SOURCE INVESTIGATIONS OF STORM DRAIN DISCHARGES CAUSING EXCEEDANCES OF BACTERIOLOGICAL STANDARDS

REPORT TO THE LEGISLATURE

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STATE WATER RESOURCES CONTROL BOARD

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

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EXECUTIVE SUMMARY

Assembly Bill (AB) 538 (Chapter 488, Statutes of 1999) enacted Water Code section 13178, which requires the State Water Resources Control Board (SWRCB), in conjunction with the California Department of Health Services (DHS) and a panel of experts, to develop source investigation protocols for identifying the sources of discharges from storm drains that exceed the State's bacteriological standards (Appendix 1). The protocols must include methods for identifying the location and biological origins of sources of bacteriological contamination and require source investigations if (1) bacteriological standards are exceeded in any three weeks of a four-week period, or (2) bacteriological standards are exceeded 75 percent of testing days in areas where testing is done more than once a week. Section 13178 also requires the SWRCB, in conjunction with the DHS, to report to the Legislature on the methods by which the SWRCB intends to conduct source investigations of storm drains that produce exceedances of bacteriological standards. The report must also include: (1) the approximate number of public beaches expected to be affected by the exceedance of standards, (2) the estimated costs for source investigation of the storm drains affecting those public beaches, and (3) a timeline for completion of source investigation.

The SWRCB contracted with Dr. Michael K. Stenstrom, Professor of Civil and Environmental Engineering, University of California, Los Angeles, to develop source investigation protocols. A well known scientist with a special interest in urban storm water runoff problems, Professor Stenstrom's achievements include the development of a land-use and drainage model for the Santa Monica Bay watershed. To accomplish the AB 538 tasks, Professor Stenstrom and his associates interviewed health officials responsible for monitoring beach water quality, reviewed documents of previous source investigations and sources of contamination, and studied existing methods used to identify human and animal fecal contamination. Professor Stenstrom's findings are summarized in this report, and his report to the SWRCB is attached as Appendix 2.

During the year 2000, approximately 160 public beaches were either closed or posted with a warning for no body-contact recreation statewide at one time or another due to the exceedance of bacteriological standards. There are many reasons for this large number of beach closures and postings. California has a very extensive bacteriological monitoring program and a very rigid set of bacteriological standards for contact recreation. If the standard is exceeded in a single water sample, the beach is posted. The number of beaches closed or posted varies from year to year depending on the sources and causes of the exceedance of bacteriological standards. Most of the beach closures and postings are in the Southern California. An assessment of the year 2000 monitoring data indicates that 36 beaches would qualify for source investigation required by section 13178.

There are many sources of bacteriological contamination. Due to the obvious human health threats, the most important source is domestic sewage, which could be from septic tanks, broken sewer lines, and vessel waste discharges, among others. Direct input from animals and humans are other potential sources. It is essential to determine the biological origin of bacteriological contamination to assess the potential human health risks, as well as to implement measures to address the problem. For instance, birds using wetland areas can excrete indicator bacteria in

densities that would suggest a potential human health problem. However, birds do not generally carry the same types of pathogens as humans, and therefore the risk of illness to people is assumed to be much lower when the indicator bacteria comes from animals instead of humans. Indicator bacteria are those standardized bacteria that are monitored to indicate the probable existence of a contamination problem even though the indicator bacteria may not cause a direct human health threat. Investigators have tried various simple and advanced microbiological and chemical methods to determine the biological origin of microbiological contamination. These methods are described in Professor Stenstrom's report. Currently, there is no easy, rapid, low-cost method for differentiating between human and nonhuman sources of bacteriological contamination.

The chemical methods reviewed by Professor Stenstrom include monitoring natural byproducts of human metabolism (such as caffeine, sterols, and medical drug residues) or activity (such as detergents). The most frequently used and well-tested method at this time is genetic fingerprinting of indicator bacteria, multiple antibiotic resistance, and methods using a combination of indicators. However, relying on a combination of methods will probably require a longer period of analysis than relying on a single method.

Professor Stenstrom's report includes a summary of source investigation studies to identify the location (physical origin) of bacteriological contamination. There is no single standard method which could be used statewide to identify the location of bacteriological contamination. Site specific attributes, such as land use practices and the number and location of waste discharge permit facilities, play an important role in determining the appropriate method for source investigation. Point sources, such as industrial and municipal wastewater treatment facilities, are permitted under State and federal regulations for discharges to surface waters. Another primary source of coastal water pollution comes from the untreated runoff flowing from the land through storm drains and hundreds of natural stream courses. Runoff from creeks, rivers, and storm drains has been a significant source of bacteriological contamination of Southern California beaches.

This report proposes a set of source investigation protocols for storm drains that produce exceedances of bacteriological standards. The protocols were developed in conjunction with DHS and the Beach Water Quality Workgroup (Workgroup). The Workgroup was formed by the SWRCB as an ad-hoc group of scientists interested in keeping the beaches clean. Its membership includes representatives from the Regional Water Quality Control Boards (RWQCBs), other State, federal and local agencies, environmental advocacy groups, and other stakeholders.

The proposed source investigation protocols rely on a four-phased approach and include actions to be taken in these phases: planning phase, study development phase, study implementation phase, and post-implementation phase of the investigation. The Workgroup recommended that the appropriate RWQCB should take the lead in conducting the source investigations in coordination with other local, State, and federal agencies and other stakeholders (such as environmental organizations). The RWQCBs have regulatory authority for this activity pursuant to the Porter-Cologne Water Quality Control Act. Further, the RWQCBs have been performing this lead role in a similar source investigation program pursuant to the Shellfish Protection Act of

1993 (Water Code section 14950 et. seq.). Once the source(s) is identified, control measures, such as best management practices (BMPs), should be implemented by the responsible parties to mitigate the problem.

Rivers, streams, bays, and other surface water bodies which do not meet water quality standards are placed on the federal Clean Water Act (CWA) section 303(d) list of impaired water bodies. Beach waters can be placed on this list due to exceedance of bacteriological standards. In such instances, the appropriate RWQCB will take the lead in developing Total Maximum Daily Loads (TMDLs) to control the point and nonpoint source discharges of the bacteria in order to improve the water quality. Source investigation would be an integral part of the TMDL development process.

The cost and timeline for completion of a source investigation of storm drains for bacteriological contamination would vary from site to site. It depends on the extent and severity of contamination and the number and complexity of sources of contamination. Based on previous case studies, a source investigation study in California would cost a minimum of $200,000. The cost of source identification studies currently funded (fully or partially) by the SWRCB ranges from $274,000 for the San Juan Creek and $300,000 for the Morro Bay to approximately $1 million for the Mission Bay. A source identification study of medium complexity is estimated to cost from $50,000 to $100, 000 just for laboratory analysis. This does not include the cost of other essential components of the study such as personnel, sample handling and shipment, and data analysis and interpretation. The cost for a special survey (such as genetic fingerprinting) also varies, depending on the method used, number of samples needed to address the problem, and whether a library of known fecal bacterial isolates needed to be developed. The cost for the RWQCBs to take the lead role in conducting these studies is estimated at $700,000 annually, including the cost of statewide coordination by the SWRCB.

The geography of sites selected for source investigations would have a significant impact on the amount of time needed to conduct a thorough investigation. Terrain and the accessibility to essential sampling locations are important factors when considering timelines for source investigations. Another important factor influencing study length is whether or not the bacteriological exceedances are rainfall-related. If a bacterial contamination problem is linked to rainfall, the source investigation study would require a longer time than the study on dry weather exceedances. Based on the Workgroup's past experiences, on an average it takes approximately two years to complete a source investigation study.

I. INTRODUCTION

California's coastline is one of its most important natural features. It extends over 1,000 miles from the rocky cliffs of the north coast to the sandy, sun-drenched beaches in the south. Approximately 80 percent of Californians live within a 30-mile drive of its coastline. The coastal areas represent a desirable place for living and recreation. Millions of visitors come to see its beauty and play on the shores and in its waters. According to the U.S. Lifeguard Association, beach usage in California is higher than the other 49 states combined. Southern California beaches attract 175 million visitors each year, who spend more than $1.5 billion during their visits. On a statewide basis, California beaches generate $1.7 billion per year in tourism revenue.

In response to increasing public concerns with impaired beach water quality and beach closures and postings, several local, State and federal agencies have intensified their monitoring, assessment, and mitigation activities. New federal and State laws and regulations have been implemented to safeguard the health of beach goers and the economy of the local beach related businesses.

In 1997, the Legislature enacted AB 411 (Wayne, Chapter 765, Statutes of 1997), which required the DHS to adopt minimum standards for testing of waters adjacent to public beaches for total coliform, fecal coliform, and enterococci bacteria, or other microbiological indictors. The law also sets minimum requirements for testing, posting, and closing public beaches that are visited by more than 50,000 people annually and are located in an area adjacent to a storm drain that flows in the summer (sections 115880, 115885, and 115915 of the Health and Safety Code). These regulations are referred to as AB 411 regulations or AB 411 standards throughout this report.

In 1999, AB 538 was enacted, which added section 13178 to the Water Code. It requires the SWRCB to: (1) develop by September 30, 2000, source investigation protocols for use in conducting source investigations of storm drains that produce exceedances of bacteriological standards, and (2) report to the Legislature, by March 31, 2001, on the methods by which the SWRCB intends to conduct source investigations of storm drains that produce exceedances of bacteriological standards. Subsequent legislation, AB 2886 (Chapter 727, Statutes of 2000), extended the date for developing the source investigation protocols to June 30, 2001, and the date for the report to December 1, 2001.

The law requires that the SWRCB develop the source investigation protocols in conjunction with the DHS and a panel of experts established by the SWRCB. The protocols must include methods for identifying the location and biological origins of sources of bacteriological contamination. To accomplish this task, the SWRCB entered into a contract with Dr. Michael K. Stenstrom, Professor of Civil and Environmental Engineering, University of California, Los Angeles. Professor Stenstrom's expertise includes process development for water and wastewater treatment systems, including mathematical modeling and optimization. He has developed a land-use and drainage model for the Santa Monica Bay watershed to predict pollutant emissions to the Bay and how changes in land-use regulations will affect pollutant emissions.

The protocols developed by Professor Stenstrom were reviewed by the Beach Water Quality Workgroup (Workgroup). The Workgroup was formed by the SWRCB as an ad-hoc group of scientists interested in keeping the beaches clean. Its membership includes representatives from State (including DHS), local (such as County Health Officers), federal agencies (such as the U.S. Environmental Protection Agency [USEPA]), environmental advocacy groups (such as Heal the Bay), sanitation agencies, and other stakeholders, such as the Southern California Coastal Water Research Project (SCCWRP).

The law also requires the SWRCB to include in its report to the Legislature the approximate number of public beaches expected to be affected by the exceedance of bacteriological standards, costs expected for source investigations of the storm drains affecting those public beaches, and a timeline for completion of source investigations. This report includes proposed source investigation protocols (Chapter 2), California's beach water quality monitoring information for bacteriological indicators for year 2000 (Chapter 3), methods for identifying location of sources of bacteriological contamination (Chapter 4), methods for identifying the biological origins of sources of bacteriological contamination (Chapter 5), a discussion of costs and timelines for completion of source investigations (Chapter 6), and a brief conclusion (Chapter 7).

II. PROPOSED SOURCE INVESTIGATION PROTOCOLS

AB 538 requires the SWRCB, in conjunction with the DHS and a panel of experts, to develop source investigation protocols for use in conducting source investigations of storm drains that produce exceedances of bacteriological standards. There is no single standard method which could be used statewide to identify the biological origin and physical location of bacteriological contamination. Site specific attributes, such as land use practices and the related polluted runoff from those areas and the number and location of wastewater treatment plants's discharge points, would play an important role in determining which method or methods are most appropriate and effective.

The protocols proposed in this report are based on Professor Stenstrom's report which includes a review of 21 published source investigations and five California-specific case studies. Professor Stenstrom interviewed health officials responsible for monitoring beach water quality, reviewed documents of previous source investigations and sources of contamination, and studied existing methods used to identify human and animal fecal contamination. The protocols were peer reviewed by the Workgroup.

The proposed source investigation protocols are grouped into four general phases: planning, study design, implementation, and post-implementation. Various tasks to be accomplished in each phase are specified. It is suggested by the Workgroup that the appropriate RWQCB should take the lead in the source investigation, since the RWQCBs have regulatory authority for this activity pursuant to the Porter-Cologne Water Quality Control Act. Further, the RWQCBs have been performing this lead role in a similar program pursuant to the Shellfish Protection Act of 1993 (Water Code section 14950 et. seq.). However, this approach can be tailored for an individual site with a careful consideration of local conditions. For instance, in a case where a sewage spill has occurred that contributed to the contamination, the responsible party (such as the sanitation district) could assume the lead in this process.

Planning Phase:

1. The County Health Office notifies appropriate agencies and individuals of the source investigation study due to exceedance of bacteriological standards.
2. The RWQCB forms a Technical Advisory Committee (TAC) with membership including one representative from each of the following: the SWRCB, RWQCB, the DHS, Coastal Commission, county health office, sanitation district, county public works department, potential point and nonpoint source dischargers, environmental groups, and public groups. Make sure that all potential sources of fecal contamination to the storm drain are represented on the TAC.
3. Assign roles and responsibilities of TAC members.
4. Review existing data to determine whether additional investigations are needed.
5. If additional monitoring is needed, form a subcommittee of the TAC to develop a study design.

Study Design Phase:

1. Identify the storm drain(s) which is causing exceedance of bacteriological standards in beach water. This step would be easy to accomplish if monitoring stations are near the mouth of the storm drains.
2. Determine whether the exceedances occurred in wet or dry weather.
3. Beginning at the mouth of the storm drain, conduct a creek walk/drive to visually survey for sewage spills or obvious sources of fecal contamination.
4. Identify all potential sources of bacteriological contamination within the appropriate watershed. Note all land use practices within the watershed.
5. Establish sampling locations along the storm drain, ensuring that samples are collected both upstream and downstream of all potential contamination sources.
6. Determine what water quality parameters (such as indicator bacteria, pH, dissolved oxygen, turbidity, and ammonia) will be measured during the course of the study. Include flow rate so that a fecal load can be calculated.
7. Determine sampling frequency.
8. Determine which fecal source identification method (such as genetic fingerprinting) might be used to determine biological origin of bacteriological contamination.
9. Develop a work plan which includes: (a) statement of problem, (b) sampling design, (c) budget and funding sources, and (d) interim and final products.
10. Present the workplan to the TAC for its comment and approval.

Study Implementation Phase:

1. Collect and analyze water samples for bacteriological indicators at selected locations and time intervals. Ensure that sample collection, handling and storage follows procedures outlined in section 9060 of "Standard Methods for the Examination of Water and Wastewater," prepared and published jointly by the American Public Health Association, American Water Works Association and the Water Environment Federation (20th Edition, 1998).
2. Collect ancillary water quality (such as pH, dissolved oxygen, and turbidity) using portable meters with specific probes.
3. Collect fecal samples from all known biological sources (such as humans, domestic animals, wildlife) within the watershed. These samples will be used to develop a source library if the selected source identification method (such as genetic fingerprinting) requires this step.
4. Review data to determine if source(s) has been identified.
5. Report results to the TAC and appropriate agency.

Post-implementation Phase:

1. If the source of bacteriological contamination is identified, implement short- and long-term structural and nonstructural BMPs to mitigate the problem and improve beach water quality. These may include sewer maintenance program, septic tank removal/replacement, cleaning and inspection ordinances for grease blockage, installation of screens, inserts, and other structural BMPs.

2. If the source could not be identified in the initial study, design a more intensive (Phase II) study using the preliminary information collected in the first phase.
3. Keep the record of all information collected during the source investigation, preferably in a Geographic Information System (GIS) database, for future use.
4. With the help of the TAC, develop a proactive program to characterize all storm drains. This would make implementation of future source investigations and mitigation measures easier and cost-effective.

The goal of the source investigation and mitigation protocols should be to expeditiously detect the problem and find the solution to the problem with the cooperation and coordination of local expertise. This would avoid the need of placing the contaminated beach on the federal CWA section 303(d) list of impaired waters due to exceedance of bacteriological standards. Once the water body is listed, the RWQCB is required to develop TMDLs to control point and nonpoint sources of bacteriological discharges to achieve water quality standards. For example, the Santa Ana RWQCB adopted a pathogen TMDL in 2000 for the Newport Bay because its coliform bacteria contamination levels exceeded the DHS' standards, resulting in the loss of beneficial uses. TMDL is a contentious, time-consuming and costly process that includes investigation of the sources of contamination.

III. BEACH WATER QUALITY MONITORING FOR BACTERIOLOGICAL INDICATORS

Presence of a variety of pathogenic microorganisms could potentially impair beach water quality, impacting the health of the beach goers when they are exposed to the contaminated water through skin contact (swimming or surfing) or ingestion. Fever, flu-like symptoms, ear infection, respiratory illness, gastroenteritis, cryptosporidiosis, hepatitis, and other illnesses have been associated with waterborne pathogens. Table 1 lists a number of pathogenic bacteria, protozoa, and viruses, their observed effects on exposed population, and the diseases commonly associated with them. A 1996 epidemiological study sponsored by the Santa Monica Bay Restoration Project and partially funded by the SWRCB validated the cause and effect relationship between elevated levels of bacteria in beach water and health problems observed in exposed beach goers.

Indicator Organisms:

Since identification and enumeration of pathogens, such as viruses in water, are difficult, time consuming and expensive, laboratory methods have been developed to measure the presence and density of "indicator" organisms. The indicator organisms may not have direct human health impacts, but their presence indicates the potential for water contamination with other pathogens that are harmful, such as bacteria, viruses and protozoa. Indicator bacteria are carried to coastal waters in a variety of ways, typically from sewage spills, overflows of sewage treatment plants and sanitary sewers, and storm water runoff from urban, suburban, and rural areas.

An ideal indicator organism could be found only when disease-causing agents were present at densities that could cause human health problems. Since the coliform bacteria group is found in the intestines and feces of warm-blooded animals, their presence indicates that pathogens from untreated or partially treated sewage or contaminated runoff may be present in water. Other advantages of using coliform bacteria group as indicator organisms include: (1) they are easily detected by simple laboratory methods, (2) their presence indicates recent or ongoing bacterial contamination because of their short life span outside of a warm-blooded host environment, (3) their concentration in water can be correlated with the extent of contamination, and (4) they are safe to work with in the laboratory.

Bacteriological Water Quality Standards:

The State of California has adopted stringent bacteriological water quality standards for protection of human health from body-contact recreation and shellfish consumption. The SWRCB's California Ocean Plan has bacteriological standards for water-contact sports and shellfish harvesting. For water-contact sports, the total coliform count should be less than 1,000 per 100 ml; provided that not more than 20 percent of the samples at any sampling station in any 30-day period, may exceed 1,000 per 100 ml, and provided that no single sample, when verified by a repeat sample taken within 48 hours, shall exceed 10,000 per 100 ml. Further, the fecal coliform density based on a minimum of not less than five samples for any 30-day period shall not exceed a geometric mean of 200 per 100 ml nor shall more than 10 percent of the total

Table 1. Waterborne Pathogens, Diseases they Cause, and their Effects on Exposed Population

Pathogen		Disease	Effects
Bacteria	*Escherichia coli* (enteropathogenic)	Gastroenteritis	Vomiting, diarrhea, death in susceptible populations
	Legionella pneumophila	Legionellosis	Acute respiratory illness
	Leptospira	Leptospirosis	Jaundice, fever (Weil's disease)
	Salmonella typhi	Typhoid fever	High fever, diarrhea, ulceration of the small intestine
	Salmonella	Salmonellosis	Diarrhea, dehydration
	Shigella	Shigellosis	Bacillary dysentery
	Vibrio cholerae	Cholera	Extremely heavy diarrhea, dehydration
	Yersinia enterolitica	Yersinosis	Diarrhea
Protozoans	*Balantidium coli*	Balantidiasis	Diarrhea, dysentery
	Crytosporidium	Cryptosporidiosis	Diarrhea
	Entamoeba histolytica	Amedbiasis (amoebic dysentery)	Prolonged diarrhea with bleeding, abscesses of the liver and small intestine
	Giardia lamblia	Giardiasis	Mild to severe diarrhea, nausea, indigestion
	Naegleria fowleri	Amoebic meningoencephalitis	Fatal disease; inflammation of the brain
Viruses	Adenovirus (31 types)	Respiratory disease	
	Enterovirus (67 types, e.g., polio, echo, and Coxsackie viruses)	Gastroenteritis	Heart anomalies, meningitis
	Hepatitis A	Infectious hepatitis	Jaundice, fever
	Norwalk agent	Gastroenteritis	Vomiting, diarrhea
	Reovirus	Gastroenteritis	Vomiting, diarrhea
	Rotavirus	Gastroenteritis	Vomiting, diarrhea

samples during any 60-day period exceed 400 per 100 ml. In areas where shellfish are harvested for human consumption, the total coliform density shall not exceed 70 per 100 ml, and not more than 10 percent of the samples shall exceed 230 per 100 ml. These standards are used to calculate effluent limits in the permits of point source discharges to the ocean. For nonpoint source discharges, these standards apply in the receiving waters.

The DHS has promulgated fecal coliform criteria for commercial shellfish growing waters in California. The geometric mean should not exceed 14 per 100 ml within the approved shellfish growing waters classification, and the 90th percentile should not exceed 43 per 100 ml within the approved or conditionally approved classification. The RWQCBs use these standards to control bacteriological discharges to shellfish growing areas in the coastal waters.

AB 538 refers to the bacteriological standards established pursuant to subdivision [c] of section 115880 of the Health and Safety Code. These standards (Table 2) were established pursuant to AB 411.

Bacteriological Monitoring:

As required by AB 411, local health officers began in 1999 to conduct weekly bacterial (coliform, fecal coliform, and enterococci) testing between April 1 and October 31 of waters adjacent to public beaches which have more than 50,000 visitors annually and are near storm drains that flow in summer. If any one of these indicator organisms exceeds the AB 411 standards, the county health officer is required to post warning signs at the beach and make a determination whether to close that beach in the case of extended exceedances. Ten coastal counties (San Mateo, Sonoma, Santa Cruz, Monterey, San Luis Obispo, Santa Barbara, Ventura, Orange, Los Angeles, and San Diego) and one city (Long Beach) meet the AB 411 monitoring criteria.

According to the AB 411 bacteriological monitoring data for year 2000, approximately 160 public beaches were either closed or posted with warning signs statewide at one time or another due to the exceedance of bacteriological standards for water-contact sports. Table 3 presents the names of the counties and the beaches which were impacted. Most of the beach closures and postings are in Southern California. The number of beaches closed or posted varies from year to year depending on the sources and causes of the exceedances of bacteriological standards.

An assessment of the year 2000 beach water quality monitoring data indicates that 36 beaches exceeded the bacteriological standards in any three weeks of a four-week period, or where testing was done more than once a week, 75 percent of testing days produced an exceedance of those standards (Table 4). Source investigations are required for these beaches pursuant to AB 538.

Table 2. California Department of Health Services Bacteriological Standards for Water-Contact Sports.

Sample Type	**Bacteria**	**Standard** (organism or colony forming unit per 100ml of water)
Single	Total Coliform	10,000
	Fecal Coliform	400
	Enterococci	104
	Total to Fecal Coliform Ratio (when total is 1,000)	10
30-day Log Mean	Total Coliform	1,000
	Fecal Coliform	200
	Enterococci	35

Table 3. California Beaches Closed or Posted in 2000[1]

COUNTY	NUMBER OF BEACHES	BEACH NAME
Mendocino	1	Virginia Creek Beach
Sonoma	1	Campbell Cove State Beach
San Mateo	8	Fitzgerald Marine Reserve
		Montara Beach
		Pacifica State Beach (San Pedro Beach)
		Pillar Point Beach
		San Gregorio State Beach
		Sharp Park Beach
		Surfer's Beach
		Venice Beach
Santa Cruz	5	Cowell Beach
		Lighthouse Beach
		Mitchell's Cove Beach
		Rio del Mar Beach
		Seacliff State Beach
Monterey	6	Del Monte Beach
		Heritage Harbor
		Lover's Point
		San Carlos Beach
		Spanish Bay Beach
		Stillwater Cove
San Luis Obispo	1	Shell Beach
Santa Barbara	19	Arroyo Burro Beach
		Arroyo Quemada Beach
		Butterfly Beach
		Carpinteria City Beach
		Carpinteria State Beach
		East Beach at Mission Creek
		East Beach at Sycamore Creek
		El Capitan State Beach
		Gaviota State Beach
		Goleta Beach
		Guadalupe Dunes
		Hammond's Beach
		Hope Ranch Beach
		Jalama Beach
		Leadbetter Beach
		Ocean Beach
		Refugio State Beach
		Rincon Beach
		Sands Beach at Coal Oil Point

[1] NOTE: Not all coastal counties reported in 2000.

COUNTY	NUMBER OF BEACHES	BEACH NAME
Ventura	33	County Line Beach (#49000)
		Deer Creek Beach (#48000)
		Hobson County Park (#5000)
		Mandalay County Park - Channel Way (#29500)
		Mandos Cove Beach (#8000)
		Marina Park Beach (#22000)
		McGrath St. Bch- .5 mi. N of Gonzales Rd. (#26000)
		McGrath St. Bch. - S. end McGrath Lake (#28000)
		McGrath State Beach - Gonzales Rd. (#27000)
		Oil Piers Beach (#4000)
		Ormond Beach - Arnold Rd. (#44000)
		Ormond Beach - Industrial Drain (#43000)
		Ormond Beach - J St. (#42000)
		Oxnard State Beach - Starfish Dr. (#33000)
		Peninsula Beach (#23000)
		Port Hueneme Beach Park (#41000)
		Promenade Park - Figueroa St. (#14000)
		Promenade Park - Holiday Inn (#17000)
		Promenade Park - Oak St. (#16000)
		Promenade Park - Redwood Apts. (#15000)
		Rincon Beach - creek mouth (#1000)
		Rincon Beach - flagpole (#1050)
		Rincon Beach - footpath (#1100)
		San Buenaventura St. Bch. - San Jon Rd. (#19000)
		San Buenaventura St. Bch. - Weymouth Ln. (#21000)
		San Buenaventura State Bch. - Dover Ln. (#20000)
		San Buenventura St. Bch. - Kalorama St. (#18000)
		Silverstrand Beach - San Nicolas Ave. (#38000)
		Silverstrand Beach - Sawtelle Ave. (#40000)
		Solimar Beach (#9000)
		South Jetty Beach (#24000)
		Staircase State Beach (#50000)
		Surfer's Point - "Stables" (#13000)
Los Angeles	26	Abalone Cove
		Avalon Beach
		Basin H
		Big Rock Beach
		Bluff Cove
		Cabrillo Beach
		Cabrillo Inner
		Corral Beach
		Dan Blocker Beach
		Dockweiler Beach
		Hermosa Beach
		Latigo Shore
		Malibu Pier

COUNTY	NUMBER OF BEACHES	BEACH NAME
		Malibu Point
		Manhattan Beach
		Marina del Rey Beach
		Nicholas Canyon Beach
		Paradise Cove
		Redondo Beach
		Santa Monica Beach
		Surfrider Beach
		Topanga Beach
		Torrance Beach
		Venice Beach
		Will Rogers State Beach
		Zuma Beach
Long Beach (city)	21	10th Place Beach
		16th Place-Beach
		1st and Bayshore
		2nd St Bridge & Bayshore
		36th Place-Beach
		3rd Place Beach
		54th Place Beach
		56th Place on Bayside
		5th Place Beach
		62nd Place Beach
		72nd Place Beach
		Belmont Pier - Eastside
		Belmont Pier - Westside
		Colorado Lagoon-Center
		Colorado Lagoon-North
		Colorado Lagoon-South
		Coronado Ave Beach
		Granada Ave Beach
		Molino Ave Beach
		Mothers Beach
		Prospect Ave Beach
Orange	13	Aliso Beach
		Aliso County Beach Park
		Capistrano County Beach
		Dana Point Harbor
		Doheny State Beach Park
		Huntington Harbour
		Laguna Beach
		Monarch Beach
		Newport Bay
		Newport Beach
		Poche Beach
		San Clemente City Beach

COUNTY	NUMBER OF BEACHES	BEACH NAME
		Seal Beach
San Diego	26	Agua Hedionda Lagoon
		Border Field State Park
		Cardiff State Beach
		Coronado City Beach
		Coronado Municipal Beach
		Del Mar City Beach
		Encinitas City Beach
		Imperial Beach City Beach
		La Jolla Community Beach
		La Jolla Shores
		La Jolla Shores Beach
		Mission Bay
		Ocean Beach
		Oceanside / Carlsbad border
		Oceanside City Beach
		Pacific Beach
		San Diego Bay
		San Onofre State Beach
		Silver Strand State Beach
		Solana Beach City Beach
		South Carlsbad State Beach
		Sunset Cliffs Park
		Tijuana Slough Nat'l Wildlife Refuge Shoreline
		Torrey Pines City Beach
		Torrey Pines State Beach
		Tourmaline Surf Park

Table 4. California Beaches Meeting AB 538 Source Investigation Criteria Based on Year 2000 Monitoring Information

COUNTY	NUMBER OF BEACHES	BEACH NAME
San Mateo	3	Pacifica State Beach (San Pedro Beach)
		Surfer's Beach
		Venice Beach
Santa Barbarba	13	Arroyo Burro Beach
		Carpinteria State Beach
		East Beach at Mission Creek
		East Beach at Sycamore Creek
		El Capitan State Beach
		Gaviota State Beach
		Goleta Beach
		Hammond's Beach
		Hope Ranch Beach
		Jalama Beach
		Leadbetter Beach
		Refugio State Beach
		Rincon Beach
Ventura	3	Rincon Beach - creek mouth (#1000)
		Rincon Beach - flagpole (#1050)
		San Buenventura St. Bch. - Kalorama St. (#18000)
Los Angeles	13	Avalon Beach
		Big Rock Beach
		Cabrillo Beach
		Dockweiler Beach
		Malibu Pier
		Marina del Rey Beach
		Paradise Cove
		Redondo Beach
		Santa Monica Beach
		Surfrider Beach
		Topanga Beach
		Venice Beach
		Will Rogers State Beach
Long Beach (city)	3	Colorado Lagoon-Center
		Colorado Lagoon-North
		Colorado Lagoon-South
Orange	1	Dana Point Harbor

IV. METHODS FOR IDENTIFYING THE LOCATION OF SOURCES OF BACTERIOLOGICAL CONTAMINATION

In general, it is difficult to pinpoint the exact physical location of the source of bacteriological contamination of beaches. There is no single standard method which could be used for source investigation on a statewide basis. The method has to be tailored for individual site with a careful consideration of local conditions. Various methods have been used in the past by researchers to identify the physical location of the source of contamination.

Professor Stenstrom's report summarizes five previous source investigation studies conducted in California. Results of these studies suggest that planning plays an important role in source investigation of storm drains. The first step is to meet with all the agencies/organizations that would be involved with source investigations. This would include the SWRCB, RWQCBs, local health agencies, sanitation districts, public works departments, permitted dischargers, local governments, as well as any other parties that might have a stake in coastal water quality. The purpose of meeting with these groups would be to establish who would have the authority to initiate an investigation and who will be responsible for carrying out that investigation. The next step in the planning process should be to identify the most likely sources of contamination in the watersheds where an investigation might be expected to occur and identify the individuals (or entities) that are responsible for those locations. This information will be used in the early stages of a source investigation. It will also be useful to determine essential points (i.e., convergence of tributaries, discharge points, etc.) where sampling will help to quickly narrow the search for the contamination source.

Land use practices within the watershed are a factor in determining possible sources. Information on land use can be used to select monitoring sites that bracket potential sources. Bacteriological monitoring sites can be placed upstream and downstream of the potential sources of storm drains. Statistical methods (such as paired t-test) can be used to determine if there are significant differences in bacteriological levels between sampling sites. Because of the inherent variability of bacteriological testing, numerous sampling events may be necessary. This would add to the cost and time of the investigation. This method, based on land use practices, may be able to identify the area of bacteriological contamination but not the specific source. For instance, if a farm site with numerous animals is identified as the source of bacteriological contamination, the possible sources at the farm site could be on-site sewage facilities or animal wastes. The methods used to identify the specific source (i.e., the biological origin of bacteriological contamination) are summarized in the next chapter.

V. METHODS FOR IDENTIFICATION OF BIOLOGICAL ORIGINS OF SOURCES OF CONTAMINATION

Information on the human or animal origin of fecal pollution gives an indication of the types of pathogens that may be expected, the risk of infection, and the treatment that may be required to control the transmission of disease. Animal fecal pollution is not without risks and, while many of the risks are unknown, it is generally believed that animal sources pose less risk. Many waterborne pathogens are difficult to detect and quantify, and specific methodology to detect them in environmental water samples is in the developmental stages.

Professor Stenstrom's report includes a description of many microbiological and chemical methods used for identifying biological sources of fecal contamination. The microbiological methods rely on the bacteriological and viral indicators found in the intestines of warm-blooded animals. Chemical indicators are natural byproducts of human metabolism or activity. The field and laboratory complexity and cost of these methods vary considerably. Following is a brief description of a few simple and promising methods.

Microbiological Methods:

The Ratio of Fecal Coliforms to Fecal Streptococci or Total Coliforms: Human fecal material may be distinguishable from animal fecal material using an old method, the ratio of fecal coliforms to fecal streptococci. Fecal streptococci are more abundant in animal feces than in humans; in contrast, fecal coliforms are more abundant in human feces than in animals. Therefore, the fecal coliform to fecal streptococci ratio has been used to differentiate human fecal contamination from that of other warm-blooded animals. The ratio of fecal coliforms to fecal streptococci greater than four is associated with human fecal sources while a ratio of less than 0.7 is associated with animal fecal sources.

Although this is an inexpensive and practical method, it is not reliable due to the variable survival rates of fecal streptococci species.

Multiple Antibiotic Resistance (MAR) Analysis: The patterns of antibiotic resistance have been used to identify sources of fecal pollution in water. This approach is based on the fact that bacteria from wildlife species are generally lacking in antibiotic resistance, while strains from humans and domestic animals exhibit varying multiple antibiotic resistance. In this procedure, either E. coli or fecal streptococci from different animal species are analyzed to determine the resistance pattern for several different types and strengths of antibiotics.

The MAR method for differentiating between fecal sources is promising. This method may successfully differentiate between human fecal pollution and animal fecal pollution, and even differentiate among the sources from different animals. However, this method is time consuming for the field and laboratory work and its laboratory procedure is complicated and costly.

Ribotype Analysis/Genetic Fingerprinting: Genetic testing has been found to be very effective in matching DNA patterns in microorganisms to their sources. Genetic fingerprinting uses collections of E. coli, which are easily modified and adapt to various host environments, leading to changes in genetic material that are thought to be specific to these host environments. As such, the genetic variability of E. coli can be used to identify their host organisms. The DNA patterns from each of these isolates, known as a ribotype, are used to match specific strains of E. coli from a contaminated site to potential sources. This method involves expensive laboratory analysis.

Human Enteric Viruses: Human enteric viruses can be used to confirm the presence of human fecal material. Human enteric virus groups include rotavirus, hepatitis A virus, adenovirus, and enterovirus. They are potentially good human source indicators. However, the methodologies involved in their detection and enumeration tend to be costly and time consuming. Many researchers are working on developing reliable and less expensive ways of finding viruses in seawater.

Chemical Methods:

Fecal Sterols: Fecal sterol, such as coprostanol, has been proposed as an alternative measure of fecal pollution by a large number of researchers. Coprostanol is formed in the gut of human and higher mammals by enzymatic hydrogenation or by bacterial reduction of cholesterol. Fecal sterol analysis has been extended to differentiate human and animal sources of pollution.

This method requires expensive gas chromatography and requires up to 10 liters of samples to be filtered through a glass fiber filter to concentrate particulate stanols. Nevertheless, it is an appropriate method for specific studies investigating the proportion of human and animal fecal contamination.

Long-Chain Alkylbenzenes: Long-chain alkylbenzenes (LABs) are widely used for anionic surfactants in commercial detergents. LABs are purely synthetic and are derived solely from direct industrial discharges and domestic wastes. They are therefore strongly indicative of human sources. However, they may or may not be related to industrial pollution. They are also generally present up to one order of magnitude lower than the corresponding human fecal sterol. They are therefore regarded as complimentary to the fecal sterols in determining that domestic sewage is the source of contamination.

Caffeine: Caffeine is a compound that is present in several beverages, such as coffee, tea, and carbonated drinks, and in pharmaceutical products. Caffeine and its metabolites are excreted in the urine of individuals who have consumed beverages and pharmaceuticals containing caffeine. It has been speculated that caffeine could be used as an indicator of human fecal pollution if the population being studied uses caffeine.

In conclusion, there is no easy, low cost method for differentiating between human and nonhuman sources of bacterial contamination. No single indicator or approach is likely to represent all the facets and issues associated with fecal contamination of waterways. At present, the best hope of distinguishing fecal pollution of human and animal origin is an appropriate

combination of indicators. Statistical analyses of appropriate groups of methods offer the best possibility of identifying human sources. Unfortunately, relying on a combination of methods would probably require a longer period of analysis than relying on a single method. A combination of methods may be useful to determine sources in chronic situations as opposed to episodic events.

VI. COST AND TIMELINE FOR COMPLETION OF SOURCE INVESTIGATIONS

Many factors determine the cost and the timeline for completion of a source investigation study. In some cases, no physical or biological source could be identified as the major contributor of bacteriological contamination. It would require more intensive follow-up studies (Phase II studies) which would build up on the information developed from the initial (Phase I) studies and therefore would require more time and resources.

Cost of Completing Source Investigations:

The cost of completing source investigations varies, depending on the extent and severity of contamination and the number and complexity of sources of contamination. Assuming a "creek walk/drive" method is selected, the necessary personnel, equipment, and their associated costs are listed below:

1. Three people per investigation team (costs will depend on the personnel available and the training necessary; costs will also vary by county)
2. One van for transportation and also serving as a portable laboratory ($30,000 to $35,000)
3. Various probes used to measure simple water quality parameters such as pH, temperature, and salinity ($5,000)
4. Laptop computer and software, including GIS ($4,000)
5. Geographic Positioning System (GPS) unit for location of sampling sites ($1,000)
6. Expendables for laboratory supplies ($200 to $400 per day)

One advantage of this investigation method is that many of the costs listed above are one-time capital outlays necessary to begin a source investigation program. Much of the hardware could be used to conduct many investigations at more than one site.

The cost of conducting a special survey (such as genetic fingerprinting of fecal coliforms) varies, depending on the method used, number of samples needed to address the problem, and whether a library of known fecal bacterial isolates needs to be developed. These special survey methods require samples to be analyzed in such a way that individual bacterial colonies (isolates) can be selected and tested. Typically, two-five isolates are selected from each sample. Based on the experiences from ongoing studies, costs per isolate vary from $10.00 per isolate (antibiotic resistance) to $75.00 per isolate (genetic fingerprinting). In addition to the source identification costs, routine bacterial analyses, quality assurance/quality control samples, and library development costs must be factored in. A source identification study of medium complexity is estimated to cost from $50,000 to $110,000 for laboratory analyses only. This does not include essential components such as personnel, sample shipment costs, and data analyses. On an average, a minimum of $200,000 would be required for a source investigation study in California. The cost of source identification studies currently funded (fully or partially) by the SWRCB ranges from $274,000 for the San Juan Creek and $300,000 for the Morro Bay to over

$1 million for the Mission Bay. The estimated annual cost for the RWQCBs to take the lead role in conducting these studies is $700,000 (one permanent, full-time position per Region for six coastal RWQCBs and a statewide coordinator at the SWRCB).

Timeline for Completing Source Investigations:

The geography of sites selected for source investigations would have a significant impact on the amount of time needed to conduct a thorough investigation. Terrain and the accessibility to essential sampling locations are important factors when considering timelines for source investigations. Another important factor influencing study length is whether or not the bacteriological exceedances are rainfall related. In theory, a source investigation study on dry weather exceedances could be completed within a six-month time period. However, if a bacterial contamination problem is linked to rainfall, the study period must include both dry weather (to determine background conditions) and several normal rainfall events. This often requires that the study period span several winters. Based on the past source investigation case studies completed in California, it is safe to say that on an average a minimum period of two years is needed to complete a source investigation study.

A three-person team conducting a "creek walk/drive" investigation could sample approximately 12 to 24 locations in a typical workday (assuming two hours to set up and shut down and sampling two to four locations per hour). However, most investigations will require more than one day to complete, and the number of days will depend, as previously stated, on the size and geography of the study area. Laboratory time for processing water samples should also be included into the timeline for the study. In addition, the frequency of water quality violations will affect the time needed for the investigation. Sites with chronically poor conditions should require less time to determine the source than sites with more sporadic occurrences. The reasoning behind this is that chronically poor sites should have more of a continuously "warm" trail for the investigators to follow. The amount of time required to complete a special survey using more advanced methods such as genetic fingerprinting could considerably lengthen the duration of the study.

It is essential that for every site that requires source investigation, a GIS database is set up initially to store information that can be used for future source investigations. If the data layers are readily available, approximately four months would be required to collect and input the data. However, if the GIS data layers are not available, it will take up to a year for organizations to develop a GIS database.

Sixty-seven water bodies adjacent to California beaches are on the 1998 CWA section 303(d) list of impaired water bodies due to exceedance of bacteriological standards or beach closures (Table 5). Most of these beaches are under the jurisdiction of the Los Angeles RWQCB. The RWQCBs are required to develop TMDLs for the bacteriological parameters to mitigate the water quality of the listed beaches. Source investigation of bacteriological contamination will be an integral part of the TMDL development process. The RWQCBs have proposed schedules for developing these TMDLs based on their priorities and availability of resources.

Table 5. California Beaches on the 1998 Clean Water Act Section 303(d) List Based on Pathogens or Beach Closure

RWQCB	NUMBER OF BEACHES	NAME OF WATER BODY
Central Coast	1	Pacific Ocean at Point Rincon
Los Angeles	49	Abalone Cove Beach
		Big Rock Beach
		Bluff Cove Beach
		Cabrillo Beach (Inner) LA Harbor Area
		Cabrillo Beach Outer
		Carbon Beach
		Castlerock Beach
		Dan Blocker Memorial (Coral) Beach
		Dockweiler Beach
		Escondido Beach
		Flat Rock Point Beach Area
		Hermosa Beach
		Inspiration Point Beach
		La Costa Beach
		Las Flores Beach
		Las Tunas Beach
		Leo Carillo Beach (South of County Line)
		Long Point Beach
		Lunada Bay Beach
		Malaga Cove Beach
		Malibu Beach
		Malibu Lagoon Beach (Surfrider)
		Mandalay Beach
		Manhattan Beach
		Marina Del Rey Harbor Beach
		McGrath Beach
		Nicholas Canyon Beach
		Palo Verde Shoreline Park Beach
		Paradise Cove Beach
		Point Dume Beach
		Point Fermin Park Beach
		Point Vicente Beach
		Portugese Bend Beach
		Puerco Beach
		Redondo Beach
		Resort Point Beach
		Robert H Meyer Memorial Beach
		Rocky Point Beach
		Royal Palms Beach
		Santa Clara River Estuary Beach/Surfers Knoll
		Santa Monica Beach

RWQCB	NUMBER OF BEACHES	NAME OF WATER BODY
		Sea Level Beach
		Topanga Beach
		Torrance Beach
		Trancas Beach (Broad Beach)
		Venice Beach
		Whites Point Beach
		Will Rogers Beach
		Zuma (Westward Beach)
San Diego	17	Pacific Ocean, Aliso
		Pacific Ocean, Buena Vista
		Pacific Ocean, Coronado
		Pacific Ocean, Dana Point
		Pacific Ocean, Escondido Creek
		Pacific Ocean, Laguna Beach
		Pacific Ocean, Loma Alta
		Pacific Ocean, Lower San Juan
		Pacific Ocean, San Clemente
		Pacific Ocean, San Diego
		Pacific Ocean, San Dieguito
		Pacific Ocean, San Luis Rey
		Pacific Ocean, San Marcos
		Pacific Ocean, Scripps
		Pacific Ocean, Tijuana
		San Diego Bay, Lindbergh
		San Diego Bay, Telegraph

VII. CONCLUSIONS

In the year 2000, approximately 160 public beaches in California, mainly in the southern part of the State, were either closed or posted with warnings for water-contact recreation due to the presence of indicator bacteria in excess of the water quality standards. The main reasons for this large number are that California has a stringent set of bacteriological standards and the beaches are extensively monitored. According to the year 2000 beach water quality bacteriological monitoring data, only 36 beaches meet the AB 538 criteria for source investigation. AB 538 requires that, at a minimum, source investigations of storm drains should be conducted when bacteriological standards are exceeded in any three weeks of a four-week period, or for areas where testing is done more than once a week, 75 percent of testing days produces exceedance of those standards.

It should be noted that a source investigation of storm drains that produce exceedances of bacteriological standards may not be necessary in every instance for the following reasons:

1. The test bacteria may not be the right indicator of pathogens in shoreline waters.
2. The indicator bacteria assay may take 18 to 36 hours or longer to complete. During this time the beach goers may be potentially exposed to harmful pathogens. By the time a beach is posted based on the monitoring data, the indicator bacteria may no longer be present in the shoreline waters. Thus, a beach may potentially be open when it is contaminated and posted when it is clean.
3. There are many sources of variability in shoreline bacteriological monitoring. Research conducted by SCCWRP revealed that different laboratories reported different bacterial counts for the same sample (inter-laboratory variability). Water samples collected from very close locations in the surf zone had different bacterial counts (spatial variability). Further, water samples collected from the same location but at different times of the day had different bacterial counts (temporal variability).

The Governor's Clean Beach Initiative provided $1.5 million to the SWRCB in Fiscal Year (FY) 2001-02 to develop simple, rapid, and inexpensive indicators to provide timely information to beach goers on the quality of water for recreational activities. This would solve the problem listed in Item 2 above.

Much research is underway to develop a reliable method to differentiate between human and nonhuman sources of fecal contamination of surface waters. Currently, the two most widely used methods are genetic fingerprinting and multiple antibiotic resistance technique. The SWRCB is partially funding a comparative study of these two methods. Although both of these methods seem promising, neither is considered to be a reliable source identification method at this time.

The FY 2001-02 Budget Act has appropriated $32,298,000 from Proposition 13 (Safe Drinking Water, Clean Water, Watershed Protection, and Flood Protection Bond Act of 2000) funding for local projects addressing beach water quality problems. These grant funds will be used for implementation of BMPs to mitigate the bacteriological contamination of some of the beaches that have chronic problems of beach postings and closures. Implementation of these BMPs would be the next logical step of the successful completion of source investigation studies.

APPENDIX 1 – ASSEMBLY BILL 538

Assembly Bill No. 538

CHAPTER 488

An act to add Section 13178 to the Water Code, relating to water.

[Approved by Governor September 27, 1999. Filed
with Secretary of State September 27, 1999.]

LEGISLATIVE COUNSEL'S DIGEST

AB 538, Wayne. Public beaches: bacteriological standards.

Existing law sets forth duties and responsibilities of the State Water Resources Control Board.

This bill, in addition, would require the state board, on or before September 30, 2000, in conjunction with the State Department of Health Services and a panel of experts established by the state board, to develop source investigation protocols for use in conducting source investigations of storm drains that produce exceedences of specified bacteriological standards.

The bill also would require the state board, on or before March 31, 2001, in conjunction with the State Department of Health Services, to report to the Legislature on the methods by which it intends to conduct sources investigations of storm drains that produce exceedences of bacteriological standards established, as specified.

The people of the State of California do enact as follows:

SECTION 1. Section 13178 is added to the Water Code, to read:

13178. (a) (1) On or before September 30, 2000, the state board, in conjunction with the State Department of Health Services and a panel of experts established by the state board, shall develop source investigation protocols for use in conducting source investigations of storm drains that produce exceedences of bacteriological standards established pursuant to subdivision (c) of Section 115880 of the Health and Safety Code. The protocols shall be based upon the experiences drawn from previous source investigations performed by the state board, regional boards, or other agencies, and other available data. The protocols shall include methods for identifying the location and biological origins of sources of bacteriological contamination, and, at a minimum, shall require source investigations if bacteriological standards are exceeded in any three weeks of a four-week period, or, for areas where testing is done more than once a week, 75 percent of testing days that produce an exceedence of those standards.

(2) The development of source investigation protocols pursuant to paragraph (1) is not subject to Chapter 3.5 (commencing with

Section 11340) of Part 1 of Division 3 of Title 2 of the Government Code.

(b) Notwithstanding Section 7550.5 of the Government Code, on or before March 31, 2001, the state board, in conjunction with the State Department of Health Services, shall report to the Legislature on the methods by which it intends to conduct source investigations of storm drains that produce exceedences of bacteriological standards established pursuant to subdivision (c) of Section 115880 of the Health and Safety Code. Factors to be addressed in the report shall include the approximate number of public beaches expected to be affected by the exceedence of bacteriological standards established pursuant to subdivision (c) of Section 115880 of the Health and Safety Code, as well as the costs expected for source investigation of the storm drains affecting those public beaches. The report shall include a timeline for completion of source investigations.

APPENDIX 2 – CONTRACT REPORT

FINAL REPORT AB-538 STUDY

By

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INTRODUCTION

This report is in response to assess the needs for investigations to determine causes of beach waters exceeding AB 411 standards. The study was proposed with the following tasks, which are discussed in the number report sections:

I. Review of source investigations
II. Develop decision trees
III. Review of sources of contamination
IV. Review of methods to identify human and animal fecal contamination
V. Recommend additional tools, review of beach closure data, cost estimates and timeline for source investigations.

Attending meetings was a separately identified task in the contract, and more than 20 meetings were attended. They are discussed as appropriate in each section.

The overall goal of the study was to identify methods to determine the reasons that certain beach waters exceed AB 411 standards, in the hopes that sources can be identified and the total number of closures can be reduced.

The entire issue of beach water quality monitoring is very dynamic. Changes in the attitudes of investigators, new developments methods as well as findings have occurred through the study period, and will continue for the foreseeable future. The published literature, which is usually a good source of information, was less valuable in this investigation. Activities are developing faster than they can be published.

I - REVIEW OF SOURCE INVESTIGATIONS

Generally, source investigations are not published and are therefore not accessible through normal research techniques, such as abstracting services and journal abstracts. Often no reports are written and if they are they are usually limited to "Gray Literature." The existence of such reports is generally discovered by attending meetings or personal communication. Gray literature is becoming more available when it is posted on web sites, where is sometimes picked up by search engines.

To find the results of previous investigations various members of agencies responsible for monitoring beach water quality were interviewed. The Clean Beach Task Force, which frequently meets at the Southern California Coastal Research Project's headquarters in Westminster, was an important way of collecting information and obtaining feedback. Representatives from agencies as far North as Santa Barbara and as far south as San Diego were interviewed. Also, several presentations were made at Northern California meetings. Finally, Mark Gold from Heal-the-Bay gave us his collection of reports.

Table 1 summarizes the published source investigations. The references were found from an exhaustive search of the literature using search techniques at the *Citation Index* and *Current Contents*. Another source of information on beach monitoring is an edited book by Bartram and Rees (2000) which discusses various aspects of recreational monitoring programs ranging from bacterial quality to drowning to cuts from litter.

Table 1 is organized showing indicator organism in the left column, source and location, brief conclusion and reference (see the reference section for the full citation). Where possible the main conclusion was taken directly from the publication. In most instances the conclusion reported in the table. There are 21 references, mostly from 1997 and later, from 10 different countries. It is obvious that contaminated bathing waters are an international problem. The recent growth of papers in this area suggests that it is receiving more attention. Many of the results are not general or are contradictory. There are few general conclusions. Some conclusions are only of academic interest.

The group of indicator organisms is much greater than those used in AB 411. The papers show a trend away from classical indicators such as total coliforms, and investigate new indicators such as bacteria phages. Several studies used *Clostrida perfringens*, and there is mounting evidence that is may be a better indicator in warm climates, where fecal coliforms are reported to grow in soils.

Many of the investigators found that birds contribute to indicator organisms. Total coliforms were rarely reported as useful indicators.

Table 1. Summary of Studies of Current Methods and Past Examples of Source Investigations Performed to Identify The Potential Sources and Their Contribution to Bacterial Contamination or Recreational Beaches.

Indicator	Source and Site	Summary	References
FC, Salmonella spp.	River and coastal outfall, France	Rain events caused major discharges and increased concentrations of fecal bacteria in near coastal waters	Baudart et al., 2000
E. coli	Coastal water and sediment, Australia	The decay rate of E. coli was greater in water than sediment	Craig et al., 2000
FC, E.coli, Enterococci	Beach water, stormwater discharge and sewer overflow, New Zealand	Onshore (200mm depth) sources of contamination were important contributors to microbiological indicators	Lewis et al., 2000
Fecal sterols, caffeine, fragrance materials, PAHs, n-alkanes	WW plant effluent, agricultural and urban runoff, Canada and USA	Molecular markers were useful in elucidating sources of contaminants on a watershed scale.	Standley et al., 2000
B. Fragilis	Bathing water and sediment, UK	They proposed using the B. fragilis group and their bacteriophages as an improved, European-wide, indicator of bacterial pathogens	Bradely et al., 1999
TC, FC, E.coli, Enterococci	Storm drains, CA	Swimmers near storm drains had a higher risk of contracting disease than swimmers far from storm drains	Haile et al., 1999
FC, Enterococci Somatic coilphages F-specific phages	Partially treated sewage, Boston harbor, MA	Treated sewage is a significant source of phages to the harbor. No significant difference in phage number was found between summer and winter	Ricca et al., 1999

TC: Total coliforms; FC: Fecal coliforms; FS: Fecal streptococci; E.coli: Escherichia coli; B. fragilis: Bacteroides fragilis

Table 1. Continued.

Indicator	Source and Site	Major Conclusion	References
Coprostanol, Cholesterol Cholestanol	Sediment, Hong Kong	The fecal stanol/sterol ratio 5β/(5α +cholestanol) is a suitable parameter for detecting sewage pollution in sediment	Chan et al., 1998
TC,Somatic coliphages, Enterococci , F-specific coliphages	Bird feces, MA	Birds (pigeons, herring gulls, geese) can contribute indicator organisms and phages to Boston harbor, MA.	Ricca et al., 1998
LABs , TABs	Sediment, CA	LABs are good sewage markers in sediment, if the ratio of TABs to LABs concentration is less than 10.	Zeng et al., 1998
Male-specific bacteriphages(MSB)	Animal waste, human feces, and sewage, Rhode Island	Wastewater treatment plants are the principal contributors of MSB to fresh, estuarine and marine waters.	Calci et al., 1998
TC,FC,FS,E.coli, somatic coliphges, F-RNA phages,salmonella	Beach water , Spain	The microbiological indicators in bathing water presented high counts associated with early morning, overcast skies, low and high tides, groundswell, intense turbidity and in the presence of flotsam.	Serrano et al., 1998
E.coli, Salmonella, salinity,etc.	Sea water, South Africa	Use of salinity with a suit of microbial indicators was useful indicators problem areas.	Rathbone et al., 1998
B. Fragilis phages, Somatic coilphages, F-specific phages	River and sewage, Spain	B.fragilis phages were outnumbered by the other phages during recent pollution events, and had the most resistance to natural inactivation.	Araujo et al., 1997
E.coli	River, Czech Republic	E.coli is a major indicator of fecal pollution.	Baudisova et al., 1997

Table 1. Continued.

Indicator	Source and Site	Summary	References
FC, E. coli, enterococci, C. perfringens	Stream, Hawaii	C. perfringens was the most reliable indicator of sewage contamination, presumably because it is not found in soils.	Roll et al., 1997
FC, Coprostanol, Cholesterol, 24ethylcoprostanol, 24-ethylcholesterol, C. perfringens	Sediment and lake, Australia	Native birds were found to be a major source of the fecal pollution	Leeming et al., 1997
FC,FS, Somatic coliphages, phages of B. fragilis, C. perfringens	Marine sediment and Groundwater, Spain	Clostridium and phages of B. fragilis are better indexes of fecal pollution than presently used bacterial indicators.	Lucena et al., 1996
Presumptive coliform, Presumptive E.coli , streptococci	Bathing water and stream U.K.	Concentrations of fecal indicator organisms were enhanced after high rainfall. A captive water fowl population was a potential source of fecal pollution	Wyer et al., 1995
FC,TC, enterococci	Lagoon, CA	A significant source of fecal loading via the storm drain to a lagoon was remediated.	Gersberg et al., 1995
TC, FC,FS, coliphages, F-specific phages, bacteriophages of B. fragilis	Seawater, Spain	Fecal streptococci and E coli C bacteriophages were the most appropriate indicator of fecal pollution in marine waters	Cornax et al., 1991

TC: Total coliforms; FC: Fecal coliforms; FS: Fecal streptococci; E. coli: Escherichia coli; B. fragilis: Bacteroides fragilis

Much of the worldwide experience relates to sewage pollution, as opposed to stormwater pollution. Generally, sewage is reliably treated to secondary standards in California, and discharged in ocean outfalls. There are many situations in Table 1 where the investigators were attempting to track or determine the plume or dispersion of known sewage sources. For example, in Australia, most of the coastal plants have only primary treatment. By the end of the 1990s, ocean outfalls were completed, but prior to this time primary effluent was discharged near the beaches. As a result, certain beaches were permanently closed. Nearby beaches were monitored to determine the dispersion of these known sewage source. The source of the indicators could more reliably be inferred as sewage than spurious sources such as soil or wetlands. This is a simpler task than determining the existence of a source and its nature, as required by AB-538 source investigations.

There are also chemical markers shown in Table 1. For example, coprostanol, detergents (LABs, TABs) and caffeine are abundant in sewage and treated sewage. They should generally not be present in stormwaters or receiving waters that are not contaminated. Therefore their presence suggests sewage contamination. If they are used in conjunction with other markers, such as fecal coliforms, they can help confirm the source of the indicator organisms. For example, fecal coliforms found with caffeine or other chemical markers are more likely to be from sewage sources, as opposed to spurious sources, such as soils (especially in warm climates).

The brief summaries presented in Table 1 should serve only as a guide to researchers. The original papers should be consulted if specific information or recommendations are required. The research team obtained copies of all the papers. If a copy of a particular paper is hard for a reader to obtain, they can request it via the email address shown on the cover page. The interim versions of the reports have been listed on the web site (www.seas.ucla.edu/stenstro). The final report will be listed there as well, and possibly other information as it becomes available.

Reports from local agencies that contained helpful information were also found in our searches. The reports are unpublished and can only be obtained from the agency. Libraries do not retain them, except in rare instances (SCCWRP has one of the better collections of gray literature).

At an early meeting, it was recommended to the research team that several of the reports be summarized so that the major findings would be available to others. The executive summaries of the reports are contained in Appendix A. The summaries should be viewed as examples of procedures to follow and in some cases of procedures that may not work. The summaries also contain contact information in order for the readers to obtain additional information.

The major conclusions are presented in the following list.

1. Santa Monica Canyon Storm Drain

   This study occurred in 1994/5 and examined high coliform counts from Santa Monica Canyon Storm drain, and was performed by the City of Los Angeles. The study found no consistent sources of sewage spills, and concluded non-point sources were possible problems. Intermittent discharges of soil, debris and accumulated sediment from storm drains were observed. Horses are boarded in this watershed, and the study authors

recommended that an educational program be developed to prevent non-point source pollution horse stables. No attempt was made to type the source of coliforms (animal vs. human origin) and procedures may not have been available in 1995. In Task IV, methods to identify the origin of indicators are reviewed, and such methods may have been helpful for this study.

2. Rincon Creek, Santa Barbara

This study was conducted by Heal the Ocean and Santa Barbara County and was designed to determine the sources of fecal coliforms in beach waters around Rincon Creek. It is an example of the use of advanced techniques to identify the source of organisms. The investigators found human, duck**,** dog, horse, cat, cow, and sheep matches. The study illustrates the problems of using advanced techniques, such as expense, sample preservation and transportation to a distant laboratory and communications. It is also important to note that the risk assessment data for coliforms from dogs, cats, etc. are not known, which means that health regulators must treat the risk the same as if the indicators were human origin.

3. Agua Hedionda Watershed

The study was conducted by URS Greiner Woodward Clyde, in association with Motibe Laboratory Services and the University of Washington, for the City of San Diego and Co-Permittees Stormwater Program as part of the 1998-1999 monitoring activities. The watershed is 29 square miles and contains several sub-watersheds. Ribotyping was performed to identify the source of the coliforms. Matches were found to humans, dogs, cat and wild animals. Direct analysis for pathogens was also performed and many were found. The study makes strong conclusions about the value of using several methods in combination to identify the sources of contamination.

4. San Diego Beaches

The County of San Diego Department of Environmental Health (DEH) and the City of San Diego cooperatively initiated "an investigation of the sources of Fecal Contamination of Four San Diego Beaches" in spring 1999 to determine the sources of fecal contamination at four beaches considered to be representative of coastal area throughout San Diego County. The study used ribotyping techniques and found matches for humans and other animals. The study concludes that the DNA methods were useful and their development should be continued. The study also supported continued use of BMPs.

5. Orange County J03P02 Watershed

The study was conducted with the Orange County Health Care Agency, researchers from UCI, USC, UCLA and the Southern California Coastal water Research Project (SCCWRP) to identify sources of bacteria in the J03P02 watershed. The study used the most advanced techniques, such as virus detection, toxin biomarkers, and chemical markers. The results are

inconclusive in that no markers were able to identify the source. The results of several of the methods suggested no sewage pollution.

The proceeding examples show the difficulty in performing source investigation. None of the investigations positively identified sources. All produced information that helps us understand aspects of the problems encountered in source investigations.

In defense of source investigations, it should be noted that many of the people interviewed had successfully identified sources. Most were potentially serious problems and resulted from leaks. These success stories are not written into reports, and remain somewhat unknown.

II – DECISION TREES

Based upon the information obtained in Task I and other information obtained from interviews, tools were to be developed to assist in future investigations. Two decision trees were envisioned in the initial contract.

After initial discussions with agencies involved in beach monitoring, it became clear that the important tasks in conducting source investigations, especially if there is any urgency in finding the source, is preparation. The extreme efforts in the Huntington Beach investigations in 1999 are a good example.

This example is important in part because it was one of the first investigations triggered by the new AB 411 rules, but also because it is a high profile area. It is also important because of the complex relationship among the various agencies: the Orange County Sanitation Districts, the City of Huntington Beach, the Orange County Health Care Agency and several independent contractors. The investigation was made with extreme urgency due to financial losses associated with the beach closure. In the process of this investigation, it became clear that there were overlapping and conflicting responsibilities in conducting the investigation.

The Huntington Beach experience is probably atypical in that the Sanitation Districts was able to take a leadership role with experienced personnel. Source investigations in other areas may not have this resource.

To avoid such issues in the future, it is necessary to prepare for investigations. Preparation can be done at a routine pace before problems occur, as opposed to an urgent pace after a problem is being investigated. To describe the required planning, a third decision tree was introduced.

Table 2 shows the steps that are envisioned for planning. This table was taken from the PowerPoint presentations made at several clean beach workshops, and has been extensively reviewed.

The first step is to decide who has the authority and responsibility to conduct source investigations. This may seem like a simple task but can become very complex. There are numerous opportunities for "finger pointing." It seems clear that the health departments or health care agencies, who perform the actual monitoring, will be announcing the occurrence of conditions that trigger an AB-538 source study. After this step, it not so clear how things will proceed.

The source investigations will potentially involve several agencies, as illustrated by the following list:

1. Health Care Agency - *Detection of high indicator organisms and beaching posting or closures. Decisions to increase or accelerate monitoring.*

2. Local and State water resources control boards – *May have received spill reports or other reports that may suggest a spill. Also responsible for enforcement and legal action.*

3. County departments of public works – "*owns" stormdrains and responsible for their maintenance. May have the most extensive knowledge of the stormdrain system, which may not be codified but only in the experience and memories of key personnel*.

4. Local cities – *may "own" catch basins and storm drains in the same drainage system as the county agency*. *May manage facilities such as beach piers that are potential sewage sources*. *May also operate treatment plants and have their own ocean monitoring systems and resources.*

5. Sanitation districts – *will have responsibility for local sewer system, treatment plants and may have an extensive monitoring system and capabilities*.

6. Industries – *industries with NPDES ocean discharges such as power plants and petroleum refineries are potential sources.*

7. Non Governmental Groups – *may have extensive knowledge due to long term involvement at particular sites, may also be able to influence public opinion to create a consensus for action*, *may also have volunteer scientific support or technical staff with related knowledge or ability.*

8. US Army Corp of Engineers. –*may have multiple roles.*

9. Home owners associations – *may have responsibility for maintaining BMPs, or may be the involved with septic tanks*.

The above list is by no means exclusive. There are other potential parties. Some of the parties described above have regulator roles or may motivate action. Others are responsible for action. There is potential for direct conflict, such as joint involvement in the storm drains, and there are incentives for one agency to determine that the problem belongs to another agency.

Table 2 suggests that the various procedures required to conduct source investigation be determined in advance. By determining them in advance, they will be done with less expense to the agencies or parties, and also without the possibility of "coloring" decisions with an event.

It seems clear that the health care agency will be doing the monitoring. The public works agency will probably have the most involvement with the storm drains. The regional boards will probably have to insure that the planning occurs and take overall regulatory responsibilities. Even at our meetings, with representatives of many of the parties, there has not been a consensus for delegation of responsibility and authority.

In the course of our work it also became clear that the ability to contract for services, such as lab services, maybe rate-limiting. Relationships with labs should be developed in advance.

Procedures are needed to allow the source investigation team within each participating agency, to contract for services without delay.

The fourth item in Table 2 is a badly needed tool for the source investigation team. Many public works agencies and cities use Geographic Information Systems (GIS) to manage information about their infrastructure, such as the location of sewers, water mains, storm drains, etc. There has been a continuing effort over the past 10 years to list all utilities in a GIS. Various "layers" are created, and each layer is specialized to a particular subject, such as storm drains. The GIS operator can place various layers in front of a detailed map. In this manner is possible to locate various structures relative to other structures, such as sewers and roads, etc. Alternatively, is possible to locate a structure using the GIS and a global positioning system (GPS) receiver.

The existence of these GIS systems can be very helpful to source control studies. Unfortunately most GIS systems are maintained on workstations and desktop computers, which are not available to field investigators. Also the personnel responsible for the GIS may be in a separate department from the personnel doing the source investigation.

An important and strong recommendation of this report is to create GIS systems on laptop computers for source investigators. Programs such as Arc View (ESRI, Redlands, CA) can easily run on lap top computers.

The creation of the GIS tool depends upon two factors: the first is simply the programming effort to develop the GIS; the second is more difficult, and is the actual knowledge of the locations of structures that are important to the source investigation team. Fortunately the second factor has been largely accomplished in many cities or counties, but the data may be grouped in layers that are not useful to source investigators.

To create the proposed tool, it will be necessary to create a new layer or layers that are optimized for source investigators. In the various meetings, structures such as sewers, storm drains, fire departments, beach rest room facilities, maintenance yards, have been proposed. There will be site specific issues associated with each area, but it is clear that such a tool will help source investigators.

GIS systems also have the ability to store data with spatial references. For example, the database can be programmed to appear with a structure, such as a storm drain. The user clicks on the object (storm drain) and a menu of table appears that shows previously collected data.

This sort of tool will be extremely valuable for investigators. Often the only way of detecting a spill is to note a difference in current conditions from previous conditions. Changes in flow rate are extremely important, as well as aesthetics (turbidity). The GIS tool described above can be used to compare existing conditions with previous conditions, and allow investigators to make field decisions.

The development of these tools may not be very difficult. Many cities have used consultants to develop their GIS systems and train their employees. Often, a great deal of knowledge about a city or county was obtained by a single consultant. Therefore, it should be easier for them to

integrate their various GIS layers into an optimized tool for source investigators. The same GIS tool can be used to manage new data as it is collected in the source investigation.

Table 2 also suggests "fire drills." This means that representatives from the various participating agencies need to meet and work through the procedures. Problems will be avoided if this is done.

Table 2. Planning Decision Tree

Planning – First Item on the Decision Tree

- **Determine responsibilities – coordination among agencies.**
- **Who is in charge, who makes the announcements**
- **Obtain authority to spend, and decide who pays**
- **Develop background information**
  - **GIS layers – those doing beach monitoring seldom have the most complete or efficient knowledge of local sources "in some senior person's memory, who retired last year."**
  - **Most data exists in machine-readable format, but never in one place or accessible in the field. Need a simple "beach monitoring layer" on a PC running ArcView or similar program.**
- **Agencies need to practice "fire drills" to work out their procedures.**
- **Work through areas of joint responsibility beforehand**

Table 3. Generic Plan of Action Tree

Plan of Action (Tree)

High counts trigger a source investigation

Health Dept Personnel

Notify appropriate agencies and individuals. Request system checks from the "usual suspects." Obtain approvals

Public Works Personnel

Sample the most probable sources

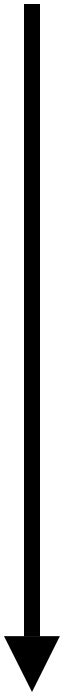
Choose a subset of sources based upon results. Select additional Markers, such as chemicals (e.g., ammonia), aesthetics (odor, color) and samples for BactT/virus analysis

Begin "creek walk" or source walk campaign using common sense and a portable lab (in a van, etc.) using real time indicators (pH, turbidity, ammonia, conductivity, UV absorption, etc)

Who is the conductor?

Table 3. Continued.

Plan of Action (Tree) Cont.



Put results into a database with a GIS front end, with layers as described before. Archive results for future surveys

Continue searching and eliminating potential sources

Create a record so that in future surveys you can see exactly what you found in previous surveys

If progress is still lacking, accelerate other types of BMPs, such as sewer replacement programs

Collect more samples that you intend to analyze. Make decisions later on whether or not to do bacT analysis on samples. Modify analysis protocols as needed to facilitate measurements.

Table 3 shows the way a source investigation may be conducted. It is generic for both wet and dry weather, but has more applicability to dry weather. The first event is the decision to trigger a source investigation, as required by AB-411. This will most likely be done by the health care agency, as shown on the table.

The actual investigation will most likely be performed by representatives from public works agencies. The first step should be to determine if a spill has occurred. There are procedures that require notification to regional boards etc. In theory this process will disseminate the information , but in practice it may not. Therefore, as part of the planning process, the source investigators should communicate with the probable sources of spills or contamination ("usual suspects"). This process may have added benefits. If a sanitation district is notified of a source investigation, it may decide to review its own operations and may find a spill or problem that it may have otherwise not noted until it became more serious. For example, sewage flow rates are so varied that only a massive spill would be detected by reduction in flow rate. If the sanitation district knows of a new source investigation, it may choose to send observers or use other means to detect spills.

The next step is to sample the most probable sources. By "probable" we mean likely sources of contamination, such as a storm drain entering a beach. These more likely sources should be eliminated from suspicion early in the process. Hopefully, analysis of one of the sources may be helpful in directing the immediate future action of the investigation.

In an ideal situation, a source investigation can begin and the team can work up the storm drain to look for spills or unusual conditions. Santa Barbara County already has such a practice and calls it a "creek walk." Similar things have been done in Los Angeles ("creek drive"). There is an opportunity to sample and eliminate tributaries from the investigation if the sampling team is equipped with a portable laboratory, such as a lab van.

It is important to sample parameters other than indicator organisms in the continuing investigation. Parameters that are easily identified or measured in the field are required, even if they appear to have no relevance to indicator organisms. The team will be looking for changes from previous conditions. Changes can be detected by conductivity, turbidity, flow rate, ammonia, pH, dissolved oxygen (DO), among others. These parameters are also easily measured in the field with kits or probes costing less than $1,000. Values obtained can be compared to the historical average, which can be obtained from the GIS tool. If no historical averages are available for that location, the values can be compared with typical values for that type of source (e.g., storm drain water quality). The GIS tool can also be used to input newly measured parameters for future use and to create a history of a particular location. It is also important to note that the reliability and precision of a parameter measured in a source investigation does not need to be the same as laboratory measurements used for compliance regulation. Less expensive and precise are suitable, and portable and rapid techniques are preferable.

It is likely that the investigation will not find the source. This is because spills and illegal discharges are episodic. Therefore it is essential that the team be able to record their progress be able to pick up where they left off, if a future event occurs. Source investigations should be

regarded as detective work, and may require considerable effort during several investigations to determine a source.

Table 3, in the second page, makes an important recommendation for situations where investigations are not successful in determining the problem. If AB-411 conditions are routinely violated, and source surveys cannot determine the problem, other BMPs, perhaps longer term BMPs, should be accelerated. The best example is sewer cleaning and repair.

Tables 2 and 3 were presented at several clean beach workshops and in some version been on our web site for review and comment since March, 2001. At the last clean beach workshop attended on June 20, 2001, Figure 1 and Tables 4, 5, 6 and 7 were presented as final versions of the decision trees. The process described in Figure 1 includes the previously cited concepts and captures the sense that many of problems with high indicator organism counts will become chronic problems.

Tables 4 through 6 are similar to the previously reviewed concepts and presents them in more precise form. Table 7 presents the concept of accelerating BMPs, especially long-term BMPs in areas that chronically violate AB-411 standards. Sewer maintenance programs are one of the important recommendations in Table 7. Communities in financial difficulty often defer sewer maintenance, and this can be a successful strategy in times of short-term financial difficulty. Continued neglect of sewers will always result in problems, which include blockages and subsequent overflows, possibly to storm drains.

Leaking sewers are another problem. Normally sewers leak from the outside to the inside, which usually called infiltration. Infiltration results in excessive wet season flow to the treatment plant.

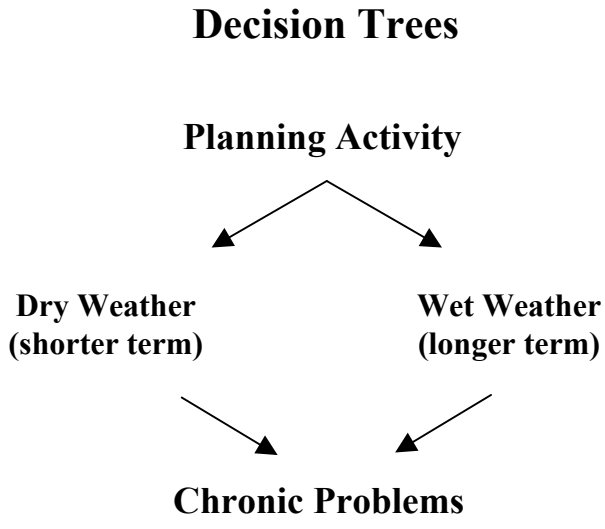


Figure 1. Overview of Final Decision Trees.

Table 4. Planning Phase

Planning Phase

- **Determine responsibilities among agencies**
- **Define lead positions**
- **Establish budget, authority to spend, write general PO's to labs or consultants, do other preparations**
- **Create GIS layers of monitored areas, suitable for use on laptop computers**
- **Train key personnel and establish continuing training program**

Table 5. Dry Weather Tree

Dry Weather Tree

- **Continuing high counts trigger a source investigation**
- **Key personnel in HD notifies appropriate agencies and individuals**
- **Check likely sources (phone calls placed to RWQB, sewer maintenance agencies, sanitation districts, etc.)**
- **Increase beach monitoring to track problem (if it disappears, modify source identification program)**
- **Begin sampling program to identify sources. Measure indicators and WQ parameters (NH4, UV, pH, conductivity, turbidity), observe/measure flow rates, and other observations**
- **Record inputs to GIS/Database for real-time observations**
- **Continue investigation with "creek walk" program until source found or spill ends**

Table 6. Wet Weather Tree.

Wet Weather Tree

- **Continuing high counts trigger a source investigation (HD responsibility)**
- **Key person in HD notifies appropriate agencies and individuals**
- **Review existing monitoring data to reveal a problem or strategy, and compare to previous investigations**
- **Meet to establish a longer term monitoring program of important sources and tributaries**
- **Collect data and input to GIS/data base to map the problem. Perform simple modeling such as done by Wong et al. (1987), LA County DWP, SCCWRP, etc. to establish mass balances**
- **Rank drains, tributaries and other sources**
- **Refine monitoring program to understand highly ranked sources**
- **Rerank and define BMPs**

Table 7. Long Term BMP Acceleration Program.

Long Term BMP Acceleration

- **Sewer Maintenance Program**
- **Septic Tank removal or reduction program**
  - **Cleaning/Inspection ordnances**
  - **Removal/replacement schedule**
  - **Will require additional legislative "teeth"**
- **Hydrology improvement program (upstream retention, etc)**
- **Other structural BMPs (screens, inserts, accelerated cleaning, etc.)**

Excessive wet weather flow can cause the treatment plant to bypass the excessive flow. Routine maintenance is required, and sanitation districts usually televise sewers by dragging cameras through sewers. Large districts often own this equipment and have trained personnel. Others have use contractors. Sewer maintenance is a large business and the techniques can be applied to storm drains as well.

Another long-term issue is septic tank replacement. Septic tanks are often excellent methods for sewage disposal. In rural areas with appropriate soil type and ground water table, they present no environmental risk, for municipal sewage. As communities develop, housing density increases and the assimilative capacity of the area are exceeded. The nature of disposal changes and essentially raw sewage may escape to the environment. Under these circumstances, septic tanks must be replaced by technologies that require less infiltration.

III - REVIEW OF SOURCES OF CONTAMINATION

There are many sources of bacterial contamination. The most important source is domestic sewage. Human wastewater contains between 10^7 and 10^{10} total coliforms per 100 ml. It is useful to consider the magnitude of this potential contamination using well-established design criteria (Metcalf and Eddy, 1992).

Per capita sewage flow ranges from 45 to 65 gallons per day, for low income to luxury homes, respectively. Apartments and condominiums are approximately 40 gallons per capita-day. Unsewered areas average 55 gallons per capita-day. Fecal solids, which are the source of most of the pathogens and bacteria, average 0.26 pounds per capita day. Using an average occupancy rate of 2.8 persons per household, a typical sewage flow rate is 154 gallons per day. Therefore the untreated sewage from a single household could contaminate 58 million to 58 billion gallons of beach water to a concentration of 1000 MPN/100ml. This is a staggering number. To put into perspective, it is useful to relate this number to the length of beachfront. If the "ankle deep" surf zone, where most monitoring occurs, extends 1000 feet wide by 5 foot deep, each linear foot of surf zone contains 5000 ft^3 or 37,000 gallons. If we divide this volume into the above numbers, we obtain 0.3 to 300 miles. This is an extraordinary volume of beach water that can be contaminated by a single household. The example is simplistic in that it assumes all the wastewater reaches the beach and is evenly distributed, and that no decay processes occur. Never the less, it is useful to show how important sewage sources are to beach pollution.

Sewage sources include septic tanks, broken sewer lines and boats, which have on-board bathroom facilities. The calculation suggests that an enclosed harbor, such as Avalon's, could be contaminated to beyond AB-411 standards by a single boat. When agencies are trying to reduce beach contamination, elimination of sewage sources has to be the first priority.

Abandoned or aging infrastructure is of particular importance. In conducting interviews, several instances of contamination from abandoned sewer facilities were found. In these cases, the sanitation district assumed there was no risk because the pump station had been removed from service. Unfortunately, the removal was incomplete and sporadic leaks occurred that were not related to rainfall. Abandoned sewer lines, pump stations and toilets in public areas are an important source, and should be investigated and eliminated as sources early in any source investigation.

Direct input from animals and humans are another potential source. Indicator organisms from animals were identified in several studies described in Task I in the gray literature section. Again, knowing that the source of indicator organisms is from dogs or horses etc. is of primary value in developing the BMPs, as opposed to knowing risk. Indicator organisms such as fecal coliforms and enterococci are found in most farms animals, and especially pigs, at densities equal to or greater than the occurrences in humans (Bartram and Rees, 2000, Chapter 9).

Birds are an especially important animal source, because of their abundance near beaches. No quantitative information is available to compare to the above example calculations. A comparison could be made if the mass of fecal material and bacterial densities are measured. The review performed in Task I shows that many investigators implicated birds in their

investigations of high indicator organisms in beach waters. The knowledge that the source is birds is of limited value. It helps in choosing BMPs, but does not reduce the risk. It is the consensus of the health professionals interviewed in the project that indicator organisms from birds must be treated the same as indicators from humans, until further information is developed.

Grant et al. (2001) implicated bird feces, at least partially, in their study of Huntington Beach postings. Marsh water exposed to wet bird feces had enterococci counts from 9,000 to 24,000,000 MPN/100mls (10 trials). Marsh water exposed to dry feces had counts from 100 to 242,000 MPN/100 ml (10 trials). The conditions and other information (e.g., bird densities, tidal velocities, etc.) they report could be used as a first approximation for bird contributions at other locations.

The source book for the American Veterinary Medical Association (1996) reports demographics for dogs, cats, horses and birds. Nationally they report increases in cat ownership, stable dog owner ship, decreasing horse ownership and fluctuating bird ownership. They report that fewer households own greater numbers of pets. Pet ownership is decreasing in California. They estimated 3.6 million and 3.4 million California households owned dogs and cats, respectively. Approximately 70% own a single pet, with less than 9% owning more than 3 pets.
It is probably not a surprise that they do not report the amount of fecal material and its pathogenic content. Therefore it is difficult to make a quantitative estimate of the risk to beach waters. Cats generally use indoor litter boxes, while most dogs are walked. Most communities require dog owners to clean up after walking their dog. This is a good policy and should be universally required. If dogs presented only 10% of the risks of humans on an individual basis, it would still be high. Inputs from dogs are probably more important in wet weather. In dry weather, there is no transport mechanism from grassy areas and parks, preferred by dog owners, to beaches.

Horses ownership is California is much lower than dog and cat ownership. Only 1.7 California households owned horses, totaling 676,000 animals. Ownership is probably very localized, with rural areas, like the San Joaquin having the greater ownership. Horse ownership on a specific basis probably presents greater risk to beaches. The mass of fecal material from dogs and cats can be easily disposed with municipal refuse. Horses create much greater mass and are most often allowed to free range, which disperses the fecal material. Again, the risk is probably a wet weather phenomenon, due to transport mechanisms. Managing equestrian areas to prevent stormwater pollution is probably worthy of special BMPs development and enforcement.

Generally one should assume that animals represent the same risk to beach water as humans. Indicator bacteria densities are similar to human densities. Pathogens have rarely been measured but should be assumed to be the same until further information is known.

IV - REVIEW OF METHODS TO IDENTIFY HUMAN AND ANIMAL FECAL CONTAMINATION

Appendix B contains an extensive review of methods for identifying human and animal fecal contamination in stormwaters. The review is divided into chemical methods, bacteriological methods and virus methods. There is no easy, low cost method for differentiating between human and non-human sources of bacterial contamination. No single indicator or approach is likely to represent all the facets and issues associated with contamination of waterways with fecal matter.

Genetic typing of E. Coli has been used in several Southern California source investigations. Often the result just verifies what investigators already suspected – the coliform sources are varied, from different animals and humans. In the case of Rincon Creek (Santa Barbara County), genetic typing was considered successful in showing that septic tanks were an important source of human indicator organisms. This type of source investigation may have greater value in developing a body of evidence to force changes in the watershed, than to actually identify the source; investigators may already know the source from their previous experience and a large body of antidotal information.

At present, the best hope of distinguishing fecal pollution of human and animal origin is an appropriate combination of indicators. Statistical analyses of appropriate groups of methods offer the best possibility of identifying human sources. Unfortunately, relying on a combination of methods will probably require a longer period of analysis than relying on a single method. A combination of methods may be useful to determine sources in chronic situations as opposed to episodic events.

Many promising methods have been identified in this review. None, at least at the time of this writing, has been demonstrated in a full-scale monitoring program. Most techniques have been limited to research laboratories, or a few special monitoring programs. Such demonstrations are needed to demonstrate the utility of the methods. Also, commercial laboratories will need assurances that the required investments in training and equipment are justified and recoverable.

A good course of action to further this technology would be to conduct several, long-term investigations, where an advanced method is used in parallel with existing monitoring techniques. Monitoring agencies will need to be involved, in order to evaluate the required retraining and adjustments in their current procedures.

It is useful to make several observations after the literature. The first relates to enterococcus for monitoring. The closures at Huntington Beach generated a number of special monitoring efforts and task forces to interpret the results. Professor Stanley of UCI analyzed and presented the enterococcus data at several clean beach meetings. An important finding is the diurnal variability in counts. The enterococci concentration (per 100 ml) routinely exceeded 100 during the dark hours of the May 4 to May 17, 2000 monitoring period. During daylight, 100 was exceed only twice in the same period. The references reported in Table 1 also noted increased enterococci counts on cloudy days. This suggests that UV radiation is destroying organisms, which is

generally well known, and has been developed into a technology for disinfection at water and wastewater treatment plants. The variability has important implications for AB-411 monitoring.

The times for monitoring are not explicitly stated in AB-411, and it is probably impractical for agencies to monitor at specific times. It is also possible for an agency to choose times which minimize enterococcus counts in order to avoid beach closures and posting. A further question relates to the activity of the organisms themselves. There are "dark period" recovery mechanisms for enterococcus and other bacteria. It is possible that diurnal fluctuations observed at Huntington Beach are due in part to the ability to detect UV disinfected or "wounded" indicator organisms, that eventually will recover during dark periods. This suggests further research is required.

Another observation is that most beach postings are due to high enterococcus counts, and that most are for a single day. It takes approximately 24 hours for to sample, analyze, and post a beach. Therefore a number of interviewed health professionals felt that they were not providing a public service by posting a beach after high enterococcus counts, only to discover that analysis for the next day showed the beach was within standards. This is discussed later with the statistical observations.

Both phenomena suggest that more research needs to be done to improve the monitoring procedures using enterococcus. A real-time procedure is badly needed. The diurnal variation suggests the need for composite samples.

Most of the health professionals interviewed believed that low total to fecal coliform ratios were good markers for sewage pollution.

Also discovered in the review is a different philosophy for beach grading. In New Zealand and Australia, they have a great deal of experience in monitoring beaches. This may be in part because they do not have the benefit of required secondary treatment. Their ranking procedure (9^{th} draft, dated May 30, 2001) is provided in Appendix C. It has useful ideas and deals with the inability to measure beach contamination in real-time.

V – ADDITIONAL TOOLS, REVIEW OF BEACH CLOSURE DATA, COST ESTIMATES AND TIMELINE FOR SOURCE INVESTIGATIONS

Additional Tools

Additional tools are clearly required to facilitate source investigations. Source investigations will generally not be single events that lead to definite conclusions. They will be a series of individual investigations when combined gradually create knowledge about a watershed that will allow investigators to eliminate sources and pint point contamination and problematic areas. A great deal of work has already been performed and most obvious sources have already been identified.

The most important needed tool is a GIS/data base to allow investigators to reference source studies to known structures and human activities. It will also be used to catalog previous data to allow field comparisons to current data. Source investigators will use changes in water quality data and flow rates to locate sources. Water quality parameters in additional to bacterial quality will be useful. Easily observed parameters (e.g., turbidity) will be among the most valuable. This tool will be valuable even if the types of indicator organisms or standards change. It could also be valuable for other functions such as scheduling maintenance.

The GIS tool can be developed by civil engineering consultants who can access the existing datasets that many cities have already developed. The key requirements are:

1. Portability – must be usable from a laptop computer in the field
2. Accessibility – previous data must be easily accessible by "clicking" on an object
3. Updateable – the database of water quality parameters must be updateable in the field. QA/QC can be performed after the laptop is returned to the home office. After QA/QC the master database can be created on a server that can be used to refresh the copies on laptop computers.
4. Training – after database development, it will probably be necessary to train users for 1 to 2 weeks in how to use and maintain the portable database. A database professional will be periodically needed to maintain the master copy and update or refresh portable copies.

The next most important tool is a better way of measuring indicator organisms. The current situation, whereby the time of the sample may determine if exceedences occur, is not acceptable. If the enterococci concentration varies at all beaches as it does at Huntington Beach, then the purpose and philosophy of AB-411 monitoring could be largely defeated by collecting samples only in the early to mid afternoon. Compositing may be a good alternative, but requires development and additional resources from monitoring agencies. Perhaps compositing could be used for beaches that have high frequency of 1-day exceedences of AB-411 standards.

Almost everyone interviewed suggested that a faster or real-time indicator is needed. Real-time is defined in control engineering and other fields in terms of process. A variable measured in real-time is measured in time to take needed control action, which in this case may be posting a beach or collecting additional samples. An indicator that provides a response in 1 to 2 hours was recommended.

This is difficult to do with current methods, which are based upon an organism's ability to growth under specific conditions (i.e., substrate, temperature, etc.). Organisms have finite growth rates and 24 to 48 hours are required. Chemical methods that do not require organism growth for detection are necessary. No methods or proposed methods were found that fulfill this requirement. Clearly additional research, development and field-testing are needed.

There is also interest in determining the distribution of various Enterococci species. Ent. Faecalis and Ent. Faecium are most often associated with fecal contamination. It would be useful to determine if the exceedences of AB-411 are caused by these two subspecies or if Ent. Casseliflavusm is also responsible for exceedences. Ent. Casseliflavusm is less strongly associated with fecal contamination.

Review of Beach Closure Data

Beach closure and posting data were from the various reports obtained from the State Water Resources Control Board and others were for the years 1992 to 1998. This data should be viewed with caution because monitoring methods were not standardized and each county had slightly different standards for posting and closures. Also the number of closures varies because of beach definition.

San Diego County had by far the greatest number of closures. Beach closures ranged from 40 to 70 per year and beach-closure days (number of beaches times the number of days each is closed) ranged from a low of 400 to more than 1200. San Mateo, Los Angeles and Orange and Santa Barbara followed in the number of closures. Monterey, Ventura, Santa Cruz and San Luis Obisbo had few to no closures. Data were not available for other counties.

The number of beach closures was proportional to rainfall, with greater numbers of closures occurring in years with greater rainfall. Normalizing the number of closures in each county by the average rainfall in the county had little change on the ordering of closures. San Diego still had the greatest number of closures, but the other closures in the other counties were nearly equal.

It is important not to conclude that the water quality in San Diego is worse than the other counties. The way data were collected do not allow for such a conclusion. Counties with greater numbers of closures may have had more thorough monitoring programs. Also, it was not possible to standardize the closures by the number of beaches in each county. San Diego has by far the greatest number of separately defined beaches, which may account for the greater number.

The most valuable conclusion from this analysis is that beach closures during this time are proportional to rainfall. This indicates that stormwater itself is causing closures (a generally held belief among everyone interviewed in this study) or that stormwater causes spills or upsets to sewers or wastewater treatment plants. A second observation is that the increased monitoring frequency and stricter rules associated with AB-411 will increase the frequency of exceedences, even if the beach water quality does not decline.

Staff from the Southern California Coastal Water Research Project (SCCWRP) are active in analyzing beach water quality statistics, and have been analyzing various aspects of contamination on coastal waters for 30 years. Leecaster and Weisberg (2001) examined sampling data from 24 sites in Los Angeles County over the five years (1995 to 1999). These stations were sampled five days per week. They found that 70% of the water quality exceedences were single day events and less than 10% lasted 3 days or longer. Only 0.1% of the exceedences were attributed to sewer leaks. They also performed a simulation using the data set. By choosing a reduced frequency (i.e., 2 per week, 1 per week, 1 per month, etc.), they showed that the number of exceedences was reduced by approximately the sampling frequency i.e., sampling only 3 times per week should miss 45% of the exceedences. This result should be expected if most events were single day events. Less frequent sampling would still catch longer events.

They also predicted that the monitoring required to meet AB-411 will increase the number of exceedences. Therefore, an increase in exceedences from pre to post AB-411 techniques should not be interpreted as declining beach water quality.

These results are useful in understanding the need for more rapid analysis. The chronology of a single, one-day exceedences might proceed as follows:

1. On the morning of the first day, a sample is collected and sent to the laboratory for analysis.
2. On the morning or afternoon of the second day, a high result is obtained, the beach is posted or closed, and a second sample is taken.
3. On the morning of the third day, the second sample is analyzed, a low result is obtained and the beach is unposted or opened.

This simple chronology demonstrates the concern of many of those interviewed during the study. In the 24 hours following the collection of a sample that exceeds the standards, the beach is unposted. During the 24 hours following the collection of a sample that does not exceed standards, the beach is posted. Clearly there is need for faster analysis, which can avoid this problem.

There are several alternatives that are possible using existing technology. Simply collecting and analyzing duplicates and averaging results will help reduce sampling and laboratory-created variability. Alternatively, additional samples can be collected later in the day from the first sample. These samples are not analyzed unless there is a problem with the first sample. Another possibility, which can be performed with both MPN and membrane counting techniques, is to observe the progress of fermentation or colony growth before the required elapsed time. It may not be necessary to wait the required time to determine that an exceedence will occur. For samples that are showing positive results early in the incubation, a second sample can be collected earlier the next day, or another sample can be analyzed. To further develop these ideas, representative groups from monitoring agencies, regulators and laboratories will need to meet and determine the value and difficulty of alternative techniques.

Cost and Timelines

The cost of source investigations will be highly variable. There will be costs associated with laboratories, survey personnel, and permanent equipment. The work will be episodic and smaller organizations may need to share responsibilities with workers who have other primary responsibilities. Only in the largest organizations will it be possible to have dedicated personnel for source studies.

Earlier it was noted that a GIS/database tool was needed to assist in source investigations. This sort of project has been performed in our research group and should take 1,000 person-hours or less. The time consuming part of this will be collecting the data and adapting it for this special application. This will need to performed with the database developer (i.e., consultants) and the organization that will do the investigations (i.e., departments of public works). It will most likely proceed as a series of meetings with information exchange followed by periods of data input by the consultant and data collection by public works. For organizations that already have GIS layers, the process should take 4 to 6 months. For smaller groups that have no GIS layers, it may take much longer and include collection of raw data (surveys, ground truthing, etc.). This data collection will have value in addition to this purpose, and should be justified by its overall value, not just its survey value. One potential problem is that Civil and Environmental engineering consultants, public works organizations, and sanitation districts are dramatically understaffed at present, due to the lack of new graduates.

The cost of doing a creek walk or creek drive as described earlier is estimated using a team of three people. Safety is a concern, which is one reason for selecting 3 people. A van equipped with a small mobile laboratory is needed. Portable instrumentation such as DO, pH, ammonia, temperature, salinity/conductivity, turbidity probes and flow meters along with a GPS GIS/database computer, camera and communications system (cell phones) will be necessary. The van will cost in the range of $30,000 to $35,000. $4,000 is a good estimate for database computer and software. The various probes may cost another $5,000 with the GPS costing another $1,000. The direct capital cost will be in the range of $40,000 to $45,000. Each agency will have an indirect cost rate that may vary as much as 30 to 100%. Expendables for lab supplies, test kits and sample bottles will be in the order of $200 to $400 per day. Training will also be necessary and includes safety, laboratory techniques and database/GIS techniques. It is possible to specialize the team (driver/sampler, chemist, computer operator)

A single team working an 8-hour day, should be able to sample 12 to 24 locations. This assumes 2 hours devoted to set up and shut down and 2 to 4 samples per hour. The terrain will dictate speed as well. The team will need the assistance of a traditional laboratory, which will receive samples at the end of the day for other analysis. The mobile team will need to be trained to decide when to take additional samples for analysis.

It is important to understand that most source investigations will not be simple one-day affairs. Experience with interviewees suggests that many of the sources are hard to find, episodic or multiple sources, and that many days may be required to locate them.

The costs of doing special surveys, such as genetic typing of fecal coliforms or virus analysis will be site-specific. Few of these techniques are routinely performed, and even fewer are done by commercial laboratories. Most of the previously cited surveys were done by university

researchers or research labs. The time will also be variable, since a survey may be part of a student's research project. Asking commercial laboratories to do this special work will be extremely expensive, since they will have only a few clients to absorb the capital and startup costs. In lieu of no other information, $50,000 is a good starting estimate for doing a single study using one of the advanced techniques.

VI CONCLUSIONS

The following conclusions and observations are made:

1. More than 10 examples of previous source investigations were reviewed in the hopes of determining which methods are most successful. Investigations have either found an immediate and obvious cause of beach water contamination (and usually no report is written) or have been inconclusive, meaning that no source was found. The report contains an executive summary of these investigations in the hopes that future investigators can learn from previous work, and contact others who have had similar problems.
2. Wet and dry weather decision trees, or action plans, have been developed and versions were reviewed at several workshops. The trees are general as opposed to site-specific, and should be considered an outline for decision trees at affected locations. A strong and important recommendation is for various involved agencies to determine appropriate responsibilities and budgeting authority before they need to conduct source investigations. Another recommendation is the development of tools for investigators. A geographic information system (GIS) with database created especially for source investigations is needed. The information in many cases will already exist in GIS form, but needs to be edited and arranged in such a way that it can be used by field investigators on a laptop computer, and can store previously collected water quality and flow data. This is an area where a demonstration project among cooperating agencies and a civil engineering consultant/GIS consultant would be useful. The demonstration project could be used as a model for other areas.
3. A brief review of bacterial sources is provided. Simple calculations show that human fecal contamination from a single family can contaminate 0.3 linear miles of surf water. There is interest in determining the origin of indicator organisms (animal or human), but there is no quantitative knowledge of the risks of each source. Therefore, even if source is non-human, the indicators should be treated as if they were from human origin. Knowledge of the origin (human or animal) will be useful in determining BMP application.
4. An extensive review of new methods was undertaken. More than 10 methods are promising, but none are currently useful for regulating beaches. It is unlikely that new methods will become available in the next three years. There is an opportunity for the State or other agencies to fund pilot investigations using new methods. The pilot studies should not be performed in response to a particular problem, but in a fashion that maximizes accurate and precise results. The pilot studies should have as a secondary goal the developing of research and monitoring expertise among California institutions, companies or agencies.
5. The costs of most of the proposed new techniques are currently prohibitive, but will dramatically decline if popularly used.
6. Enterococcus is the most sensitive of currently used indicators, and causes the most beach postings. The time for analysis is 24 hours or more. Recent evidence suggests strong temporal variations, which cast doubt in using this indicator as it is currently used (single measurements). There is sufficient reason to consider changing the current protocol for

postings using enterococcus. Composite samples or multiple exceedences may be a better strategy. In areas that have chronic occurrences of enterococcus, further analysis to determine species is warranted.

7. 70% of the exceedences of standards in the past 5 years (Leecaster and Weisberg, 2001) were one-day exceedences. A simple example in the text shows that for these cases, the beach is not posted directly after the exceedence but is posted after the beach has returned to standards.
8. Approximate cost estimates are provided. These should be used as a guide and each agency considering source investigations should develop their own estimates.
9. At the end of this project we found a beach grading system being developed for the beaches around Auckland, New Zealand. It is included as an appendix. It has several alternative ways of grading beaches, that tends reduce day-to-day variations in grading and produce longer term average grading. Aspects of this alternative system may be useful for California.

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Appendix A. Executive Summaries of Previous Source Investigations

1. Santa Monica Canyon Storm Drain

Background

The Santa Monica Canyon Task Force was formed in June, 1994 by the Stormwater Management Division of the City of Los Angeles, Department of Public Works, Bureau of Engineering. It was formed to investigate and identify the sources of the high bacterial counts in the Santa Monica Canyon Storm Drain, and to provide recommendations that may reduce the bacterial counts in the storm drain. In the report, the Task Force recommended that test results at Station No.4 located 50 yards south of Santa Monica Canyon storm drain be monitored and that a public education program targeting the local residents be implemented. The Santa Monica Canyon Task Force reconvened in May 1995 to conduct a more extensive study of the storm drain channels that are tributary to the Santa Monica Canyon. Phase II of the study was focused on the collecting storm drain samples, coordinating laboratory resources, coordinating miscellaneous activities, analyzing sample results, and preparing a report of its findings. The task Force includes two Civil Engineering Assistants (Ritchie Yee and Roberto de Leon) and two Industrial Waste Inspectors (Robert Maldonado and George Payba), all from the Stormwater Management Division.

Study Area

The Santa Monica Canyon Channel discharges at the Will Rogers state Beach. Mandeville Canyon Channel, Sullivan Canyon Channel, and Rustic Canyon Creek are tributary to the Santa Monica Canyon storm drain. Sampling stations were chosen at upstream and downstream location within each creek or channel. The Phase II study consisted of six regular sampling points. The sampling location and corresponding descriptions are follows:

MCDS:	Mandeville Canyon downstream just before the Sullivan Canyon/Mandeville Canyon confluence;
SCDS:	Sullivan Canyon downstream just before the Mandeville Canyon/Sullivan Canyon confluence;
SMUS:	Santa Monica Canyon upstream near Sunset Boulevard and Westcove Drive;
SMDS:	Santa Monica Canyon downstream just before the Santa Monica Canyon/Rustic Canyon confluence;
RCDS:	Rustic Canyon downstream just before the Rustic Canyon/ Santa Monica canyon Channel;
SMSD2:	Santa Monica Canyon Storm Drain just after the Santa Monica Canyon/Rustic Canyon confluence. Due to an unexpected discharge, one sampling location was added during the Phase II sampling period.
SUNS:	Storm drain outlet that discharges into the Santa Monica Canyon just upstream of SMUS.

Study Design and Methods

A total of forty-four samples were collected from each location during the two months, from May 16,1995 to July 14, 1995. All water samples were tested in accordance with *Standard*

Methods for the Examination of Water and Wastewater (18th edition, 1992) consistent with EPA standards. In the Phase I study, the Task Force used the following guideline in determining the possibility of sanitary sewer or septic tank leak into the storm drain: 100,000 CFU/100ml for total coliform and a total to fecal coliform ratio of less than or equal to 2:1. In reviewing the Phase I report, the Task Force adjusted the total-to fecal coliform ratio to less than or equal to 3:1. Ammonia-nitrogen, MBAS (detergent) and potassium were added as parameters to ascertain possible sources of human sewage. These parameters may not individually provide sufficient information to identify the specific sources of pollutants, but it is possible to distinguish between sources by comparing a variety of parameters.

Results

None of the six sampling stations had bacterial levels high enough to suspect any sewage contamination. Test results from each sampling location, except SUNS storm drain, indicated non-detectable amounts of ammonia and detergents with background levels of potassium. The test results appeared to indicate that the contamination may have come from diffused sources.

The samples from the SUNS storm drain on July 7, 1995 had the following results: 2,900,000CFU for total coliform; 1,500,000 CFU for fecal coliform; 1,000,000 CFU for enterococcus; and a total-to-fecal coliform ratio approximately 2:1. The result of the SUNS storm drain sampling indicated possible sewage contamination and therefore necessitated further investigation. The Task Force conducted a CCV inspection of the SUNS storm drain to investigate possible illegal sewer or septic tank connections on October 13, 1995. It did not reveal any illegal connections to the storm drain line, but confirmed that the line was partially filled with soil and organic debris. A major source for the presence of high bacterial counts, ammonia, detergents and potassium at this location was due to the intermittent discharge of contaminated sediment and debris in the storm drain line. One source of the contamination could be the large horse population in the area.

Conclusively, no point source discharges were identified by the Task Force as the cause of bacterial contamination in the Santa Monica Canyon storm drain. In addition, no consistent leaks or septic tank seepage were found.

Recommendations

The Public Affairs office (PAO) intends to enhance the horse owner outreach in the Santa Monica canyon drainage area. The Department of Animal Regulations has provided a list of licensed horse owners in the City of Los Angeles. The bureau of sanitation is in the process of compiling a database of city residents that requested extra containers for horse waste disposal. A database of the horse owners in the City of Los Angeles will be developed by combining data from the Bureau of Sanitation and the Department of Animal Regulations. The PAO plans to utilize these databases to continue the horse owner and equine industry outreach.

Contacts

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2. Rincon Creek, Santa Barbara

Background

In order to better understand the sources of water contamination at Rincon Creek, Heal the Ocean and Santa Barbara County agencies combined efforts to fund a detailed study that focused on identifying the sources of bacteriological contamination in the lower Rincon Creek Watershed. The study addressed two primary objectives:

(1) Identify the sources of coliform bacteria in discharges from dry weather flows and
(2) Characterize the sources of *E.coli* and the presence of human pathogens in runoff from the Rincon watershed

Study Area

The Rincon Creek watershed begins in the Los Padres National Forest located in both Santa Barbara and Ventura Counties. The beach area around Rincon Creek has experienced elevated bacterial levels on a consistent basis even during periods of low flow. The lower portion of Rincon Creek watershed forms a lagoon that is under tidal influence, which creates an interface between freshwater, brackish water and ocean water. Three sample locations were chosen in an attempt to isolate the upper and middle sections of the watershed from the lower residential section:

RC-007 (culvert), which is just upstream of the lagoon and residential areas, represents everything down the watershed up until that point
RC-002 (lagoon), which is in the lower end of the lagoon and represents the mixing of salt and freshwater as well as any contributions from the lower residential area to the watershed.
RC-OC (Ocean surfzone), which is located in the surfzone, at the mouth of the creek, and represents the watershed's contribution to the oceans.

Study Design and Methods

The following investigation were conducted for this study:
1. Major mammal and avian species were identified
2. Water samples were collected from three discrete locations in the watershed
3. Sources of fecal coliform present in the water sample were determined by comparing *E.coli* genetic material extracted from the water samples to previously established genetic ribotyping of *E.coli* bacteria

Under base flow condition, 50 discrete water samples were collected from each location for a total of 150 water samples. Water samples were analyzed at the Santa Barbara County Health Laboratory and then shipped the day after sample submission for overnight delivery to Dr. Samadpour's DNA laboratory at the University of Washington for the further analysis. A total of 208 fecal samples were collected, of which 80 were bird species and 128 were mammal species. Source samples were boxed and shipped to Dr. Samadpour's DNA laboratory.

Results

After membrane filtration and culturing, 138 water samples of the total 150 water samples produced a minimum of 2-19 bacterial colonies. Unfortunately, due to communication errors between the laboratories, 30 filter plates were not processed, leaving isolates from only 108 of a possible 150 water samples. 184 *E. coli* isolates were processed and produces a total of 124 matches.

Matches to human species showed the highest percentage (20%; 25/124) but were noted in only the lagoon (14 matches) and the surfzone (11 matches) sample locations. However, 40%(10) of the human matches occurred during one sampling event. No human matches were noted in the area of the culvert. Duck species were the second most prevalent match and match distributed over all sampling locations. Domestic species (human, dog, horse, cat, cow, and sheep) accounted for 46%(57) of the total matches.

Recommendations

Although all study goals were achieved, there still remain obstacles to reliance on this technique for widespread usage.

<u>Some of the problems</u>

- Potential interference from many sources in the watershed. It is critical that the scope of the application in a natural environment be as focused as possible.
- Relatively high expense for ongoing monitoring or extensive studies.
- Protracted timeframe between sample submission and test results.
- Inability to address potential public health risk based on finding

<u>The study was effective in</u>

- Identifying sources of fecal coliform pollution.
- Providing a rough assessment of location within the watershed of these sources contributions within the scope of this study work plan.
- Providing focus and guidance to potential source reduction strategies.
- Increasing experience of local staff with source and water collection techniques.
- Development of the first component of a local database for *E.coli* ribotypes if future studies and technique application are contemplated.
- Providing reassurance to the local community that conversion of septic systems, in this particular watershed, to a sanitary sewer collection system will likely educe the amount of human waste in the watershed.

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3. Agua Hedionda Watershed

Background

This microbiological study was conducted in the Agua Hedionda Watershed by URS Greiner Woodward Clyde, in association with Motile Laboratory Services and the University of Washington, for the City of San Diego and Co-Permittees Stormwater Program as part of the 1998-1999 monitoring activities. The objective of the study was to evaluate the following:

- Sources of fecal coliforms in waterways (Agua Hedionda Creek and tributaries) during dry weather and during storm events;
- Sources and persistence of fecal coliforms in the receiving waters of this watershed, the coastal Agua Hedionda lagoon;
- Presence of potential human pathogens (bacteria and protozoa) as assessed by direct detection

Study Area

The Agua Hedionda watershed affords the benefits of a representative, moderate-size watershed (approximately 29 square miles), with relatively homogeneous land uses in several accessible subwatersheds, and with good stream branching. Sampling stations were established at four creek locations in the watershed and in a lagoon transect.

Station AH-Os is the uppermost station which drains approximately 1% of the total watershed, mostly agricultural area and open space.
Station AH-Co is located on Buena Creek which drains approximately 17 % of the total watershed, mostly from residential areas.
Station AH-Re is located on Buena Creek which drains approximately 22% of the total watershed, mostly residential area
Station AH1 is located on Agua Hedionda Creek which drains 81% of the total watershed Agua Hedionda Lagoon

Study Design and Methods

Creek water samples for fecal coliform counts and isolation of *E.coli* clonal strains were sampled at 5 minutes intervals, during two dry weather monitoring events and three wet weather (rainstorm) events. Lagoon samples were collected at 30-meter intervals along a transect across the eastern end of the lagoon, one or seven days after a storm event.

The source species samples were collected from the Encina sewage treatment plant in the Agua Hedionda watershed and from animals that reside in the San Diego region. All source samples were shipped directly to Dr. Samadpour's DNA laboratory at the University of Washington for DNA ribotyping.

For *Giardia* and *Cryptosporidium* monitoring, approximately 10 gallons of creek water collected at each site using grab sampling technique during dry weather in June 1999. *Giardia* cysts and *Cryptosporidium* oocysts were detected by immunomicroscopy.

Bacterial pathogens were sampled in the Agua Hedionda watershed during dry weather using Moore swabs. The samples were shipped to Dr. Samadpour's DNA laboratory for analysis. Polymerase Chain Reaction (PCR) techniques applied to environmental samples provided useful information regarding the presence or absence of *Salmonella, Shigella, Yersinia, Listeria, Campylobacter, Helicobacter, Legionella, Mycobacterium, Vibrio cholerae, Vibrio vulnificus, Staphylococcus aureus,* and three variants of pathogenic *E.coli*.

Results

Dry-weather creek samples, characterized by low turbidity and high conductivity, contained relatively low numbers of fecal coliforms (several hundred to several thousand colony forming units per 100ml).

Wet weather samples had high turbidity, low conductivity, and elevated fecal coliform counts. The first storm event monitored yielded samples with very high fecal coliform counts (up to 12million cfu/100ml), and the densities of fecal coliforms diminished as the storm season progressed.

Agua Hedionda water samples provided bacteria for 656 *E. coli* isolates, 417(63.6%) of which could be matched to a known source among a variety of warm–blooded animals. The three dominant groups of source organisms were domestic pets (dogs and cats), birds, and human. Coyotes, raccoons,, and opossums were the leading wildlife sources.

Following enrichment of bacteria from Moore Swabs deployed in the creeks during dry weather, *Listeria* and S*almonella* were detected most often, in 9 and 8(of 12) swab enrichments respectively. The DNA laboratory conducted enrichments and PCR analysis for 65 of the fecal samples collected from animals in the San Diego Regions. *Salmonella* was the most prevalent pathogen regardless of source species. *Giardia* cysts and *Cryptosporidium* oocysts were detected by immunomicroscopy in an all four creeks stations at a similar concentration range of 20-60 cysts per 100 liter. There was no apparent difference between stations.

Results of this study indicated no apparent difference between creek stations in the assemblages of *E.coli* source species, the presence of bacterial pathogens, or the number of protozoan cysts (the only exception was the high proportions of *E.coli* from human origin at one station during one rain event). Thus, none of the stations appears to represent clear background conditions, none appears to be more contaminated than others.

Recommendations

For the future work, it is recommended to utilize a combination of source tracking and pathogen detection techniques. The values of data generated for pathogen reservoirs and occurrence in

water will be strongly enhanced by performance of ribotyping to the same fecal source samples and to water samples collected in conjunction with swab deployment during storm events. Essentially, the ribotype distribution can serve as indicator of movement of fecal bacteria, including pathogens, in the watershed.

In addition, it is recommended to augment the creek information with information from other compartments of the lagoon system, i.e., water, mussels, and sediment. Future efforts should focus on water sampling in areas used for contact recreation, collection of mussel samples (they are very effective integrators of potential pathogens), and analysis of sediments (which are known to be a sink and a source of fecal bacteria)

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4. San Diego Beaches

Background

The County of San Diego Department of Environmental Health (DEH) and the City of San Diego cooperatively initiated " an investigation of the sources of Fecal Contamination of Four san Diego Beaches" in spring 1999 to determine the sources o fecal contamination at four beaches considered to be representative of coastal area throughout San Diego County. The study addressed three primary objectives:

(1) Identify the sources contributing to elevated fecal coliform bacterial levels at local beaches,
(2) Identify management actions needed to control these sources, and
(3) Identify additional information needed to further characterize the problem

Study Area

Four beaches located entirely within the City if San Diego in the central coastal portion of San Diego County were investigated.

Avenida de la Playa (La Jolla Drainage Basin)
Windansea Beach (Windansea Drainage Basin)
Tourmaline Surf Park (Tourmaline Drainage Basin)
Dog Beach (San Diego River Watershed)

Representative sampling points for each of these areas were established. Each of these sites was chosen because it is used for a variety of recreational water contacts activities and has a history of beach closures due to elevated bacteria levels. Three of the four sites convey runoff exclusively from basins of less than 1,100 acres located entirely within coastal communities, and the fourth (dog Beach) drains a larger portion of the San Diego River watershed.

Study Design and Methods

This study was designed to identify the potential sources of *E. coli*. These bacteria make up approximately 80 % of the coliform bacteria in normal intestinal flora. *E. coli* is easily modified and adaptive to various host environments leading to changes in genetic material that are thought to be specific to these host environments, as such, their genetic variability can be used to identify their host organisms.

275 water samples were collected at four samples sites for laboratory analysis, 105 during dry weather and 270 during wt weather conditions. Fecal specimens were also colleted from fifty-six animal suspected to be fecal coliform contributors. The County of San Diego Public Health Laboratory processed the water samples for analysis of fecal coliform bacteria. Plated colonies and fecal specimens were shipped to Dr. Samadpour's DNA laboratory at the University of Washington, School of Public Health where they were further processed for ribosomal analysis of *E. coli* using polymerase chain reaction (PCR) and agarose gel electrophoresis. Sample

results were then compared to a library of DNA fingerprints from known sources at the University of Washington.

Results

Of 550 possible *E.coli* colonies, 489 colonies were produced and positively verified as *E.coli*. Three hundred and fifty-three (353) were matched to 12 source groups; 179 isolates were reported as unknown. Human isolates were responsible for the highest percentage of matches during dry weather conditions, but were absent in all wet weather samples. In contrast, dog and bird isolates were generally the most abundant groups in wet weather samples. Matches for dogs were particularly abundant, according for more than 30% of wet weather matches at all sites, while birds accounted for more than 24% of isolates at all but Dog Beach site.

Results were generally similar at the Avenida de la Playa, Windansea, and Tourmaline sites. Human were responsible for the highest percentages of matches during dry weather conditions (31.6 to 44.7%), followed by dogs (2.6 to 20.0%), cats (0 to 17.5%), and indigenous mammals (2.5 to 13.2%). Conversely, humans were not represented during wet weather, but dogs accounted for the highest percentage of matches (31.4 to 36.4%), followed closely by birds (24.3 to 39.0%) which were unrepresented during dry weather. Results at the Dog Beach site were very different. Unlike the other three sites, only 1 dry weather isolate was matched to a human source. Avian matches were also relatively predominant during dry weather (40.7%) in contrast to the complete absence of birds in dry weather results at the other sites.

It is useful to consider the degree to which specific sources are controllable, as well as the types of controls which may be appropriate and feasible for each. As a rule it should be noted that a variety of non-structural best management practice (BMPs) can appropriately be applied to all of thee source groups identified in this study but indigenous animals (birds and small mammals). Dogs and cats were the most consistently represented domestic animals throughout the study, and therefore should be a primary focus of management efforts. Humans are also important, but more information is needed on the specific sources of these inputs. In most cases, public education appears to be the most broadly applicable BMP since many people are still unaware of their role in preventing water quality contamination. It is therefore likely that effective public awareness campaigns can measurably change the attitudes and behaviors of many citizens.

Recommendations

As a whole, the results of this study support the continued use of conventional stormwater and urban runoff management practices and the collection of additional data to further characterize risks to human users. With this in mind, the following are recommended.

<u>Source Management Recommendations</u>

1 Continue the use of non-structural BMPs as a general control measure.
2. Continue the use of diversion/interceptor systems for dry weather control where appropriate and feasible.
3. Develop performance measures for best management practices (BMPs).

Monitoring/Research Recommendations

4. Conduct sanitary survey to further characterize specific sources of human fecal mater during dry weather conditions
5. Identify specific pathogens and risks to human recreational users.
6. Periodically re-evaluate study against updated DNA Library.
7. Develop standard protocols with measures of statistical significance for DNA source studies.

Contacts

County of San Diego
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5. Orange County J03P02 Watershed

Background

In March, June, and September of 2000 the Co-permittees met with representatives of the Orange County Health Care Agency and researchers from UCI, USC, UCLA and the Southern California Coastal water Research Project (SCCWRP) to develop a study to identify sources of bacteria in the J03P02 watershed. A three-phase experiment was initiated in late April.

Phase 1 Investigation

The purpose of this study was to determine if there were any patterns in the flow rate from the 72 inch J03P02 dissipater pipe that corresponded to anticipated peak sewage flow periods, generally the periods before and after the workday. The secondary purpose was to provide information in order to assistant in the design of the sampling for Phase 2 of the experiment and to subsequently allow an assessment o the "flux" of bacteria at different times of the study.

On April 27, 2000 a flume and recording water level gage were installed in the 72inch pipe just upstream of the outlet to the dissipater basin. More than 25,000 instantaneous discharge rate measurements were recorded during the following months. Each discharge measurement and its corresponding time were evaluated.

Nine daily reoccurring peaks were identified. Six of this peaks occurred between 4:20 a.m. and 8:45 a.m. It should be noted that these peaks however represented only minor (4 - 14%) increase in the discharge rates. The greatest discharges during the day occurred between 2:00 a.m. and 10 a.m. with a maximum between 6:00 and 7:00 a.m.

Phase 2 Investigation

The purpose of this study was to evaluate the hourly bacterial flux from the pipe, to determine what proportion of discharge from the pipe could be attributed to groundwater, and to provide information needed for the design of the Phase 3 investigation.

On May 24, 2000 the Co-permittees conducted a 24-hr chemical and bacteriological assessement of the discharge from the 72inch pipe. Street runoff from the lower watershed was samples in the morning and at noon.

The chemical data suggest that groundwater comprises a significant portion of the total discharge from J03P02. The average contribution for this 24-hr period was approximately 34%. The concentration of bacteria in the samples varied throughout the day. The period of greatest bacteriological flux from the pipe was between the hours of 4:00 a.m. and 8:00 a.m. Monitoring of the street runoff in the lower J03P02 watershed showed that the greatest concentration and flux of bacteria occurred in the early morning (3:00 a.m. to 6:00 a.m.). These finding suggested that the Phase 3 investigation be conducted between 3:00 and 8:00 a.m.

Phase 2B Investigation

Because these findings may have been influences by the light precipitation that occurred during the Phase 2 study, a follow-up study was conducted on August 16,2000. This study hereinafter referred to as the Phase 2B study showed that the water discharges rate from J03P02 on August 16th was not as variable as was measured on may 24th. On August 16th the highest average hourly discharge rate occurred between 7:00 and 8:00 a.m. On May 24th the highest average discharge rate occurred between 8:00 and 9:00 a.m.

The groundwater contribution to the total discharge from the 72inch pipe was estimated to be 31% compared to 34% from the May study. The greatest bacteriological concentrations in the discharges from the 72-inch pipe occurred between 6:00 and 7:00 a.m. The greatest bacteriological flux was also measured during this period.

Phase 3 Investigation

The Phase study was conducted to address the following question:

Are there any indications that the fecal coliform bacteria found in the samples collected from J03P02 are of human origin?

On September 27, 2000 the Co-permittees conducted a one-hour chemical, bacteriological and microbiological assessment of the discharges from the 72-inch pipe as well as two underground laterals to J03P02 and one catchbasin in the lower J03P02 watershed. In addition to the normal suite of bacteriological tests (total coliform, fecal coliform, *E. Coli*, and *Enterococcus*) conducted in the previous phages, analysis for coprostanol, Linear Alkyl Benzenes, human enteric viruses, and bacterial biomarkers were also conducted. The preliminary results have not been reviewed by the full panel of researchers.

Chemical Markers in Sewage: Ken Schiff, Principal scientists at SCCWRP, reported that the results from all analyses indicated that LABs were not detected in any of the submitted samples. Indira Venkatesan, research geochemist at UCLA, reported that the results indicate that none of the water samples contained fecal sterol.

Human Adenovirus: Sunny Jiang, professor at University of California Irvine, used a nested polymerase chain method to detect the presence of viral genetic material in the water samples collected from each of the four locations. The preliminary results indicated that human adenovirus was not present in any of the four samples in J03P02.

Human Entero- and hepatitis A Viruses: Professor Sunny Jiang, at University of California Irvine, analyzed the sample for human entero-and hepatitis A viruses. Positive detection of human enterovirus genetic material occurred in three of the four sample locations. Weak signals of hepatitis a virus genetic material were also observed but the result was inconclusive during this preliminary study.

Toxin Genes as Biomarker: Dr. Olson, professor at University of California Irvine has developed three biomarkers using simple PCR to identify wastes in waterways based on the occurrence of toxin genes in E.Coli populations that cause disease in a specific host animal. The markers of human waste, cattle and pig waste are host specific and therefore provide a simple presence or absence result. All samples were negative for human and pig wastes. The only positive result was determined for cattle at the lateral on Alicia Parkway and Kite Hill All other location had negative for cattle.

The results from the Phase3 investigation have not yet been reviewed by the full research panel. LAB and Coprostanol data from SCCWRP and UCLA preliminary suggest a finding of no sewage system cross-contamination, which is supported by the non-detection of adenoviruses by Dr. Jiang and non-detection of toxic gene sequences fro human E.coli in Dr. Olson's evaluation. Further research would be required to confirm whether the findings were transient or replicable, or to isolate the specific sources.

Appendix B - Methods to Identify Human and Animal Fecal Pollution in Water: A Review

Methods to Identify Human and Animal Fecal pollution in Water: A Review

ABSTRACT

This paper reviews methods to detect human fecal pollution and differentiate it from other sources such as animals. Microbial methods, especially those using molecular biology, and chemical methods are reviewed. The conclusion is that no single method can provide definitive answers, as least not with our current understanding or experience. Additional testing with some of the reviewed methods may provide the required experience and confidence. It is much more likely that a combination of methods can be used to accurately identify human fecal pollution. Unfortunately, a combination of procedures will be more expensive and most likely be no faster than existing techniques.

Key words: adenovirus, antibiotic resistance analysis, Bacteriodes fragilis, Clostridium perfringens, Fecal coliform, Fecal sterols, Fecal streptococci, Escherichia coli, F-specific RNA coliphages, linear alkyl benzenes (LABs), rep-PCR DNA technique, ribotyping technique

INTRODUCTION

Fecal bacterial contamination from human and animal waste is a major cause of deteriorating water quality in receiving waters and has direct economic impacts to coastal communities through the loss of shellfisheries and restrictions of recreational uses. The possible sources of fecal contamination are point sources, such as industrial and municipal effluents, or nonpoint sources, such as surface runoff, direct animal and human input, failing or inadequate septic systems, and sewer overflows. In recent years nonpoint pollution has surpassed point sources as the major source of Fecal contamination to surface water. Management of this problem depends on knowing which sources of Fecal matter are the cause. A method that could distinguish sources would be the first step to solving this problem.

Methods for distinguishing between human and animal. Fecal pollution are necessary for assessing the overall protection of water supplies and implementing effective remediation for epidemiological studies, and even for legal purposes when it is necessary to determine the source of environmental contamination. Animal Fecal pollution is not without risks, and many of the risks are unknown, but it is generally thought that animal sources pose less risk. Furthermore, knowing the source will help in identifying and eliminating the problems.

Information on the human or animal origin of Fecal pollution gives an indication of the types of pathogens that maybe expected, the risk of infection, and the treatment that may be required to control the transmission of disease. Many waterborne pathogens are difficult to detect and quantify, and specific methodology to detect them in environmental water samples has still to be developed.

Bacterial indicator organisms such as Fecal coliforms have been used to test water samples for Fecal pollution, but such indicators do not provide specific information on the specific source of pollution. These bacteria may be found in a variety of warm-blooded animals and are not unique to the human intestinal flora.

Since the early 1900s there have been various attempts to develop methods that differentiate the source of Fecal pollution. Traditionally, efforts have concentrated on determining Fecal pollution of human origin. It is now is also important to distinguish between animal sources of Fecal pollution as well as human source because animals can carry potentially harmful human pathogens. If animals are the source of indicator organisms, control measures and management practices will be different. Ribotyping analysis is one encouraging method that may be able to differentiate sources.

This review paper discusses some of the current methods to identify human sources from nonhuman sources of Fecal contamination in surface water. This review is divided into sections on microbiological and chemical approaches for identifying sources of Fecal contamination. Microbiological approaches cover bacterial and viral indicators found in the intestines of warm-blooded animals. Chemical approaches cover natural byproducts of human metabolism or human activity. Microbiological approaches include the measurement of the ratio of Fecal coliforms to Fecal streptococci or total coliforms, the detection of bacteriophages of bacteroides fragilis HSP40 and some serotypes of F-specific RNA coliphages, antibiotic resistance analysis, ribotype analysis, rep-PCR DNA technique, and use of human enteric viruses. Chemical approaches include Fecal sterol fingerprinting technique and the presence of contaminants normally associated with sewage, such as detergents. This review provides a short description of each method, some examples of studies that used the method, and a discussion of advantage and disadvantage of each method if possible.

MICROBIOLOGICAL METHODS

The ratio of Fecal coliforms (FC) to Fecal streptococci (FS) or total coliforms (TC)

Human fecal material may be distinguishable from animal fecal material using an old method, the ratio of fecal coliforms to fecal streptococci (FC/FS). Fecal streptococci have received widespread acceptance as useful indicators of Fecal pollution in natural aquatic ecosystem. Fecal streptococci are more abundant in animal feces than in humans; in contrast, Fecal coliforms are more abundant in human feces than in animals (see Table 1). Therefore, Fecal coliform to Fecal streptococci ratio have been used to differentiate human Fecal contamination from that of other warm-blooded animals (Geldreich and Kenner, 1969; Feachem, 1975). The ratio of Fecal coliforms to Fecal streptococci (FC/FS) greater than four were associated with human Fecal sources while a ratio of less than 0.7 was associated with animal Fecal sources.

If this ratio were reliable it would an inexpensive and practical method. However, the application of this method is now considered unreliable due to the variable survival rates of Fecal streptococci species. Furthermore, the ratio is affected by the methods for enumerating Fecal streptococci and by disinfection of wastewater (APHA, 1998).

Therefore, to use this method to provide information on possible Fecal pollution source we have to consider its limits: (1) Sampling needs to occur soon after waste contamination (within 24 hours if possible) because the Fecal bacteria may die off at different rates; (2) it becomes difficult to distinguish Fecal streptococci in waters from Fecal streptococci that are naturally present in soil and water when fewer than 100 Fecal streptococci/100ml are present, and (3) the water pH needs to be between 4 and 9 because Fecal coliforms die off quicker than Fecal streptococci in more acid or alkaline water (Geldreich and Kenner,1969; Coyne and Howell,1994).

Many attempts have been made to use the ratio to determine the sources of Fecal bacteria. For example, Jagals et al. (1995) showed that the ratio of Fecal coliforms to Fecal streptococci was close to unity in streams and rivers, which were upstream of the settlements, and were exposed to Fecal pollution predominantly of domestic animal origin. However, downstream of settlements which were exposed predominantly to human Fecal pollution, the ratio increased to 3.5 to 4.7.

Coyne and Howell (1994) measured FC/FS from two watersheds typical of agricultural use in Kentucky with some success. They concluded that the FC/FS ratio suggests the probable source of Fecal contamination, but considered their conclusions tentative. This method is an inexpensive and moderately complicated laboratory procedure (Sargeant, 1999). The result of this method taken alone must be quite carefully evaluated. If the method were used with some other method, such as the detection of bacteriophages, the result will be more reliable.

Fecal (thermotolerant) coliforms constitute a subset of total coliforms. These bacteria conform to all the criteria used to define total coliforms, but in addition they grow and ferment lactose with production of gas and acid at 44.5 ± 0.2 C within the first 48 hours of incubation. The ratio of Fecal coliforms to total coliforms (FC/TC) is used to show the percentage of the total coliforms comprised of Fecal coliforms, i.e., coming from the guts of warm-blooded animals. If the Fecal coliforms to total coliforms ratio exceeds 0.1 (fecal coliforms comprise10% or more than the total coliform group) suggests the presence of human fecal contamination.

Hiraishi et al. (1984) measured TC, FC, and BOD from the Tamagawa River and its tributaries in Tokyo. Geometric means of the fecal coliforms to total coliforms ratios ranged from 0.007 to 0.069 in streams, which were located on the upstream of human contamination sources, but downstream of human sources, the ratio ranged from 0.21 to 0.26. Noble et al. (2000) measured FC/TC in a regional survey of the microbiological water quality along the shoreline of the Southern California. Although they used two total/fecal ratio criteria, more than 0.1 and more than 0.2, the results were very similar. Poor water quality was found at the point-zero freshwater outlets sites where 21.8 % of the shoreline-miles exceeded 0.1 at the ratio of Fecal coliforms to total coliforms.

This method roughly shows the possibility of Fecal pollution but this method is not for distinguishing human from animal–derived Fecal matter. One of this method's shortcomings is the potential growth of Fecal coliforms in soils in tropical areas. As a result, its application in tropical areas is questionable (Bartram and Rees, 2000). The method should not be discarded for tropical areas, since it maybe useful in conjunction with other methods.

Table1. Bacterial densities in warm-blooded animals feces.
(Sources: Pitt, 1998; Godfrey, 1992;Geldrich et al., 1962)

Source	Fecal coliform (density/gram)	Fecal streptococci (density/gram)	Ratio FC/FS
Human	1.3×10^7	3.0×10^6	4.33
Cats	7.9×10^6	2.7×10^7	0.29
Dogs	2.3×10^7	9.8×10^8	0.02
Rats	1.6×10^3	4.6×10^7	0.003
Cows	2.3×10^3	1.3×10^7	0.02
Ducks	3.3×10^7	5.4×10^7	0.61

Bacteroides fragilis (strain HSP40)

Bacteroides fragilis is one of about 11 species, which are loosely placed together in the 'B. fragilis' group. They are gram-negative, anaerobic, pleomorphic rods. Tartera and Jofre (1987) tested twelve strains of different Bacteriodes species and found that one B. fragilis strain, HSP40, was detected in feces of 10 % of 40 human Fecal samples and was never detected in feces of other animal species. They suggested that the detection of Bacteriophages by strain HSP40 of B. fragilis could be used to distinguish between Fecal pollution of human and animal origin. This observation confirmed by Grabow et al. (1995). They investigated the Fecal excretion of somatic and male-specific coliphages and phages of B. fragilis strain HSP40 by human and a variety of animals. Bacteriodes fragilis phages were detected in only 13 % of 90 human stool samples but not in any animal or birds feces.

Many researchers have investigated the detection of bacteriophages infecting strain HSP40 of bacteroides fragilis. Table 2 summarizes levels of bacteriophages of B. fragilis HSP40 found in different countries. Tartera et al. (1989) reported that phage infecting B. fragilis HSP40 have the same origin as human viruses and were able to multiply under anaerobic conditions, but did not replicate significantly in the environment. Jofre et al. (1989) found a significant correlation between the numbers of B. fragilis phages and human enteric viruses. Jagals et al. (1995) investigated a stream and river exposed to predominately Fecal pollution of domestic animal origin and to run-off. B. fragilis HSP40 phages were not detected by direct plaque assays in any of their samples. They concluded that more sensitive detection methods were required for the phages. Sun et al. (1997) reported that bacteriophages of Bateroides fragilis have been proven as specifically present in human feces and have relationships with water contamination by eterovirus. The researcher reported that the MPN method appeared to be more sensitive than that of PFU as reported previously Ajaujo et al. (1993) and Tartera et al. (1988).

Puig et al. (1997) tested 115 strains of B. fragilis isolated from humans and 6 of the strains were examined in feces from various animal species and in slaughterhouse wastewater. The strain HSP 40 and RYC4023 were similar in number of phages in urban sewage, but phages were not present in animal feces. Their study was performed in different countries (Puig et al., 1999). Strain HSP40 was not detected in slaughterhouse wastewater of the different geographical areas. Counts ranging from 0 to 4.5×10^4 pfu per 100ml were found in urban sewage from different

geographical areas (see Table 2). Bradley et al. (1999) reported that the numbers of B. fragilis bacteriophages, were higher than the other bacteriophages, including F+ bacteriophages in their sampling site but they failed to isolate B. fragilis HSP40. They pointed to a lack of these bacteriophages in sewage in their study area and a need to concentrate the samples before assay.

Table 2. Levels of active bacteriophages against B. fragilis HSP40 in wastewater and surface water.

Samples	Country	Range (mean) pfu or cfu or MPN/100 ml	% positive samples	Reference
Sewage	Spain	(8.9×10^3)	100	Tartera et al. 1988
Highly polluted river		(4.8×10^3)	100	
Moderately polluted river		(6.5×10^3)	59	
Pollted sea water		(7.3 x 10)	44	
Non-sewage polluted water		0	0	
Raw Sewage1[a]	Spain	2.1×10^2 - 4.6×10^4 (5.3×10^3)	100	Tartera et al. 1989
Raw Sewage2[b]		2.3×10^2 - 4.6x103 (1.3×10^3)	100	
Slaughterhouse wastewater1		0 - 1, 2	20	
Slaughterhouse wastewater2		0	0	
River		0 - 43 (6.7)	46	
Non-sewage polluted water		0	0	
Sewage	Spain	1.0×10^3 - 2.6×10^5 (2.5×10^4)	100	Comaz et al. 1991
Seawater				
10 m from sewage discharge		2.9×10^3 - 1.0×10^5 (1.8×10^4)	100	
100 m from sewage disch.		<10 - 1.0×10^4 (3.4×10^2)	70	
1000 m from sewage disch.		< 10 - 7.9×10^2 (< 10)	80	
Non-sewage polluted seawater		< 10 (< 10)	100	
Highly polluted river	Spain	10 - 1.6×10^3 (1.2×10^2)	100	Joffe et al. 1995
Low-pollute river		BLDc - 1.0×10^2 (10)	36.4	
Stream and river water	S. Africa		0	Jagals et al. 1997
Sewage (influent)	France	$\geq 4.4\times10^4$	100	Sun et al. 1997
Sewage (effluent)		$\geq 4.4\times10^3$	75	
River (downstream treatment)		$\geq 1.6\times10^3$	100	
River (upstream treatment)		< 40	50	
Sewage, river water	Spain	(2.3×10^3)	100	Araujo et al. 1997
River water		(2.3×10^1)	83.3	
Stream	PA, US	(2.21×10^2 - 3.9×10^2)		Brenner et al. 1999
Marine bathing water	UK		0	Bradley et al. 1999

Table 2. Continued.

Samples	Country	Range (mean) pfu or cfu or MPN/100 ml	% positive samples	Reference
Sewage (different countries)	Netherlands	$1.0x10^2$ - $2.6x10^2$	0	Puig et al. 1999
	Ireland	$1.4x10^2$ - $1.6x10^2$		
	Austria	$0.5x10^2$ - $8.5x10^2$		
	Portugal	0 - $0.4x10^2$		
	Germany	$1.3x10^2$ - $2.2x10^2$		
	Sweden	$0.9x10^2$		
	France	$3.1x10^3$		
	S. Africa			
Slaughterhouse wastewater	different countries[d]	$1.1z10^4$ - $4.5x10^4$		

[a] Samples were colleted from Colector Prim which receives mainly domestic sewage.
[b] Samples were collected from de Levante, which receives a mixture of domestic and industrial waste water
[c] BDL, below detection limit
[d] The countries are Netherlands, Ireland, Denmark, Portugal, Germany, South Africa

The use of bacteriophages of B.fragilis HSP40 has the advantage of their high specificity of human Fecal pollution. Strain HSP40 detects numbers of phages up to 10^5 per 100ml of urban sewage and polluted water in some areas. However they are present in low or zero concentrations both in sewage and in natural polluted water in some countries (Jagals et al., 1995; Bradley et al., 1999; Puig et al., 1999). Therefore the use of B. fragilis HSP40 for phages detection may limit their usefulness as a universal method.

F-specific RNA coliphages subgroups

The use of male-specific RNA (FRNA) coliphages has been proposed as potential sewage pollution indicator (Havelaar and Hogeboom 1984; Havelaar et al., 1990; Furuse, 1987). The gastrointestinal tract of warm-blooded animals and domestic sewage are major habitats for these viruses (Furuse et al., 1978). FRNA phages may also be source-specific. FRNA phages fall into four distinct subgroups (groups I, II, III, and IV). Groups I and II are related, and together form major group A. Subgroups III and IV are very similar and are together called major group B. The subgroup identity of FRNA phages from environmental samples may help distinguish between human and animal waste.

Either serotyping or genotyping may achieve identification of the FRNA phage subgroups. Usually, identification of FRNA phages as members of one of the subgroups is achieved by serotyping (Osawa et al., 1981; Furuse, 1987; Havelaar et al., 1990).

Osawa et al. (1981) showed that FRNA phages belonging to group I were only detected in feces or gastrointestinal contents of domestic farm and feral zoo animals. FRNA phages isolates from pigs belonged to group I and II and those from humans groups II and III. Phages belonged to group III were exclusive to humans. Furuse (1987) found that subgroup II and III tend to be isolated from human faeces; subgroup I is usually isolated from the faces of non-human mammals and subgroup IV phages are mixed origin. Havelaar et al. (1990) serotyped 178 FRNA phage strains from faeces and 206 from wastewater. FRNA phages occur rarely in faces.

FRNA phages strain from Fecal source belonged to either group I or IV with one exception. The group I and IV also predominated in wastewater samples in particular from slaughterhouse wastewater and gray water. Domestic and hospital wastewater samples sometimes yield group II and III phages. Subgroup II phages were abundant in wastewater of human origin but rare in feces. They suggested that FRNA phage should be considered as indicators of sewage pollution rather than Fecal pollution.

However, serotyping is ambiguous and too time consuming for routine assay since it requires propagation of individual plaques, preparation, titration and maintenance of antiphage sera as well as neutralization assays (Beekwilder et al., 1996). Some researchers investigated genotyping method as an alternative approach to distinguishing the four groups of FRNA phages (Hsu et al., 1995; Beekwilder et al., 1996; Griffin et al., 2000). The method employs specific gene probes to differentiate between the four subgroups of FRNA phages.

Hsu et al. (1995) developed a genotyping methods to group F-specific coliphages by nucleic acid hybridization with nonradioactive oligonucleotide probes, and compared this method with serotyping. Of the 203 isolates of FRNA phages from environmental samples, wastewater and shellfish, 99.5 and 96.6% could be classified into each group by serotyping and genotyping, respectively. Beekwilder et al. (1996) reported that identification of organisms by nucleic acid hybridization is genome-targeted and therefore has a high probability of exposing true relationships between organisms. Furthermore, it is easily performed and it appears to be quantitative and highly specific. Griffin et al. (2000) demonstrated that F-specific RNA coliphages genotyping provide confirmatory data to determine the sources of Fecal contamination. In their study FRNA phage analysis indicated that Fecal contamination at a park and surrounding areas in Florida, influenced by animal and non-human sources and 86% of the isolated FRNA phages from wastewater treatment plants were subgroup II - human in origin.

The use of FRNA phages is limited because FRNA phages are found in low occurrence in humans, although FRNA phages occur at reasonably high rates in sewage. (These phages may be poor indicators of human contamination in nonpoint source areas.) Sharing serotype between human and animals such as pigs is also a problem.

Clostridium perfringens

Spores of Clostridium Perfringens are largely Fecal in origin (Sorensen et al., 1989). They are ubiquitous in sewage sludge at concentrations several orders of magnitude higher than in soil or sediments (Fujioka and Shizumura, 1985). Contamination of deep-water disposal sites has been confirmed by the distribution of Clostridium perfringens in sediments (Hill et al., 1993 and 1996). The concentration of Clostridium perfringens as well as fecal sterols (discussed in detail in Chemical method section) in the Antarctic sediments have been used to investigate the contamination from untreated sewage outfall (Edwards et al., 1998).

A combined C. perfringens and fecal coliforms data can be used to differentiate between native birds and domestic pets (Leeming et al., 1997). Dog and cat faeces contain roughly equal and higher numbers (10^6 - 10^8 cfu/g) of both fecal coliforms and C. perfringens pore, whereas the feces of native birds (seagulls, swans, rosellas, magpies and ducks) contained 10^6 - 10^8 cfu/g of fecal coliforms and generally less than 10^2 cfu /g of C. perfringens spores. Therefore, the

relatively higher ratio of Clostridium perfringens spores found in dog and cat feces may be a useful indicator when fresh fecal contamination is being investigated (Leeming et al., 1998). Furthermore, C. perfringens was found to significantly correlate to other pathogens like Giardia and Aeromonas from sewage-impacted waters (Ferguson et al., 1996).

Use of Enterococci diversity

The Enterococcus group is a subgroup of the fecal streptococci. The enterococci portion of the fecal streptococcus groups are being used as bacterial indicator for detecting the extent of fecal contamination of recreational surface waters (APHA, 1998). Water quality guidelines based on enterococci were incorporated in State of Californnia, Assembly Bill 411 (35 enterococci/100ml based on 30 day geometric mean). In the closure of Huntington Beach in Orange County, CA, during the summer of 1999, enterococci were the most frequent bacterial indicator that exceeded the thresholds. Enterococci are found inboth human and animal faeces and vegetation. Therefore identifying the specific sources of enterococci may be a good approach to indentify fecal pollution.

In New Zealand, enterococci were specified and characterized to a sub-specific level to address the possible influence of these non-Fecal sources enterococci in a beach environment (seawater, sand, seaweed, stream water, sediment)(Anderson and Lewis, 2001). The genotypic diversity of Ent. faecium and Ent. Fecalis, and the phenotypic diversity of Ent. casseliflavus were examined using randomly amplified polymorphic DNA (RAPD)-PCR fingerprinting and biochemical screening, respectively. In their initial study, calculation of similarity coefficients from the sub-species revealed a complexity of associations between beach environmental sources. There were limited relationships among specific enterococci strains and specific environments. Similarity coefficients from Ent. Fecalis and Ent. casseliflavus were found. For example, seaweed: sand; marine water: stream water; seaweed: marine water. A high similarity value suggests but does not confirm a biological or ecological association.

Further research on the using enterococci species and sub-species is required to use identification to identify the specific sources of fecal pollution (Anderson and Lewis, 2001). This technique may be a promising method and deserves future development.

Multiple Antibiotic Resistance (MAR) Analysis

The patterns of antibiotic resistance have been used to identify sources of Fecal pollution in water. This approach is based on the fact that bacteria from wildlife species are generally lacking in antibiotic resistance, while strains from humans and domestic animals exhibit varying multiple antibiotic resistance (Sargeant, 1999). For this procedure either Escherichia coli or Fecal streptococci from different animal species are analyzed to determine the resistance pattern for several different types and strengths of antibiotics.

There have been several reports of the use of antibiotic resistance profiles to determine sources of E. coli. Krumperman (1983) showed that the multiple antibiotic resistance index of E. coli from wild animals was generally low, while human and poultry isolates had higher MAR indices. Kaspar and Burgess (1990) reported that there were larger multiple antibiotic resistance of E. coli isolated in urban areas than from rural areas, and postulated that human isolates are may present. Knudtson and Hartman (1993) measured antibiotic resistance of Fecal enterococci

isolates from humans, pigs, and natural waters but found only slight differences among the various sources. Although the studies have measured antibiotic resistance of Fecal isolates from various sources, it has been difficult to use that information to identify the sources of Fecal pollution (Wiggins, 1996).

Wiggins (1996) demonstrated that Discriminant Analysis (DA) of antibiotic resistance patterns of Fecal streptococci is a useful tool for differentiating human and animal sources of Fecal pollution in water. Discriminant function analysis is a variation of multivariate analysis of variance and can be used to classify individuals into groups on the basis of the values of several classification variables (Tabachnick and Fidell, 1983; Hair et al., 1998). In the study an average of 74% of the known isolates were correctly classified into one of six possible sources (beef, chicken, dairy, human, turkey, or wild). 92% of human isolates were correctly classified. Human versus animal isolates were correctly classified at an average rate of 95%. In their recent study, more than 10,000 Fecal streptococci isolates were obtained from 236 samples of human sewage and septage, cattle feces, poultry feces, and pristine waters (Wiggins et al.1999). The average rates of correct classification into one of four possible groups (human, cattle, poultry, and wildlife) ranged 64 to 78 %. Parveen (1998) reported that DA of MAR profiles of E. coli isolates from the Apalachicola National Estuarine Research Reserve, Florida, classified 82 % and 68% of human sources and nonhuman isolates, respectively

Hagedorn et al. (1999) affirmed the work by Wiggins (1996) with the addition of Cluster Analysis. Patterns of antibiotic resistance in Fecal streptococci were analyzed in a rural Virginia watershed. They used 13 antibiotics and more than 7000 isolates from 147 samples obtained from humans, dairy cattle, beef cattle, chickens, deer, and waterfowl. Correct classification into one of the six groups was above 87 %. Fecal streptococci from their study site were classified as being predominantly from cattle (>78% of isolates)

The MAR method for differentiating between Fecal sources is promising. This method may successfully differentiate between human Fecal pollution and animals and even differentiate between animals. However, this method is time intensive for the field and laboratory work and its laboratory procedure is complicated and costly (Sargeant, 1999). Parveen et al. (1999) said the antibiotic resistance patterns of bacteria are influenced by selective pressure and thus may be different in other geographical areas and may vary over time.

DNA-based approach

More recently DNA-based approaches have been evaluated to determine whether they can be used to differentiate among sources of Fecal contamination of water. Genotype is considered to be more reliable than phenotypic biochemical reaction. Genotypic approaches differ with respect to the level of resolution of individual bacterial species or strains into distinct categories (Versalovic et al., 1998). Genotypic bacterial typing methods are shown in Table 3. We will provide short description and some work of Rep-PCR technique, and Ribotyping method only.

Ribotype Analysis

Genetic testing has been found to be very effective in matching DNA patterns in microorganisms to their sources. Genetic fingerprinting uses collections of E. coli, which are easily modified and adapt to various host environments, leading to changes in genetic material that are thought to be

specific to these host environments. As such, the genetic variability of E. coli can be used to identify their host organisms. The DNA patterns from each of these isolates, known as a ribotype, are used to match specific strains of E. coli from a contaminated site to potential sources.

Table 3. List of bacterial genotypic typing methods according to ability to distinguish genus/species or subspecies/strains (Versalovic et al., 1998).

Genus/Species	*Subspecies/Strain*
Ribotyping	ARDRA
tRNA-PCR	Chromosomal RFLP
ITS-PCR	ITS Sequencing
16S rRNA sequencing	Plasmid RFLP
	Pulsed-field gel electrophoresis (PFGE)
	Randomly amplified polymorphic DNA (RAPD)
	Rep-PCR

ARDRA: Amplified ribosomal DNA Restriction Analysis
ITS: Internal Transcribed Spacer
RFLP: Restriction fragment length polymorphisms
PCR: Polymerase chain reaction

Ribotyping has been used to determine the sources of E. coli contaminating Little Soos Creek in Washington State (Samadpour and Chechowitz, 1995). In this study, 71% of the source matches belonged to 57 identified strains, leaving 29% unmatched. Samadpour also conducted DNA analysis for an investigation of the sources of fecal contamination of four San Diego beaches (CSDDEH, 1999) and the Agua Hedionda watershed (URS, 1999). From the 489 total isolates collected from San Diego beaches, storm drains and a river during wet and dry weather, 353(72.2%) were matched to 12 source groups; 179 isolates were reported unknown. Human isolates were responsible for the highest percentage of matches during dry weather conditions but were completely absence in wet weather samples; dog and bird isolates were generally the most abundant groups in wet weather samples. In the Agua Hedioda watershed, San Diego, the water samples provided bacteria for 656 E. coli isolates, 417 (63.6%) of which could be matched to a known source among a variety of warm-blooded animals (URS, 1999). The three dominant groups of source organisms were domestic pets (dogs and cat), birds, and human.

Parveen et al. (1999) analyzed Ribotype profiles of 238 E. coli isolates from human sources and nonhuman sources. Human and nonhuman source isolates showed 41 and 61 RT profiles, respectively. Ribotyping profiles with discriminant analysis showed that 97% of the nonhuman source isolates and 100% of the animal Fecal isolates were correctly classified.

Dombek et al. (2000) said ribotyping methods tend to require extensive manipulation of DNA and the use of labeled gene probes. Grouping may be influenced by a strain's prior exposure to antibiotics. Sargeant (1999) concluded this is an excellent method for determining some of the sources of Fecal contamination in a watershed, but laboratory analysis is expensive. Only a portion of receiving water isolates can be identified, leaving a significant percentage of unknown origin.

Rep-PCR DNA technique

Repetitive sequence-based polymerase chain reaction (rep-PCR) was introduced by Versalovic et al. (1991) and yields DNA fingerprints comprised of multiple, differently-sized DNA amplicons. The rep-PCR method has been useful for DNA fingerprinting of a large variety of prokaryotic and eukaryotic microorganisms (Versalovic et al., 1994; de Bruijn et al., 1995; Louws et al., 1996). Versalovic et al. (1998) reported that key advantages of rep-PCR based chromosomal typing include its speed, reproducibility, convenience, and modest resource requirements. The required equipment is often available in molecular biology laboratories.

Dombek et al. (2000) investigated the rep-PCR DNA fingerprint technique, which uses repetitive intergenic DNA sequences, to differentiate E. coli strains obtained from human and animal sources (geese, ducks, cows, pigs, chicken, and sheep). BOX and REP primers were used to generate DNA fingerprints. Their studies revealed that DNA fingerprints obtained with the BOX primer were more effective for grouping E. coli strains than with REP primers. Jackknife analysis of the similarity coefficients revealed that 100% of the chicken and cow isolates, 83 % of the human isolates and between 78 and 90 % of the other animal isolates were assigned to the correct source groups. Genotypic analysis such as rep-PCR is considered less subject to environmental effects than phenotypic analysis. Other advantages of rep-PCR are its simplicity, accuracy, and speed, which are desirable for high-throughput analysis (Versalovic et al., 1994; Dombek et al., 2000). In addition, in the rep-PCR analyses performed in their study, DNA fingerprints were generated by using whole cell suspensions, which eliminated the need for DNA purification.

Use of human enteric viruses

Human enteric viruses can be used to confirm the presence of human Fecal material. Human enteric virus groups include Norwalk virus, rotavirus, hepatitis A virus, adenovirus, and enterovirus. Adenoviruses are the only human enteric viruses that contain DNA rather than RNA and are substantially more stable than either poliovirus or hepatitis A virus in tap water and seawater (Enriquez et al., 1995). They are potentially good human source indicators. However, the methodologies involved in their detection and enumeration tend to be intensive, costly and time consuming (Sinton, 1998). Many researchers are trying to develop less expensive and reliable ways of finding viruses in seawater.

Worldwide, only a few laboratories are capable of tracking viruses in ocean water. Sassaroli et al. (2000) collected 43 samples of raw sewage and sewage-polluted creek water near San Paulo, Brazil. Adenoviruses were detected in 25(58.1%) samples by PCR using the primers hexAA1885 and hexAA1913. Jiang et al. (2001) tested human adenovirus with Coliphages and bacterial indicators (TC, FC, enterococci) in coastal waters of Southern California. Human adenoviruses were successfully detected in 4 of the 12 samples using the nested PCR method. They suggested that the detection of adenovirus could be used as an index for human fecal pollution, and the presence of other human viruses.

Host-specific molecular markers

Unlike antibiotic resistance and ribotyping analysis, which require culturing indicator organisms, detection of host-specific molecular markers does not require culturing and holds promise as a precise, rapid method for identifying sources of fecal contamination (Bernhard and Field,

2000b). Several researchers have suggested that members of the genera Bacteroides could be used an alternative fecal pollution indicator (Allsop and Stickler, 1985 and Kreader, 1995). Members of these genera are strict anaerobes, are restricted to warm-blooded animals, and make up a significant portion of fecal bacteria. The use of these organisms as indicators however, has been limited because strict anaerobes are often difficult to grow. Using molecular methods rather culture-based methods to detect them can circumvent the difficulty of growing strict anaerobes.

Bernhard and Field (2000a) identified host-specific Bacteroides-Prevotella 16S rDNA markers for human and cows by screening fecal DNAs by length heterogeneity PCR (LH-PCR) and terminal restriction fragment length polymorphism (T-RFLP) analysis. LH-PCR (Suzuki et al., 1998) and T-RFLP (Bruce, 1997;Clement et al., 1998; Liu et al., 1997) are methods that are used to analyze differences in the lengths of gene fragments due to insertions and deletions and to estimate the relative abundance of each fragment. Following the study, they identified additional clones, recovered from water samples, and developed cluster-specific primers that can discriminate between human and ruminant feces using the sequences from fecal and water clones (Bernhard and Field, 2000b). They believe that these PCR assays provide a promising diagnostic tool for identifying nonpoint sources of fecal pollution, although extensive field-testing is required to determine the efficiency of the assays and the geographic distribution of the host-specific markers.

In the past, most of the DNA related technologies involved characterization of individual isolates. Since we are limited in the number of isolates we can test, there is always a statistical problem if a high abundance of target organism present in the contaminated water (Jiang, 2001). This method could overcome conventional culture-dependent technique's problem that mentioned above, it is promising, and further investigation of this approach is required.

CHEMICAL METHODS

Fecal sterols

The term ''Fecal sterol''is a broad term covering the various A-C27, C28, and C29 cholestane-based sterols found in Fecal material (Sinton et al., 1998). Using Fecal sterol such as coprostanol has been proposed as an alternative measure of Fecal pollution by a large number of researchers (Walker et al., 1982). Coprostanol is formed in the gut of human and higher mammals by enzymatic hydrogenation of choleserol or by stereo-specific bacterial reduction of cholesterol (MacDonald et al., 1983). Therefore it is present in sewage effluent and sewage contaminated waters. Several studies have highlighted the usefulness of coprostanol for examining sewage pollution in many diverse environments (Venkatesan and Kaplan, 1990; Venkatesan and Mirsadeghi, 1992). Fecal sterols analysis has been extended to differentiate human and animals sources of pollution. The distribution of sterols found in faeces and hence their source specificity is largely determined by the following three factors (Leeming et al., 1994): (1) The animal's diet. For example, humans, cows, and dogs, respectively, are omnivorous or herbivorous or carnivorous. Each type of diet contains a different sterol profile and the proportions of sterol precursors entering the digestive tract are different; (2) irrespective of dietary habits, many animals can biosynthesize sterols, and (3) anaerobic bacteria in the

digestive tract of some animals biohydrogenate sterols to stanols of different isomeric configurations. This is probably the major factor in dictating the composition and characteristics of the sterols fingerprint. Because of this feature, Fecal sterols offer an advantageous approach that can help distinguish among sources of Fecal pollution.

Fecal sterol such as coprostanol, which constitutes ~60% of the total sterols found in human faces, has been successfully used to trace sewage in many countries. Coprostanol is produced in the intestine of humans and some higher mammals by bacterial biohydrogenation of cholesterol to the 5β(H)-stanol. Coprostanol profiles from a wide variety of animals show difference in the presence/absence or relative amounts of individual sterols (Venkatesan, 1995; Leeming et al, 1996; DNRP, Broward County, 1998). Figure 1 shows coprostanol content (ug /g) in Fecal matter from various sources.

Venkatesan (1995) analyzed Fecal sterols from humans, several animals and influent and effluent samples of Hyperion Plant, and four storm drains. The relative and absolute amounts of coprostanol were much higher in human feces compared to the animals and avian species. For example, the human specimen contained at least 10 times as much coprostanol than the specimens from carnivores and 20-100 times the specimens from herbivores. She suggested that coprostanol in conjunction with other specific sterols parameters can be used to distinguish input of humans from domestic animals and birds.

Leeming et al. (1996) examined the Fecal sterols from humans and 14 species of animals common to rural or urban environments. Human faeces contained ten times more coprostanol on a dry weight basis than faeces from cats and pigs. Herbivores such as cows, sheep and horse feces contained some coprostanol, but their sterol profiles were dominated by C_{29} sterols (24-ethylcoprostanol and 24-ethylcholesterol). They concluded the 'Sterol fingerprints' of the feces of humans and animals are sufficiently distinctive to be of diagnostic value in determining whether Fecal pollution in water samples are of human or animal origin. They also studied the use of a wider range of Fecal sterols, in combination with conventional bacterial indicators, to distinguish the source of Fecal pollution in Lake Tuggerah, Australia, and found that native birds were a major source of the Fecal pollution using an empirical basis and sterol ratio (Leeming et al., 1997).

A Fecal sterol study was conducted in Florida to determine coprostanol, epicoprostanol, cholestanol and epicholesterol from surface water, feces and sediment (DNRP, Broward county, 1998). From the initial data they suggested that it was possible to tell the difference between fresh Fecal samples of human and nonhuman origin based upon the concentration ratios of two of the Fecal sterols. The coprostanol/cholestanol concentration ratio was shown to be greater than 1.0 in human sources and less than 1.0 in non-human feces.

More study is needed on this method if it is to be used for nonpoint sources. This method requires expensive gas chromatography and requires up to 10 liters of samples to be filtered through a glass fiber filter to concentrate particulate stanols. Nevertheless, it is an appropriate method for specific studies investigating the proportion of human and animal Fecal contamination (Bartram and Rees, 2000).

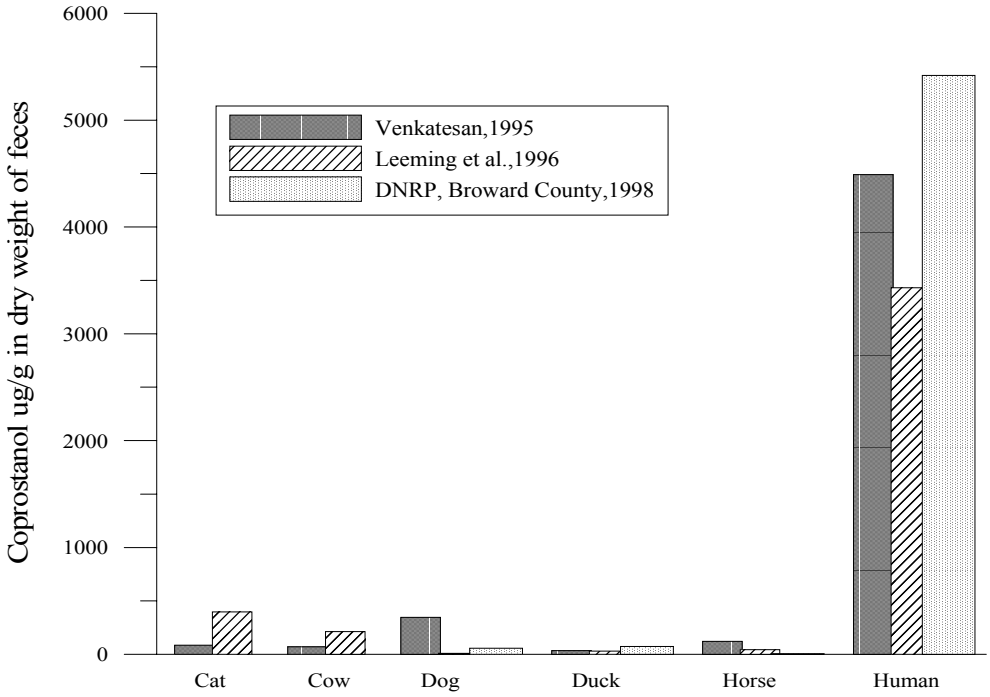

Figure 1. Comparison of coprostanol content in various Fecal samples, ug/g

Long-Chain Alkylbenzenes

Long-chain alkylbenzens (LABs) having C_{10}–C_{14} normal alkyl chains are sulfonated in the industrial production of linear alkylbenzene sulfonates. They are widely used as anionic surfactants in commercial detergents (Eganhouse et al., 1983). A number of studies have found LABs in the waters and sediments exposed to sewage. Observations have been made worldwide and especially in Southern California. Table 4 shows the occurrence of LABs in different California locations. LABs are purely synthetic and are derived solely from direct industrial discharges and domestic wastes (Eganhouse, 1986). They are therefore strongly indicative of human sources. However, they may not be related to sewage such as industrial pollution (Bartram and Rees, 2000). They are also generally present up to one order of magnitude lower than the corresponding Fecal sterol in human derived wastes (Sinton et al.,1998). They are therefore regarded as complimentary to the Fecal sterols as domestic sewage pollution.

Caffeine

Caffeine is a compound that is present in several beverages, coffee, tea, and carbonated drinks, and in pharmaceutical products. Caffeine and its metabolites are excreted in the urine of individuals who have consumed beverages and pharmaceuticals containing caffeine. It has been speculated that caffeine has promise as an indicator of human fecal pollution if the population being studied uses caffeine, is uniquely and unambiguously associated with human activity. Caffeine has been detected in domestic wastewater effluent, environmental surface water samples, ground water and finished drinking water in several locations worldwide. Caffeine was present in municipal wastewaters of populations that use caffeine at levels between 20 and 300 ug/L (Rogers et al., 1986). C affeine has also been detected in Los Angels County wastewater treatment plant effluent samples, 1980-1981, at 40 ug/L (Spectrum Laboratories, 1998).

Concentrations of caffeine in water along the main stem of the Mississippi River, from Minneapolis, Minnesota, to New Orleans, Louisiana, ranged from 0.01 to 0.07 ug/L (Barber et al., 1995). Caffeine was present in samples collected from wastewater treatment plant effluent, and agricultural and urban runoff in Canada and United States, in both dissolved and particulate phases at concentrations up to 0.115 ug/L and 0.044ug/L, respectively (Standley et al., 2000). Although caffeine has been extensively detected in environments exposed to human wastes, there are only a small number of studies that can be used to estimate the probable concentrations of caffeine that might result from sewage spills. Also, because caffeine is extensively metabolized; only 3 percent of ingested caffeine is excreted unmetabolized in the urine (Tang-Liu et al., 1983), its sensitivity as a marker of human fecal pollution is unknown. Therefore, further investigations are required.

Table 4. Levels of LABs in the state of California.

Samples	Area of study	Characteristic	Concentration[a]	Reference
Treated wastewater effluent	CSDOC[b]		8.2 ± 1.8	Phillips et al., 1997
(ug/L)	PLWTP[c]	Particulates	1.92 - 2.76	Zeng et al., 1997
River water	Tijuana river runoff, San Diego	Particulates	0.057-0.714	Zeng et al., 1997
Sewage sludge (ng/g)	JWPCP[d]	Primary-secondary	200,000	Bayona et al., 1997
Sediments (ng/g)	Santa Monica basin	Box-core (0-1, 0-4 cm)	236 ± 124	Bayona et al., 1997
	Santa Ana river		10 ± 7	Phillips et al., 1997
	Newport bay		18 ± 12	Phillips et al., 1997
	Off the coast of San Diego		ND[e] - 39.2	Zeng et al., 1997

[a] Arithmetic mean of total concentrations ± standard deviation or range of concentration.
[b] CSDOC: County Sanitation Districts of Orange County.
[c] PLWTP : Point Loma Wastewater Treatment Plant.
[d] JWPCP: Joint Water Pollution Control Plan.
[e] ND: not detectable.

CONCLUSIONS

There is no easy, low cost method for differentiating between human and non-human sources of bacterial contamination. No single indicator or approach is likely to represent all the facets and issues associated with contamination of waterways with Fecal matter.

At present, the best hope of distinguishing Fecal pollution of human and animal origin is an appropriate combinations of indicators. Statistical analyses of appropriate groups of methods offer the best possibility of identifying human sources. Unfortunately, relying on a combination of methods will probably require a longer period of analysis than relying on a single method. A combination of methods may be useful to determine sources in chronic situations as opposed to episodic events.

Many promising methods have been identified in this review. None, at least at the time of this writing, has been demonstrated in a full scale monitoring program. Most techniques have been limited to research laboratories. Such demonstrations are needed to demonstrate the utility of the methods. Also, commercial laboratories will need assurances that the required investments in training and equipment are justified and recoverable.

A good course of action to further this technology would be to conduct several, long-term investigations, where an advanced method is used in parallel with existing monitoring techniques. Monitoring agencies will need to be involved, in order to evaluate the required retraining and adjustments in their current procedures.

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