Mission Bay Bacterial source Identification Study

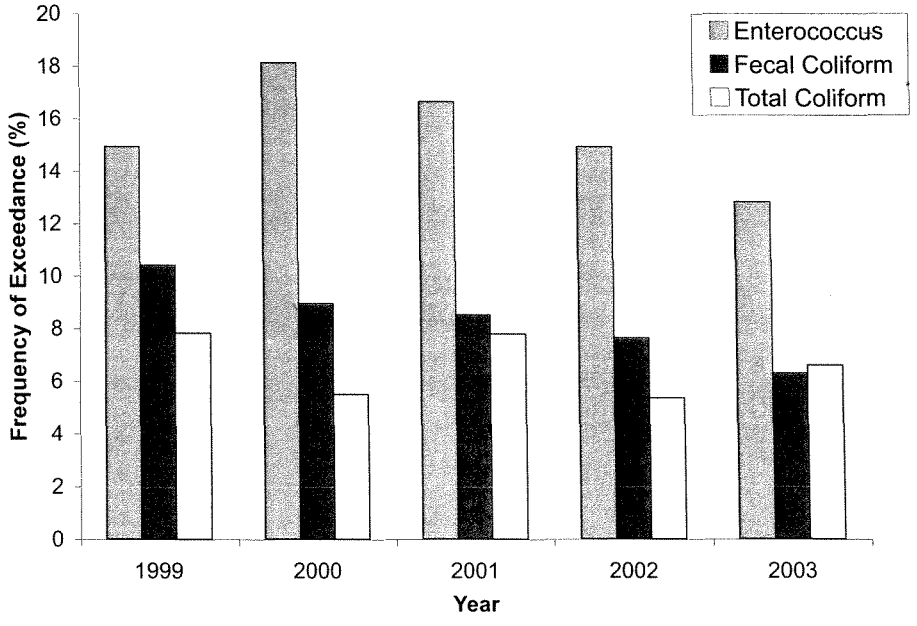
A Clean Beaches Initiative grant helps track causes of contamination.

By Stephen J. Gruber, Lisa Marie Kay, Ruth Kolb, and Karen Henry

Mission Bay, located in the city of San Diego, CA, is used by millions of people each year for a variety of recreational activities. The bay encompasses numerous smaller bays, coves, inlets, and stretches of beach that make it one of the city's most desirable places for aquatic recreation. Unfortunately, elevated levels of indicator bacteria (total coliform, fecal coliform, and enterococcus) have affected water quality in some areas of Mission Bay. Historically, the bay has had more beach postings and closures as a result of elevated bacterial levels than other beaches in San Diego County. As a result, the entire bay was listed as an impaired water body in 1998 under Section 303(d) of the Clean Water Act for exceedances of indicator bacterial standards.

Although high levels of indicator bacteria in the bay have been well documented, the sources of the bacteria have remained elusive. To address this problem, the City of San Diego obtained a Clean Beaches Initiative grant (funded under Proposition 13) to conduct the Mission Bay Bacterial Source Identification Study. The purpose of the two-year study was to identify sources of bacterial contamination in Mission Bay and recommend appropriate actions and activities to eliminate the input of those sources. The study was prepared for the California State Water Resources Control Board by the City of San Diego and Weston Solutions Inc.

The city recognizes Mission Bay as a precious civic resource and for years has taken action to protect its water quality. These efforts span decades and continue today. The city's Metropolitan Wastewater Department has renewed its infrastructure, including sewer main replacements, trunk sewers, and pump station upgrades within the Mission Bay area at a cost of over $120 million between 1985 and 1996. In the early 1990s, the city constructed the Mission Bay Sewage Interceptor System, a $10 million state-of-the-art low-flow storm drain diversion system that encircles the bay. The system diverts dry-weather flows, typically with high bacterial densities, from existing storm drains to the sanitary sewer system for treatment. In 2002, the City of San Diego's Storm Water Pollution Prevention Program received a $3 million grant from the State Water Resources Control Board for water-quality improvements in Mission Bay. A total of $1.3 million was appropriated for this study, and the remainder was used for continued infrastructure improvements within the Mission Bay watershed. In addition, the program created the Mission Bay Water Quality



Average percentage of bacterial analyses in Mission Bay that exceeded single-sample criteria from 1999 through 2003.

Management Plan to better manage and coordinate the water-quality projects being conducted in Mission Bay.

These efforts have been effective in reducing exceedances of water-quality standards for bacteria in the bay, and in recent years the number of exceedances has decreased. In addition, many of the recreational beach areas in Mission Bay do not suffer from bacterial water-quality exceedances, suggesting that input of bacteria to the bay is site specific. Identifying the sources of elevated bacterial levels throughout this complex coastal embayment is a high priority for the city and the primary focus of this study.

Mission Bay investigation sites.

Major Tasks of the Study

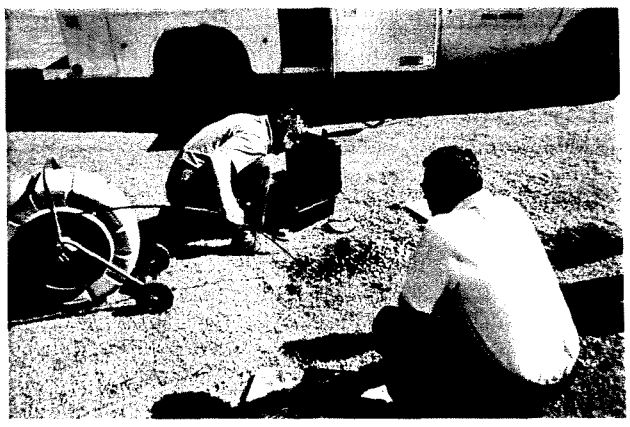
The overall goal of this study was to identify the sources of bacterial contamination to Mission Bay. There were six major tasks designed to achieve this goal. Tasks 1 through 3 were conducted in Phase I from July 2002 through June 2003, and Tasks 4 through 6 were conducted in Phase II from July 2003 through June 2004.

- *Task 1:* Investigate potential sources of human sewage from park restroom infrastructure.
- *Task 2:* Investigate potential sources of human sewage from moored or anchored boats.
- *Task 3:* Conduct visual observations and bacterial assessments of other potential sources in the park.
- *Task 4:* Identify the host origin (human, avian, etc.) of bacteria using molecular source tracking techniques.
- *Task 5:* Determine if bacteria are being transported from the grassy areas of Mission Bay Park to the receiving waters of the bay via groundwater.
- *Task 6:* Determine if the sediments in Mission Bay act as a source of bacteria to the receiving waters at area beaches.

Twelve sites with persistently elevated bacterial densities were identified for the study.

Task 1: Sources of Human Sewage From Park Infrastructure

In Task 1, 16 comfort stations (restrooms) around the 12 investigation sites were evaluated to determine if leaking infrastructure from these facilities was a source of bacteria. The lateral lines of the comfort stations, which carry sewage to the sewer mains, were visually inspected with a closed-circuit television (CCTV) system to assess their physical condition. The inspections revealed that the integrity of the lateral lines of all of the comfort stations investigated was intact and was not a likely source of bacteria. The sewer mains themselves were not inspected because they had been replaced within the last two decades.



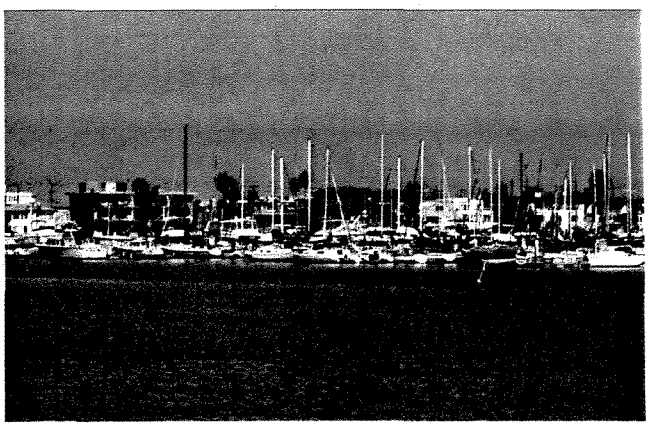
A typical CCTV investigation of a sewer lateral line in Mission Bay Park.

Task 2: Sources of Human Sewage From Moored Boats

In Task 2, illicit discharge of sewage from boat holding tanks was investigated at three locations in Mission Bay where boats moor or anchor. At each site, samples were collected for bacterial analyses in surface waters surrounding the moored or anchored boats and from a beach location where routine monitoring is conducted. Each site was sampled on three separate days. Very low densities of all three bacterial indicators were detected throughout the study at all three sites. In most cases, the densities were below or just above the detection limits. The lack of elevated levels of indicator bacteria from any of the samples collected indicates illegal discharge of sewage from moored and anchored boats was not occurring during the time of sampling. The results also suggest illegal sewage dumping from moored and anchored boats is not a likely chronic source of bacterial contamination. However, the illegal discharge of sewage holding tanks from moored boats is inherently episodic, and results of the study do not rule out the potential for isolated events.

Task 3: Visual Observations

Task 3 was designed to assess the numerous potential sources of bacteria other than leaking comfort station infrastructure and illicit discharge from moored and anchored boats. The poten-



Boats at the Mission Bay Yacht Club.

tial sources assessed included fecal matter from birds and feral and wild animals that inhabit the park; the homeless population; the behavior of some park visitors; and park management practices, such as comfort station cleaning and irrigation procedures. Task 3 included comprehensive visual observations conducted in conjunction with samples taken for analysis of indicator bacteria. Observations and sampling took place during three periods between mid-August and mid-October 2002: low-use, medium-use, and high-use. Within each of these periods, the study included three days of observation (sunrise to sunset). During each day of observation, samples for bacterial analyses (total coliform, fecal coliform, and enterococcus) were taken at 12 sampling locations three times per day. The results were compared to California standards for indicator bacteria. In addition, "spot sampling" was conducted at areas where bacterial influx to the bay was expected (e.g., flowing storm drains).

Approximately 1,300 man-hours of visual observations were made during the nine days of the study (more than 100 hours per site). More than 500 samples from receiving waters and suspected sources were collected and analyzed for indicator bacteria.

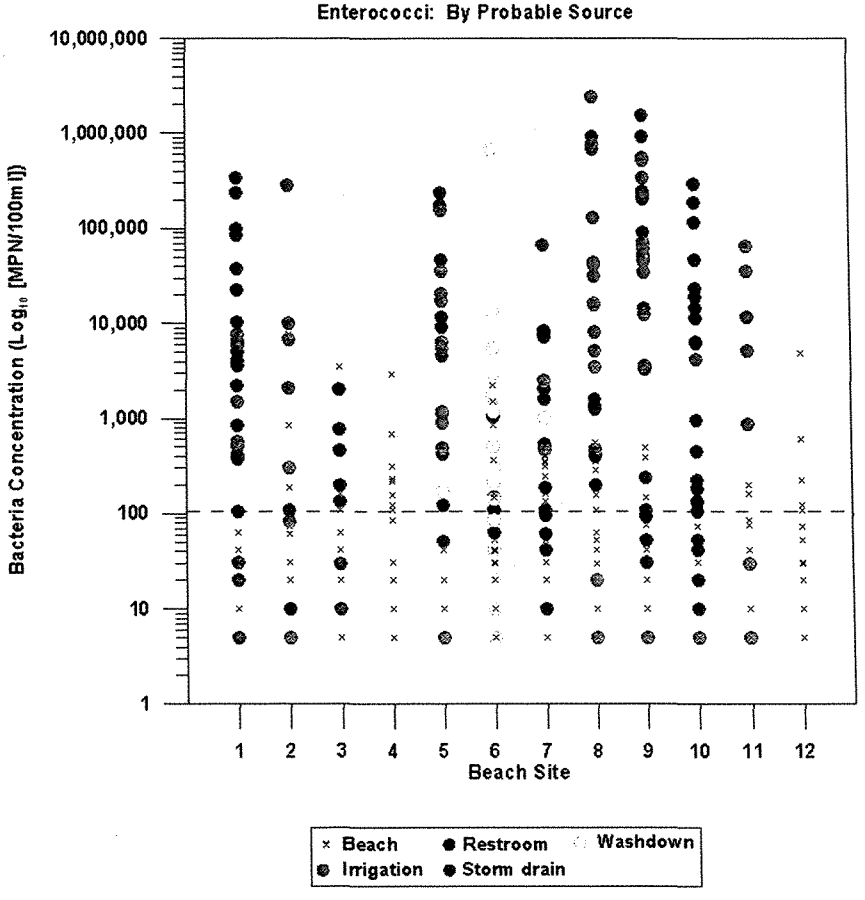
After all of the spot samples had been assessed, the data were categorized by probable source and summarized by site. It was clear from the analysis that each of the 12 sites had a unique set of potential bacterial sources. For instance, most of the samples taken at Site 1 were from drainage around comfort stations, most samples at Site 10 were from flowing drains, and most samples from Site 6 were from boat washdown. The other sites had a mixture of potential sources.

It was clear from the results of Phase I that each of the 12 sites examined had a unique set of characteristics related to potential bacterial sources. At many sites assessed in Phase I, potential bacterial sources initially identified were found not to have an impact on bacterial densities in the receiving waters. The list included illicit discharge of sewage from boats, comfort station infrastructure, the homeless, and RV pump-out stations. In addition, management actions initiated by the city allowed for the removal of potential sources such as comfort station washdown and pet waste at most sites. The results of Phase I were also important in focusing attention on the more likely sources of bacterial influx to the bay identified at the end of the study, such as birds, storm drains, groundwater, and irrigation runoff.

Task 4: Microbial Source Tracking

One of the major goals of this study was to identify the host origin (human, avian, etc.) of the indicator bacteria found in Mission Bay. To this end, two molecular source tracking techniques were employed: ribotyping and the polymerase chain reaction (PCR) technique.

A ribotype is the unique genetic fingerprint of a single bacterial cell, also known as an isolaten (Simpson et al. 2002). Ribotyping analysis relies on a comparison of the fingerprint from bacteria collected from the site (Mission Bay receiving water, storm drain effluent, etc.) to a library database of DNA fingerprints derived from known or confirmed host fecal specimens. The results

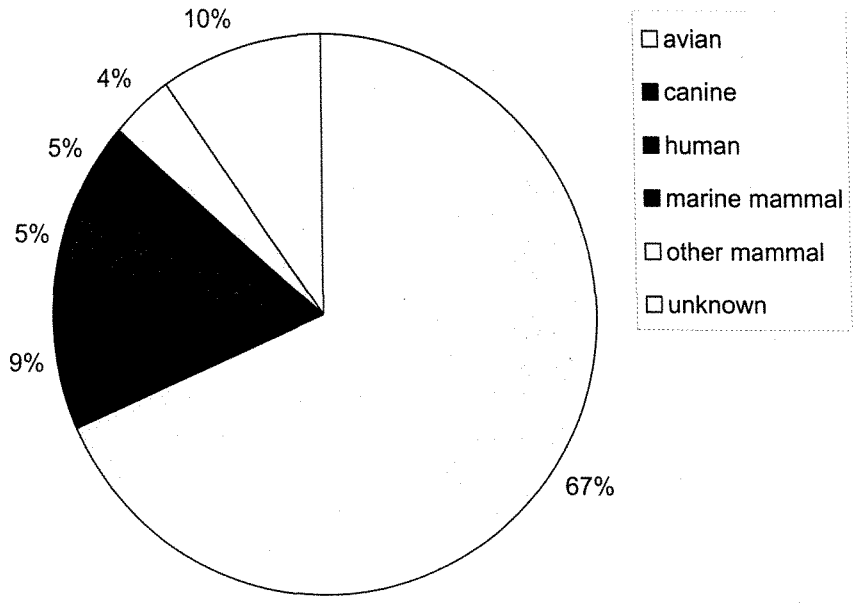


Plot of enterococcus density by site and probable source during the visual observations task. The dashed line represents the AB411 single-sample criteria of 104 MPN/100 mL.

of the ribotyping assessment allow us to determine the host origin (human, avian, canine, etc.) of bacteria in the receiving waters as well as the suspected conduit from which the bacteria were derived (e.g., storm drains, sediments, organic debris).

The PCR technique takes advantage of host-specific genetic differences in an anaerobic bacterium, *Bacteroides*, a major bacterial resident present in feces of warm-blooded animals (Bernhard and Field 2000). The PCR assay provides a rapid first step in tracking bacterial host origin and allows us to determine the presence or absence of human fecal contamination.

The results of Phase I were used to focus the efforts of Task 4 on sites with the highest number of exceedances of California AB411 criteria. A total of 1,097 receiving water isolates was analyzed. The results of the ribotyping analysis indicate birds are the dominant source of the indicator bacteria. Avian sources accounted for 67% of all the bacterial isolates collected, followed by unknown and canine. The percentage of bacterial isolates that originated from human sources was very small, accounting for only 5% of the total number of isolates.



Results of ribotyping analysis of receiving-water samples from all sites studied in Mission Bay between July 2003 and April 2004 show host origins of bacteria.

The results of the PCR analyses strongly support the ribotyping results. Of the 175 receiving-water samples analyzed with the PCR assay, only 9% contained bacterial DNA from human origin.

Because each of the sites assessed in Mission Bay had different characteristics related to bacterial sources, the dominant suspected source (e.g., storm drain effluent) or sources at each site were assessed using MST along with the receiving waters. In this way, the origin of bacteria in a storm drain, for instance, could be assessed. A summary of the bacterial host origin in receiving waters and major suspected sources is presented in Table 1. Results are shown for isolates from the major hosts identified in receiving water and suspected source water (e.g., storm drains). The dominant host origin in each sample type is shown in red.

It is clear from the results that a large majority of the indicator bacteria in Mission Bay receiving waters and major sources (e.g., storm drains) originates from birds. This was a consistent observation at all sites. In addition, the proportion of bacteria from human origin was very small in the receiving waters and particularly in the storm drains. The very low percentage in the storm drains suggests the small amount of bacteria from human origin present in the receiving waters originates on the beach rather than from Mission Bay Park or upstream.

In addition to identifying the host origin of bacteria, the genetic fingerprint provided by the ribotyping assay was used to determine the proportion

of the bacteria in the receiving waters also found in the effluent from suspected sources. A high degree of similarity between the genetic fingerprints of bacteria in the receiving water and those in storm drain effluent, for instance, suggests the storm drain is a source of bacteria. This assessment was completed for the sites in Mission Bay, and in general, there was good agreement between genetic fingerprints of bacteria in the storm drains and those in receivingwaters. These results suggest storm drains are a source of indicator bacteria at the sites assessed.

Table 1. Summary of Ribotyping Results

Site	Sample Type	Isolates From Major Hosts (%) Avian	Canine	Marine Mammal	Other Mammal	Unknown	Human
Bonita Cove	Receiving Water	75	7	2	5	5	6
	Storm Drain	68	10	0	12	10	0
Fanuel Park	Receiving Water	69	7	10	3	4	7
	Storm Drain	60	20	0	15	5	0
Campland	Receiving Water – Dry	79	8	1	7	1	4
	Receiving Water – Wet	69	10	5	1	13	2
De Anza Cove	Receiving Water – Dry	64	5	4	6	12	9
	Storm Drain – Dry	48	19	0	29	0	4
	Receiving Water – Wet	80	3	8	2	5	2
	Storm Drain – Wet	49	29	0	19	3	0
Visitor's Center	Receiving Water	66	14	8	4	6	2
	Spring/Storm Drain	49	27	0	13	11	0
	Cudahy Creek	66	23	0	3	7	1
Leisure Lagoon	Receiving Water	46	16	1	3	29	5
	Storm Drain	58	16	0	22	4	0

Dry refers to samples collected from July 1, 2003, through November 10, 2003. *Wet* refers to samples collected from November 11, 2003, through April 7, 2004.

Task 5: Bacterial Fate and Transport

During the Phase I investigations, very high densities of indicator bacteria were found in the grassy areas of Mission Bay

Park. Excessive irrigation at some sites facilitated the transport of the bacteria through the Mission Bay Park storm drains. The primary goal of Task 5 was to determine if bacteria in the grass are also being transported to the receiving waters via groundwater (Lo et al. 2002). Two types of assessments were conducted:

1. Assessment of bacterial densities in soil beneath the grassy areas of Mission Bay Park
2. Assessment of bacterial densities in groundwater at the same locations and at the beach face springs

Three sites were assessed in Task 5. At each site, a series of three wells was drilled along a transect in line with the beach face spring, perpendicular to the bay. At each well, three sampling probes attached to sterile tubing were inserted into the soil at depths of 4, 7, and 12 feet below the surface. Groundwater was extracted from each of the wells using a peristaltic pump, and fecal coliform and enterococcus bacteria were enumerated.

The results of the study revealed the grassy areas of the three sites assessed (and likely other areas in Mission Bay Park) contain a large reservoir of both fecal coliform and enterococcus bacteria. The origin of the bacteria was determined to be predominantly avian. However, an analysis of bacterial density with depth from the soil core samples indicated the migration of bacteria from the park surface to the groundwater is limited to the upper 18 inches of soil by layers of clay and other fine-grained material. Virtually no indicator bacteria were found in the groundwater wells or beach face springs. The results indicated the grassy areas of the park and the soil directly beneath it contain a large reservoir of indicator bacteria, but the bacteria are not transported to the receiving waters via groundwater seepage. However, the bacteria can be transported to the bay via excessive irrigation and subsequent flow through the park storm drains. The observations made in Phase I suggested that this is occurring at several sites in Mission Bay.

Task 6: Sediment Investigation

The primary goal of Task 6 was to determine if the sediments in Mission Bay act as a source of bacteria to the receiving waters. Investigations were conducted to determine the potential for receiving-water bacterial contamination originating from two types of sediments:

1. Sediments at the mouths of the three major drainages that discharge to Mission Bay that might contaminate adjacent beaches via tidal currents (Solo-Gabrielle et al. 2000)
2. Intertidal sediments that might contaminate receiving water via resuspension (Steets and Holden 2003)

For Task 6, two surveys were conducted: dry season (October 2003) and wet season (January 2004). During both surveys, sediment cores were taken and analyzed for fecal coliform and enterococcus bacteria from surface sediments and at a depth of 4 inches. In addition, bacteria from the surficial sediments and receiving waters at AB411 monitoring sites adjacent to the beaches were analyzed to determine the bacteria host origin.

During the dry-weather survey, fecal coliform and enterococcus densities were generally low. During the wet-weather survey, the mean enterococcus density in surficial sediments increased dramatically at all three sites. The most remarkable differences between the two surveys were in enterococcus densities at depth. At Rose Creek, the mean enterococcus density at depth [4,703 most probable number per gram (MPN/g)] was significantly greater than the dry-weather mean at depth and an order of magnitude higher than any other value measured in either survey. Enterococcus densities at two of the three samples collected at depth from Cudahy Creek were also extremely high (3,047 and 1,375 MPN/g) and similar in magnitude to samples collected at depth at Rose Creek.

Ribotyping analysis from Tecolote Creek samples indicated the majority of the bacteria in the sediment and receiving waters during the wet season originated from avian sources. When the ribotypes from the receiving water were compared to the sediment, 45% of the isolates matched, which suggests the delta sediment at Tecolote Creek may act as a source of bacteria. However, the receiving-water samples were collected during extremely high tides when currents that would transport bacteria were maximal. When the data were applied to a simple transport model, the results suggested that under most conditions, current velocities are insufficient to transport bacteria to the receiving-water monitoring sites. Thus, sediments in the deltas of major drainages to Mission Bay are unlikely sources of bacteria under most conditions.

To assess the extent to which intertidal sediments on the beach impact bacterial densities, two types of assessments were conducted:

1. Beach face transects, which provided a profile of bacterial densities in the intertidal sediments from the high- to low-tide marks
2. Sediment resuspension analysis, which provided a measure of the extent to which resuspension of beach sediments contributed to bacterial levels in the receiving water

The results of the beach face transect assessment indicated there was a strong spatial pattern of bacterial densities along the beach face. Bacteria in beach face sediment samples collected in the upper intertidal zone were typically an order of magnitude greater than those in the lower intertidal zone. Thus, the beach face sands in the upper intertidal zone act as a reservoir for fecal coliform and enterococcus bacteria. The sediment resuspension assessment was designed to determine if bacteria associated with the upper intertidal beach face sediments were a source of bacteria to the receiving waters when the sediments are disturbed (e.g., by swimmer activity).

The results of the resuspension study indicate the bacterial reservoir maintained in the beach face sediments within the upper intertidal zone are released to the receiving waters when they are disturbed. This pattern was not observed when the experience was repeated in the lower intertidal zone.

Bacterial Amplifiers

Toward the completion of the six investigative tasks, two additional investigations were carried out. They were based on observations that organic debris (eel grass, algae, etc.) washed up on beaches and deposited in some storm drains appeared to be associated with elevated bacterial densities at some sites.

Two studies were conducted to assess the extent to which organic debris contributed to elevated bacterial densities as has been shown elsewhere (Whitman et al. 2003):

1. Field study, which investigated the wrack line (primarily organic debris, such as eel grass and algae) that is deposited on some beaches in the upper intertidal zone
2. Laboratory study, which investigated the potential for growth of indicator bacteria under conditions typically

Wrack line on beach at Riviera Shores.

found in a tidally influenced storm drain

The objective of both studies was to assess the extent to which these two areas amplified the indicator bacterial load in Mission Bay.

In the wrack line investigation, samples were collected over an 11-day period after wrack had been deposited on the beach by a high spring tide. Bay water did not make contact with the wrack during the sampling period. Wrack samples were collected from two sites and analyzed for fecal coliform and enterococcus bacteria. The results indicated bacterial densities were maintained at elevated levels for the entire 11-day period, suggesting the wrack line acted as a bacterial reservoir.

At the end of the initial sampling period, receiving-water samples were collected over a tidal cycle as the subsequent spring tide washed over the wrack line. Bacterial densities were low during low tide at the beginning of the tidal cycle before the water made contact with the wrack line. As the tide rose, bacterial densities increased, peaking when the water made maximal contact with the wrack, then decreased as the tide receded. These results strongly suggest the indicator bacteria retained in the wrack line are released during high tide when the bay water makes contact with the wrack. In this way, the wrack amplifies the initial bacterial load. This mechanism is thought to be an important source of indicator bacteria at several sites, particularly in areas where no other bacterial sources have been identified.

The second investigation of bacterial amplification simulated the conditions inside a tidally influenced storm drain. Flasks containing clumps of sterilized eel grass and varying dilutions of sterilized seawater were inoculated with indicator fecal coliform and enterococcus bacteria. The flasks were maintained in the dark under controlled conditions. Bacterial densities were then monitored over a 27-day period.

The results of this simulation show indicator bacteria can survive for an extended period of time in the presence of an organic substrate (eel grass) in 100% seawater (salinity of 32 parts per thousand) and 70% seawater (23 ppt). Survival was reduced in the absence of eel grass.

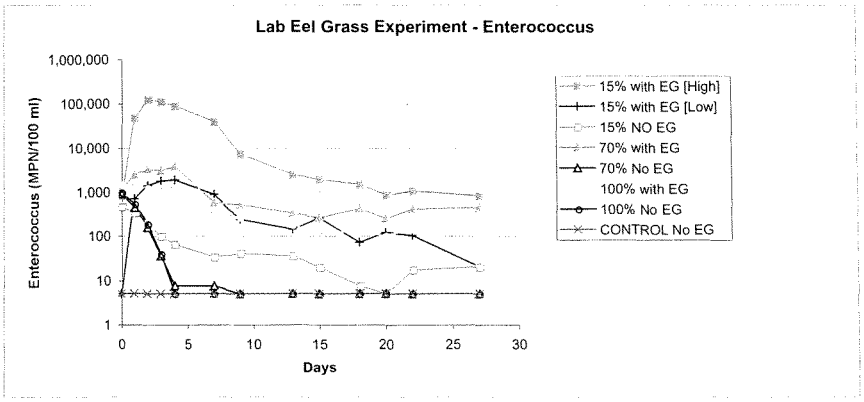
Results of the storm drain simulation experiment suggest both fecal coliform and enterococcus bacteria can survive for prolonged periods of time in coastal storm drains, particularly in the presence of an organic substrate. When fresh water is present in the storm drain, as is often the case due to groundwater intrusion, bacterial densities can increase by several orders of magnitude within a few days of the initial deposition. In this way, storm drains that discharge to Mission Bay can act as bacterial incubators, amplifying the original bacterial load.

Findings and Recommendations

The study is summarized in the conceptual model on page 51.

Overall, the results of this study suggest the majority of the indicator bacteria in Mission Bay originates from birds and that the initial load generated from avian sources can then be amplified by irrigation runoff, storm drains, intertidal sediments, and the wrack line.

Because little can be done about the number of birds in Mission Bay, recommendations on reducing bacterial densities in the bay receiving waters focused on these four areas. The city is actively pursuing management actions to address these recommendations.



Enterococcus densities over time under simulated storm drain conditions. *EG* refers to flasks containing eel grass. *[High]* and *[Low]* refer to initial bacterial inoculant densities.

Acknowledgements

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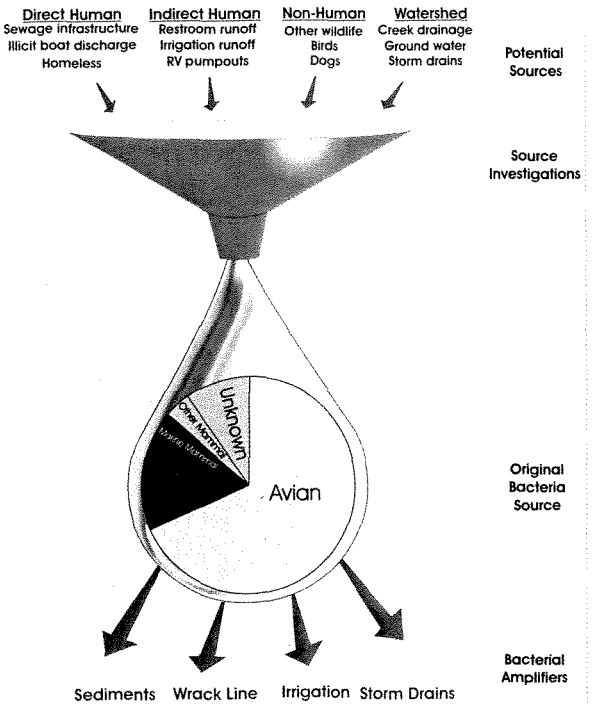

Summary of findings showing original bacteria sources and bacterial amplifiers.

References

Bernhard, A.E., and K.G. Field. 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. *Appl Environ Microbiol* 66:4571-4574.

Lo, K.W., Y.C. Jin, and T. Viraraghavan. 2002. Transport of bacteria in heterogeneous media under leaching conditions. *J Environ Eng Sci* 1:383-395.

Simpson, J.M., J.W. Santo Domingo, and D.J. Reasoner. 2002. Microbial source tracking: State of the science. *Environ Sci Technol* 36:5279-5288.

Solo-Gabrielle, H.M., M.A. Wolfert, T.R. Desmarais, and C.J. Palmer. 2000. Sources of *Escherichia coli* in a coastal subtropical environment. *Appl Environ Microbiol* 66:230-237.

Steets, B.M., and P.A. Holden, 2003. A mechanistic model of runoff-associated fecal coliform fate and transport through a coastal lagoon. *Water Res* 37:589-608.

Whitman, R.C., D.A. Shively, H. Pawlik, M.B. Nevers, and M.N. Byappanahalli. 2003. Occurrence of *Escherichia coli* and enterococci in cladophora (Clorophyta) in nearshore water and beach sand of Lake Michigan. *Appl Environ Microbiol* 69(8): 4714-4719.

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