it is the cumulative effects of multiple stressors that are responsible for degraded aquatic habitat and water quality. Imperviousness, while not a perfect yardstick, appears to be a useful predictor of ecological condition. However, a range of stream conditions can be associated with any given level of imperviousness. In general, only streams that retain a significant proportion of their natural vegetative land-cover <u>and</u> have very low levels of watershed imperviousness appear to retain their natural ecological integrity. It is this change in watershed land-cover that is largely responsible for the shift in hydrologic regime from a sub-surface flow dominated system to one dominated by surface runoff.

While the decline in ecological integrity is relatively continuous and is consistent for all parameters, the impact on physical conditions appears to be more pronounced earlier in the urbanization process than chemical degradation. It is generally acknowledged, based on field research and hydrologic modeling, that it is the shift in hydrologic conditions that is the driving force behind physical changes in urban stream-wetland ecosystems.

Multiple scales of impact operate within urbanizing watersheds: landscape-level impacts, including the loss of natural forest cover and the increase in impervious surface area throughout the watershed; riparian corridor-specific impacts such as encroachment, fragmentation, and loss of native vegetation; and local impacts such as water diversions, exotic vegetation, stream channelization, streambank hardening, culvert installation, and pollution from the widespread use of pesticides and herbicides. All of these stressors contribute to the overall cumulative impact.

The researchers found that there is no clear threshold of urbanization below which there exists a "no-effect" condition. Instead, there appears to be a relatively continuous decline in almost all measures of water quality or ecological integrity. Losses of integrity occur from the lowest levels of TIA and are already pronounced by the point that TIA reaches 5 percent.

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[11] Horner, R. R., C. W. May, (2002) *The Limitations of Mitigation-Based Stormwater Management in the Pacific Northwest and the Potential of a Conservation Strategy based on Low-Impact Development Principles*. (Proceedings of the American Society of Engineers Stormwater Conference, Portland, OR).

[12] Horner, R.R., E. H. Livingston, C. W. May, J. Maxted, (2006) *BMPs, Impervious Cover, and Biological Integrity of Small Streams*. (Proceedings of the Eighth Biennial Stormwater Research and Watershed Management Conference, Tampa, FL).

Similarly, the Alliance for the Chesapeake Bay[13] reports that small-watershed studies by the Maryland Department of Natural Resources Biological Stream Survey have shown that some sensitive species are affected by even low amounts of impervious cover. In one study, no brook trout were observed in any stream whose watershed had more than 2 percent impervious cover, and brook trout were rare in any watershed with more than 0.5 percent impervious cover.

III. Ventura County's watersheds include biologically-significant water bodies

The literature discussed above is relevant to the watersheds of Ventura County, which contain rivers and streams that currently or historically support a variety of beneficial uses that may be impaired by water quality degradation and stream hydromodification as a result of storm water runoff from impervious land cover. Unlike some Southern California watersheds, Ventura County still has many natural stream systems with a high degree of natural functionality.

For instance, the Ventura River watershed in northwestern Ventura County "supports a large number of sensitive aquatic species,"[14] including steelhead trout, a federally-listed endangered species. Although "local populations of steelhead and rainbow trout have nearly been eliminated along the Ventura River" itself, the California Department of Fish and Game has "recognized the potential for the restoration of the estuary and enhancement of steelhead populations in the Ventura River."[15] Steelhead may also be present in tributaries such as San Antonio Creek.[16] Thriving rainbow trout populations exist in tributaries of the Ventura River including Matilija Creek and Coyote Creek.[17] The Ventura River either does or is projected to support the following beneficial uses: warm freshwater habitat; cold freshwater habitat; wildlife habitat; rare, threatened, or endangered species; migration of aquatic organisms; and spawning and reproduction.[18] Furthermore, the Ventura River Estuary also supports commercial fishing, shellfish harvesting, and wetland habitat.[19] The Ventura River receives municipal storm drain discharges from Ojai, San Buenaventura, and unincorporated areas of Ventura County.[20]

The Santa Clara River watershed in northern Ventura County "is the largest river system in southern California that remains in a relatively natural state."[21] Sespe Creek is one of the Santa Clara's largest tributaries, and "supports significant steelhead spawning and rearing habitat."[22] Other creeks in the Santa Clara River watershed that support steelhead are Piru Creek and Santa Paula Creek. Sespe Creek and the Santa Clara River also provide spawning habitat for the Pacific lamprey. Rainbow trout populations exist in tributaries of the Santa Clara River including Sespe Creek.[23] The creeks and the Santa Clara river do or are projected to support the following beneficial uses: warm freshwater habitat; cold freshwater habitat; wildlife habitat; preservation of biological habitats rare, threatened, or endangered species; migration of aquatic organisms; and spawning and reproduction.[24] Los Padres National Forest covers much of the Santa Clara River watershed, but increasing development in floodplain areas has been

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[13] Karl Blankenship, BAY JOURNAL,"It's a hard road ahead for meeting new sprawl goal: States will try to control growth of impervious" (July/August 2004), at http://www.bayjournal.com/article.cfm?article=66.
[14] Los Angeles Region Water Quality Control Plan (1994) p. 1-18 ("Basin Plan").
[15] Basin Plan, p. 1-16; Ventura County Environmental & Energy Resources Division, "Endangered Steelhead Trout in Ventura County: Past, Present, and Future," available at http://www.wasteless.org/Eye_articles/steelhead.htm.
[16] Ventura County Environmental & Energy Resources Division, "Steelhead Spawning in Ventura County," (2005), available at http://www.wasteless.org/Eye_articles/steehead2005.html.
[17] Ventura County Environmental & Energy Resources Division, "Endangered Steelhead Trout in Ventura County: Past, Present, and Future," available at http://www.wasteless.org/Eye_articles/steelhead.htm.
[18] Basin Plan, Table 2-1.
[19] Basin Plan, Table 2-4.
[20] Ventura County Watershed Protection District, *Report of Waste Discharge* (January 2005) at p. 3.
[21] Basin Plan, p. 1-16.
[22] Basin Plan, p. 1-16.
[23] Ventura County Environmental & Energy Resources Division, "Endangered Steelhead Trout in Ventura County: Past, Present, and Future," available at http://www.wasteless.org/Eye_articles/steelhead.htm.
[24] Basin Plan, Table 2-1.

identified as a threat to the river system's water quality.[25] Furthermore, the Santa Clara estuary supports the additional beneficial uses of shellfish harvesting and wetlands habitat.[26] The Santa Clara River receives municipal storm drain discharges from Fillmore, Oxnard, San Buenaventura, Santa Paula, and unincorporated areas of Ventura County.[27]

The Calleguas Creek watershed "empties into Mugu Lagoon, one of southern California's few remaining large wetlands."[28] It supports or is projected to support the following beneficial uses: estuarine habitat; marine habitat; wildlife habitat; preservation of biological habitats; rare, threatened, or endangered species; migration of aquatic organisms; spawning and reproduction; shellfish harvesting; and wetlands habitat.[29] Historically, Calleguas Creek drained largely agricultural areas. But this watershed has been under increasing pressure from sedimentation due to increased surface flow from municipal discharges and urban wastewaters, among other sources.[30] Increasing residential developments on steep slopes has been identified as a substantial contributing factor to the problem of accelerated erosion in the watershed (and sedimentation in the Lagoon). Calleguas Creek receives municipal storm drain discharges from Camarillo, Moorpark, Simi Valley, Thousand Oaks, and unincorporated areas of Ventura County.[31]

Ventura County's coastal streams also support a variety of beneficial uses:[32]

- Little Sycamore Canyon Creek in southern Ventura County (warm freshwater habitat; wildlife habitat; rare, threatened or endangered species; and spawning and reproduction);
- Lake Casitas tributaries (warm freshwater habitat; cold freshwater habitat; wildlife habitat; rare, threatened or endangered species; spawning and reproduction; and wetland habitat);
- Javon Canyon and Padre Juan Canyon (warm freshwater habitat; cold freshwater habitat; wildlife habitat; and spawning and reproduction); and
- Los Sauces Creek in northern Ventura County (warm freshwater habitat; cold freshwater habitat; wildlife habitat; migration of aquatic species; and spawning and reproduction).

IV. Conclusion

In order to protect the biological habitat, physical integrity, and other beneficial uses of the water bodies in Ventura County, effective impervious area should be capped at no more than three percent.

[25] Basin Plan, pp. 1-16, 1-18.
[26] Basin Plan, Table 2-4.
[27] Ventura County Watershed Protection District, *Report of Waste Discharge* (January 2005) at p. 3.
[28] Basin Plan, p. 1-18.
[29] Basin Plan, Table 2-1.
[30] Basin Plan, pp. 1-16, 1-18.
[31] Ventura County Watershed Protection District, *Report of Waste Discharge* (January 2005) at p. 3.
[32] Basin Plan, Table 2-1.

ATTACHMENT B

POLLUTANT CONCENTRATIONS FOR URBAN SOURCE AREAS (HERRERA ENVIRONMENTAL CONSULTANTS, INC. UNDATED)

Source Area	Study	Location	Sample Size (n)	TSS (mg/L)	TCu (ug/L)	TPb (ug/L)	TZn (ug/L)	TP (mg/L)	Notes
Roofs									
Residential	Steuer, et al. 1997	MI	12	36	7	25	201	0.06	2
Residential	Bannerman, et al. 1993	WI	~48	27	15	21	149	0.15	3
Residential	Waschbusch, et al. 2000	WI	25	15	n.a.	n.a.	n.a.	0.07	3
Residential	FAR 2003	NY		*19*	*20*	*21*	*312*	*0.11*	4
Residential	Gromaire, et al. 2001	France		*29*	*37*	*493*	*3422*	*n.a.*	5
Representative Residential Roof Values				**25**	**13**	**22**	**159**	**0.11**	
Commercial	Steuer, et al. 1997	MI	12	24	20	48	215	0.09	2
Commercial	Bannerman, et al. 1993	WI	~16	15	9	9	330	0.20	3
Commercial	Waschbusch, et al. 2000	WI	25	18	n.a.	n.a.	n.a.	0.13	3
Representative Commercial Roof Values				**18**	**14**	**26**	**281**	**0.14**	
Parking Areas									
Res. Driveways	Steuer, et al. 1997	MI	12	157	34	52	148	0.35	2
Res. Driveways	Bannerman, et al. 1993	WI	~32	173	17	17	107	1.16	3
Res. Driveways	Waschbusch, et al. 2000	WI	25	34	n.a.	n.a.	n.a.	0.18	3
Driveway	FAR 2003	NY		*173*	*17*		*107*	*0.56*	4
Representative Residential Driveway Values				**120**	**22**	**27**	**118**	**0.66**	
Comm./ Inst. Park. Areas	Pitt, et al. 1995	AL	16	110	116	46	110	n.a.	1
Comm. Park. Areas	Steuer, et al. 1997	MI	12	110	22	40	178	0.2	2
Com. Park. Lot	Bannerman, et al. 1993	WI	5	58	15	22	178	0.19	3
Parking Lot	Waschbusch, et al. 2000	WI	25	51	n.a.	n.a.	n.a.	0.1	3
Parking Lot	Tiefenthaler, et al. 2001	CA	5	36	28	45	293	n.a.	6
Loading Docks	Pitt, et al. 1995	AL	3	40	22	55	55	n.a.	1
Highway Rest Areas	CalTrans 2003	CA	53	*63*	*16*	*8*	*142*	*0.47*	7
Park and Ride Facilities	CalTrans 2003	CA	179	*69*	*17*	*10*	*154*	*0.33*	7
Comm./ Res. Parking	FAR 2003	NY		*27*	*51*	*28*	*139*	*0.15*	4
Representative Parking Area/Lot Values				**75**	**36**	**26**	**97**	**0.14**	

Landscaping/Lawns									
Landscaped Areas	Pitt, et al. 1995	AL	6	33	81	24	230	n.a.	1
Landscaping	FAR 2003	NY		*37*	*94*	*29*	*263*	n.a.	4
Representative Landscaping Values				**33**	**81**	**24**	**230**	**n.a.**	
Lawns - Residential	Steuer, et al. 1997	MI	12	262	n.a.	n.a.	n.a.	2.33	2
Lawns - Residential	Bannerman, et al. 1993	WI	~30	397	13	n.a.	59	2.67	3
Lawns	Waschbusch, et al. 2000	WI	25	59	n.a.	n.a.	n.a.	0.79	3
Lawns	Waschbusch, et al. 2000	WI	25	122	n.a.	n.a.	n.a.	1.61	3
Lawns - Fertilized	USGS 2002	WI	58	n.a.	n.a.	n.a.	n.a.	2.57	3
Lawns - Non-P Fertilized	USGS 2002	WI	38	n.a.	n.a.	n.a.	n.a.	1.89	3
Lawns - Unfertilized	USGS 2002	WI	19	n.a.	n.a.	n.a.	n.a.	1.73	3
Lawns	FAR 2003	NY	3	*602*	*17*	*17*	*50*	*2.1*	4
Representative Lawn Values				**213**	**13**	**n.a.**	**59**	**2.04**	

Notes:

Representative values are weighted means of collected data. Italicized values were omitted from these calculations.

1 - Grab samples from residential, commercial/institutional, and industrial rooftops. Values represent mean of DETECTED concentrations

2 - Flow-weighted composite samples, geometric mean concentrations

3 - Geometric mean concentrations

4 - Citation appears to be erroneous - original source of data is unknown. Not used to calculate representative value

5 - Median concentrations. Not used to calculate representative values due to site location and variation from other values.

6 - Mean concentrations from simulated rainfall study

7 - Mean concentrations. Not used to calculate representative values due to transportation nature of land use.

INITIAL INVESTIGATION OF THE FEASIBILITY AND BENEFITS OF LOW-IMPACT SITE DESIGN PRACTICES ("LID") FOR THE SAN FRANCISCO BAY AREA

Richard R. Horner[†]

ABSTRACT

The Clean Water Act NPDES permit that regulates municipal separate storm sewer systems (MS4s) in the San Francisco Bay Area, California will be reissued in 2007. The draft permit includes general provisions related to low impact development practices (LID) for certain kinds of development and redevelopment projects. Using six representative development project case studies, based on California building records, the author investigated the practicability and relative benefits of LID options for the majority of the region having soils potentially suitable for infiltration either in their natural state or after amendment using well recognized LID techniques. The results showed that (1) LID site design and source control techniques are more effective than conventional best management practices (BMPs) in reducing runoff rates; and (2) in each of the case studies, LID methods would reduce site runoff volume and pollutant loading to zero in typical rainfall scenarios.

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INTRODUCTION

The Assessment in Relation to Municipal Permit Conditions

This purpose of this study is to investigate the relative water quality and water reuse benefits of three levels of storm water treatment best management practices (BMPs): (1) basic "treat-and-release" BMPs (e.g., drain inlet filters, CDS units), (2) commonly used BMPs that expose runoff to soils and vegetation (extended-detention basins and biofiltration swales and filter strips), and (3) low impact development (LID) practices. The factors considered in the investigation are runoff volume, pollutant loading, and the availability of water for infiltration or other reuse. In order to assess the differential impact of storm water reduction approaches on these factors, this study examines six case studies typical of development covered by the proposed Municipal Regional Urban Runoff Phase I NPDES Stormwater Permit (MRP).

This report covers locations in the Bay Area most amenable to soil infiltration of stormwater runoff, those areas having soils in Natural Resources Conservation Service (NRCS) Hydrologic Soil Groups A, B, or C as classified by the Natural Resources Conservation Service (http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx). Depending on site-specific conditions, A and B soils would generally effectively infiltrate water without modification, whereas C soils could require organic amendments according to now standard LID methods. This report does not cover locations with group D soils, which are generally not amenable to infiltration, again depending on the specific conditions on-site. A subsequent report will examine options in these locations, which include other LID techniques (e.g., roof runoff harvesting for irrigation or gray water supply) and state-of-the-art conventional stormwater management practices. A minority but still substantial fraction of the Bay Area has group D soils (39.3, 68.0, 18.3, and 50.1 percent of the mapped areas of Alameda, Contra Costa, San Mateo, and Santa Clara Counties, respectively). Regarding any mapped soil type, it is important to keep in mind that soils vary considerably within small distances. Characteristics at specific locations can deviate greatly from those of the major mapped unit, making infiltration potential either more or less than may be expected from the mapping.

Low impact development methods reduce storm runoff and its contaminants by decreasing their generation at sources, infiltrating into the soil or evaporating storm flows before they can enter surface receiving waters, and treating flow remaining on the surface through contact with vegetation and soil, or a combination of these strategies. Soil-based LID practices often use soil enhancements such as compost, and thus improve upon the performance of more traditional basins and biofilters. The study encompassed vegetated swales (channels for conveyance at some depth and velocity), vegetated filter strips (surfaces for conveyance in thin sheet flow), and bioretention areas (shallow basins with a range of vegetation types in which runoff infiltrates through soil either to groundwater or a subdrain for eventual surface discharge). Application of these practices in a low impact site design mode requires either determination that existing site soils can support runoff reduction through infiltration or that soils will be amended using accepted LID techniques to attain this objective. Finally, the study further broadened implementation options to include water harvesting (collection and storage for use in, for example, irrigation or gray water systems), roof downspout infiltration trenches, and porous pavements.

The investigation also considered whether typical development patterns and local conditions in the Bay Area would enable LID implementation as required by a new standard proposed for the 2007 Ventura County Municipal Storm Water Permit. This standard requires management of effective impervious area (EIA), limiting it to 5%, as well as other impervious area (what might be termed Not-Connected Impervious Area, NCIA), and pervious areas.

Where treatment control BMPs are required to manage runoff from a site, Volume or Flow Hydraulic Design Bases commonly used in California were assumed to apply. The former basis applies to storage-type BMPs, like ponds, and requires capturing and treating either the runoff volume from the 85th percentile, 24-hour rainfall event for the location or the volume of annual runoff to achieve 80 percent or more volume treatment. The calculations in this analysis used the 85th percentile 24-hour rainfall event basis. The Flow basis applies to flow-through BMPs, like swales, and requires treating the runoff flow rate produced from a rain event equal to at least 0.2 inches per hour intensity (or one of two other approximately equivalent options).

Scope of the Assessment

With respect to each of the six development case studies, three assessments were undertaken: a baseline scenario incorporating no stormwater management controls; a second scenario employing conventional BMPs; and a third development scenario employing LID stormwater management strategies.

To establish a baseline for each case study, annual stormwater runoff volumes were estimated, as well as concentrations and mass loadings of four pollutants: (1) total suspended solids (TSS), (2) total recoverable copper (TCu), (3) total recoverable zinc (TZn), and (4) total phosphorus (TP). These baseline estimates were based on the anticipated land use and cover with no stormwater management efforts.

Two sets of calculations were then conducted using the parameters defined for the six case studies. The first group of calculations estimated the extent to which basic BMPs reduce runoff volumes and pollutant concentrations and loadings, and what impact, if any, such BMPs have on recharge rates or water retention on-site.

The second group of calculations estimated the extent to which commonly used soil-based BMPs and LID site design strategies ameliorate runoff volumes and pollutant concentrations and loadings, and the effect such techniques have on recharge rates. When evaluating LID strategies in the context of the EIA concept employed in the draft Ventura County MS4 permit, it was presumed that EIA would be limited to three percent. It was also assumed that pervious surfaces on a site receiving runoff from other areas on the site would be sized and prepared to manage (through infiltration or storage) the volume directed there in addition to precipitation falling directly on those areas. The assessment of basins, biofiltration, and low impact design practices analyzed the expected infiltration capacity of the case study sites. It also considered related LID techniques and practices, such as source reduction strategies, that could work in concert with infiltration to serve the goals of: (1) preventing increase in annual runoff volume from the pre- to the post-developed state, (2) preventing increase in annual pollutant mass loadings between the two development states, and (3) avoiding exceedances of the Water Quality Control Plan for the San Francisco Bay Basin (Basin Plan) criteria for copper and zinc.

The results of this analysis show that:

- A full-range of typical development categories common in the Bay Area, from single family residential to restaurants, housing developments, and commercial uses like office buildings, can feasibly implement standard LID techniques to achieve no stormwater discharge during rain events equal to, and in some cases greater than, design storm conditions. This conclusion is based on an analysis that used actual building records in California and annual rainfall records in two rainfall zones in the Bay Area to show that site conditions support this level of performance. In addition, site conditions typical at a wide range of development projects are more than sufficient to attain compliance with a three percent EIA limit, as is being contemplated in other MS4 re-issuance proceedings in California presently.

- Developments implementing no post-construction BMPs result in storm water runoff volume and pollutant loading that are substantially increased, and recharge rates that are substantially decreased, compared to pre-development conditions.

- Developments implementing basic post-construction treatment BMPs achieve reduced pollutant loading compared to developments with no BMPs, but stormwater runoff volume and recharge rates are similar to developments with no BMPs.

- Developments implementing traditional basins and biofilters, and even more so low impact post-construction BMPs, achieve significant reduction of pollutant loading and runoff volume as well as greatly enhanced recharge rates compared to both developments with no BMPs and developments with basic treatment BMPs.

This report covers the methods employed in the investigation, data sources, and references for both. It then presents the results, discusses their consequences, draws conclusions, and makes recommendations relative to the feasibility of utilizing low-impact development practices in Bay Area developments.

CASE STUDIES

Six case studies were selected to represent a range of urban development types considered to be representative of the Bay Area. These case studies involved: a multi-family residential complex (MFR), a relatively small-scale (23 homes) single-family residential development (Sm-SFR), a restaurant (REST), an office building (OFF), a relatively large (1000 homes) single-family residential development (Lg-SFR), and a single home (SINGLE).[1]

Parking spaces were estimated to be 176 sq ft in area, which corresponds to 8 ft width by 22 ft length dimensions. Code requirements vary by jurisdiction, with the tendency now to drop below the traditional 200 sq ft average. About 180 sq ft is common, but various standards for full- and compact-car spaces, and for the mix of the two, can raise or lower the average.[2] The 176 sq ft size is considered to be a reasonable value for conventional practice.

Roadways and walkways assume a wide variety of patterns. Exclusive of the two SFR cases, simple, square parking lots with roadways around the four sides and square buildings with walkways also around the four sides were assumed. Roadways and walkways were taken to be 20 ft and 6 ft wide, respectively.

Single-family residences were assumed each to have a driveway 20 ft wide and 30 ft long. It was further assumed that each would have a sidewalk along the front of the lot, which was calculated to be 5749 sq ft in area. Assuming a square lot, the front dimension would be 76 ft. A 40-ft walkway was included within the property. Sidewalks and walkways were taken to be 4 ft wide. For each case study the total area for all of these impervious features was subtracted from the total site area to estimate the pervious area, which was assumed to have conventional landscaping cover (grass, small herbaceous decorative plants, bushes, and a few trees).

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[1] Building permit records from the City of San Marcos in San Diego County provided data on total site areas for the first four case studies, including numbers of buildings, building footprint areas (including porch and garage for Sm-SFR), and numbers of parking spaces associated with the development projects. While the building permit records made no reference to features such as roadways, walkways, and landscaping normally associated with development projects, these features were taken into account in the case studies using assumptions described herein. Larger developments and redevelopment were not represented in the sampling of building permits from the San Marcos database. To take these types of projects into account in the subsequent analysis, the Lg-SFR scenario scaled up all land use estimates from the Sm-SFR case in the ratio of 1000:23. The single home case (SINGLE) was derived from Bay Area records obtained at http://www.ppic.org/content/other/706EHEP_web_only_appendix.pdf, which showed 8000 ft^2 as a rough average for a single home lot in the region. As with the other cases, these hypothetical developments were assumed to have roadways, walkways, and landscaping, as described herein.

[2] J. Gibbons, *Parking Lots*, NONPOINT EDUCATION FOR MUNICIPAL OFFICERS, Technical Paper No. 5 (1999) (http://nemo.uconn.edu/tools/publications/tech_papers/tech_paper_5.pdf).

Table 1 summarizes the characteristics of the six case studies. The table also provides the recorded or estimated areas in each land use and cover type.

Table 1. Case Study Characteristics and Land Use and Land Cover Areas

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
No. buildings	11	23	1	1	1000	1
Total area (ft^2)	476,982	132,227	33,669	92,612	5,749,000	8,000
Roof area (ft^2)	184,338	34,949	3,220	7,500	1,519,522	2114
No. parking spaces	438	-	33	37	-	-
Parking area (ft^2)	77,088	-	5808	6512	-	-
Access road area (ft^2)	22,212	-	6097	6456	-	-
Walkway area (ft^2)	33,960	10,656	1362	2078	463,289	518
Driveway area (ft^2)	-	13,800	-	-	600,000	835
Landscape area (ft^2)	159,384	72,822	17,182	70,066	3,166,190	4533

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; SINGLE—single-family home

METHODS OF ANALYSIS

Annual Stormwater Runoff Volumes

Annual surface runoff volumes produced were estimated for both pre- and post-development conditions for each case study site. Runoff volume was computed as the product of annual precipitation, contributing drainage area, and a runoff coefficient (ratio of runoff produced to rainfall received). For impervious areas the following equation was used:

$$\mathbf{C = (0.009)\,\mathit{I} + 0.05}$$

where I is the impervious percentage. This equation was derived by Schueler (1987) from Nationwide Urban Runoff Program data (U.S. Environmental Protection Agency 1983). With I = 100 percent for fully impervious surfaces, C is 0.95.

The basis for pervious area runoff coefficients was the Natural Resource Conservation Service's (NRCS) Urban Hydrology for Small Watersheds (NRCS 1986, as revised from the original 1975 edition). This model estimates storm event runoff as a function of precipitation and a variable representing land cover and soil, termed the curve number (CN). Larger events are forecast to produce a greater amount of runoff in relation to amount of rainfall because they more fully saturate the soil. Therefore, use of the model to estimate annual runoff requires selecting some event or group of events to represent the year. The 85th percentile, 24-hour rainfall event was used in the analysis here for the relative comparison between pre- and post-development and applied to deriving a runoff coefficient for annual estimates, recognizing that smaller storms would produce less and larger storms more runoff.

A memorandum titled Rainfall Data Analysis and Guidance for Sizing Treatment BMPs (http://www.cccleanwater.org/construction/Publications/CCCWPBasinSizingMemoFINAL_4-20-05.pdf) prepared for the Contra Costa Clean Water Program demonstrated a linear relationship between unit basin storage volume for 80 percent capture (which is related to the 85th percentile event) and mean annual precipitation. Rainfall for Bay Area 85th percentile, 24-hour events could thus be determined from locations where events have been established in direct proportion to mean annual rainfall.

In order to obtain appropriate regional estimates of annual precipitation, rainfall records were obtained from a number of sites in the four counties, plus the city of Vallejo, covered by the permit.[3] The mean annual range is from 13.73 to 24.30 inches, with quantities close to either 14 or 20 inches predominating. The study was performed for both of these rainfall totals. These figures were used in conjunction with 85th percentile, 24-hour event amounts of 0.75 for Los Angeles and 0.92 for Santa Rosa (http://ci.santa-rosa.ca.us/pworks/other/SW/SRSWManualFinalDraft.pdf), respectively, and mean annual totals of 12 and 31 inches for the respective cities to estimate 85 percentile, 24-hour event quantities of 0.77 and 0.82 inch for the 14 and 20-inch Bay Area rainfall zones, respectively.

Pre- and post-development runoff quantities were computed with selected CN values and the 0.77- and 0.82-inch rainfalls. The CN choices based on tabulated data in NRCS (1986) and professional judgment were 83 before development and 86 after land modification. Estimate runoff amounts were then divided by the rainfall totals to obtain runoff coefficients. The results were about the same for the two rainfall zones at 0.07 and 0.12 before and after development, respectively. Finally, total annual runoff volumes were estimated based on the two average annual precipitation figures.

Stormwater Runoff Pollutant Discharges

Annual pollutant mass discharges were estimated as the product of annual runoff volumes produced by the various land use and cover types and pollutant concentrations typical of those areas. Again, the 0.75-inch precipitation event was used as a basis for volumes. Stormwater pollutant data have typically been measured and reported for general land use types (e.g., single-family residential, commercial). However, an investigation of low impact development practices of the type this study sought to conduct demands data on specific land coverages. The literature offers few data on this basis. Those available and used herein were assembled by a consultant to the City of Seattle for a project in which the author participated. They appear in Attachment A (Herrera Environmental Consultants, Inc. undated).

Pollutant concentrations expected to occur typically in the mixed runoff from the several land use and cover types making up a development were estimated by mass balance; i.e., the concentrations from the different areas of the sites were combined in proportion to their contribution to the total runoff.

The Effect of Conventional Treatment BMPs on Runoff Volume, Pollutant Discharges, and Recharge Rates

The first question in analyzing how BMPs reduce runoff volumes and pollutant discharges was, What BMPs are being employed in Bay Area developments under the permit now in force? These county permits provide regulated entities with a large number of choices and few fixed requirements regarding the selection of stormwater BMPs. (See Contra Costa County NPDES Municipal Stormwater Permit, Order No. 99-058; see also Santa Clara County NPDES Municipal Stormwater Permit, Order No. 01-024, at C.3.a.). Clean Water Program Available options presumably include manufactured BMPs, such as drain inlet inserts (DIIs) and continuous deflective separation (CDS) units. Developments may also select such non-

[3] http://www.census.gov/stab/ccdb/cit7140a.txt,
http://www.acwd.org/dms_docs/76d0b026b60d97830492079a48b1cb88.pdf,
http://www.ci.berkely.ca.us/aboutberkeley/weather.html, http://www.usbr.gov/dataweb/dams/ca10168.htm,
http://www.redwoodcity.org/about/weather.html.

proprietary devices as extended-detention basins (EDBs) and biofiltration swales and filter strips. EDBs hold water for two to three days for solids settlement before releasing whatever does not infiltrate or evaporate. Biofiltration treats runoff through various processes mediated by vegetation and soil. In a swale, runoff flows at some depth in a channel, whereas a filter strip is a broad surface over which water sheet flows. Each of these BMP types was applied to each case study, although it is not clear that these BMPs, in actuality, have been implemented consistently within the Bay Area to date.

The principal basis for the analysis of BMP performance was the California Department of Transportation's (CalTrans, 2004) BMP Retrofit Pilot Program, performed in San Diego and Los Angeles Counties. One important result of the program was that BMPs with a natural surface infiltrate and evaporate (probably, mostly infiltrate) a substantial amount of runoff, even if conditions do not appear to be favorable for an infiltration basin. On average, the EDBs, swales, and filter strips lost 40, 50 and 30 percent, respectively, of the entering flow before the discharge point. DIIs and CDS units do not contact runoff with a natural surface, and therefore do not reduce runoff volume.

The CalTrans program further determined that BMP effluent concentrations were usually a function of the influent concentrations, and equations were developed for the functional relationships in these cases. BMPs generally reduced influent concentrations proportionately more when they were high. In relatively few situations influent concentrations were constant at an "irreducible minimum" level regardless of inflow concentrations.

In analyzing the effects of BMPs on the case study runoff, the first step was to reduce the runoff volumes estimated with no BMPs by the fractions observed to be lost in the pilot study. The next task was estimating the effluent concentrations from the relationships in the CalTrans report. The final step was calculating discharge pollutant loadings as the product of the reduced volumes and predicted effluent concentrations. As before, typical pollutant concentrations in the mixed runoff were established by mass balance.

Estimating Infiltration Capacity of the Case Study Sites

Infiltrating sufficient runoff to maintain pre-development hydrologic characteristics and prevent pollutant transport is the most effective way to protect surface receiving waters. Successfully applying infiltration requires soils and hydrogeological conditions that will pass water sufficiently rapidly to avoid overly-lengthy ponding, while not allowing percolating water to reach ground-water before the soil column captures pollutants.

The study assumed that infiltration would occur in surface facilities and not in below-ground trenches. The use of trenches is certainly possible, and was judged to be an approved BMP by CalTrans after the pilot study. However, the intent of this investigation was to determine the ability of pervious areas to manage the site runoff. This was accomplished by determining the infiltration capability of the pervious areas in their original condition for each development case study, and further assessing the pervious areas' infiltration capabilities if soils were modified according to low impact development practices.

The chief basis for this aspect of the work was an assessment of infiltration capacity and benefits for Los Angeles' San Fernando Valley (Chralowicz et al. 2001). The Chralowicz study posited providing 0.1-0.5 acre for infiltration basins to serve each 5 acres of contributing drainage area. At 2-3 ft deep, it was estimated that such basins could infiltrate 0.90-1.87 acre-ft/year of runoff in San Fernando Valley conditions. Soils there are generally various loam textures with infiltration rates of approximately 0.5-2.0 inches/hour. Loams are also common formations in the portion of the Bay Area covered by this report, those areas with Hydrologic

Soil Groups A, B, and C,[4] thus making the conclusions of the San Fernando Valley study applicable for these purposes. This information was used to estimate how much of each case study site's annual runoff would be infiltratable, and if the pervious portion would provide sufficient area for infiltration. For instance, if sufficient area were available, the infiltration configuration would not have to be in basin form but could be shallower and larger in surface area. This study's analyses assumed the use of bioretention areas rather than traditional infiltration basins.

Volume and Pollutant Source Reduction Strategies

As mentioned above, the essence of low impact development is reducing runoff problems before they can develop, at their sources, or exploiting the infiltration and treatment abilities of soils and vegetation. If a site's existing infiltration and treatment capabilities are inadequate to preserve pre-development hydrology and prevent runoff from causing or contributing to violations of water quality standards, then LID-based source reduction strategies can be implemented, infiltration and treatment capabilities can be upgraded, or both.

Source reduction can be accomplished through various LID techniques. Soil can be upgraded to store runoff until it can infiltrate, evaporate, or transpire from plants through compost addition. Soil amendment, as this practice is known, is a standard LID technique.

Upgraded soils are used in bioretention cells that hold runoff and effect its transfer to the subsurface zone. This standard LID tool can be used where sufficient space is available. This study analyzed whether the six development case study sites would have sufficient space to effectively reduce runoff using bioretention cells, assuming the soils and vegetation could be amended and enhanced where necessary.

Conventional pavements can be converted to porous asphalt or concrete or replaced with concrete or plastic unit pavers or grid systems. For such approaches to be most effective, the soils must be capable of infiltrating the runoff passing through, and may require renovation.

Source reduction can be enhanced by the LID practice of water harvesting, in which water from impervious surfaces is captured and stored for reuse in irrigation or gray water systems. For example, runoff from roofs and parking lots can be harvested, with the former being somewhat easier because of the possibility of avoiding pumping to use the water and fewer pollutants. Harvesting is a standard technique for Leadership in Energy and Environmental Design (LEED) buildings.[5] Many successful systems of this type are in operation, such as the Natural Resources Defense Council office (Santa Monica, CA), the King County Administration Building (Seattle, WA), and two buildings on the Portland State University campus (Portland, OR). This investigation examined how water harvesting could contribute to stormwater management for case study sites where infiltration capacity, available space, or both appeared to be limited.

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[4] http://gis.ca.gov/catalog/BrowseCatalog.epl?id=108, http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx

[5] New Buildings Institute, Inc., *Advanced Buildings* (2005) (http://www.poweryourdesign.com/LEEDGuide.pdf).

RESULTS OF THE ANALYSIS

1. *"Base Case" Analysis: Development without Stormwater Controls*

Comparison of Pre- and Post-Development Runoff Volumes

Table 2 presents a comparison between the estimated runoff volumes generated by the respective case study sites in the pre- and post-development conditions, assuming implementation of no stormwater controls on the developed sites. On sites dominated by impervious land cover, most of the infiltration that would recharge groundwater in the undeveloped state is expected to be lost to surface runoff after development. This greatly increased surface flow would raise peak flow rates and volumes in receiving water courses, raise flooding risk, and transport pollutants. Only the office building, the plan for which retained substantial pervious area, would lose less than 40 percent of the site's pre-development recharge.

Table 2. Pre- and Post-Development without BMPs: Distribution of Surface Runoff Versus Recharge to Groundwater (annual volume in acre-ft)

Distribution	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
14 Inches/Year Rainfall:						
Precipitation[b]	12.8	3.54	0.90	2.47	154	0.21
Pre-development runoff[c]	0.89	0.25	0.07	0.17	10	0.02
Pre-development recharge[d]	11.9	3.29	0.83	2.30	144	0.19
Post-development impervious runoff[c]	8.07	1.51	0.42	0.57	66	0.09
Post-development pervious runoff[c]	0.51	0.24	0.06	0.23	10	0.01
Post-development total runoff[c]	8.58	1.75	0.48	0.80	76	0.10
Post-development recharge[d]	4.22	1.79	0.42	1.67	78	0.11
Post-development recharge loss (% of pre-development)	7.68 (65%)	1.50 (46%)	0.41 (49%)	0.65 (27%)	66 (45%)	0.08 (41%)
20 Inches/Year Rainfall:						
Precipitation[b]	18.2	5.06	1.29	3.54	220	0.30
Pre-development runoff[c]	1.28	0.35	0.10	0.24	15	0.03
Pre-development recharge[d]	16.9	4.71	1.19	3.30	205	0.27
Post-development impervious runoff[c]	11.5	2.16	0.60	0.82	94	0.13
Post-development pervious runoff[c]	0.73	0.34	0.08	0.33	15	0.01
Post-development total runoff[c]	12.2	2.50	0.68	1.15	109	0.14
Post-development recharge[d]	6.0	2.56	0.61	2.39	111	0.16
Post-development recharge loss (% of pre-development)	10.9 (65%)	2.15 (46%)	0.58 (49%)	0.91 (27%)	94 (45%)	0.11 (41%)

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; SINGLE—single family home
[b] Volume of precipitation on total project area
[c] Quantity of water discharged from the site on the surface
[d] Quantity of water infiltrating the soil; the difference between precipitation and runoff

Pollutant Concentrations and Loadings

Table 3 presents the pollutant concentrations from the literature and loadings calculated as described for the various land use and cover types represented by the case studies. Landscaped areas are expected to release the highest TSS concentration, although relatively low TSS mass loading because of the low runoff coefficient. The highest copper concentrations and loadings are expected from parking lots. Roofs, especially commercial roofs, top the list for both zinc concentrations and loadings. Landscaping would issue by far the highest phosphorus, although access roads and driveways would contribute the highest mass loadings. With expected concentrations being equal in the two rainfall zones, mass loadings in the 20 inches/year zone would be higher than those in the 14 inches/year zone in the same proportion as the ratio of rainfall quantities.

Table 3. Pollutant Concentrations and Loadings for Case Study Land Use and Cover Types

Land Use	Concentrations				Loadings			
	TSS (mg/L)	TCu (mg/L)	TZn (mg/L)	TP (mg/L)	Lbs. TSS/ acre-year	Lbs. TCu/ acre-year	Lbs. TZn/ acre-year	Lbs. TP/ acre-year
14 Inches/Year Rainfall:								
Residential roof	25	0.013	0.159	0.11	75	0.039	0.477	0.330
Commercial roof	18	0.014	0.281	0.14	54	0.042	0.844	0.420
Access road/driveway	120	0.022	0.118	0.66	360	0.066	0.354	1.981
Parking	75	0.036	0.097	0.14	225	0.108	0.291	0.420
Walkway	25	0.013	0.059	0.11	75	0.039	0.177	0.330
Landscaping	213	0.013	0.059	2.04	81	0.005	0.022	0.774
20 Inches/Year Rainfall:								
Residential roof	25	0.013	0.159	0.11	107	0.056	0.683	0.472
Commercial roof	18	0.014	0.281	0.14	77	0.060	1.207	0.601
Access road/driveway	120	0.022	0.118	0.66	515	0.094	0.507	2.834
Parking	75	0.036	0.097	0.14	322	0.155	0.417	0.601
Walkway	25	0.013	0.059	0.11	107	0.056	0.253	0.472
Landscaping	213	0.013	0.059	2.04	135	0.008	0.037	1.291

The Basin Plan freshwater acute criteria for copper and zinc are 0.013 mg/L and 0.120 mg/L, respectively (http://www.swrcb.ca.gov/rwqcb2/basinplan/web/BP_CH3.html). All developed land uses are expected to discharge copper at or above the criterion, based on the mass balance calculations using concentrations from Table 3. Any surface release from the case study sites would just meet or violate the criterion at the point of discharge, although dilution by the receiving water would lower the concentration below the criterion at some point. Even if copper mass loadings are reduced by BMPs, any surface discharge would equal or exceed the criterion initially, but it would be easier to dilute below that level. In contrast, runoff from land covers other than roofs would not violate the acute zinc criterion. Because of this difference, the evaluation considered whether or not the zinc criterion would be exceeded in each analysis, whereas there was no point in this analysis for copper. There are no equivalent water quality criteria for TSS and TP; hence, their concentrations were not further analyzed in the different scenarios.

Table 4 shows the overall loadings, as well as zinc concentrations, expected to be delivered from the case study developments should they not be fitted with any BMPs. As Table 4 shows, all cases are forecast to exceed the 0.120 mg/L acute zinc criterion. Because of its size, the large residential development dominates the mass loading emissions.

Table 4. Case Study Pollutant Concentration and Loading Estimates without BMPs

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
14 Inches/ Year Rainfall:						
TZn (mg/L)	0.127	0.123	0.128	0.133	0.123	0.121
Lbs. TSS/year	1254	328	119	230	14249	20
Lbs. TCu/year	0:44	0.070	0.030	0.043	3.04	0.004
Lbs. TZn/year	2.94	0.576	0.165	0.286	25.04	0.034
Lbs. TP/year	6.24	2.27	0.68	1.69	98.55	0:14
20 Inches/ Year Rainfall:						
TZn (mg/L)	0.127	0.123	0.128	0.133	0.123	0.121
Lbs. TSS/year	1864	501	180	360	21781	30
Lbs. TCu/year	0.63	0.102	0.043	0.063	4.44	0.006
Lbs. TZn/year	4.22	0.833	0.238	0.417	36.2	0.050
Lbs. TP/year	9.60	3.55	1.05	2.71	154	0.22

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; SINGLE—single-family home

2. "Conventional BMP" Analysis: Effect of Basic Treatment BMPs

Effect of Basic Treatment BMPs on Post-Development Runoff Volumes

The current set of regional permits allows regulated parties to select from a range of BMPs in order to treat or infiltrate a given quantity of annual rainfall. The administrative draft of the proposed MRP is also non-specific regarding the role of LID in satisfying permit conditions. The range of BMPs includes drain inlet inserts, CDS units, and other manufactured BMPs, detention vaults, and sand filters, all of which isolate runoff from the soil; as well as basins and biofiltration BMPs built in soil and generally having vegetation. Treatment BMPs that do not permit any runoff contact with soils discharge as much stormwater runoff as equivalent sites with no BMPs, and hence yield zero savings in recharge. As mentioned above, the CalTrans (2004) study found that BMPs with a natural surface can reduce runoff by substantial margins (30-50 percent for extended-detention basins and biofiltration).

With such a wide range of BMPs in use, runoff reduction ranging from 0 to 50 percent, and a lack of clearly ascertainable requirements, it is not possible to make a single estimate of how much recharge savings are afforded by maximal implementation of the current permits or the Municipal Regional Permit (MRP), if issued as now proposed. We made the following assumptions regarding implementation of BMPs. Assuming natural-surface BMPs perform at the average of the three types tested by CalTrans (2004), i.e., 40 percent runoff reduction, the estimate can be bounded as shown in Table 5. The table demonstrates that allowing free choice of BMPs without regard to their ability to direct water into the ground forfeits substantial groundwater recharge benefits when hardened-surface BMPs are selected. Use of soil-based conventional BMPs could cut recharge losses from half or more of the full potential to about one-quarter to one-third or less, except with the highly impervious commercial development. This analysis shows the wisdom of draining impervious to pervious surfaces, even if those surfaces are not prepared in any special way. But as subsequent analyses showed, soil amendment can gain considerably greater benefits.

Table 5. Pre- and Post-Development with Conventional BMPs: Distribution of Surface Runoff Versus Recharge to Groundwater (annual volume in acre-ft)

Distribution	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
14 Inches/Year Rainfall:						
Precipitation[b]	12.8	3.54	0.90	2.47	154	0.21
Pre-development runoff[c]	0.89	0.25	0.07	0.17	10	0.02
Pre-development recharge[d]	11.9	3.29	0.83	2.30	144	0.19
Post-development impervious runoff[e]	4.84-8.07	0.90-1.51	0.25-0.42	0.34-0.57	39-66	0.05-0.09
Post-development pervious runoff[e]	0.30-0.51	0.14-0.24	0.04-0.06	0.13-0.23	6.3-10	0.006-0.01
Post-development total runoff[e]	5.15-8.58	1.05-1.75	0.29-0.48	0.48-0.80	46-76	0.06-0.10
Post-development recharge[d, e]	4.22-7.60	1.79-2.49	0.42-0.62	1.67-2.00	78-108	0.11-0.15
Post-development recharge loss (% of pre-development)[e]	4.29-7.68 (36-65%)	0.80-1.50 (24-46%)	0.80-0.41 (26-49%)	0.30-0.65 (13-27%)	34-66 (24-45%)	0.05-0.08 (24-41%)
20 Inches/Year Rainfall:						
Precipitation[b]	18.2	5.06	1.29	3.54	220	0.30
Pre-development runoff[c]	1.28	0.35	0.10	0.24	15	0.03
Pre-development recharge[d]	16.9	4.71	1.19	3.30	205	0.27
Post-development impervious runoff[e]	6.92-11.5	1.29-2.16	0.35-0.60	0.49-0.82	56-94	0.08-0.13
Post-development pervious runoff[e]	0.44-0.73	0.20-0.34	0.05-0.08	0.19-0.33	9.0-15	0.006-0.01
Post-development total runoff[e]	7.36-12.2	1.50-2.50	0.41-0.68	0.68-1.15	65-109	0.08-0.14
Post-development recharge[d, e]	6.0-10.8	2.56-3.56	0.61-0.88	2.39-2.86	111-155	0.16-0.22
Post-development recharge loss (% of pre-development)[e]	6.1-10.9 (36-65%)	1.14-2.15 (24-46%)	0.31-0.58 (26-49%)	0.44-0.91 (13-27%)	49-94 (24-45%)	0.07-0.11 (24-41%)

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; SINGLE—single-family home. Ranges represent 40 percent runoff volume reduction, with full site coverage by BMPs having a natural surface, to no reduction, with BMPs isolating runoff from soil.
[b] Volume of precipitation on total project area
[c] Quantity of water discharged from the site on the surface
[d] Quantity of water infiltrating the soil; the difference between precipitation and runoff [e] Ranging from the quantity with hardened bed BMPs to the quantity with soil-based BMPs

Effect of Basic Treatment BMPs on Pollutant Discharges

Table 6 presents estimates of zinc effluent concentrations and mass loadings of the various pollutants discharged from four types of conventional treatment BMPs. The loading reduction results show the CDS units always performing below 50 percent reduction for all pollutants analyzed, and most often in the vicinity of 20 percent, with zero copper reduction.

Table 6. Pollutant Concentration and Mass Loading Reduction Estimates with Conventional BMPs

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
Effluent Concentrations:						
CDS TZn (mg/L)[a]	0.095	0.095	0.098	0.102	0.095	0.094
EDB TZn (mg/L)[a]	0.085	0.086	0.084	0.084	0.086	0.084
Swale TZn (mg/L)	0.055	0.054	0.055	0.056	0.054	0.053
Filter strip TZn (mg/L)	0.039	0.039	0.039	0.041	0.039	0.038
Mass Loading Reductions—14 Inches/Year Rainfall:						
CDS TSS reduction	15.7%	19.9%	22.0%	24.0%	19.9%	20.2%
CDS TCu reduction	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
CDS TZn reduction	22.7%	22.4%	22.9%	23.1%	22.4%	22.5%
CDS TP reduction	30.6%	41.5%	40.7%	45.9%	41.5%	42.0%
EDB TSS reduction	68.1%	73.7%	79.0%	81.1%	73.7%	74.3%
EDB TCu reduction	61.9%	55.7%	66.2%	63.0%	55.7%	55.8%
EDB TZn reduction	59.7%	59.6%	60.4%	61.9%	59.6%	59.8%
EDB TP reduction	61.9%	69.7%	69.1%	72.9%	69.7%	70.1%
Swale TSS reduction	68.8%	71.1%	73.1%	73.9%	71.1%	71.3%
Swale TCu reduction	72.5%	68.5%	78.2%	73.3%	68.5%	68.5%
Swale TZn reduction	78.4%	78.1%	84.3%	78.8%	78.1%	78.2%
Swale TP reduction	66.3%	70.7%	67.2%	76.2%	70.7%	71.1%
Filter strip TSS reduction	69.9%	75.4%	80.6%	82.6%	75.4%	76.0%
Filter strip TCu reduction	74.4%	69.1%	78.2%	75.4%	69.1%	69.1%
Filter strip TZn reduction	78.3%	77.9%	78.4%	78.7%	77.9%	78.1%
Filter strip TP reduction	48.4%	53.1%	63.7%	59.8%	53.1%	53.5%

Table 6 continued

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
Mass Loading Reductions—20 Inches/Year Rainfall:						
CDS TSS reduction	18.8%	25.0%	26.3%	30.5%	25.0%	25.4%
CDS TCu reduction	0.7%	1.9%	1.1%	3.0%	1.9%	2.0%
CDS TZn reduction	23.1%	23.3%	23.6%	24.7%	23.3%	23.4%
CDS TP reduction	35.4%	46.6%	44.8%	51.8%	46.6%	47.1%
EDB TSS reduction	68.8%	74.6%	79.6%	81.6%	74.6%	75.1%
EDB TCu reduction	61.8%	55.6%	66.0%	62.7%	55.6%	55.7%
EDB TZn reduction	59.6%	59.3%	60.2%	61.5%	59.3%	59.6%
EDB TP reduction	63.0%	70.4%	69.7%	73.4%	70.4%	70.7%
Swale TSS reduction	69.1%	71.4%	73.6%	74.1%	71.4%	71.6%
Swale TCu reduction	72.5%	68.4%	77.9%	73.1%	68.4%	68.5%
Swale TZn reduction	78.3%	78.0%	84.1%	78.6%	78.0%	78.1%
Swale TP reduction	67.6%	71.9%	68.2%	77.1%	71.9%	72.3%
Filter strip TSS reduction	70.6%	76.3%	81.2%	83.1%	76.3%	76.8%
Filter strip TCu reduction	74.4%	69.0%	78.0%	75.1%	69.0%	69.1%
Filter strip TZn reduction	78.2%	77.8%	78.3%	78.5%	77.8%	77.9%
Filter strip TP reduction	49.9%	54.6%	66.3%	61.0%	54.6%	55.0%

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; SINGLE—single family home; CDS— continuous deflective separation unit; EDB—extended-detention basin

When treated with extended-detention basins, swales, or filter strips, effluents from each development case study site are expected to fall below the Basin Plan acute zinc criterion. These natural-surface BMPs, if fully implemented and well maintained, are predicted to prevent the pollutant masses generated on the six case study development sites from reaching a receiving water in both rainfall zones, which do not differ appreciably. Only total phosphorus reduction falls below 50 percent for three case studies. Otherwise, mass loading reductions range from about 60 to above 80 percent for the EDB, swale, and filter strip. These data indicate that draining impervious to pervious surfaces, even if those surfaces are not prepared in any special way, pays water quality as well as hydrologic dividends.

3. LID Analysis

(a) Hydrologic Analysis

The LID analysis repeats the analysis above, focusing here on the performance of LID techniques in reducing or eliminating runoff from the six development case studies. In addition to assessing the total runoff that would be expected, the analysis also considered whether LID techniques would be sufficient to attain compliance with a performance standard being considered by the Los Angeles Regional Water Quality Control Board for Ventura County, California. This standard limits EIA (Effective Impervious Area) to five percent (but our analysis further assumed EIA would be ultimately reduced to three percent). All runoff from NCIA (Not-Connected Impervious Area) was assumed to drain to vegetated surfaces.

One goal of this exercise was to identify methods that reduce runoff production in the first place. It was hypothesized that implementation of source reduction techniques could allow all of the case study sites to infiltrate substantial proportions, or all, of the developed site runoff, advancing the hydromodification mitigation objective of the Draft Permit. When runoff is dispersed into the soil instead of being rapidly collected and conveyed away, it recharges groundwater, supplementing a resource that maintains dry season stream flow and wetlands. An increased water balance can be tapped by humans for potable, irrigation, and process water supply. Additionally, runoff volume reduction would commensurately decrease pollutant mass loadings.

Accordingly, the analysis considered the practicability of more than one scenario. In one option, all roof runoff is harvested and stored for some beneficial use. A second option disperses runoff into the soil via roof downspout infiltration trenches. The former option is probably best suited to cases like large commercial and office buildings, while distribution in the soil would fit best with residences and relatively small commercial developments. The analysis was repeated with the assumptions of harvesting OFF roof runoff for some beneficial use and dispersing roof runoff from the remaining four cases in roof downspout infiltration systems.

Expected Infiltration Capacities of the Case Study Sites

The first inquiry on this subject sought to determine how much of the total annual runoff each property is expected to infiltrate, since infiltration is a basic (although not exclusive) LID technique. Based on the findings of Chralowicz et al. (2001), it was assumed that an infiltration zone of 0.1-0.5 acres in area and 2-3 ft deep would serve a drainage catchment area in the size range 0-5 acres and infiltrate 0.9-1.9 acre-ft/year. The conclusions of Chralowicz et al. (2001) were extrapolated to conservatively assume that 0.5 acre would be required to serve each additional five acres of catchment, and would infiltrate an incremental 1.4 acre-ft/year (the midpoint of the 0.9-1.9 acre-ft/year range). According to these assumptions, the following schedule of estimates applies:

Pervious Area Available for Infiltration	Catchment Served acres	Infiltration Capacity
0.5 acres	0-5 acres	1.4 acre-ft/year
1.0 acres	5-10 acres	2.8 acre-ft/year
1.5 acres	10-15 acres	4.2 acre-ft/year
(Etc.)	...	...

As a formula, infiltration capacity ≈ 2.8 x available pervious area. To apply the formula conservatively, the available area was reduced to the next lower 0.5-acre increment before multiplying by 2.8.

As shown in Table 7, in both rainfall zones all six of the sites have adequate or greater capacity to infiltrate the full annual runoff volume expected from NCIA and pervious areas where EIA is limited to three percent of the total site area. Indeed, five of the six development types have sufficient pervious area to infiltrate *all* runoff, including runoff from EIA areas. These results are based on infiltrating in the native soils with no soil amendment. For any development project at which infiltration-oriented BMPs are considered, it is important that infiltration potential be carefully assessed using site-specific soils and hydrogeologic data. In the event such an investigation reveals a marginal condition (e.g., hydraulic conductivity, spacing to groundwater) for infiltration basins, soils could be enhanced to produce bioretention zones to assist infiltration. Notably, the five case studies with far greater than necessary infiltration capacity would offer substantial flexibility in designing infiltration, allowing ponding at less than 2-3 ft depth.

Table 7. Infiltration and Runoff Volume (With 3 Percent EIA and All NCIA Draining to Pervious Areas)

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
14 Inches/Year Rainfall:						
EIA runoff (acre-ft/year)	0.36	0.10	0.03	0.07	4.4	0.01
NCIA + pervious area runoff (acre-ft/year)	**8.20**	**1.64**	**0.45**	**0.73**	**71.3**	**0.08**
Total runoff (acre-ft/year)	8.56	1.74	0.48	0.80	75.7	0.09
Pervious area available for infiltration (acres)	3.66	1.67	0.39	1.61	72.7	0.10
Estimated infiltration capacity (acre-ft/year)[b]	**9.8**	**4.2**	**1.4**	**4.2**	**203**	**0.28**
Infiltration potential[c]	>100%	>100%	>100%	>100%	>100%	>100%
20 Inches/Year Rainfall:						
EIA runoff (acre-ft/year)	0.52	0.14	0.04	0.10	6.2	0.01
NCIA + pervious area runoff (acre-ft/year)	**11.7**	**2.34**	**0.64**	**1.04**	**101.7**	**0.14**
Total runoff (acre-ft/year)	12.2	2.48	0.68	1.14	108.0	0.15
Pervious area available for infiltration (acres)	3.66	1.67	0.39	1.61	72.7	0.10
Estimated infiltration capacity (acre-ft/year)[b]	**9.8**	**4.2**	**1.4**	**4.2**	**203**	**0.28**
Infiltration potential[c]	84%	>100%	>100%	>100%	>100%	>100%

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; SINGLE—single family home;
[b] Based on Chralowicz et al. (2001) according to the schedule described above
[c] Compare runoff production from NCIA + pervious area (**row 3**) with estimated infiltration capacity (**row 6**)

As Table 7 shows, each of the six case study sites have the capacity to infiltrate *all* or substantially all of the runoff produced onsite annually by draining impervious surfaces to pervious areas on native soils or, in some soil regimes, soils amended with organic matter. If these sites were designed as envisioned in this analysis, no runoff discharge is expected in storms as large as, and probably larger than, the design storm event—using infiltration only. Discharge would be anticipated only with exceptionally intense, large, or prolonged rainfall that saturates the ground at a faster rate than water can infiltrate or evaporate. Even runoff from the area assumed to be EIA could be infiltrated in most cases based on the amount of pervious area available in typical development projects. Therefore, this analysis shows that the EIA performance standard being considered for Ventura County, California, or one more stringent, can be met readily in development projects occurring on A, B, and C soils in the San Francisco Bay Area.

Additional Source Reduction Capabilities of the Case Study Sites: Water Harvesting Example

As noted, infiltration is one of a wide variety of LID-based source reduction techniques. Where site conditions such as soil quality or available area limit a site's infiltration capacity, other source LID measures can enhance a site's runoff retention capability. For example, soil amendment, which improves infiltration, is a standard LID technique. Water harvesting is another. Such practices can also be used where infiltration capacity is adequate, but the developer desires greater flexibility for land use on-site. Table 8 shows the added LID implementation flexibility created by subtracting roof runoff by harvesting it or efficiently directing it into the soil through downspout dispersion systems, further demonstrating the feasibility and robust performance of LID options for reducing or eliminating runoff in most expected conditions. Specifically, all development types studied could readily infiltrate and/or retain all expected annual precipitation.

Table 8. Infiltration and Runoff Volume Reduction Analysis Including Roof Runoff Harvesting or Disposal in Infiltration Trenches (Assuming 3 Percent EIA and All NCIA Draining to Pervious Areas)

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
14 Inches/Year Rainfall:						
EIA runoff (acre-ft/year)	0.36	0.10	0.03	0.07	4.4	0.01
Roof runoff (acre-ft/year)	4.68	0.89	0.08	0.19	38.5	0.05
Other NCIA + pervious area runoff (acre-ft/year)	**3.52**	**0.75**	**0.37**	**0.54**	**32.7**	**0.04**
Total runoff (acre-ft/year)	8.56	1.74	0.48	0.80	75.6	0.10
Pervious area available for infiltration (acres)	3.66	1.67	0.39	1.61	72.7	0.10
Estimated infiltration capacity (acre-ft/year)[b]	**9.8**	**4.2**	**1.4**	**4.2**	**203**	**0.28**
Infiltration capacity[c]	>100%	>100%	>100%	>100%	>100%	>100%
20 Inches/Year Rainfall:						
EIA runoff (acre-ft/year)	0.52	0.14	0.04	0.10	6.2	0.01
Roof runoff (acre-ft/year)	6.67	1.27	0.12	0.28	55.1	0.08
Other NCIA + pervious area runoff (acre-ft/year)	**5.03**	**1.07**	**0.52**	**0.76**	**46.7**	**0.06**
Total runoff (acre-ft/year)	12.2	2.48	0.68	1.14	108.0	0.15
Pervious area available for infiltration (acres)	3.66	1.67	0.39	1.61	72.7	0.10

Table 8 continued

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
Estimated infiltration capacity (acre-ft/year)[b]	**9.8**	**4.2**	**1.4**	**4.2**	**203**	**0.28**
Infiltration capacity[c]	>100%	>100%	>100%	>100%	>100%	>100%

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; SINGLE—single family home;
[b] Based on Chralowicz et al. (2001) according to the schedule described above
[c] Comparison of runoff production from NCIA + pervious area **(row 3)** with estimated infiltration capacity **(row 6)**

Effect of Full LID Approach on Recharge

Table 9 shows the recharge benefits of preventing roofs from generating runoff and infiltrating as much as possible of the runoff from the remainder of the case study sites. The data show that LID methods offer significant benefits relative to the baseline (no stormwater controls) in all cases. These benefits are particularly impressive in developments with relatively high site imperviousness, such as in the MFR case.

Table 9. Comparison of Water Captured Annually (in acre-ft) from Development Sites for Beneficial Use with a Full LID Approach Compared to Development With No BMPs

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
14 Inches/Year Rainfall:						
Pre-development recharge[b] (acre-ft)	11.9	3.29	0.83	2.30	144	0.19
No BMPs—						
Post-development recharge[b] (acre-ft)	4.22	1.79	0.42	1.67	78	0.11
Post-development recharge lost (acre-ft)	7.68	1.50	0.41	0.65	66	0.08
Post-development % recharge lost	65%	46%	49%	27%	45%	41%
Full LID approach—						
Post-development runoff capture (acre-ft)[c]	11.9	3.29	0.83	2.30	144	0.19
Post-development recharge lost (acre-ft)	0	0	0	0	0	0
Post-development % recharge lost	0%	0%	0%	0%	0%	0%

Table 9 continued

	MFR[a]	Sm-SFR[a]	REST[a]	OFF[a]	Lg-SFR[a]	SINGLE[a]
20 Inches/Year Rainfall:						
Pre-development recharge[b] (acre-ft)	16.9	4.71	1.19	3.30	205	0.27
No BMPs—						
Post-development recharge[b] (acre-ft)	6.0	2.56	0.61	2.39	111	0.16
Post-development recharge lost (acre-ft)	10.9	2.15	0.58	0.91	94	0.11
Post-development % recharge lost	65%	46%	49%	27%	45%	41%
Full LID approach—						
Post-development runoff capture (acre-ft)[c]	16.9	4.71	1.19	3.30	205	0.27
Post-development recharge lost (acre-ft)	0	0	0	0	0	0
Post-development % recharge lost	0%	0%	0%	0%	0%	0%

[a] MFR—multi-family residential; Sm-SFR—small-scale single-family residential; REST—restaurant; OFF—office building; Lg-SFR—large-scale single-family residential; SINGLE—Single family home
[b] Quantity of water infiltrating the soil; the difference between precipitation and runoff
[c] Water either entirely infiltrated in BMPs and recharged to groundwater or partially harvested from roofs and partially infiltrated in BMPs. EIA was not distinguished from the remainder of the development, because these sites have the potential to capture all runoff.

(b) Water Quality Analysis

It was assumed that any site discharges would be subject to treatment control. For purposes of the analysis, treatment control was assumed to be provided by conventional sand filtration. This choice is appropriate for study purposes for two reasons. First, sand filters can be installed below grade, and land above can be put to other uses. Pervious area should be reserved for receiving NCIA drainage, and using sand filters would not draw land away from that service or other site uses. A second reason for the choice is that sand filter performance data equivalent to the data used in analyzing other conventional BMPs are available from the CalTrans (2004) work. Sand filters may or may not expose water to soil, depending on whether or not they have a hard bed. This analysis assumed a hard bed, meaning that no infiltration would occur and thus there would be no additional recharge in sand filters. Performance would be even better than shown in the analytical results if sand filters were built in earth.

Pollutant Discharge Reduction Through LID Techniques

The preceding analyses demonstrated that in each of the six case studies, *all* stormwater discharges could be eliminated at least under most meteorological conditions by dispersing runoff from impervious surfaces to pervious areas. Therefore, pollutant additions to receiving waters would also be eliminated.

SUMMARY AND CONCLUSIONS

This paper demonstrated that common Bay Area residential and commercial development types subject to the Municipal NPDES Permit are likely, without stormwater management, to reduce groundwater recharge from the pre-development state by approximately half in most cases to a much higher fraction with a large ratio of impervious to pervious area. With no treatment, runoff from these developments is expected to exceed Basin Plan acute copper and zinc criteria at the point of discharge and to deliver large pollutant mass loadings to receiving waters.

Conventional soil-based BMP solutions that promote and are component parts of low impact development approaches, by contrast, regain about 30-50 percent of the recharge lost in development without stormwater management in Bay Area locations having NRCS Hydrologic Soil Groups A, B, and C. It is expected the soil-based BMPs generally would release effluent that meets the acute zinc criterion at the point of discharge, although it would still exceed or just barely meet the copper limit. Excepting phosphorus, it was found that these BMPs would capture and prevent the movement to receiving waters of the majority of the pollutant loadings considered in the analysis.

It was found that by draining all site runoff to pervious areas with A, B, or C soil types, runoff can be eliminated entirely in most development categories. It follows that a three percent Effective Impervious Area standard can be met in typical developments, as well. This result was reached assuming the use of native soils or well recognized soil enhancement techniques (typically, with compost). Draining impervious surfaces onto these soils, in connection with limiting directly connected impervious area to three percent of the site total area, should eliminate storm runoff from some development types and greatly reduce it from more highly impervious types. Adding roof runoff elimination to the LID approach (by harvesting or directing it to downspout infiltration trenches) provides an additional tool, increasing flexibility and confidence that no discharge in most meteorological conditions is a feasible performance expectation. Even in the development scenarios involving the highest relative proportion of impervious surface, losses of rainfall capture for beneficial uses could be reduced from the untreated scenario when draining to pervious areas was supplemented with water harvesting. These results demonstrate the basic soundness of the concept of using LID techniques to reduce stormwater pollution in the Bay Area, and further show that limiting directly connected impervious area and draining the remainder over pervious surfaces, as contemplated by some Regional Water Boards in California, is also feasible.

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ATTACHMENT A

POLLUTANT CONCENTRATIONS FOR URBAN SOURCE AREAS (HERRERA ENVIRONMENTAL CONSULTANTS, INC. UNDATED)

Source Area	Study	Location	Sample Size (n)	TSS (mg/L)	TCu (ug/L)	TPb (ug/L)	TZn (ug/L)	TP (mg/L)	Notes
Roofs									
Residential	Steuer, et al. 1997	MI	12	36	7	25	201	0.06	2
Residential	Bannerman, et al. 1993	WI	~48	27	15	21	149	0.15	3
Residential	Waschbusch, et al. 2000	WI	25	15	n.a.	n.a.	n.a.	0.07	3
Residential	FAR 2003	NY		*19*	*20*	*21*	*312*	*0.11*	4
Residential	Gromaire, et al. 2001	France		*29*	*37*	*493*	*3422*	*n.a.*	5
Representative Residential Roof Values				**25**	**13**	**22**	**159**	**0.11**	
Commercial	Steuer, et al. 1997	MI	12	24	20	48	215	0.09	2
Commercial	Bannerman, et al. 1993	WI	~16	15	9	9	330	0.20	3
Commercial	Waschbusch, et al. 2000	WI	25	18	n.a.	n.a.	n.a.	0.13	3
Representative Commercial Roof Values				**18**	**14**	**26**	**281**	**0.14**	
Parking Areas									
Res. Driveways	Steuer, et al. 1997	MI	12	157	34	52	148	0.35	2
Res. Driveways	Bannerman, et al. 1993	WI	~32	173	17	17	107	1.16	3
Res. Driveways	Waschbusch, et al. 2000	WI	25	34	n.a.	n.a.	n.a.	0.18	3
Driveway	FAR 2003	NY		*173*	*17*		*107*	*0.56*	4
Representative Residential Driveway Values				**120**	**22**	**27**	**118**	**0.66**	
Comm./ Inst. Park. Areas	Pitt, et al. 1995	AL	16	110	116	46	110	n.a.	1
Comm. Park. Areas	Steuer, et al. 1997	MI	12	110	22	40	178	0.2	2
Com. Park. Lot	Bannerman, et al. 1993	WI	5	58	15	22	178	0.19	3
Parking Lot	Waschbusch, et al. 2000	WI	25	51	n.a.	n.a.	n.a.	0.1	3
Parking Lot	Tiefenthaler, et al. 2001	CA	5	36	28	45	293	n.a.	6
Loading Docks	Pitt, et al. 1995	AL	3	40	22	55	55	n.a.	1
Highway Rest Areas	CalTrans 2003	CA	53	*63*	*16*	*8*	*142*	*0.47*	7
Park and Ride Facilities	CalTrans 2003	CA	179	*69*	*17*	*10*	*154*	*0.33*	7
Comm./ Res. Parking	FAR 2003	NY		*27*	*51*	*28*	*139*	*0.15*	4
Representative Parking Area/Lot Values				**75**	**36**	**26**	**97**	**0.14**	

Landscaping/Lawns										
Landscaped Areas	Pitt, et al. 1995	AL	6	33	81	24	230	n.a.	1
Landscaping	FAR 2003	NY		*37*	*94*	*29*	*263*	n.a.	4
Representative Landscaping Values				**33**	**81**	**24**	**230**	**n.a.**	
Lawns - Residential	Steuer, et al. 1997	MI	12	262	n.a.	n.a.	n.a.	2.33	2
Lawns - Residential	Bannerman, et al. 1993	WI	~30	397	13	n.a.	59	2.67	3
Lawns	Waschbusch, et al. 2000	WI	25	59	n.a.	n.a.	n.a.	0.79	3
Lawns	Waschbusch, et al. 2000	WI	25	122	n.a.	n.a.	n.a.	1.61	3
Lawns - Fertilized	USGS 2002	WI	58	n.a.	n.a.	n.a.	n.a.	2.57	3
Lawns - Non-P Fertilized	USGS 2002	WI	38	n.a.	n.a.	n.a.	n.a.	1.89	3
Lawns - Unfertilized	USGS 2002	WI	19	n.a.	n.a.	n.a.	n.a.	1.73	3
Lawns	FAR 2003	NY	3	*602*	*17*	*17*	*50*	*2.1*	4
Representative Lawn Values				**213**	**13**	**n.a.**	**59**	**2.04**	

Notes:

Representative values are weighted means of collected data. Italicized values were omitted from these calculations.

1 - Grab samples from residential, commercial/institutional, and industrial rooftops. Values represent mean of DETECTED concentrations

2 - Flow-weighted composite samples, geometric mean concentrations

3 - Geometric mean concentrations

4 - Citation appears to be erroneous - original source of data is unknown. Not used to calculate representative value

5 - Median concentrations. Not used to calculate representative values due to site location and variation from other values.

6 - Mean concentrations from simulated rainfall study

7 - Mean concentrations. Not used to calculate representative values due to transportation nature of land use.

SUPPLEMENTARY INVESTIGATION OF THE FEASIBILITY AND BENEFITS OF LOW-IMPACT SITE DESIGN PRACTICES ("LID") FOR THE SAN FRANCISCO BAY AREA

Richard R. Horner[†]

ABSTRACT

The Clean Water Act NPDES permit that regulates municipal separate storm sewer systems (MS4s) in the San Francisco Bay Area, California will be reissued in 2007. The draft permit includes general provisions related to low impact development practices (LID) for certain kinds of development and redevelopment projects. Using eight representative development project case studies, based on California building records, the author investigated the practicability and relative benefits of LID options for the portion of the region having soils potentially limiting to infiltration. The principal LID option applicable in this situation is roof runoff harvesting, supplement by dispersion of the roof water in single-home sites. Other site runoff would be treated by conventional stormwater best management practices (BMPs), as specified in the permit. The results showed that effectively managing roof runoff and treating the remainder with conventional BMPS can: (1) reduce annual runoff volumes by almost half to more than 3/4, depending on land use characteristics, with much of the water saved available for a beneficial use; and (2) decrease mass loadings of pollutants to receiving waters by 63 to over 90 percent, depending on pollutant and land use.

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INTRODUCTION

Background

A report titled Initial Investigation of the Feasibility and Benefits of Low-Impact Development Practices ("LID") for the San Francisco Bay Area used six representative development project case studies, based on California building records, to investigate the practicability and relative benefits of LID options for the majority of the region having soils potentially suitable for infiltration either in their natural state or after amendment using well recognized LID techniques. The results demonstrated that: (1) LID site design and source control techniques are more effective than conventional best management practices (BMPs) in reducing runoff rates; and (2) in each of the case studies, LID methods would reduce site runoff volume and pollutant loading to zero in typical rainfall scenarios.

For a broad regional assessment of relatively large scale use of soil-based, infiltrative LID practices, the initial report covered areas having soils in Natural Resources Conservation Service (NRCS) Hydrologic Soil Groups A, B, or C as classified by the Natural Resources Conservation Service (http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx). Depending on site-specific conditions, A and B soils would generally effectively infiltrate water without modification, whereas C soils could require organic amendments according to now standard LID methods. This supplementary report covers locations with group D soils, which are generally not amenable to infiltration, again depending on the specific conditions on-site. A minority but still substantial fraction of the Bay Area has group D soils (39.3, 68.0, 18.3, and 50.1 percent of the mapped areas of Alameda, Contra Costa, San Mateo, and Santa Clara Counties, respectively). Regarding any mapped soil type, it is important to keep in mind that soils vary considerably within small distances. Characteristics at specific locations can deviate greatly from those of the major mapped unit, making infiltration potential either more or less than may be expected from the mapping. The soil survey data are regarded as appropriate for use in broad-scale assessments such as underlie this and the initial report, but once site-specific implementation begins, it is important to verify site conditions.

General Assessment Methods

The assessment for group D soils reported herein emphasizes the use of LID practices appropriate in areas with relatively restrictive soils to the greatest possible extent, supplemented by conventional stormwater management practices implemented at fully practicable, high levels of effectiveness. The assessment was performed in a manner analogous to the analysis for the other soil groups and as described in the initial report. To recap briefly, with respect to each of several development case studies, three assessments were undertaken: a baseline scenario incorporating no stormwater management controls; a second scenario employing conventional BMPs; and a third development scenario employing LID stormwater management strategies. In each assessment, annual stormwater runoff volumes were estimated, as well as concentrations and mass loadings (the products of concentrations times flow volumes) of four pollutants: (1) total suspended solids (TSS), (2) total recoverable copper (TCu), (3) total recoverable zinc (TZn), and (4) total phosphorus (TP). The results of the second and third assessments were expressed in terms of the extent to which the management practices would reduce pollutant concentrations and loadings and runoff volumes, converting stormwater discharge a potential beneficial use (direct consumption or, in the case of group A, B, C soil areas, groundwater recharge).

Six case studies were selected to represent a range of urban development types considered to be representative of the Bay Area. These case studies involved: a multi-family residential complex (MFR), a relatively small-scale (23 homes) single-family residential development (Sm-SFR), a restaurant (REST), an office building (OFF), a relatively large (1000 homes) single-family residential development (Lg-SFR), and a single home (SINGLE). The land cover types for these various land uses were derived from building permit and other public records from the Bay Area or elsewhere in California.

Adaptation of Methods for Areas with Group D Soils

A key LID technique in a setting with soils relatively restrictive to infiltration is water harvesting, which can be applied at larger scales in commercial and light industrial developments and at smaller residential scales using cisterns or rain barrels. Harvesting has been successful in reducing runoff discharged to the storm drain system and conserving water in applications at all scales. For example, in downtown Seattle the King County Government Center collects enough roof runoff to supply over 60 percent of the toilet flushing and plant irrigation water requirements, saving approximately 1.4 million gallons of potable water per year (http://www.psat.wa.gov/Publications/LID_studies/rooftop_rainwater.htm, http://dnr.metrokc.gov/dnrp/ksc_tour/features/features.htm). A much smaller public building in Seattle, the Carkeek Environmental Learning Center, drains roof runoff into a 3500-gallon cistern to supply toilets (http://www.harvesth2o.com/seattle.shtml). Collecting drainage from individual dwellings for household use is a standard technique around the world, particularly in areas deficient in rainfall and without affordable alternative sources.

An additional general category of LID practices for poorly infiltrating locations, applicable especially at single homes and other relatively small-scale developments, is runoff dispersion for storage in vegetation and soil until evapotranspiration and some infiltration occurs. Section C.3.c of the California Regional Water Quality Control Board San Francisco Bay Region "Administrative Draft" NPDES Municipal Regional Stormwater Permit ("the Permit") requires all single-family home projects that create and/or replace 5,000 square feet or more of impervious surface to implement one or more stormwater lot-scale BMPs from a selection of: (1) diverting roof runoff to vegetated areas; (2) directing paved surface runoff flow to vegetated areas; and/or (3) installing driveways, patios, and walkways with pervious material such as pervious concrete or pavers. Another way of distributing and dissipating roof runoff used successfully in varied soils in the state of Washington is the downspout dispersion system, consisting of a splash block or gravel-filled trench serving to spread roof runoff over a vegetated area (Washington Department of Ecology 2005 [Volume III, Section 3.1.2]).

The basis of the group D soils assessment was harvesting roof runoff to the maximum possible degree, supplemented in smaller-scale developments by runoff dispersion methods. The report asserts that, through these LID BMPs, it is practicable to prevent the entrance of any roof runoff into the municipal storm drain system in any soils setting in the Bay Area. In group D soils, infiltration likely cannot be relied upon to reduce runoff from other portions of developments, such as walkways, driveways, parking lots, access roads, and landscaping. Some water loss would undoubtedly occur, especially through evapotranspiration and at least some infiltration of runoff generated on or directed to landscaping. The analysis presented in this report does not take account of these losses and hence is somewhat conservative in estimating benefits.

As required by the Permit, any runoff not attenuated by harvest, evapotranspiration, or infiltration would be subject to quantity and quality controls. The analysis assumes that extended-detention basins (EDBs) with water residence times up to 72 hours would provide this control. EDBs are one of several general-purpose, conventional stormwater BMPs available for this service, others being wet ponds, constructed wetlands, sand or other media filters, and biofiltration swales and filter strips. The California Department of Transportation (Caltrans, 2004) tested the performance of all of these practices in its BMP Retrofit Pilot Program, conducted in San Diego and Los Angeles Counties. The initial report investigating LID for A, B, and C soils presented estimates of benefits for EDBs, swales, and filter strips, along with continuous deflective separation (CDS) units, a practice that effectively captures only large particulate pollutants. For brevity, this follow-up report focuses on just EDBs as the supplement to LID. In performance, EDBs tend to fall between swales and filter strips for total suspended solids, slightly lower than the other two BMP types for metals, and either between the two or comparable to swales for total phosphorus.

These practices were applied to the same six case studies used in the initial analysis and described in Table 1 of the first report. Two additional case studies were defined for the assessment reported here: a sizeable commercial retail installation (COMM) and an urban redevelopment (REDEV). The hypothetical COMM scenario consists of a building with a 2-acre footprint and 500 parking spaces. Parking spaces were estimated to be 176 sq ft in area, which corresponds to 8 ft width by 22 ft length dimensions. A simple, square parking lot with roadways around the four sides and a square building with walkways also around the four sides were assumed. Roadways and walkways were taken to be 20 ft and 6 ft wide, respectively. The REDEV case was taken from an actual project in Berkeley involving a remodel of an existing structure, built originally as a corner grocery store with apartments above and a large side yard, and the addition of a new building on the same site to create a nine-unit, mixed-use, urban infill project. Table 1 summarizes the characteristics of these two case studies. The table also provides the recorded or estimated areas in each land use and cover type.

Table 1. Characteristics and Land Use and Land Cover Areas of Added Case Studies

	COMM[a]	REDEV[a]
No. buildings	1	1
Total area (ft^2)	226,529	5,451
Roof area (ft^2)	87,120	3,435
No. parking spaces	500	2 uncovered
Parking area (ft^2)	88,000	316 uncovered
Access road area (ft^2)	23,732	-
Walkway area (ft^2)	7,084	350
Driveway area (ft^2)	-	650
Landscape area (ft^2)	20,594	700

[a] COMM—retail commercial; REDEV—commercial/residential infill

The assessment for group D soils employed the same methods as the earlier analysis to estimate annual stormwater runoff volumes and pollutant discharges. Please refer to the initial report for details on those methods. The Natural Resource Conservation Service (NRCS, 1986) methodology cited in that report was applied to estimate that infiltration in group D soils would be roughly 60 percent of the amount through landscaping or the bed of a conventional BMP in C soils, which were the basis for establishing runoff coefficients in the first analysis. While that initial analysis was performed for both 14- and 20-inch average annual runoff zones, typical of different Bay Area locations, this supplementary work covered only the former condition. This simplification was made in the interest of brevity in this report, given that the first analysis showed almost no difference in conclusions between the two situations.

RESULTS OF THE ANALYSIS

Table 2 provides a comprehensive summary of the results. Rows shaded in gray compare runoff and pollutant discharges with and without treatment by CDS units, which can capture relatively large solids but have no mechanisms for dissolved substances and the finer particles. Having no soil contact and very limited residence time for evaporation, this BMP cannot reduce runoff volume at all. It can achieve some substantial reductions in TSS and TP for land uses relatively high in landscaped area but little removal of metals, especially copper.

The blue-shaded rows show the performance of conventional EDBs. In the group D soils considered in this analysis, they were estimated to reduce annual runoff volumes by 13-23 percent, the higher values for land uses with relatively small impervious footprints (OFF and REST). These BMPs can capture the majority of the long-term mass loading of most pollutants from most land uses in these soils, falling below 50 percent in reducing metals in stormwater flowing from residential developments.

Rows shaded in green present the results of applying LID BMPs appropriate for group D soils, roof runoff harvesting supplemented by dispersion in single-home land uses, plus treating the remaining runoff with EDBs. Comparing annual runoff volumes with and without LID, it can be seen that removing roof runoff from the storm drain system affords very significant benefits in reducing surface discharge and putting much of that water to productive use. Compared to directing all site runoff to EDBs, LID is expected to reduce volume by almost 10 times in the REDEV case, by about five times for the various residential land uses, 3.6 times for the large commercial development, and around twice for the OFF and REST cases. This management strategy can recover over 3/4 of the stormwater that would otherwise go down the drain in the intense redevelopment case, approximately 2/3 for the multi- and single-family residential cases, over half in the COMM development, and almost half in the office and restaurant cases with relatively small roof footprints.

Reduction of volume translates to decreases in pollutant loadings also. The combination of LID and EDB treatment is estimated to raise copper and zinc reductions to about 70 to over 90 percent in all except the developments with relatively low roof proportions (60-65 percent in these cases). TSS predictions come in at a quite consistent 75-82 percent across land uses. Total phosphorus estimates are a similarly consistent 63-71 percent, a bit higher in the highly impervious REDEV case.

Effectively managing roof runoff gives a way out of the dilemma posed by group D soils in the Bay Area. The analysis has demonstrated that harvesting this runoff stream, supplemented by ground dispersion techniques with sufficient space, shows strong promise to reduce the majority of flow inputs to municipal storm drain systems while conserving water. Moreover, this strategy can also stem the majority of solids, copper, zinc, and phosphorus transport to receiving waters.

Table 2. Runoff Volume and Pollutant Loading Reductions with Conventional and Low-Impact Development (LID) Best Management Practices (BMPs) for Eight Land Use Case Studies in Hydrologic Group D Soils

	COMM[a]	OFF[a]	REST[a]	REDEV[a]	MFR[a]	Lg-SFR[a]	Sm-SFR[a]	SINGLE
Total annual runoff with no BMPs (ac-ft)	5.29	0.80	0.47	0.12	8.57	75.66	1.74	0.10
Total annual runoff with CDS units[b] (reduction)	5.29 (0.0%)	0.80 (0.0%)	0.47 (0.0%)	0.12 (0.0%)	8.57 (0.0%)	75.66 (0.0%)	1.74 (0.0%)	0.10 (0.0%)
Total annual runoff with EDBs[b] (reduction)	4.43 (16.3%)	0.63 (21.3%)	0.36 (23.2%)	0.11 (8.1%)	7.48 (12.7%)	65.27 (13.7%)	1.50 (13.7%)	0.09 (13.3%)
Total annual runoff with LID[b] (reduction)	2.22 (58.0%)	0.44 (45.0%)	0.28 (40.4%)	0.03 (78.9%)	2.80 (67.3%)	26.72 (64.8%)	0.61 (64.8%)	0.04 (65.7%)
CDS TSS reduction[b, c]	19.4%	44.8%	33.9%	22.1%	27.1%	37.1%	37.1%	37.7%
CDS TCu reduction[b, c]	0.4%	11.0%	4.2%	0.9%	2.7%	7.3%	7.3%	7.6%
CDS TZn reduction[b, c]	25.3%	29.1%	25.5%	25.5%	24.1%	25.6%	25.6%	25.9%
CDS TP reduction[b, c]	25.9%	63.7%	54.3%	35.7%	46.7%	57.6%	57.6%	58.2%
EDB TSS reduction[b, c]	64.7%	78.1%	74.9%	66.5%	62.8%	70.3%	70.3%	70.9%
EDB TCu reduction[b, c]	57.9%	51.6%	56.4%	53.2%	51.4%	43.5%	43.5%	43.6%
EDB TZn reduction[b, c]	57.6%	49.6%	48.9%	58.1%	48.5%	47.7%	47.7%	48.0%
EDB TP reduction[b, c]	44.4%	67.6%	63.3%	52.8%	56.3%	64.4%	64.4%	64.7%
LID + EDB TSS reduction[b, c, d]	74.6%	80.3%	77.0%	81.5%	79.4%	81.3%	81.3%	81.8%
LID + EDB TCu reduction[b, c, d]	71.9%	60.3%	62.2%	82.3%	73.8%	68.9%	68.9%	69.5%
LID + EDB TZn reduction[b, c, d]	79.7%	65.1%	60.9%	92.3%	78.9%	76.4%	76.4%	77.0%
LID + EDB TP reduction[b, c, d]	63.1%	69.8%	66.0%	75.2%	69.4%	70.8%	70.8%	71.1%

[a] COMM—retail commercial; OFF—office building; REST—restaurant; REDEV—commercial/residential redevelopment; MFR—multi-family residential; Lg-SFR—large-scale single-family residential; Sm-SFR—small-scale single-family residential; SINGLE—single family home

[b] CDS— continuous deflective separation; EDBs—extended-detention basins; reduction—comparison with no BMPs

[c] TSS—total suspended solids; TCu—total recoverable copper; TZn—total recoverable zinc; TP—total phosphorus

[d] LID + EDB—roof runoff harvesting for COMM, OFF, REST, REDEV, AND MFR; harvesting supplemented by dispersion of roof runoff for Lg-SFR, Sm-SFR, and SINGLE; treatment of remaining runoff by EDBs

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July 23, 2010

Mr. Ivar Ridgeway
Los Angeles Regional Water Quality Control Board
320 W. 4th Street, Suite 200
Los Angeles, CA 90013

Submitted electronically to LAMS42012@waterboards.ca.gov

RE: **Comments on Greater Los Angeles County MS4 Permit Draft Tentative Oder No. R4-2012-XXXX, NPDES No. CAS004001, Dated June 6, 2012**

Dear Mr. Ridgeway:

This letter provides comments on the hydromodification provisions in the subject draft tentative order.

My experience includes ten years of research and practice in the field of urban hydromodification impacts and management, and co-authorship of the report released earlier this year entitled: Hydromodification Assessment and Management in California[1], prepared for the State Water Resources Control Board.

Section VI.D.6.c.v.(1)(c)(ii) Interim Hydromodification Control Criteria for project disturbing 50 acres or more within natural drainage systems, provides three alternative options that are each presumed to meet pre-development hydrology:

1. The site infiltrates on-site at least the runoff from a 2-year, 24-hour storm event
2. The runoff flow rate, volume, velocity, and duration for the post-development condition does not exceed the pre-development condition for the 2-year, 24-hour rainfall events
3. The Erosion Potential (Ep) in the receiving water channel will approximate 1, as determined by a Hydromodification Analysis Study and the equation presented in Attachment J.

The proposed criteria are inconsistent with current scientific understanding. These three options represent very different levels of control and will not provide equivalent protection of downstream resources for the following reasons:

A. Options 1 and 2 are *event*-based criteria, whereas current scientific understanding of hydromodification impacts is that a *range* of moderately frequent, "geomorphically significant" flows transport the majority of the sediment over the long term[2,3,4] and are the most influential in determining channel form. Rather than focusing on a single event, hydromodification control requirements should therefore address this critical range of flows. This scientific understanding has been reflected in other hydromodification regulations in the State of California by establishing flow-control criteria that require pre-project flow rates, volumes and durations to be matched across a *range* of flows (e.g., 0.5Q2 through Q10).

B. I am unaware of any studies that have evaluated the use of the 2-yr, 24-hr storm event (as either an infiltration volume or as a basis for matching flow rates, volumes and durations) to determine its equivalence to an Erosion Potential metric or to a flow control criteria using a range of geomorphically significant flows. Options 1 and 2 do not appear to have any basis in the scientific literature.

C. The use of a flow-control criterion assumes this range of flows to be appropriate for all receiving waters, and had been provided in other permits as an easy-to-implement option for smaller development projects. However, field research and modeling show that characteristics of the receiving stream channel (such as cross-section, slope, and sediment size) *will* influence the in-stream impacts of a changed runoff regime. This is why larger developments have been required to use an Erosion Potential metric, as it accounts for in-stream characteristics, as well as the full range of flows, to establish design criteria to minimize excess erosion and channel instability.

The Ventura County MS4 Permit (finalized by the Los Angeles Regional Board in January 2010) contains requirements for a Hydromodification Analysis Study (HAS) for projects disturbing 50 acres or greater. The HAS must demonstrate that post development conditions approximate pre-project erosive effects in receiving waters through the incorporation of an Erosion Potential or equivalent metric. I recommend that the Board modify the draft tentative order for Los Angeles County to be consistent with the Ventura County Permit hydromodification control criteria for projects of 50 acres or greater.

I also suggest that Attachment J be modified to indicate that the Work equation shown is just one of several equations that could be used to calculate an Erosion Potential. Other options include sediment transport function such as the Brownlie equation or the Meyer-Peter and Muller equation[5]. Allowing additional options supported by the scientific literature will permit the use of equations most appropriate for the characteristics of the receiving channel.

I am available to discuss these comments in more detail with Regional Board staff if desired.

Sincerely,

Felicia Federico, D.Env.
Executive Director, UCLA La Kretz Center

[1]Stein, E.D., Federico, F., Booth, D.B., Bledsoe, B.P., Bowles, C., Rubin, Z., Kondolf, G.M., Sengupta, A., 2012. *Hydromodification Assessment and Management in California.* Southern California Coastal Water Research Project Technical Report 667, April 2012.

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[4]Roesner, L. and B. Bledsoe. 2003. Physical Effects of Wet Weather Flows on Aquatic Habitats: Present Knowledge and Research Needs. Water Environment Research Foundation Report 00-WSM-4Rep.

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