This page intentionally left blank

Enterovirus detection in storm drain-impacted waters along the shoreline of the Southern California Bight

Rachel T. Noble

ABSTRACT

Assays for the detection of enteroviruses by reverse transcriptase-polymerase chain reaction (RT-PCR) were performed on coastal seawater samples taken at 15 randomly selected freshwater outlet sites along the shoreline of the Southern California Bight (SCB). All of the sites from which these samples were taken are influenced year-round by urban runoff and many are proximal to high-use sandy beaches that are recreational "hot spots" in southern California. Reverse transcriptase-PCR (RT-PCR) is a primer-based molecular biology technique that can be used to detect specific groups or types of viruses based upon the complementarity of primer sequences with conserved sequences in viral genomes. Pan-enterovirus primers were used for the detection of enteroviruses, the family of which includes poliovirus, Coxsackievirus, and echovirus. The results of our analyses showed that the concentration methods and RT-PCR protocol can be used consistently to detect enterovirus genomes from 20l samples of coastal seawater. Results were presence-absence for enterovirus detection. In addition, a most probable number (MPN) approach was used to estimate the number of viral genomes present in each sample. Of the 15 samples taken, 7 were positive for enteroviruses. Results of the virus estimates did not demonstrate a strong significant logistical or rank correlation to total and fecal coliforms, or enterococci ($p > 0.05$). However, a weak logistical correlation was observed between fecal coliforms and the detection of enteroviruses. Our results demonstrate that enteroviruses, which are indicative of local human fecal contamination, are found in nearly half of the freshwater outlets in southern California. Since enteroviruses are known to be causative agents of disease in humans from recreational water contact, the analysis of their presence will assist managers in protecting the public health at high-risk locations (e.g., high-use sandy beaches) or during certain seasons of the year.

INTRODUCTION

The beaches of southern California are popular destinations for recreation, water contact sports, and relaxation. However, serious concerns have been raised by the public, government agencies, and the scientific community about ocean water quality, particularly in areas heavily impacted by urban runoff. In southern California, the main sources of urban runoff are freshwater outlets (more specifically, the concrete-lined drains and rivers that empty the watershed of the entire region). Many of these freshwater outlet locations directly impact "high-use" sandy beaches that are vital to the economy of the region. Although waterborne illnesses in humans can be caused by protozoa, bacteria, and viruses, methodological limitations have until recently forced monitoring to focus only on the use of bacterial indicators such as total and fecal coliforms and enterococci. However, the time required to produce results with bacterial indicator analysis can delay management action (e.g., beach closure or posting) following a deterioration in water quality from activities such as a storm event or illicit dumping. Also, indicator bacteria cannot always be cultured effectively when introduced into aquatic environments; traditional plate counts often dramatically underestimate actual bacterial concentrations (Byrd *et al.* 1991). As a result, human pathogenic viruses are not monitored routinely in coastal waters and no guidelines or thresholds have been established for viral indicators.

Viruses have been shown to cause a significant portion of the waterborne diseases transmitted to humans through water contact, mostly from the ingestion of sewage-contaminated water and contaminated seafood (Fogarty *et al.* 1995). Large numbers of viruses are excreted in the

feces and urine of infected individuals, which even at low concentrations can cause a range of diseases when ingested (Moore *et al.* 1994). These diseases include meningitis, vomiting, gastrointestinal distress, diarrhea, myocarditis, and infectious hepatitis. However, virus detection in the past was based upon infectivity assays, which have limitations. Cell-culture methods are labor intensive, slow (taking days to weeks), insensitive to certain types of viruses, and not suitable as a basis for management decisions. However, infectivity-based methods are not the only way to detect human pathogenic viruses in seawater. Molecular genetic techniques have been developed in recent years, such as reverse transcriptase-polymerase chain reaction (RT-PCR), that detect viral genetic material (Metcalf *et al.* 1995). Tests in southern California (Noble and Fuhrman 2000, Tsai *et al.* 1993, Tsai *et al.* 1994), Florida (Griffin *et al.* 1999, Rose *et al.* 1997), and Europe (Pina *et al.* 1998) have detected the genetic material of enterovirus, hepatitis A virus (HAV), rotavirus, and adenovirus genomes in seawater.

The PCR-based assays detect only viral nucleic acid, so they have the capability to detect inactive as well as active viruses (Ma *et al.* 1994, Sobsey *et al.* 1998). However, viruses have been shown to degrade rapidly in seawater (Wommack and Colwell 2000, Noble and Fuhrman 1997, Noble and Fuhrman 1999), and a finding of inactive viruses in marine plankton would probably indicate the co-occurrence of active viruses, even at a lower level. Also, these assays are based upon the detection of viral genomes of picornaviridae, a family of viruses whose nucleic acid composition is single-stranded RNA. Single-stranded RNA is much more labile and likely to degrade than DNA. Also, free viral RNA has been shown to be extremely unstable in seawater, surviving for a period of only hours to days (Tsai *et al.* 1995). Therefore, while a positive RT-PCR result appears to be a reasonable indicator of a potential health hazard warranting caution and/or further investigation, it does not constitute a known health hazard.

Tangential flow filtration (TFF) was used to concentrate viruses from seawater, and pan-enterovirus "universal" primers were used for total enterovirus nucleic acid amplification (these primers target multiple related pathogens) with RT-PCR. Other concentration methods and PCR primer sets have been used by other laboratories; e.g., for the detection of HAV, rotavirus, and adenovirus (Griffin *et al.* 1999, Tsai *et al.* 1993, Tsai *et al.* 1994).

The goal of this study was to use molecular approaches for enterovirus detection at freshwater outlet sites along the shoreline of the Southern California Bight (SCB). A regional approach was used for the sampling design to provide a "snapshot" of the presence of potentially infectious viruses enteroviruses throughout the SCB, while at the same time developing and optimizing methods to make virus screening faster, more convenient, and more affordable.

METHODS

Sampling Design

This study was a component of the 1998 Southern California Bight Shoreline Microbiology Regional Monitoring Program (Noble *et al.* 1999). The study was conducted during summer to coincide with the period of maximum beach bathing usage. The study area extended 690 miles from Point Conception in Santa Barbara County, California, to Punta Banda in Baja California, just south of Ensenada, Mexico. A total of 81 freshwater outlets were identified and differentiated as perennial or ephemeral based upon whether water flowed year-round or seasonally, respectively. The freshwater outlets selected for inclusion in the Summer 1998 Study are those outlets that account for 99% of the total shoreline runoff flow inputs to the SCB. Of those perennial freshwater outlets selected, 15 were chosen randomly for enterovirus analysis.

To determine bacterial indicator levels, each participating laboratory used its standard method for processing samples from the 15 randomly selected freshwater outlets. Sampling efforts were coordinated to ensure that samples for bacteriological and viral analyses were taken simultaneously and at the same location. More detailed information on the methods used by all participants can be found in Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1995 (APHA 1995).

Sample Collection and Concentration

Twenty liters of seawater were collected in a plastic carboy from the wavewash at point zero (the point where the outlet flow reaches the beach) for each freshwater outlet site. Samples were placed on ice and returned immediately to the laboratory at the University of Southern California where they were pressure filtered (15 psi) through two 142 mm diameter stainless steel filtration units. The first unit housed a glass fiber filter (Whatman, nominal pore size of 1 mm), and the second unit housed a 0.22 mm Durapore filter. While still on ice, the filtrate was ultraconcentrated with a spiral cartridge filtration system (molecular weight cutoff of 30 kDa, SY130, Millipore, Inc.) to a final volume of ca. 150 mL. This sample was further concentrated using Centriprep-30 centrifugal concentration units (Amicon, Inc.). The Centriprep-30 concentration units were centrifuged at 5,000 x g for 30 min at 4° C, then the filtrate was poured off and the remaining concentrate was added to the units until the volume reached approximately 5

mL. The Centricon-30 concentration units were spun in a Sorvall SS-34 rotor at 5,000 x g at 10° C to further concentrate the material to approximately 100 µL. This volume of concentrate was selected because it is sufficient for many amplifications and dilution studies of the sample (each uses 2 µL).

ReverseTranscriptase-Polymerase Chain Reaction

Detection of enterovirus genomes was by RT-PCR. With one primer pair, this method can detect a whole group of related viruses, the human enteroviruses (Hyypia *et al.* 1989). Selection of a target RNA sequence for the primers depends upon identification of regions within the enterovirus genome that show maximum sequence conservation at the RNA level among the serotypes. Enterovirus detection by RT-PCR essentially followed that reported by Tsai *et al.* (1993), using pan-enterovirus "universal" primers for total enterovirus nucleic acid amplification. The amplification was performed with a GeneAmp RNA PCR kit (PE Applied Biosystems, Inc., Foster City, CA) with slight modifications. Specific amplification of enteroviruses was performed using specific primers (Tsai *et al.*1993), EV-L and EV-R. The upstream primer sequence was 5'-CCTCCGGCCCCTGAATG-3', and the downstream primer sequence was 5'-ACCGGATGGCCAATCCAA-3' (DeLeon *et al.* 1990). This primer set can amplify at least 25 enteroviral types, and the relatively short length of the PCR amplicon ensures efficient amplification. Perkin Elmer's RNA PCR core kits were used for the RT-PCR. Also, poliovirus Type 1 strain Lsc was used for positive controls. Positive RT-PCR reactions for enteroviruses produced amplified DNA fragments of 196 bp. Visualization of amplified DNA products was performed by electrophoresis on 2% SeaKem agarose stained with ethidium bromide (0.5 ug/mL) and illumination with ultraviolet light. Lane markers of 100 bp increments were used for size comparison. Sensitivity assays were performed by spiking ocean samples with known amounts of poliovirus and subsequently amplifying the sample with pan-enterovirus primers. Our preliminary results suggest that there are natural variations in the concentration of potentially interfering substances in seawater. The detection limit of the RT-PCR assay ranged from 0.1-10.0 infectious units and was comparable to detection limits reported in similar studies (Tsai *et al.* 1993, Rose *et al.* 1997). Triplicate analyses were run for each sample by using the RT-PCR protocol for each dilution. Negative and positive signals observed on agarose gels were recorded, and quantitative results were calculated using a most probable number (MPN) approach. Basically, for each sample, five replicate RT-PCR tests were run on each of five serial dilutions. Using an MPN table, the number of enterovirus genomes present in the original sample could be estimated.

Negative and positive controls were performed for each RT-PCR run. For the negative controls, 2µL of deionized water was added to the PCR mixture rather than the seawater sample. A positive control for the RT-PCR kit was performed each time a new kit was used, and involved the amplification of a given target RNA with random hexamer primers. A positive control for the poliovirus amplification was performed by adding known amounts of high-titer stock poliovirus to the RT-PCR mixture, with amplification using the EV-L and EV-R primer pair.

RESULTS

Seven of the 15 samples examined for human enteric virus genetic material were positive (Table 1). The number of enterovirus genomes detected ranged from 4 to 75 MPN/ 100 µL of concentrate, as determined by the MPN approach. Inhibitory substances, as evidenced by higher concentrations in more dilute samples in serial dilutions, were present in only a single sample from the Los Angeles River.

Correlations between human enteric virus genomes and each of the bacterial indicators (total coliforms, fecal coliforms, and enterococci) were statistically insignificant using rank correlation analysis. A significant logistical correlation was found between fecal coliform concentration and the presence or absence of human enteric virus genomes. In 73% of the samples, the presence of human enteric virus genomes coincided with the exceedence of the fecal coliform threshold of 400 cfu/100 mL.

DISCUSSION

Our results demonstrated that genetic material from members of the enterovirus family was found in nearly 50% of the freshwater outlets in the SCB during August 1998. Several dangerous viruses can be contracted by swimming or diving in contaminated waters (Cabelli *et al.* 1982, Seyfried *et al.* 1985 a and b). Recently, several anecdotal stories have been reported in the media based upon interviews with swimmers who claimed they were infected with serious intestinal viruses after swimming in Los Angeles County waters. Previous scientific studies have found that coliform bacteria standards are not adequate for predicting the virological quality of water for bathing, and that outbreaks of gastroenteritis have been caused by swimming in water with acceptable coliform counts (Cabelli *et al.* 1982). Our results support these findings. In addition, Haile *et al.* (1999) specifically discuss the increased risk of disease for

TABLE 1. Human enteric virus genome and bacterial indicator concentrations at virus sampling sites. Bold face type indicates a threshold exceedence for that bacterial indicator (nm = not measured).

Freshwater Outlet Site	Sampling Date	Virus Genomes per 2 L seawater sample	Total Coliforms (MPN or cfu/100 mL)	Fecal Coliforms (MPN or cfu/100 mL)	Enterococci (MPN or cfu/100 mL)
Tijuana River	8/03/98	75	30	8	10
Los Penosquitos Lagoon	8/03/98	75	8	4	2
San Luis Rey River	8/10/98	14	800	80	24
Los Angeles River	8/18/98	4	9,000	**1,700**	2
Aliso Creek	8/17/98	66	140	20	64
Ballona Creek	8/31/98	75	5,000	**1,600**	**170**
Malibu Creek	8/31/98	75	1,353	**616**	**175**
San Diego River	80/3/98	0	36	38	54
Moonlight Beach	8/10/98	0	3,000	230	nm
San Juan Creek	8/17/98	0	160	70	20
Goleta Creek	8/24/98	0	314	314	20
Mission Creek	8/24/98	0	240	85	10
Arroyo Burro	8/24/98	0	**24,192**	**589**	99
Carpinteria Creek	8/24/98	0	41	20	10
Calleguas Creek	8/31/98	0	1,100	170	**140**

those swimming in waters near storm drains. Although the microorganism responsible for inducing symptoms of disease in infected swimmers is often difficult to determine, it is probable that some of the symptoms encountered during the study of Haile *et al.* (1999) were caused by enteroviruses similar to those detected in our study.

Enteroviruses, unlike most bacterial indicators, are direct indicators of the presence of human fecal contamination. This study focused upon the detection of the genetic material specific to enteroviruses, a family of viruses that includes 67 human serotypes including poliovirus, Coxsackie virus, echovirus, and other poorly defined viruses. Even though the vaccine strain of poliovirus genomes is an attenuated version of the virus and thus poses no risk to the public health, the viruses and their genetic material detected using our RT-PCR technique are still a direct indicator of human fecal contamination. Vaccine-strain poliovirus may be found in elevated quantities in fecal material from children, as it is actively shed by those that have been recently vaccinated. Other viruses found in human fecal material that are capable of posing serious health risks, include astrovirus, adenovirus, Norwalk virus, coronavirus, rotavirus, and HAV. These viruses were not pursued as part of this study. While enteroviruses are responsible for a variety of illnesses or symptoms, including upper respiratory tract infections, meningitis, myocarditis, and hemorrhagic conjunctivitis, the measurement techniques used in this study do not provide direct information about the infectivity of the observed virus particles. The RT-PCR identifies the presence of viral RNA based upon conserved sequences of RNA found within the viral genome of specific virus families, in this case enteroviruses, without distinction as to whether the viral RNA is free or contained within an intact, infective virus particle. This technique is valuable for detecting the viral material found in human fecal contamination and thus distinguishing between human and animal waste. Work is under way to define whether a relationship exists between the detection of the genetic material of a virus and the presence of infectivity. To this end, RT-PCR must be combined with other measures, such as direct plating of coliphages or cell culture techniques, to assess infectivity.

Our study is not the first to examine the presence of enteroviruses in the coastal waters of the SCB. A pilot study performed in Santa Monica Bay in 1989 using cell culture techniques revealed the presence of infective human enteric viruses in 11 of 15 samples taken at a single storm drain in Santa Monica Bay, and repeat testing in 1990 revealed positive results in 3 of 4 samples (Gold *et al.* 1990). In another study in 1991, human enteric viruses were detected in all five of the storm drain samples tested in Santa Monica Bay (Gold *et al.* 1992). In this study, one of the enterovirus types identified by antibody neutralization was Coxsackie B virus, a known etiological agent. More recently, in an epidemiological study in Santa Monica Bay in 1995, human enteric viruses were detected in all three of the storm drain systems tested (Haile *et al.* 1999). Our findings demonstrate the positive detection of enterovirus genomes at both of the Santa Monica Bay storm drains tested, where positive detection for enteroviruses has been demonstrated previously (e.g., Haile *et al.* 1999; Table 1), with quantitative results suggesting that the levels of human

enteric virus genomes at these sites were among the highest of the sites studied. However, our study is the first to examine storm drains in the entire southern California region. By using a random site selection process, we were able to provide a regional perspective for the presence of enteroviruses, rather than focusing purely on storm drains that are considered to be "problem areas." Throughout the entire SCB, enterovirus genomes were detected at nearly 50% of the freshwater outlets tested.

Our finding that bacterial indicator levels do not correlate with the presence of human pathogenic viruses is supported by previous studies (Dufour 1984, Elliott and Colwell 1985). Analyzing samples from southern California through funding from USC Sea Grant, Noble and Fuhrman (2000) examined the incidence of enterovirus genomes by RT-PCR over 5 years in over 50 coastal seawater samples, and found that 35% of the samples were positive, with no significant statistical relationship to any of the standard bacterial indicators (total coliforms, fecal coliforms, and enterococci; but only about one-third of the samples had corresponding enterococcus counts for comparison). The findings of this study are also consistent with those of earlier related reports. For example, the analysis of wild shellfish from the Atlantic coast of France indicated no significant correlation between fecal coliforms and enteroviruses or HAV (LeGuyader *et al.* 1993, Leguyader *et al.* 1994), and viruses have sometimes been found in oysters without coliform contamination (Goyal *et al.* 1984, Yamashita *et al.* 1992). The lack of reliable standards for safe or unsafe levels of viruses in ocean waters has sparked an ongoing debate about the best way to use bacterial indicators); however, the weak relationship between positive bacterial and viral indicators suggests that the health risks of viral exposure are not reliably predicted by bacterial indicators.

Although a weak correlation was found between the presence of enterovirus genomes and fecal coliforms, the correlation did not extend to all of the other bacterial indicators. The poor relationship between bacterial and viral indicators may indicate the substantial influence of non-human sources of bacterial contamination. All of the samples from this study were taken in the surf zone immediately adjacent to the freshwater outlets. Many of these outlets drain lagoonal systems inhabited by waterfowl that can contribute large amounts of animal wastes. High bacterial counts in the absence of human enteric virus genomes were found at Calleguas Creek, Moonlight Beach, and Arroyo Burro, where hundreds of birds were observed near the storm drain at the time of sampling. If animal wastes are a significant source of viral contamination to the coastal waters, then bacterial indicators may provide an overly conservative estimate of microbiological water quality conditions, since animal waste does not typically contain pathogens of concern to humans.

An alternative explanation for the poor correlation between bacterial and viral indicators is the difference in the survival rates of pathogens in seawater (McNeill 1992). Many complex factors that influence the persistence of pathogenic microorganisms, among them sedimentation, turbulence, sunlight intensity, temperature, and predation. Under some circumstances, viral pathogens can survive longer in the marine environment than indicator bacteria as they adsorb to solids that can protect them from inactivation by biological, chemical, and physical factors (U.S. EPA 1985). Conversely, McNeill (1992) has shown that coliforms and enterococci not only persist but, in warmer water temperatures such as those found in tropical areas, can also grow in the marine environment. Understanding the relative degradation rates between bacterial indicators and the viral pathogens of human health concern, and how various environmental factors such as temperature affect their relative rates of attenuation, is essential to knowing how well bacterial indicators predict human health threats in marine waters.

The poor relationship between bacterial indicators and the presence of human enteric viruses leads to the conclusion that viral monitoring is probably advisable. Unfortunately, current genetic virus tests are technically demanding, expensive, and not particularly quantitative, so they are not yet suitable for routine monitoring. Such monitoring ideally would use relatively inexpensive methods, not require very large samples, permit analysis of several samples at a time, be quantitative, and be readily amenable to quality control. The RT-PCR technique presented here provides advancements toward these goals. The RT-PCR radically improves upon the time required to detect the presence of human pathogens in seawater, and requires a day rather than the weeks required for conventional cell culture techniques. Additionally, RT-PCR can be used to detect a variety of human pathogenic viruses not detectable by cell-culture techniques. The cost of RT-PCR, however, remains 50 times higher than that for bacterial indicator measurements. Further refinements to reduce cost and improve quality control will be required before the technique is feasible on a routine basis for addressing management decisions about local coastal health hazards.

LITERATURE CITED

American Public Health Association (APHA). 1992. Standard Methods for the Examination of Water and Wastewater. 17th Edition. Washington, DC.

Ansari, S.A., V.S. Springthorpe and S.A. Sattar. 1991. Survival and vehicular spread of human rotaviruses—possible relation to seasonality of outbreaks. *Review of Infectious Diseases* 13:448-461.

Byrd, J.J., H.S. Xu and R.R. Colwell. 1991. Viable but nonculturable bacteria in drinking water. *Applied Environmental Microbiology* 57:875-878.

DeLeon, R., Y.S.C. Shieh, R.S. Baric and M. Sobsey. 1990. Detection of enteroviruses and hepatitis A virus in environmental samples by gene probes and polymerase chain reaction: Advances in water analysis and treatment. Proceedings of the Water Quality Technology Conference. American Water Works Association. San Diego, CA.

Dufour, A.P. 1984. Bacterial indicators of recreational water quality. *Canada Journal of Public Health* 75:49-56.

Elliott, E.L. and Colwell, R.R. 1985. Indicator organisms for estuarine and marine waters. *FEMS Microbiology Review* 32:61-79.

Fogarty, J., L. Thornton, C. Hayes, M. Laffoy, D. O'Flanagan, J. Devlin and R. Corcoran. 1995. Illness in a community associated with an episode of water contamination with sewage. *Epidemiology of Infections* 114:289-295.

Gold, M., M. Bartlett, J.H. Dorsey and C.D. McGee. 1990. An Assessment of Inputs of Fecal Indicator Organisms and Human Enteric Viruses from Two Santa Monica Storm Drains. Santa Monica Bay Restoration Project. Monterey Park, CA.

Gold, M., M. Bartlett, C.D. McGee and G. Deets. 1992. Pathogens and Indicators in Storm Drains Within the Santa Monica Bay Watershed. Santa Monica Bay Restoration Project. Monterey Park, CA.

Goyal, S.M., W.N. Adams, M.L. O'Malley and D.W. Lear. 1984. Human pathogenic viruses at sewage sludge disposal sites in the Middle Atlantic region. *Applied Environmental Microbiology* 48:757-763.

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

Haile, R.W., J.S. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamillo, K. Barrett, M. Nides and G.-Y. Wang. 1999. The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology* 10:355-363.

Hyypia, T., P. Auvinen and M. Maaronen. 1989. Polymerase chain reaction for human picornaviruses. *Journal of General Virology* 70:3261-3268.

Kopecka, H., S. Dubrou, J. Prevot, J. Marechal and J.M. Lopez-Pila. 1993. Detection of naturally occurring enteroviruses in waters by reverse transcription polymerase chain reaction and hybridization. *Applied Environmental Microbiology* 59:1213-1219.

LeGuyader, F., V. Apaire-Marchais, J. Brillet and S. Billaudel. 1993. Use of genomic probes to detect hepatitis A virus and enterovirus RNAs in wild shellfish and relationship of viral contamination to bacterial contamination. *Applied Environmental Microbiology* 59:3963-3968.

LeGuyader, F., E. Dubois, D. Menard and M. Pommepuy. 1994. Detection of hepatitis a virus, rotavirus, and enterovirus in naturally contaminated shellfish and sediment by reverse transcription-seminested PCR. *Applied and Environmental Microbiology* 60:3665-3671.

Ma, J.-F., T.M. Straub, I.L. Pepper and C.A. Gerba. 1994. Cell culture and PCR determination of poliovirus inactivation by disinfectants. *Applied Environmental Microbiology* 60:4203-4206.

McNeill, A.R. 1992. Recreational water quality. pp. 193-216 *in:* D.W.C.and D.W.H. (eds.), Pollution of Tropical Aquatic Systems. CRC Press Inc. Boca Raton, FL.

Melnick, J.L. 1985. Enteroviruses: Polioviruses, Coxsackieviruses, Echoviruses and Newer Enteroviruses. Virology. Raven Press. New York, NY.

Metcalf, T.G., J.L. Melnick and M.K. Estes. 1995. Environmental virology: From detection of virus in sewage and water by isolation to identification by molecular biology—a trip of over 50 years. *Annual Review Microbiology* 49:461-487.

Moore, A.C., G.F. Herwaldt, G.F. Craun, R.L. Calderon, A.K. Highsmith and D.D. Juranek. 1994. Waterborne disease in the United States, in 1991 and 1992. *American Water Works Association Journal* 86:87-99.

Noble, R.T., J.H. Dorsey, M.K. Leecaster, M. Mazur, C.D. McGee, D. Moore, V. Orozco-Borbon, D. Reid, K. Schiff, P.M. Vainik and S.B. Weisberg. 1999. Southern California Bight 1998 Regional Monitoring Program: I. Summer Shoreline Microbiology. Southern California Coastal Water Research Project. Westminster, CA.

Noble, R.T. and J.A. Fuhrman. 1999. Breakdown and microbial uptake of marine viruses and viral lysis products. *Aquatic Microbial Ecology* 20:1-11.

Noble, R.T. and J.A. Fuhrman. In press. Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: Low correlation to bacterial indicator levels. *Hydrobiologia*.

Pina, S., M. Puig, F. Lucena, J. Jofre and R. Girones. 1998. Viral pollution in the environment and in shellfish: Human adenovirus detection by PCR as an index of human viruses. *Applied and Environmental Microbiology* 64:3376-3382.

Rose, J.B., X.T. Zhou, D.W. Griffin and J.H. Paul. 1997. Comparison of PCR and plaque assay for detection and enumeration of coliphage in polluted marine waters. *Applied and Environmental Microbiology* 63:4564-4566.

Seyfried, P.L., R.S. Tobin, N.E. Brown and P.F. Ness. 1985a. A prospective study of swimming-related illness. II. Morbidity and the microbiological quality of water. *American Journal of Public Health* 75:1071.

Seyfried, P.L., R.S. Tobin, N.E. Brown and P.F. Ness. 1985b. A prospective study of swimming-related illness. I. Swimming-associated health risks. *American Journal of Public Health* 75:1068.

Sobsey, M.D., D.A. Battigelli, G.A. Shin and S. Newland. 1998. RT-PCR amplification detects sin activated viruses in water and wastewater. *Water Science and Technology* 38:91-94.

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

Tsai, Y.L., B. Tran, L.R. Sangermano and C.J. Palmer. 1994. Detection of poliovirus, hepatitis a virus, and rotavirus from sewage and ocean water by triplex reverse transcriptase PCR. *Appied and Environmental Microbiology* 60:2400-2407.

Tsai, Y.-L., B. Tran, L.R. Sangermano and C.J. Palmer. 1995. Analysis of viral RNA persistence in seawater by reverse transcriptase-PCR. *Appied and Environmental Microbiology* 61:363-366.

United States Environmental Protectin Agency (U.S. EPA). 1985. Drinking Water Criteria Document for Viruses. Draft PB86-118270. U.S. EPA, National Technical Information Service. Springfield, VA.

Wommack, K.E. and R.R. Colwell. 2000. Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Review* 64:69-114.

Yamashita, T., K. Sakae, Y. Ishihara and S. Isomura. 1992. A 2-year survey of the prevalence of enteric viral infections in children compared with contamination in locally harvested oysters. *Epidemiology of Infections* 108:155-163.

ACKNOWLEDGEMENTS

Thanks to Dr. Jed A. Fuhrman for the use of laboratory facilities and thoughtful comments in review of this article. Thanks to Alison A. Davis for molecular biology expertise, and John F. Griffith for his assistance with field sampling. Thanks to the dedicated individuals at the organizations that participated in the Summer 1998 Shoreline Microbiology Study.