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Appendix 3

Pathogens and Indicators in Storm Drains Within the Santa Monica Bay Watershed

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by

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EXECUTIVE SUMMARY

Based on the public's perception that recreational activities in Santa Monica Bay increase their health risks, the Santa Monica Bay Restoration Project (SMBRP) has decided that quantification of the health risks of swimming in nearshore waters is a priority area of concern. Previous studies completed under the auspices of the SMBRP demonstrated that high densities of indicator bacteria were present in ankle depth waters were found 100 yards from the Pico-Kenter storm drain. In addition, human enteric viruses were found on two consecutive years in runoff from the same storm drain.

This year's research was designed to further evaluate dispersion of indicator bacteria around the Pico-Kenter storm drain after the completion of a 600 foot pipe extension. Also, further testing for the presence of human enteric virus was completed at the Pico-Kenter and Herondo storm drains, and Malibu Lagoon.

Surf zone indicator bacteria distribution - Low densities of indicator bacteria in the surf zone around the Pico-Kenter storm drain seldom were found when the 600 foot pipe extension was functioning as designed. These results were significantly different from the previous year's study on indicator bacteria distribution that was completed before the pipe extension was completed. When the effluent was discharged further off shore, dilution and near shore currents were effective in reducing indicator bacterial densities in the surf zone. Although drain extensions do not reduce pollution loads to nearshore waters, a properly designed and maintained extension can prove effective in reducing health risks to bathers and swimmers.

Enteric virus prevalence - Human enteric viruses were found at all five virus sampling sites: Pico-Kenter and Herondo storm drains; and all three virus sampling locations within the Malibu Lagoon. The only specific viruses identified were Coxsackie B viruses which can cause illnesses ranging from gastroenteritis to, on rare occasions, pericarditis and meningitis. The results confirm that human fecal contamination of storm drains is far more prevalent than originally assumed. Even though the storm drain system in Los Angeles County is completely separate from the sewer system, the storm drains are not free of human fecal inputs.

Based on the results of the virus studies, recommendations for further research include:
1) An investigation of the health risks associated with various levels of indicators of water quality or with swimming at various distances from flowing drains should move forward;
2) A human specific indicator that correlates with the densities and presence or absence of human pathogens is needed;
3) Tools need to be developed for the easy detection and quantification of specific human pathogens. Models could then be used to demonstrate health risks associated with the specific pathogen; and
4) A sanitary survey needs to be developed and implemented to trace low to moderate sewage contamination in addition to large sewer leaks and spills. A concerted effort to identify and abate the sources of pathogens to the watersheds should be undertaken immediately.

I. INTRODUCTION

A primary goal of the Santa Monica Bay Restoration Project (SMBRP) is to quantify the health risks of swimming in the Bay's recreational waters. The issue of safe swimming in the Bay has been questioned by the public and environmental managers based on numerous anecdotal reports of illnesses attributed to swimming in the Bay, and from measurements of elevated densities of indicator bacteria from around storm drains where their effluents enter the surf zone (Southern California Association of Governments, 1988; City of Los Angeles Environmental Monitoring Division (CLA EMD), 1989, 1990, 1991, SMBRP 1990). Ultimately, these concerns could be addressed through an epidemiology study associating rates of illness with swimming in the Bay.

A pilot study on levels of indicator microorganisms in the surf zone near two storm drains and human enteric viruses in the storm drains was completed during the first year of a multi-year study (Gold et al., 1990). This study revealed that indicator microorganism densities in the surf zone frequently exceeded California Ocean Plan bacterial water quality objectives (State Water Resources Control Board (SWRCB), 1990) in the vicinity of both the Pico-Kenter and Ashland Avenue storm drains. In this and subsequent studies, bacterial "levels of concern" were based on three water quality objectives presented in the State's Ocean Plan. Levels of concern were exceeded when:

a. total coliforms were greater than 1000 colony forming units per 100 ml of water (cfu/100 ml); or

b. fecal coliforms were greater than 200 cfu/100 ml; or

c. enterococci were greater than 24 cfu/100 ml.

The SWRCB numerical limits for bacterial densities were applied as individual values for each sample instead of geometric means for samples collected over a 30 day period as stated in the Ocean Plan. The indicator bacteria standards in the Ocean Plan are predominantly used for compliance monitoring for permitted dischargers, whereas the research performed by the SMBRP was not designed to determine compliance.

In addition to surf zone sampling, water in storm drains was analyzed for densities of a bacteriophage, F-male specific coliphage. Coliphage are currently being considered as an indicator of pathogenic virus survival in marine waters. Standards have not been set for coliphage.

During the first study, human enteric viruses were found in the Pico-Kenter storm drain in 1989 on 11 out of 15 sampling dates (Gold et al,. 1990) and in 1990 during the second study, on three out of four dates (Gold et al., 1991). These viruses are pathogenic, causing illnesses like gastroenteritis, and are specific to human waste (Lennette and Schmidt, 1969). Their presence demonstrated that water in the drain had been contaminated by human sewage. Since

the release of the 1990 study, the City of Santa Monica has kept the beach closed, 100 yards north and south of the Pico-Kenter drain, and implemented measures to reduce or eliminate ponding of runoff on the beach.

A number of questions still need to be addressed prior to deciding whether or not to conduct a large scale epidemiology study. This study should be conducted on recreational bathers in waters with large variability in indicator bacteria densities adjacent to flowing storm drains. These conditions would provide information on the health risks from swimming in waters contaminated only by urban runoff.

In this report, results are presented from two studies performed during the third year of the project. They were designed to:

1. Further evaluate dispersion of indicator bacteria around the Pico-Kenter storm drain after the completion of the 600 foot pipe extension; and

2. Testing for the presence of human enteric virus in the Pico-Kenter and Herondo storm drains, and Malibu Lagoon.

Further work was done in the first study to refine our knowledge on the distribution of indicator bacteria around the Pico-Kenter drain. Gaining an understanding of indicator bacteria distribution in the surf zone is an essential component in deciding which portion of the beach and the swimming public is exposed to excessive indicator densities resulting from storm drain effluent. In this case, the Pico-Kenter drain was chosen because of the historically high levels of indicator bacteria measured in the surf adjacent to the drain. The drain was also chosen because it offered a unique opportunity to test the efficacy of storm drain extensions in reducing indicator bacteria densities in the surf zone.

In the second study, we continued to survey various storm drain effluents for the presence of human enteric viruses. The two storm drains and lagoon studied, like the sampling locations from previous studies, were chosen because they historically have been associated with high densities of indicator bacteria in the surf zone when the drains flow directly to the ocean. This year's virus study was the first time that such a large section of Santa Monica Bay was covered. The information from this study will be used to help determine the potential sites and need for an epidemiology study .

II. STUDY SITES

A. Pico-Kenter Storm Drain

The Pico-Kenter drain is located where Pico Boulevard meets the beach (See figure 1). The storm drain system drains a large area that includes much of Santa Monica and part of West L.A. and Brentwood. There are two drains that discharge to the beach: one is owned by Los

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Angeles County Department of Public Works, and the other by Cal-Trans. Dry weather flow is usually transported 600 feet off shore through a pipe extension. When the pipe is clogged, runoff flows across the beach. The storm drain flows year round with a typical dry flow of approximately 0.2 cubic feet per second (Greene, pers. comm., 1991).

The Pico-Kenter storm drain was sampled again for human enteric virus to determine if viruses were present for the third consecutive year. Samples of runoff samples from the drain were collected where the Promenade crosses the storm drain channel, at a point approximately 200 yards from the surf zone.

In August, 1991, a pipe extension was activated to convey dry weather runoff from the drain to a point 600 ft offshore. This structure was designed to prevent pooling of effluent on the beach, and direct discharge of dry weather flow beyond the surf zone. The distribution of total and fecal coliforms and enterococcus bacteria in the surf zone was further characterized to better define the distribution of these bacteria. Bacterial densities around the drain prior to building this pipe (Gold et al., 1990) will be compared with distribution patterns after activation of the pipe; this "before and after" comparison will be presented in the Discussion section of this report.

B. Herondo Storm Drain

The end of the drain is located at a popular surfing beach where Herondo Blvd. intersects the ocean on the Hermosa Beach-Redondo Beach border (See figure 1). Samples were collected at the intersection of Herondo Blvd. and Harbor Drive from a manhole on the bike path on the west side of Harbor Drive. The storm drain system drains a large area in Hermosa Beach, Redondo Beach and Torrance which has predominantly residential and commercial land uses. Flow exits from a small concrete structure located near the mean high tide line and then drains across the beach. The puddle that forms on the beach is brackish with extensive tidal input. The storm drain flows intermittently throughout the year (Mitchell, pers. comm., 1991).

C. Malibu Creek

The Malibu Creek watershed, containing over 105 square miles, is the largest watershed draining into Santa Monica Bay. Parts of five cities are contained within the watershed: Malibu, Calabasas, Thousand Oaks, Agoura Hills and Westlake Village. Malibu Creek is a soft bottomed stream terminating in Malibu Lagoon. This lagoon is the only remaining brackish water lagoon adjacent to Santa Monica Bay (See figure 1).

All bacteria and virus samples were collected in the 13 acre Malibu Lagoon, adjacent to one of the most popular surfing beaches in California, Surfrider Beach. There were four different sampling sites in the lagoon (See figure 2):

1) The location near Surfrider Beach where the sand berm is typically breached artificially by the California Department of Parks and Recreation (Breach);

2) At the bridge over the C-channel which is the western most channel of the lagoon, next to the Malibu Colony (C-channel);

3) In the soft bottomed rivulet (approximately 150,000 gallons per day) formed by the discharge of treated contaminated groundwater from the Texaco gas station treatment facility and runoff from the adjacent Cross Creek Road shopping center parking lot. The sampling point was three to five yards north of Malibu Creek, just east of the Pacific Coast Highway bridge (Texaco); and

4) At a point on the north bank of the creek, 100 yards upstream of the Texaco discharge (Upstream).

III. SAMPLING

A. Indicator Bacteria

1. Sampling Design and Frequency

The study was carried out over a six month period from mid- May to mid-November, 1991. Ideally, sampling would have occurred during weekends when the most people were using the beach. However, because of the logistical requirements of the microbiology laboratories, sampling was conducted during morning hours on weekdays. All bacterial samples were tested within six hours of sampling.

Sampling in the surf zone around the Pico-Kenter drain occurred on seventeen days over a five week period. Samples for bacterial analyses were collected at the same ten sites as the 1990 surf zone study (Figure 3):

1. Seven stations were positioned at ankle depth at 0, 25, 50, and 100 yard intervals; the "0" position was located directly west of the drain.

2. Three stations were positioned at chest depth at 0, and 25 yard intervals.

All samples were taken from the incoming breaking surf. The ankle depth samples were taken as the surf foam reached the sample bottle at the height of the sampler's ankle. The chest samples were taken where the breaking waves reached the chest height (approximately 3 to 4 ft) of a medium sized adult. Chest depth sampling usually occurred between 30 and 50 yards further away from the drain than ankle depth sampling.

Samples of drain effluent were only collected for bacterial analyses on each of the thirteen enteric virus sampling days. Because of logistical problems after the pipe was extended 600 feet, there was no sampling directly from the Pico-Kenter drain on days where surf zone

bacterial monitoring took place. Likewise, bacterial samples were collected during each day of viral sampling at the Herondo drain and Malibu Lagoon.

2. Analyses

Samples were collected in either 125 ml or 1 liter, high-density, sterile polypropylene bottles. After collection, samples were placed on ice and transferred to the Environmental Monitoring Division's (EMD) microbiology laboratory at the Hyperion Treatment Plant.

Densities of total and fecal coliforms were determined according to Standard Methods (American Public Health Association (APHA), 1985) using the membrane filtration techniques (Section 909). The nutrient enrichment procedure was used for total coliform analyses as recommended in Standard Methods Section 909a. Tests for enterococci followed recommended Environmental Protection Agency (USEPA, 1985) procedures using m-E and Esculin Iron Agar media. Results were reported in colony forming units (cfu/100 ml).

B. Viruses

1. Sampling Design and Frequency

The virus sampling period was from June 3 to October 23, 1991. In addition to enteric virus, samples were analyzed for total and fecal coliforms, enterococcus indicator bacteria, and F-male specific coliphage.

Virus sampling occurred on five different days at Herondo drain, thirteen days at the Pico Kenter drain, and twenty six days at Malibu Lagoon. At Malibu Lagoon, nine virus samples each were taken at the C-channel bridge and the upstream stations. Eight samples were taken from the breach location.

A single sample, was collected on each day. Field sampling was conducted by the Hyperion Environmental Monitoring Division (EMD) and Heal the Bay.

2. Seed Study

Seed studies were performed at the two drains and Malibu Creek using adsorption and elution techniques described in Standard Methods, modified Section 913-A (APHA, 1985). A minimum of three seed experiments were completed at each study location, and these were separated temporally by a minimum of two weeks from field studies in order to prevent the possibility of contamination of field samples with laboratory virus. These studies were done to measure the effectiveness (i.e. percent of recovery) of the process to concentrate and analyze for virus and secondarily, to test toxic effects of the storm drain effluent on virus. Samples for the seed experiments were collected in November, 1991 and the seed studies were all completed by the Orange County Sanitation Districts (OCSD) staff.

Two 35 gal containers were filled with storm drain effluent. The effluent was adjusted to pH 3.5, $AlCl_3$ was added to a final concentration of 0.005 M, and a known amount of attenuated poliovirus (vaccine strain) was added to each container. Triplicate grab samples were taken at the beginning and end of the filtration period. Each grab consisted of 1 ml of sample diluted with 9 ml of sterile Hank's Balanced Salt Solution (HBSS) to minimize any toxic effect to the virus by the drain effluent. The HBSS contained 2% fetal bovine serum which helps prevent virus inactivation when samples are frozen and thawed. Effluent in each container then was run through a concentrator (described below).

Grab samples before and after concentration were analyzed for densities of the poliovirus. Concentrations of virus in the first set of grab samples yielded baseline amounts. Any toxic effects of the effluent then could be assessed by comparing differences in viral concentrations between the first and second set of grab samples. The percent of recovery was measured by comparing virus levels in the final concentrate with baseline levels. The results of the seed study were used to give recovery estimates, not to adjust the densities of enteric viruses recovered from field samples.

3. Sampling and Analysis

a. F-Male Specific Coliphage

Grab samples were obtained on 14 days at the Pico-Kenter drain, five days at the Herondo drain, 25 days at the Malibu Lagoon breach site, 24 days at the C-channel site, 23 days at the Texaco site, and 26 days at the upstream site (from splits of the bacterial samples). Nearly all of the samples were collected on the same dates that virus sampling occurred. The samples were analyzed for F male-specific coliphage by the Orange County Sanitation Districts' (OCSD) virology laboratory. The F-male specific coliphage assay methods and E. coli host bacteria were obtained from Dr. V. Cabelli as described in Appendix I.

b. Enteric Viruses

Enteric viruses were sampled at the storm drain sites using a modified version of Standard Method 913-A (APHA, 1985) and "The USEPA Manual of Methods for Virology," 1984 (EPA-600/4-84-013. Adsorption conditions were pH 3.5 with an $AlCl_3$ molarity of 0.005. The eluent was 3% beef extract adjusted to pH 7.2. Approximately 50 to 140 gallons of effluent were filtered per sample. A detailed description of the enteric virus sampling protocol is in "The USEPA Manual of Methods for Virology" (1984).

Travel set up and the long field processing time (approximately 1.5 hours) required that sampling began in the morning and continued until noon. Only one sample was taken per day at each of the drains.

One-liter eluates from the field sample were delivered to the laboratory and reconcentrated in the afternoon using an organic flocculation procedure (Katzenelson et al.,

1976). Final concentrates were detoxified prior to assay (Glass et al.,1978).

All of the samples were initially analyzed for human enteric viruses on Buffalo green monkey kidney cells (BGMK) by the plaque assay technique. Ten percent of the sample was tested in this manner to determine if there was a quantifiable number of viruses present. If the sample was negative for Plaque Forming Units (PFU), then the remainder of the sample was analyzed by using the liquid overlay technique known as the cytopathic effect assay (CPE) (Lennette and Schmidt, 1969). If the samples were positive in the PFU assay, then the remainder of the sample was not analyzed by using the CPE assay. The CPE assay generally detects a greater number of viruses but is not quantitative. Samples exhibiting CPE were confirmed as viral by re-infecting another flask of BGMK cells with a portion of the supernatant from the original flask. In all cases, CPE positive flasks were confirmed by testing some of the original flask supernate as well as passage into liquid overlay cultures. A detailed description for cell culture and virus assay is presented by EPA (1984).

Fractions of samples positive for enteric virus were sent to the Centre de Recherche en Virologie in Laval, Quebec for identification by the laboratory of Dr. Pierre Payment. Identification by the Lim Pool antisera neutralization technique were not successful. Isolates were eventually identified using monospecific antisera.

C. Physical Measurements

Conductivity, temperature, and pH of the runoff were measured when virus samples were collected. Lagoon heights were measured because there is a common perception that there is the potential for septic system failure in the nearby Malibu Colony area when the water table is high and the lagoon height is over 3.5 feet.

D. Quality Assurance and Quality Control (QA/QC)

The QA/QC protocols established by Standard Methods (APHA,1985) and USEPA (1984, 1985) were followed. Also, the OCSD' primary effluent was assayed for coliphage periodically for quality control purposes. In addition the QA/QC project plan for the study was approved by Ken Kitchingman, a QA officer for EPA Region IX.

E. Data Analyses

Geometric means and 95% confidence intervals were calculated for bacterial indicators. Geometric means were calculated for F-male specific coliphage. Values that were greater than or less than the countable range were not used to calculate geometric means of indicator microorganisms. Arithmetic means were calculated for pH, temperature, and conductivity data.

The percentage of days where surf zone samples exceeded levels of concern was calculated with all raw data. If any replicate on any day exceeded the level of concern, the day was considered above the level of concern for that station.

Analysis of variance (ANOVA) was used to test for significant differences among means from stations at ankle and chest depths. Each station mean was calculated from all data gathered over the study period. The geometric means from the 1990 bacterial distribution study were compared to the 1991 data using separate t-tests.

ANOVA with posteriori multiple comparisons were run to test for site differences in geometric means. Tukey's studentized range test (HSD method; SAS, 1990, Steel and Torrie, 1960) was used for the geometric mean comparisons.

IV. RESULTS

A. Bacterial Indicators

1. Bacterial Distribution

Densities of indicator bacteria were low at all ankle and chest depth stations in the surf zone (Table 1) and extremely high in the Pico-Kenter effluent (Table 2). During each sampling period, indicator densities in drain effluent exceeded levels of concern for all three bacterial groups (Figure 4). In the surf zone, however, levels of concern were seldom exceeded at all ankle and chest depth stations for these indicators. The geometric means of the bacterial densities at all surf zone sites were not significantly different from each other. Ankle depth geometric means were not significantly different from chest depth means.

2. Bacterial Densities in Runoff

The geometric means and 95% Confidence Intervals of bacterial densities measured in runoff samples collected from the Malibu Lagoon, and the Pico-Kenter and Herondo storm drains are given in Table 2. Bacterial densities in the Pico-Kenter drain were approximately three to five times higher than the Herondo drain and one to two orders of magnitude higher than the densities at the four Malibu Lagoon sites. This trend held for all three bacterial indicators. In Malibu Lagoon, the densities were generally lowest to highest from C-channel to the breach to Upstream to Texaco.

The geometric mean densities at the Malibu Lagoon C-Channel and Breach stations were significantly different ($P \leq 0.5$) from the other four stations for total coliforms and enterococcus (Table 2). The geometric means for the Breach and C-Channel stations were not significantly different ($P \geq 0.5$) for all three indicators. The geometric means for the Pico-Kenter and Herondo drains were not significantly different from each other, but they were significantly higher than all other sampling sites for total coliforms and than the C-Channel and Breach sites for enterococcus.

B. Viruses

1. Seeding Studies

Recovery of the seeded poliovirus generally was poor. Results ranged from 6.9 to 30% for Pico-Kenter drain on four days, 0.0 to 18.0% for Herondo on three days, and 2.9 to 7.7% for two days at Malibu Lagoon (Table 3).

2. F-Male Specific Coliphage

The geometric mean of the F-male specific coliphage densities at Herondo and Pico-Kenter drains were similar and they were approximately one order of magnitude greater than the mean densities measured at the Malibu Lagoon sites (Table 4 , Appendix 2). The Malibu Lagoon sites all had low to very low coliphage densities. Unlike samples from the Pico-Kenter and Herondo drains, many of the samples from the Malibu Lagoon (approximately 39%) were below the method detection limits for coliphage.

3. Enteric Virus

Human enteric viruses were detected in runoff on at least one day at all six sampling locations (Table 5, Appendix 3). Quantification of virus was completed for all ten positive virus samples (Table 5). Only three samples were positive in the plaque forming unit assay while seven samples were positive using cytopathic effect assays. The rough estimates of viral densities ranged from 1 pfu/ 2.8 gallons to 1 pfu/ 141 gallons for the plaque assays and ≥ 1 infectious unit (In)/ 10.6 gallons to $\geq$ 1 In/ 80 gallons for the cytopathic effect assays.

Confirmed virus isolates were sent to Dr. Pierre Payment at the Centre de Recherche en Virologie in Laval, Quebec for identification. Identification by the Lim Pool antisera neutralization technique was not successful.* Isolates were eventually identified using monospecific antisera. Enteric viruses were identified in nine of the ten samples that were positive for virus. Due to a laboratory accident, the virus isolate from the 6/5/91, Malibu Lagoon C-channel station sample was lost.

All isolates were identified as Coxsackie B. The isolate from Pico-Kenter on 9/25/91 contained Coxsackie B2 virus while the isolate from Pico-Kenter on 8/13/91 contained Coxsackie B4 virus. The remaining seven isolates contained Coxsackie B5 virus. Coxsackie B viruses can cause gastroenteritis and on rare occasions, pericarditis and meningitis.

C. Physical Measurements

Mean temperatures, conductivities and pHs for runoff from the six sample sites are summarized in Table 6 (All data in Appendix 4). Mean temperatures were similar at all sites ranging from 20.1 to 21.2 C. while the pH at Herondo drain was more acidic averaging 6.5, compared to the other five sites that averaged 7.7 to 8.2. The mean conductivity of runoff was low at Pico-Kenter drain (1.7 mmhos), similar to brackish water at the Herondo drain and Texaco sites, and nearly saline at the breach and C-channel sites. Other than at Pico-Kenter,

the conductivity of runoff varied over a wide range from nearly fresh water to salt water.

The height of Malibu Lagoon above sea level and the tide height at the time of sampling at the Lagoon are also listed in Appendix 4. Lagoon height ranged from two feet (the lowest possible measurement with the measuring stick near the C-Channel station) to 5.4 feet above sea level. Of the 26 days where samples were collected and analyzed for enteric virus, 11 occurred when the lagoon was at its lowest at two feet and nine occurred when the lagoon elevation was over 3.5 feet. Tidal heights ranged from plus 1.2 to six feet during virus sampling at the lagoon.

In general, the conductivity of the samples was high when the lagoon mouth was open and/or when there was a very high tide. The sample conductivity was seldom below ten mmhos at the Breach, C-Channel and Upstream stations when the tide was higher than the lagoon height. The Texaco station was less susceptible to the impacts of the tide because samples collected there were predominantly treated discharge and runoff, and the sampling site was not directly in the lagoon.

IV. DISCUSSION

A. Bacterial Dispersion

1. After Storm Drain Pipe Extension

Only 11 of the 17 sampling dates were ideal for assessing the effectiveness of the drain extension for reducing indicator bacteria densities in the surf zone. The last six days of the sampling period produced different results because a nearby sewer replacement project contributed a large volume of water to the Pico-Kenter drain. The flow from dewatering was discharged into the Pico-Kenter drain where it then flowed across the beach and into the surf zone.

Bacterial levels of concern were always exceeded in the drain itself, but rarely if ever exceeded at ankle or chest depth in the surf zone on days when the pipe extension was operating as designed. Because of the logistical problems of sampling directly from the Pico-Kenter drain after the pipe extension, samples of drain effluent were only collected on days during enteric virus sampling. These dates were not usually the same days as the bacterial dispersion study. Based on the results of the bacterial dispersion study, the runoff plume either did not travel back to shore or the plume was diluted sufficiently to cause indicator densities to be low.

2. Comparison with Storm Drain Before Pipe Extension

The results of the 1991 dispersion study were drastically different from the results of the 1990 dispersion study. In 1990, bacterial levels of concern were frequently exceeded at ankle depth stations at distances of up to 100 yards from the drain (See figure 5). Chest depth samples also exceeded levels of concern 10%, 30% and 40% for fecal coliforms, total coliforms, and

enterococci, respectively. In contrast, bacterial levels of concern were only exceeded a maximum of one out of 11 days at the 10 surf zone samples in the 1991 dispersion study. Also, unlike the 1990 data, there were no significant differences between the bacterial densities at ankle depth versus chest depth for all three indicators.

A comparison of the 1990 and 1991 geometric means and 95% Confidence Intervals over the sampling grid further demonstrates the differences in the data sets (Figures 6a-f). For all three indicators, the geometric means at nearly every surf zone site in 1991 were significantly different than the means in 1990 (Table 7).

Although the data were not representative of conditions before or after the pipe extension was completed and functioning correctly, the geometric means and the percentage of times that bacterial levels of concern were exceeded for six additional sampling dates are included in Appendix 5. The predominant source of the runoff during these six days was the dewatering operation that was occurring in conjunction with a sewer replacement nearby.

It is clear that extending the Pico-Kenter drain 600 feet from shore was effective in reducing the indicator densities of bacteria in the surf zone where potential human exposure to pathogens is highest. However, the drain extension was seldom functioning as designed because of frequent blockage of the extension pipe inlet by debris. This situation caused ponding of effluent on the beach and consequently, direct discharge to the surf zone. Overall, as a Best Management Practice (BMP) to reduce bather exposure to undiluted runoff, the pipe extension appeared to work well. However, the BMP would require some operational, maintenance and design changes to ensure that it works consistently and effectively.

3. Bacterial Densities in Runoff

As in the 1989 and 1990 studies, indicator bacterial densities in the storm drains were high. The geometric mean densities of the indicators at the Pico-Kenter and Herondo drains were two to three orders of magnitude above levels of concern. The mean densities at the Lagoon sites ranged from the low end of levels of concern to over twenty times the levels of concern.

The mean densities at the Lagoon sites further from the ocean (Texaco and Upstream) were two to ten times higher than the densities at the sampling sites close to the beach (Breach and C-Channel). This result was not surprising because of the salinity gradient in the Lagoon. Sites closer to the ocean were more influenced by the tidal flux and usually had higher conductivity than the sites on the east side of the Pacific Coast Highway (PCH) bridge. Sea water dilution of bacterial densities probably accounted for most of the differences between sites in the lagoon, although the prevalence of waterfowl in the upstream portions of the Lagoon likely augmented these differences.

For well over a decade, there has been an assumption that the Malibu Colony septic system begins to experience failure if there is heavy residential use when the water table is high and the water level within the Lagoon channels is above 3.5 feet. In this study, water level

within the Lagoon appeared to have little impact on bacterial densities in the Lagoon (Table 1 and bacterial density data). In general, bacterial densities were high all the time throughout the lagoon, regardless of the water level within the Lagoon. These results do not rule out septic systems within the watershed as a potential source of bacterial contamination, but they imply that bacterial densities are independent of the water level within the Lagoon in dry weather, when the water table is lower. These results support conclusions reached by Warshall et al.,(1992) on Malibu Lagoon water quality.

Possible other sources of indicator bacteria to the lagoon are warm blooded wild and domestic animals, vegetation and soil for total coliforms, numerous storm drains including three that discharge into the Lagoon and lower reaches of the watershed, and many other diffuse sources including campers, temporary residents, and picnickers within the watershed. Little information exists regarding potential sources of indicator bacterial contamination in this watershed. However, monthly monitoring reports on water quality in Malibu Creek are submitted to the Regional Water Quality Control Board (RWQCB) by the Las Virgenes Metropolitan Water District (LVMWD).

A review of one potential source of bacterial indicators, the LVMWD's Tapia Water Reclamation Facility, indicates that the plant could be discounted as a direct source because levels of bacteria in the effluent during the study were very low; in over 90% of the samples, total coliforms were not detected (≤ 1 Most Probable Number (MPN)/100ml (LVMWD, 1991). The highest reported value during the study period was 4 MPN/100 ml. However, the issue of bacterial regrowth in the watershed has never been investigated. In contrast to the Tapia plant's effluent, bacterial densities from Malibu Creek and its tributaries were well above levels of concern at the ten monitoring stations throughout the watershed in the Santa Monica Mountains. Total Coliform densities were high everywhere, while fecal coliform and enterococci densities were generally higher at lagoon stations than at upstream stations (LVMWD, 1991). It appears that many of the sources listed above, especially non-point sources, are contributing bacterial indicators to the lagoon.

B. Viruses

1. F-Male Specific Coliphage

Higher densities of male specific coliphage are expected in sewage contaminated waters than in waters without human fecal inputs (Cabelli, pers. comm., 1989). The trend predicted by Cabelli was not supported by these results. Enteric viruses were found at all five sampling locations despite the fact that mean coliphage densities were approximately an order of magnitude higher at the Pico-Kenter and Herondo drains than the Malibu Lagoon sites. Enteric virus was found at all three Malibu Lagoon sites, yet coliphage densities were always low in the lagoon. The coliphage densities on the days that virus was found were comparable to the mean densities. Also, virus was never found on days where coliphage densities were highest for the site.

The purpose of obtaining the coliphage data was to further investigate the efficacy of using this indicator organism to indicate the presence of human waste without the disadvantages of high cost, slow sample analysis, and extensive training associated with human pathogen detection. Field sample coliphage densities from this study demonstrate that this indicator is not a reliable measure of human pathogen contamination and underscores the need for better methods to identify human specific organisms.

2. Seeding study

Typically, virus recoveries of 20 to 30% are achieved during environmental sampling of water and wastewater (Rao and Melnick, 1986). Recoveries from all sampling sites in this study were far below expected recoveries for environmental samples with seven out of ten seed studies achieving recoveries of less than 10%.

One possible reason for the variability and low levels of recovery was that organic materials in the runoff may have hampered the efficiency of the filter. Oil, grease, and humic acid in runoff can bind to the adsorption sites on filters in the virus concentrator, thus causing the virus adsorbing filters to function poorly. Because most of the virus samples were collected in the summer and all of the seed studies were performed weeks later in the late fall, the question of whether the organic content significantly varied from month to month or between the seasons is an important consideration. The recoveries found in the seed experiments may not have accurately reflected the method recoveries on days of field sampling.

Virus was detected at every site, so the seed data were not important in determining the presence or absence of human waste. The seed data were important because they demonstrated that the procedure had a low precision under field conditions for poliovirus. However, the method may have variable recoveries for different types of enteric viruses. With such low precision in the method, it cannot be assumed that there was no human waste present on days when samples were negative. If viruses were not found at a certain sampling location, one still couldn't determine with certainty the presence or absence of enteric virus because of the low virus recoveries. The fact that virus was detected in the field with such low sample recovery rates reflects on the prevalence of human fecal inputs to Malibu Creek, Herondo and the Pico-Kenter drains.

3. Enteric viruses

Despite the low recovery of the virus concentration methods, enteric viruses were detected at all sampling locations by either the PFU or CPE methods. This result meant that water in the two storm drains, and the Malibu Lagoon, had been contaminated with human sewage. Contamination was probably at a low to moderate level given that the bacterial densities measured in the drains and lagoon sites were below those densities often seen after a sewage spill or leak (Table 2). Typically, runoff contaminated with raw sewage will have densities of total and fecal coliforms ranging from 10^6 to 10^7 cfu/100 ml with the ratio of

Total:Fecal usually ≤ 10 (City of Los Angeles, Environmental Monitoring Division, unpublished data).

Indicator bacteria are useful for sanitary surveys when there is gross or undiluted sewage contamination (e.g. large sewage spill or leak) in the storm drains. In other situations, such as the conditions seen at the two drains and Malibu Lagoon, indicator densities provide little information on the source of virus contamination. Even at Malibu Lagoon, where the Total:Fecal coliform ratio was less than four at the virus sampling locations, indicator densities provided little information on the source(s) of bacterial and viral contamination. However, the intent of this study was only to determine if there was virus contamination, not to determine sources of viral contamination. Studies designed specifically to trace the source(s) of human fecal contamination to the drains are needed.

The only type of viruses detected were the Coxsackie B viruses which are often found in sewage contaminated waters. None of the positive isolates were poliovirus, so there was no cross contamination of the samples from the seed studies. In addition, any potential cross contamination was eliminated because all field samples were collected prior to performing the seed studies. Also, the virus concentrator used for collecting samples had never been used previously for seed studies.

Sources of the contamination to the storm drains are unknown. For the two drains, sources could include illegal connections to the sewage system, illegal discharges (e.g. from mobile homes or recreational vehicles), or leaks from the sewer system. The Malibu Lagoon also could be contaminated by these sources in addition to discharges to the watershed from septic tanks and diffuse sources such as campers and picnickers.

When the Lagoon was sampled, the Tapia Water Reclamation Plant was discharging tertiary treated water into Malibu Creek, about six miles upstream from the lagoon. According to the LVMWD, flows from Tapia during sampling periods in the lagoon were 1.04 MGD and ranged from 0 to 4.6 MGD (LVMWD, 1991). As stated earlier, levels of bacteria in the effluent were very low. A study of enteric virus in water produced by the Tapia plant was conducted in 1987 using similar virus sampling and analytical techniques. Only a single virus on one sampling day (90 gallon sample when the chlorination unit was not functioning) was detected out of 25 days of sampling (James M. Montgomery Engineers, Inc., 1990). However, Tapia does not have an enteric virus monitoring program, so the efficacy of the tertiary filters in virus removal has not been determined in over four years. Although Tapia can not be ruled out as a possible source of virus, it seems unlikely that this facility would represent a source of detectable virus in the Malibu Lagoon system unless the tertiary filters were not functioning properly.

The amount of viruses per volume of water presented in Table 5 must be considered as rough estimates given the following reasons:

1. At low densities, viruses are not normally distributed throughout the sample. It is not

appropriate to extrapolate viral densities based on an aliquot taken from a sample having low concentrations. Only 10% of the sample was used in plaque screening. When that 10% was negative, the remaining sample was assayed by the more sensitive, but more qualitative CPE technique.

2. One of the basic limitations of virus testing is that no cell line can detect all of the enteric viruses that are present in the sample.

3. The results of the seeding studies demonstrated that the density of viruses in the samples were underestimated. Poliovirus recovery at the three drain locations ranged from 0% to 48%, demonstrating the large uncertainty in the accuracy of the sampling method.

4. Toxicity to cultured cells was noted in the first year study at the Ashland Avenue drain, so concentrates had to be diluted prior to assay. Toxic effects of the runoff concentrate on viability of the enteric viruses could not be assessed. The viruses may have been inactivated or experienced a loss of infectivity, so fewer, or even no viruses would have formed plaques during the test.

5. A plaque forming unit may be either one single virion or an aggregate of the virus (Bitton, 1980).

Ideally, a risk assessment of the virus data would be completed and sent to decision makers and risk managers. The enteric virus data will be given to the L.A. County Department of Health Services to perform the risk assessment. The following is a list of the limitations of the results for the purpose of performing an accurate risk assessment:

1. Problems with quantification of enteric viruses in the runoff (see above).

2. The virus analytical methods do not detect all of the enteric viral pathogens.

3. Runoff is a flowing medium that is extremely variable. Physical (flow, pH, total suspended solids, etc.), chemical (oil and grease, heavy metals, etc.) and biological (bacterial indicator densities) parameters vary greatly over time. Virus concentrations are expected to vary over time as well.

4. At low virus densities, one can not assume that the viruses are normally distributed throughout the runoff or the surf zone after discharge. Also, there are large uncertainties in developing accurate dilution factors for storm drains and runoff in the ocean.

5. Without knowing what types of viruses were in the storm drain, it is impossible to estimate the minimum infectious dose for people exposed to sea water contaminated with virus. However, for a conservative risk assessment, one could assume that exposure to

one virus would result in infection (Bitton, 1980).

6. Other than gene probes, current techniques cannot detect some of the viruses (rotavirus and Norwalk viruses) which could cause swimming associated illnesses such as gastroenteritis.

VI. CONCLUSION

Historical monitoring data were used to identify surf zone sites where indicator bacteria densities frequently exceeded levels of concern. Human enteric virus samples were taken at the sites with high bacterial densities in order to assess whether there was human waste present. Because swimming associated illnesses are generally believed to be caused by human viruses, the presence of human waste at the sites would indicate a greater risk of swimming associated illnesses than at sites without human fecal inputs (Cabelli et al 1979, 1982). The original intent of the studies was to identify sites with high indicator bacteria densities caused by flowing storm drains with no sewage inputs. These sites were needed for an epidemiology study designed to determine the health risks from swimming in storm drain contaminated water.

Three years later, human enteric viruses were found at various locations in Malibu Lagoon, Herondo Drain, and three consecutive years at the Pico-Kenter storm drain. Human viruses were not found in limited sampling at Ballona Creek, the Santa Monica Canyon drain, and the Ashland drain, but that does not preclude them from occurring in those locations. In terms of the original study design, the enteric virus assessment has done what it was intended to do; to determine the presence or absence of human waste at the study sites. The study results can not be used to provide accurate information on potential sources of human viruses to the storm drains.

Human fecal contamination of storm drains is far more prevalent than originally assumed. Even though the sewer system in Los Angeles County is completely separate from the storm drain system, the storm drains are not free of human fecal inputs.

The results of the last three years of virus monitoring in storm drains support the following recommendations:

1) An investigation of the health risks associated with various levels of indicators of water quality or with swimming at various distances from flowing drains should move forward.
2) A human specific indicator that correlates with the densities and presence or absence of human pathogens is needed.

3) Tools need to be developed for the easy detection and quantification of specific human pathogens. Models could then be used to demonstrate health risks associated with the

specific pathogen.

4) A sanitary survey needs to be developed and implemented to trace low to moderate sewage contamination in addition to large sewer leaks and spills. A concerted effort to identify and abate the sources of pathogens to the watersheds should be undertaken immediately.

Three years of bacterial indicator monitoring have provided new information on storm drain effluent plume dispersion behavior in the surf zone. When the storm water is discharged directly to the surf, bacterial densities are frequently above levels of concern as far as 100 yards in either direction of the drain. Densities are especially high in shallow water. When the effluent is discharged further off shore, dilution and near shore currents are effective in reducing indicator bacterial densities in the surf zone. Although drain extensions do not reduce pollution loads to nearshore waters, a properly designed and maintained extension can prove effective in reducing health risks to bathers and swimmers.

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Pathogens and Indicators in Storm Drains Within the Santa Monica Bay Watershed

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A document prepared for the:

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EXECUTIVE SUMMARY

Based on the public's perception that recreational activities in Santa Monica Bay increase their health risks, the Santa Monica Bay Restoration Project (SMBRP) has decided that quantification of the health risks of swimming in nearshore waters is a priority area of concern. Previous studies completed under the auspices of the SMBRP demonstrated that high densities of indicator bacteria were present in ankle depth waters were found 100 yards from the Pico-Kenter storm drain. In addition, human enteric viruses were found on two consecutive years in runoff from the same storm drain.

This year's research was designed to further evaluate dispersion of indicator bacteria around the Pico-Kenter storm drain after the completion of a 600 foot pipe extension. Also, further testing for the presence of human enteric virus was completed at the Pico-Kenter and Herondo storm drains, and Malibu Lagoon.

Surf zone indicator bacteria distribution - Low densities of indicator bacteria in the surf zone around the Pico-Kenter storm drain seldom were found when the 600 foot pipe extension was functioning as designed. These results were significantly different from the previous year's study on indicator bacteria distribution that was completed before the pipe extension was completed. When the effluent was discharged further off shore, dilution and near shore currents were effective in reducing indicator bacterial densities in the surf zone. Although drain extensions do not reduce pollution loads to nearshore waters, a properly designed and maintained extension can prove effective in reducing health risks to bathers and swimmers.

Enteric virus prevalence - Human enteric viruses were found at all five virus sampling sites: Pico-Kenter and Herondo storm drains; and all three virus sampling locations within the Malibu Lagoon. The only specific viruses identified were Coxsackie B viruses which can cause illnesses ranging from gastroenteritis to, on rare occasions, pericarditis and meningitis. The results confirm that human fecal contamination of storm drains is far more prevalent than originally assumed. Even though the storm drain system in Los Angeles County is completely separate from the sewer system, the storm drains are not free of human fecal inputs.

Based on the results of the virus studies, recommendations for further research include:
1) An investigation of the health risks associated with various levels of indicators of water quality or with swimming at various distances from flowing drains should move forward;
2) A human specific indicator that correlates with the densities and presence or absence of human pathogens is needed;
3) Tools need to be developed for the easy detection and quantification of specific human pathogens. Models could then be used to demonstrate health risks associated with the specific pathogen; and
4) A sanitary survey needs to be developed and implemented to trace low to moderate sewage contamination in addition to large sewer leaks and spills. A concerted effort to identify and abate the sources of pathogens to the watersheds should be undertaken immediately.

I. INTRODUCTION

A primary goal of the Santa Monica Bay Restoration Project (SMBRP) is to quantify the health risks of swimming in the Bay's recreational waters. The issue of safe swimming in the Bay has been questioned by the public and environmental managers based on numerous anecdotal reports of illnesses attributed to swimming in the Bay, and from measurements of elevated densities of indicator bacteria from around storm drains where their effluents enter the surf zone (Southern California Association of Governments, 1988; City of Los Angeles Environmental Monitoring Division (CLA EMD), 1989, 1990, 1991, SMBRP 1990). Ultimately, these concerns could be addressed through an epidemiology study associating rates of illness with swimming in the Bay.

A pilot study on levels of indicator microorganisms in the surf zone near two storm drains and human enteric viruses in the storm drains was completed during the first year of a multi-year study (Gold et al., 1990). This study revealed that indicator microorganism densities in the surf zone frequently exceeded California Ocean Plan bacterial water quality objectives (State Water Resources Control Board (SWRCB), 1990) in the vicinity of both the Pico-Kenter and Ashland Avenue storm drains. In this and subsequent studies, bacterial "levels of concern" were based on three water quality objectives presented in the State's Ocean Plan. Levels of concern were exceeded when:

a. total coliforms were greater than 1000 colony forming units per 100 ml of water (cfu/100 ml); or

b. fecal coliforms were greater than 200 cfu/100 ml; or

c. enterococci were greater than 24 cfu/100 ml.

The SWRCB numerical limits for bacterial densities were applied as individual values for each sample instead of geometric means for samples collected over a 30 day period as stated in the Ocean Plan. The indicator bacteria standards in the Ocean Plan are predominantly used for compliance monitoring for permitted dischargers, whereas the research performed by the SMBRP was not designed to determine compliance.

In addition to surf zone sampling, water in storm drains was analyzed for densities of a bacteriophage, F-male specific coliphage. Coliphage are currently being considered as an indicator of pathogenic virus survival in marine waters. Standards have not been set for coliphage.

During the first study, human enteric viruses were found in the Pico-Kenter storm drain in 1989 on 11 out of 15 sampling dates (Gold et al,. 1990) and in 1990 during the second study, on three out of four dates (Gold et al., 1991). These viruses are pathogenic, causing illnesses like gastroenteritis, and are specific to human waste (Lennette and Schmidt, 1969). Their presence demonstrated that water in the drain had been contaminated by human sewage. Since

the release of the 1990 study, the City of Santa Monica has kept the beach closed, 100 yards north and south of the Pico-Kenter drain, and implemented measures to reduce or eliminate ponding of runoff on the beach.

A number of questions still need to be addressed prior to deciding whether or not to conduct a large scale epidemiology study. This study should be conducted on recreational bathers in waters with large variability in indicator bacteria densities adjacent to flowing storm drains. These conditions would provide information on the health risks from swimming in waters contaminated only by urban runoff.

In this report, results are presented from two studies performed during the third year of the project. They were designed to:

1. Further evaluate dispersion of indicator bacteria around the Pico-Kenter storm drain after the completion of the 600 foot pipe extension; and

2. Testing for the presence of human enteric virus in the Pico-Kenter and Herondo storm drains, and Malibu Lagoon.

Further work was done in the first study to refine our knowledge on the distribution of indicator bacteria around the Pico-Kenter drain. Gaining an understanding of indicator bacteria distribution in the surf zone is an essential component in deciding which portion of the beach and the swimming public is exposed to excessive indicator densities resulting from storm drain effluent. In this case, the Pico-Kenter drain was chosen because of the historically high levels of indicator bacteria measured in the surf adjacent to the drain. The drain was also chosen because it offered a unique opportunity to test the efficacy of storm drain extensions in reducing indicator bacteria densities in the surf zone.

In the second study, we continued to survey various storm drain effluents for the presence of human enteric viruses. The two storm drains and lagoon studied, like the sampling locations from previous studies, were chosen because they historically have been associated with high densities of indicator bacteria in the surf zone when the drains flow directly to the ocean. This year's virus study was the first time that such a large section of Santa Monica Bay was covered. The information from this study will be used to help determine the potential sites and need for an epidemiology study .

II. STUDY SITES

A. Pico-Kenter Storm Drain

The Pico-Kenter drain is located where Pico Boulevard meets the beach (See figure 1). The storm drain system drains a large area that includes much of Santa Monica and part of West L.A. and Brentwood. There are two drains that discharge to the beach: one is owned by Los

Angeles County Department of Public Works, and the other by Cal-Trans. Dry weather flow is usually transported 600 feet off shore through a pipe extension. When the pipe is clogged, runoff flows across the beach. The storm drain flows year round with a typical dry flow of approximately 0.2 cubic feet per second (Greene, pers. comm., 1991).

The Pico-Kenter storm drain was sampled again for human enteric virus to determine if viruses were present for the third consecutive year. Samples of runoff samples from the drain were collected where the Promenade crosses the storm drain channel, at a point approximately 200 yards from the surf zone.

In August, 1991, a pipe extension was activated to convey dry weather runoff from the drain to a point 600 ft offshore. This structure was designed to prevent pooling of effluent on the beach, and direct discharge of dry weather flow beyond the surf zone. The distribution of total and fecal coliforms and enterococcus bacteria in the surf zone was further characterized to better define the distribution of these bacteria. Bacterial densities around the drain prior to building this pipe (Gold et al., 1990) will be compared with distribution patterns after activation of the pipe; this "before and after" comparison will be presented in the Discussion section of this report.

B. Herondo Storm Drain

The end of the drain is located at a popular surfing beach where Herondo Blvd. intersects the ocean on the Hermosa Beach-Redondo Beach border (See figure 1). Samples were collected at the intersection of Herondo Blvd. and Harbor Drive from a manhole on the bike path on the west side of Harbor Drive. The storm drain system drains a large area in Hermosa Beach, Redondo Beach and Torrance which has predominantly residential and commercial land uses. Flow exits from a small concrete structure located near the mean high tide line and then drains across the beach. The puddle that forms on the beach is brackish with extensive tidal input. The storm drain flows intermittently throughout the year (Mitchell, pers. comm., 1991).

C. Malibu Creek

The Malibu Creek watershed, containing over 105 square miles, is the largest watershed draining into Santa Monica Bay. Parts of five cities are contained within the watershed: Malibu, Calabasas, Thousand Oaks, Agoura Hills and Westlake Village. Malibu Creek is a soft bottomed stream terminating in Malibu Lagoon. This lagoon is the only remaining brackish water lagoon adjacent to Santa Monica Bay (See figure 1).

All bacteria and virus samples were collected in the 13 acre Malibu Lagoon, adjacent to one of the most popular surfing beaches in California, Surfrider Beach. There were four different sampling sites in the lagoon (See figure 2):

1) The location near Surfrider Beach where the sand berm is typically breached

artificially by the California Department of Parks and Recreation (Breach);

2) At the bridge over the C-channel which is the western most channel of the lagoon, next to the Malibu Colony (C-channel);

3) In the soft bottomed rivulet (approximately 150,000 gallons per day) formed by the discharge of treated contaminated groundwater from the Texaco gas station treatment facility and runoff from the adjacent Cross Creek Road shopping center parking lot. The sampling point was three to five yards north of Malibu Creek, just east of the Pacific Coast Highway bridge (Texaco); and

4) At a point on the north bank of the creek, 100 yards upstream of the Texaco discharge (Upstream).

III. SAMPLING

A. Indicator Bacteria

1. Sampling Design and Frequency

The study was carried out over a six month period from mid- May to mid-November, 1991. Ideally, sampling would have occurred during weekends when the most people were using the beach. However, because of the logistical requirements of the microbiology laboratories, sampling was conducted during morning hours on weekdays. All bacterial samples were tested within six hours of sampling.

Sampling in the surf zone around the Pico-Kenter drain occurred on seventeen days over a five week period. Samples for bacterial analyses were collected at the same ten sites as the 1990 surf zone study (Figure 3):

1. Seven stations were positioned at ankle depth at 0, 25, 50, and 100 yard intervals; the "0" position was located directly west of the drain.
2. Three stations were positioned at chest depth at 0, and 25 yard intervals.

All samples were taken from the incoming breaking surf. The ankle depth samples were taken as the surf foam reached the sample bottle at the height of the sampler's ankle. The chest samples were taken where the breaking waves reached the chest height (approximately 3 to 4 ft) of a medium sized adult. Chest depth sampling usually occurred between 30 and 50 yards further away from the drain than ankle depth sampling.

Samples of drain effluent were only collected for bacterial analyses on each of the thirteen enteric virus sampling days. Because of logistical problems after the pipe was extended 600 feet, there was no sampling directly from the Pico-Kenter drain on days where surf zone

bacterial monitoring took place. Likewise, bacterial samples were collected during each day of viral sampling at the Herondo drain and Malibu Lagoon.

2. Analyses

Samples were collected in either 125 ml or 1 liter, high-density, sterile polypropylene bottles. After collection, samples were placed on ice and transferred to the Environmental Monitoring Division's (EMD) microbiology laboratory at the Hyperion Treatment Plant.

Densities of total and fecal coliforms were determined according to Standard Methods (American Public Health Association (APHA), 1985) using the membrane filtration techniques (Section 909). The nutrient enrichment procedure was used for total coliform analyses as recommended in Standard Methods Section 909a. Tests for enterococci followed recommended Environmental Protection Agency (USEPA, 1985) procedures using m-E and Esculin Iron Agar media. Results were reported in colony forming units (cfu/100 ml).

B. Viruses

1. Sampling Design and Frequency

The virus sampling period was from June 3 to October 23, 1991. In addition to enteric virus, samples were analyzed for total and fecal coliforms, enterococcus indicator bacteria, and F-male specific coliphage.

Virus sampling occurred on five different days at Herondo drain, thirteen days at the Pico Kenter drain, and twenty six days at Malibu Lagoon. At Malibu Lagoon, nine virus samples each were taken at the C-channel bridge and the upstream stations. Eight samples were taken from the breach location.

A single sample was collected on each day. Field sampling was conducted by the Hyperion Environmental Monitoring Division (EMD) and Heal the Bay.

2. Seed Study

Seed studies were performed at the two drains and Malibu Creek using adsorption and elution techniques described in Standard Methods, modified Section 913-A (APHA, 1985). A minimum of three seed experiments were completed at each study location, and these were separated temporally by a minimum of two weeks from field studies in order to prevent the possibility of contamination of field samples with laboratory virus. These studies were done to measure the effectiveness (i.e. percent of recovery) of the process to concentrate and analyze for virus and secondarily, to test toxic effects of the storm drain effluent on virus. Samples for the seed experiments were collected in November, 1991 and the seed studies were all completed by the Orange County Sanitation Districts (OCSD) staff.

Two 35 gal containers were filled with storm drain effluent. The effluent was adjusted to pH 3.5, $AlCl_3$ was added to a final concentration of 0.005 M, and a known amount of attenuated poliovirus (vaccine strain) was added to each container. Triplicate grab samples were taken at the beginning and end of the filtration period. Each grab consisted of 1 ml of sample diluted with 9 ml of sterile Hank's Balanced Salt Solution (HBSS) to minimize any toxic effect to the virus by the drain effluent. The HBSS contained 2% fetal bovine serum which helps prevent virus inactivation when samples are frozen and thawed. Effluent in each container then was run through a concentrator (described below).

Grab samples before and after concentration were analyzed for densities of the poliovirus. Concentrations of virus in the first set of grab samples yielded baseline amounts. Any toxic effects of the effluent then could be assessed by comparing differences in viral concentrations between the first and second set of grab samples. The percent of recovery was measured by comparing virus levels in the final concentrate with baseline levels. The results of the seed study were used to give recovery estimates, not to adjust the densities of enteric viruses recovered from field samples.

3. Sampling and Analysis

a. F-Male Specific Coliphage

Grab samples were obtained on 14 days at the Pico-Kenter drain, five days at the Herondo drain, 25 days at the Malibu Lagoon breach site, 24 days at the C-channel site, 23 days at the Texaco site, and 26 days at the upstream site (from splits of the bacterial samples). Nearly all of the samples were collected on the same dates that virus sampling occurred. The samples were analyzed for F male-specific coliphage by the Orange County Sanitation Districts' (OCSD) virology laboratory. The F-male specific coliphage assay methods and E. coli host bacteria were obtained from Dr. V. Cabelli as described in Appendix I.

b. Enteric Viruses

Enteric viruses were sampled at the storm drain sites using a modified version of Standard Method 913-A (APHA, 1985) and "The USEPA Manual of Methods for Virology," 1984 (EPA-600/4-84-013. Adsorption conditions were pH 3.5 with an $AlCl_3$ molarity of 0.005. The eluent was 3% beef extract adjusted to pH 7.2. Approximately 50 to 140 gallons of effluent were filtered per sample. A detailed description of the enteric virus sampling protocol is in "The USEPA Manual of Methods for Virology" (1984).

Travel set up and the long field processing time (approximately 1.5 hours) required that sampling began in the morning and continued until noon. Only one sample was taken per day at each of the drains.

One-liter eluates from the field sample were delivered to the laboratory and reconcentrated in the afternoon using an organic flocculation procedure (Katzenelson et al.,

1976). Final concentrates were detoxified prior to assay (Glass et al.,1978).

All of the samples were initially analyzed for human enteric viruses on Buffalo green monkey kidney cells (BGMK) by the plaque assay technique. Ten percent of the sample was tested in this manner to determine if there was a quantifiable number of viruses present. If the sample was negative for Plaque Forming Units (PFU), then the remainder of the sample was analyzed by using the liquid overlay technique known as the cytopathic effect assay (CPE) (Lennette and Schmidt, 1969). If the samples were positive in the PFU assay, then the remainder of the sample was not analyzed by using the CPE assay. The CPE assay generally detects a greater number of viruses but is not quantitative. Samples exhibiting CPE were confirmed as viral by re-infecting another flask of BGMK cells with a portion of the supernate from the original flask. In all cases, CPE positive flasks were confirmed by testing some of the original flask supernate as well as passage into liquid overlay cultures. A detailed description for cell culture and virus assay is presented by EPA (1984).

Fractions of samples positive for enteric virus were sent to the Centre de Recherche en Virologie in Laval, Quebec for identification by the laboratory of Dr. Pierre Payment. Identification by the Lim Pool antisera neutralization technique were not successful. Isolates were eventually identified using monospecific antisera.

C. Physical Measurements

Conductivity, temperature, and pH of the runoff were measured when virus samples were collected. Lagoon heights were measured because there is a common perception that there is the potential for septic system failure in the nearby Malibu Colony area when the water table is high and the lagoon height is over 3.5 feet.

D. Quality Assurance and Quality Control (QA/QC)

The QA/QC protocols established by Standard Methods (APHA,1985) and USEPA (1984, 1985) were followed. Also, the OCSD' primary effluent was assayed for coliphage periodically for quality control purposes. In addition the QA/QC project plan for the study was approved by Ken Kitchingman, a QA officer for EPA Region IX.

E. Data Analyses

Geometric means and 95% confidence intervals were calculated for bacterial indicators. Geometric means were calculated for F-male specific coliphage. Values that were greater than or less than the countable range were not used to calculate geometric means of indicator microorganisms. Arithmetic means were calculated for pH, temperature, and conductivity data.

The percentage of days where surf zone samples exceeded levels of concern was calculated with all raw data. If any replicate on any day exceeded the level of concern, the day was considered above the level of concern for that station.

Analysis of variance (ANOVA) was used to test for significant differences among means from stations at ankle and chest depths. Each station mean was calculated from all data gathered over the study period. The geometric means from the 1990 bacterial distribution study were compared to the 1991 data using separate t-tests.

ANOVA with posteriori multiple comparisons were run to test for site differences in geometric means. Tukey's studentized range test (HSD method; SAS, 1990, Steel and Torrie, 1960) was used for the geometric mean comparisons.

IV. RESULTS

A. Bacterial Indicators

1. Bacterial Distribution

Densities of indicator bacteria were low at all ankle and chest depth stations in the surf zone (Table 1) and extremely high in the Pico-Kenter effluent (Table 2). During each sampling period, indicator densities in drain effluent exceeded levels of concern for all three bacterial groups (Figure 4). In the surf zone, however, levels of concern were seldom exceeded at all ankle and chest depth stations for these indicators. The geometric means of the bacterial densities at all surf zone sites were not significantly different from each other. Ankle depth geometric means were not significantly different from chest depth means.

2. Bacterial Densities in Runoff

The geometric means and 95% Confidence Intervals of bacterial densities measured in runoff samples collected from the Malibu Lagoon, and the Pico-Kenter and Herondo storm drains are given in Table 2. Bacterial densities in the Pico-Kenter drain were approximately three to five times higher than the Herondo drain and one to two orders of magnitude higher than the densities at the four Malibu Lagoon sites. This trend held for all three bacterial indicators. In Malibu Lagoon, the densities were generally lowest to highest from C-channel to the breach to Upstream to Texaco.

The geometric mean densities at the Malibu Lagoon C-Channel and Breach stations were significantly different ($P \leq 0.5$) from the other four stations for total coliforms and enterococcus (Table 2). The geometric means for the Breach and C-Channel stations were not significantly different ($P \geq 0.5$) for all three indicators. The geometric means for the Pico-Kenter and Herondo drains were not significantly different from each other, but they were significantly higher than all other sampling sites for total coliforms and than the C-Channel and Breach sites for enterococcus.

B. Viruses

1. Seeding Studies

Recovery of the seeded poliovirus generally was poor. Results ranged from 6.9 to 30% for Pico-Kenter drain on four days, 0.0 to 18.0% for Herondo on three days, and 2.9 to 7.7% for two days at Malibu Lagoon (Table 3).

2. F-Male Specific Coliphage

The geometric mean of the F-male specific coliphage densities at Herondo and Pico-Kenter drains were similar and they were approximately one order of magnitude greater than the mean densities measured at the Malibu Lagoon sites (Table 4 , Appendix 2). The Malibu Lagoon sites all had low to very low coliphage densities. Unlike samples from the Pico-Kenter and Herondo drains, many of the samples from the Malibu Lagoon (approximately 39%) were below the method detection limits for coliphage.

3. Enteric Virus

Human enteric viruses were detected in runoff on at least one day at all six sampling locations (Table 5, Appendix 3). Quantification of virus was completed for all ten positive virus samples (Table 5). Only three samples were positive in the plaque forming unit assay while seven samples were positive using cytopathic effect assays. The rough estimates of viral densities ranged from 1 pfu/ 2.8 gallons to 1 pfu/ 141 gallons for the plaque assays and $\geq$1 infectious unit (Iu)/ 10.6 gallons to $\geq$ 1 Iu/ 80 gallons for the cytopathic effect assays.

Confirmed virus isolates were sent to Dr. Pierre Payment at the Centre de Recherche en Virologie in Laval, Quebec for identification. Identification by the Lim Pool antisera neutralization technique was not successful. Isolates were eventually identified using monospecific antisera. Enteric viruses were identified in nine of the ten samples that were positive for virus. Due to a laboratory accident, the virus isolate from the 6/5/91, Malibu Lagoon C-channel station sample was lost.

All isolates were identified as Coxsackie B. The isolate from Pico-Kenter on 9/25/91 contained Coxsackie B2 virus while the isolate from Pico-Kenter on 8/13/91 contained Coxsackie B4 virus. The remaining seven isolates contained Coxsackie B5 virus. Coxsackie B viruses can cause gastroenteritis and on rare occasions, pericarditis and meningitis.

C. Physical Measurements

Mean temperatures, conductivities and pHs for runoff from the six sample sites are summarized in Table 6 (All data in Appendix 4). Mean temperatures were similar at all sites ranging from 20.1 to 21.2 C. while the pH at Herondo drain was more acidic averaging 6.5, compared to the other five sites that averaged 7.7 to 8.2. The mean conductivity of runoff was low at Pico-Kenter drain (1.7 mmhos), similar to brackish water at the Herondo drain and Texaco sites, and nearly saline at the breach and C-channel sites. Other than at Pico-Kenter, the conductivity of runoff varied over a wide range from nearly fresh water to salt water.

The height of Malibu Lagoon above sea level and the tide height at the time of sampling at the Lagoon are also listed in Appendix 4. Lagoon height ranged from two feet (the lowest possible measurement with the measuring stick near the C-Channel station) to 5.4 feet above sea level. Of the 26 days where samples were collected and analyzed for enteric virus, 11 occurred when the lagoon was at its lowest at two feet and nine occurred when the lagoon elevation was over 3.5 feet. Tidal heights ranged from plus 1.2 to six feet during virus sampling at the lagoon.

In general, the conductivity of the samples was high when the lagoon mouth was open and/or when there was a very high tide. The sample conductivity was seldom below ten mmhos at the Breach, C-Channel and Upstream stations when the tide was higher than the lagoon height. The Texaco station was less susceptible to the impacts of the tide because samples collected there were predominantly treated discharge and runoff, and the sampling site was not directly in the lagoon.

IV. DISCUSSION

A. Bacterial Dispersion

1. After Storm Drain Pipe Extension

Only 11 of the 17 sampling dates were ideal for assessing the effectiveness of the drain extension for reducing indicator bacteria densities in the surf zone. The last six days of the sampling period produced different results because a nearby sewer replacement project contributed a large volume of water to the Pico-Kenter drain. The flow from dewatering was discharged into the Pico-Kenter drain where it then flowed across the beach and into the surf zone.

Bacterial levels of concern were always exceeded in the drain itself, but rarely if ever exceeded at ankle or chest depth in the surf zone on days when the pipe extension was operating as designed. Because of the logistical problems of sampling directly from the Pico-Kenter drain after the pipe extension, samples of drain effluent were only collected on days during enteric virus sampling. These dates were not usually the same days as the bacterial dispersion study. Based on the results of the bacterial dispersion study, the runoff plume either did not travel back to shore or the plume was diluted sufficiently to cause indicator densities to be low.

2. Comparison with Storm Drain Before Pipe Extension

The results of the 1991 dispersion study were drastically different from the results of the 1990 dispersion study. In 1990, bacterial levels of concern were frequently exceeded at ankle depth stations at distances of up to 100 yards from the drain (See figure 5). Chest depth samples also exceeded levels of concern 10%, 30% and 40% for fecal coliforms, total coliforms, and

enterococci, respectively. In contrast, bacterial levels of concern were only exceeded a maximum of one out of 11 days at the 10 surf zone samples in the 1991 dispersion study. Also, unlike the 1990 data, there were no significant differences between the bacterial densities at ankle depth versus chest depth for all three indicators.

A comparison of the 1990 and 1991 geometric means and 95% Confidence Intervals over the sampling grid further demonstrates the differences in the data sets (Figures 6a-f). For all three indicators, the geometric means at nearly every surf zone site in 1991 were significantly different than the means in 1990 (Table 7).

Although the data were not representative of conditions before or after the pipe extension was completed and functioning correctly, the geometric means and the percentage of times that bacterial levels of concern were exceeded for six additional sampling dates are included in Appendix 5. The predominant source of the runoff during these six days was the dewatering operation that was occurring in conjunction with a sewer replacement nearby.

It is clear that extending the Pico-Kenter drain 600 feet from shore was effective in reducing the indicator densities of bacteria in the surf zone where potential human exposure to pathogens is highest. However, the drain extension was seldom functioning as designed because of frequent blockage of the extension pipe inlet by debris. This situation caused ponding of effluent on the beach and consequently, direct discharge to the surf zone. Overall, as a Best Management Practice (BMP) to reduce bather exposure to undiluted runoff, the pipe extension appeared to work well. However, the BMP would require some operational, maintenance and design changes to ensure that it works consistently and effectively.

3. Bacterial Densities in Runoff

As in the 1989 and 1990 studies, indicator bacterial densities in the storm drains were high. The geometric mean densities of the indicators at the Pico-Kenter and Herondo drains were two to three orders of magnitude above levels of concern. The mean densities at the Lagoon sites ranged from the low end of levels of concern to over twenty times the levels of concern.

The mean densities at the Lagoon sites further from the ocean (Texaco and Upstream) were two to ten times higher than the densities at the sampling sites close to the beach (Breach and C-Channel). This result was not surprising because of the salinity gradient in the Lagoon. Sites closer to the ocean were more influenced by the tidal flux and usually had higher conductivity than the sites on the east side of the Pacific Coast Highway (PCH) bridge. Sea water dilution of bacterial densities probably accounted for most of the differences between sites in the lagoon, although the prevalence of waterfowl in the upstream portions of the Lagoon likely augmented these differences.

For well over a decade, there has been an assumption that the Malibu Colony septic system begins to experience failure if there is heavy residential use when the water table is high and the water level within the Lagoon channels is above 3.5 feet. In this study, water level within the Lagoon appeared to have little impact on bacterial densities in the Lagoon (Table 1 and bacterial density data). In general, bacterial densities were high all the time throughout the lagoon, regardless of the water level within the Lagoon. These results do not rule out septic systems within the watershed as a potential source of bacterial contamination, but they imply that bacterial densities are independent of the water level within the Lagoon in dry weather, when the water table is lower. These results support conclusions reached by Warshall et al.,(1992) on Malibu Lagoon water quality.

Possible other sources of indicator bacteria to the lagoon are warm blooded wild and domestic animals, vegetation and soil for total coliforms, numerous storm drains including three that discharge into the Lagoon and lower reaches of the watershed, and many other diffuse sources including campers, temporary residents, and picnickers within the watershed. Little information exists regarding potential sources of indicator bacterial contamination in this watershed. However, monthly monitoring reports on water quality in Malibu Creek are submitted to the Regional Water Quality Control Board (RWQCB) by the Las Virgenes Metropolitan Water District (LVMWD).

A review of one potential source of bacterial indicators, the LVMWD's Tapia Water Reclamation Facility, indicates that the plant could be discounted as a direct source because levels of bacteria in the effluent during the study were very low; in over 90% of the samples, total coliforms were not detected ($\leq$ 1 Most Probable Number (MPN)/100ml (LVMWD, 1991). The highest reported value during the study period was 4 MPN/100 ml. However, the issue of bacterial regrowth in the watershed has never been investigated. In contrast to the Tapia plant's effluent, bacterial densities from Malibu Creek and its tributaries were well above levels of concern at the ten monitoring stations throughout the watershed in the Santa Monica Mountains. Total Coliform densities were high everywhere, while fecal coliform and enterococci densities were generally higher at lagoon stations than at upstream stations (LVMWD, 1991). It appears that many of the sources listed above, especially non-point sources, are contributing bacterial indicators to the lagoon.

B. Viruses

1. F-Male Specific Coliphage

Higher densities of male specific coliphage are expected in sewage contaminated waters than in waters without human fecal inputs (Cabelli, pers. comm., 1989). The trend predicted by Cabelli was not supported by these results. Enteric viruses were found at all five sampling locations despite the fact that mean coliphage densities were approximately an order of magnitude higher at the Pico-Kenter and Herondo drains than the Malibu Lagoon sites. Enteric virus was found at all three Malibu Lagoon sites, yet coliphage densities were always low in the lagoon. The coliphage densities on the days that virus was found were comparable to the mean densities. Also, virus was never found on days where coliphage densities were highest for the site.

The purpose of obtaining the coliphage data was to further investigate the efficacy of using this indicator organism to indicate the presence of human waste without the disadvantages of high cost, slow sample analysis, and extensive training associated with human pathogen detection. Field sample coliphage densities from this study demonstrate that this indicator is not a reliable measure of human pathogen contamination and underscores the need for better methods to identify human specific organisms.

2. Seeding study

Typically, virus recoveries of 20 to 30% are achieved during environmental sampling of water and wastewater (Rao and Melnick, 1986). Recoveries from all sampling sites in this study were far below expected recoveries for environmental samples with seven out of ten seed studies achieving recoveries of less than 10%.

One possible reason for the variability and low levels of recovery was that organic materials in the runoff may have hampered the efficiency of the filter. Oil, grease, and humic acid in runoff can bind to the adsorption sites on filters in the virus concentrator, thus causing the virus adsorbing filters to function poorly. Because most of the virus samples were collected in the summer and all of the seed studies were performed weeks later in the late fall, the question of whether the organic content significantly varied from month to month or between the seasons is an important consideration. The recoveries found in the seed experiments may not have accurately reflected the method recoveries on days of field sampling.

Virus was detected at every site, so the seed data were not important in determining the presence or absence of human waste. The seed data were important because they demonstrated that the procedure had a low precision under field conditions for poliovirus. However, the method may have variable recoveries for different types of enteric viruses. With such low precision in the method, it cannot be assumed that there was no human waste present on days when samples were negative. If viruses were not found at a certain sampling location, one still couldn't determine with certainty the presence or absence of enteric virus because of the low virus recoveries. The fact that virus was detected in the field with such low sample recovery rates reflects on the prevalence of human fecal inputs to Malibu Creek, Herondo and the Pico-Kenter drains.

3. Enteric viruses

Despite the low recovery of the virus concentration methods, enteric viruses were detected at all sampling locations by either the PFU or CPE methods. This result meant that water in the two storm drains, and the Malibu Lagoon, had been contaminated with human sewage. Contamination was probably at a low to moderate level given that the bacterial densities measured in the drains and lagoon sites were below those densities often seen after a sewage spill or leak (Table 2). Typically, runoff contaminated with raw sewage will have densities of total and fecal coliforms ranging from 10^6 to 10^7 cfu/100 ml with the ratio of

Total:Fecal usually ≤ 10 (City of Los Angeles, Environmental Monitoring Division, unpublished data).

Indicator bacteria are useful for sanitary surveys when there is gross or undiluted sewage contamination (e.g. large sewage spill or leak) in the storm drains. In other situations, such as the conditions seen at the two drains and Malibu Lagoon, indicator densities provide little information on the source of virus contamination. Even at Malibu Lagoon, where the Total:Fecal coliform ratio was less than four at the virus sampling locations, indicator densities provided little information on the source(s) of bacterial and viral contamination. However, the intent of this study was only to determine if there was virus contamination, not to determine sources of viral contamination. Studies designed specifically to trace the source(s) of human fecal contamination to the drains are needed.

The only type of viruses detected were the Coxsackie B viruses which are often found in sewage contaminated waters. None of the positive isolates were poliovirus, so there was no cross contamination of the samples from the seed studies. In addition, any potential cross contamination was eliminated because all field samples were collected prior to performing the seed studies. Also, the virus concentrator used for collecting samples had never been used previously for seed studies.

Sources of the contamination to the storm drains are unknown. For the two drains, sources could include illegal connections to the sewage system, illegal discharges (e.g. from mobile homes or recreational vehicles), or leaks from the sewer system. The Malibu Lagoon also could be contaminated by these sources in addition to discharges to the watershed from septic tanks and diffuse sources such as campers and picnickers.

When the Lagoon was sampled, the Tapia Water Reclamation Plant was discharging tertiary treated water into Malibu Creek, about six miles upstream from the lagoon. According to the LVMWD, flows from Tapia during sampling periods in the lagoon were 1.04 MGD and ranged from 0 to 4.6 MGD (LVMWD, 1991). As stated earlier, levels of bacteria in the effluent were very low. A study of enteric virus in water produced by the Tapia plant was conducted in 1987 using similar virus sampling and analytical techniques. Only a single virus on one sampling day (90 gallon sample when the chlorination unit was not functioning) was detected out of 25 days of sampling (James M. Montgomery Engineers, Inc., 1990). However, Tapia does not have an enteric virus monitoring program, so the efficacy of the tertiary filters in virus removal has not been determined in over four years. Although Tapia can not be ruled out as a possible source of virus, it seems unlikely that this facility would represent a source of detectable virus in the Malibu Lagoon system unless the tertiary filters were not functioning properly.

The amount of viruses per volume of water presented in Table 5 must be considered as rough estimates given the following reasons:

1. At low densities, viruses are not normally distributed throughout the sample. It is not

appropriate to extrapolate viral densities based on an aliquot taken from a sample having low concentrations. Only 10% of the sample was used in plaque screening. When that 10% was negative, the remaining sample was assayed by the more sensitive, but more qualitative CPE technique.

2. One of the basic limitations of virus testing is that no cell line can detect all of the enteric viruses that are present in the sample.

3. The results of the seeding studies demonstrated that the density of viruses in the samples were underestimated. Poliovirus recovery at the three drain locations ranged from 0% to 48%, demonstrating the large uncertainty in the accuracy of the sampling method.

4. Toxicity to cultured cells was noted in the first year study at the Ashland Avenue drain, so concentrates had to be diluted prior to assay. Toxic effects of the runoff concentrate on viability of the enteric viruses could not be assessed. The viruses may have been inactivated or experienced a loss of infectivity, so fewer, or even no viruses would have formed plaques during the test.

5. A plaque forming unit may be either one single virion or an aggregate of the virus (Bitton, 1980).

Ideally, a risk assessment of the virus data would be completed and sent to decision makers and risk managers. The enteric virus data will be given to the L.A. County Department of Health Services to perform the risk assessment. The following is a list of the limitations of the results for the purpose of performing an accurate risk assessment:

1. Problems with quantification of enteric viruses in the runoff (see above).

2. The virus analytical methods do not detect all of the enteric viral pathogens.

3. Runoff is a flowing medium that is extremely variable. Physical (flow, pH, total suspended solids, etc.), chemical (oil and grease, heavy metals, etc.) and biological (bacterial indicator densities) parameters vary greatly over time. Virus concentrations are expected to vary over time as well.

4. At low virus densities, one can not assume that the viruses are normally distributed throughout the runoff or the surf zone after discharge. Also, there are large uncertainties in developing accurate dilution factors for storm drains and runoff in the ocean.

5. Without knowing what types of viruses were in the storm drain, it is impossible to estimate the minimum infectious dose for people exposed to sea water contaminated with virus. However, for a conservative risk assessment, one could assume that exposure to

one virus would result in infection (Bitton, 1980).

6. Other than gene probes, current techniques cannot detect some of the viruses (rotavirus and Norwalk viruses) which could cause swimming associated illnesses such as gastroenteritis.

VI. CONCLUSION

Historical monitoring data were used to identify surf zone sites where indicator bacteria densities frequently exceeded levels of concern. Human enteric virus samples were taken at the sites with high bacterial densities in order to assess whether there was human waste present. Because swimming associated illnesses are generally believed to be caused by human viruses, the presence of human waste at the sites would indicate a greater risk of swimming associated illnesses than at sites without human fecal inputs (Cabelli et al 1979, 1982). The original intent of the studies was to identify sites with high indicator bacteria densities caused by flowing storm drains with no sewage inputs. These sites were needed for an epidemiology study designed to determine the health risks from swimming in storm drain contaminated water.

Three years later, human enteric viruses were found at various locations in Malibu Lagoon, Herondo Drain, and three consecutive years at the Pico-Kenter storm drain. Human viruses were not found in limited sampling at Ballona Creek, the Santa Monica Canyon drain, and the Ashland drain, but that does not preclude them from occurring in those locations. In terms of the original study design, the enteric virus assessment has done what it was intended to do; to determine the presence or absence of human waste at the study sites. The study results can not be used to provide accurate information on potential sources of human viruses to the storm drains.

Human fecal contamination of storm drains is far more prevalent than originally assumed. Even though the sewer system in Los Angeles County is completely separate from the storm drain system, the storm drains are not free of human fecal inputs.

The results of the last three years of virus monitoring in storm drains support the following recommendations:

1) An investigation of the health risks associated with various levels of indicators of water quality or with swimming at various distances from flowing drains should move forward.
2) A human specific indicator that correlates with the densities and presence or absence of human pathogens is needed.

3) Tools need to be developed for the easy detection and quantification of specific human pathogens. Models could then be used to demonstrate health risks associated with the

specific pathogen.

4) A sanitary survey needs to be developed and implemented to trace low to moderate sewage contamination in addition to large sewer leaks and spills. A concerted effort to identify and abate the sources of pathogens to the watersheds should be undertaken immediately.

Three years of bacterial indicator monitoring have provided new information on storm drain effluent plume dispersion behavior in the surf zone. When the storm water is discharged directly to the surf, bacterial densities are frequently above levels of concern as far as 100 yards in either direction of the drain. Densities are especially high in shallow water. When the effluent is discharged further off shore, dilution and near shore currents are effective in reducing indicator bacterial densities in the surf zone. Although drain extensions do not reduce pollution loads to nearshore waters, a properly designed and maintained extension can prove effective in reducing health risks to bathers and swimmers.

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FIGURE CAPTIONS

Figure 1. Map of the shoreline in northern Santa Monica Bay showing the three storm drain study sites.

Figure 2. Map of the four sampling sites at Malibu Lagoon.

Figure 3. Station positions for the surf zone monitoring study adjacent to the Pico-Kenter storm drain.

Figure 4. Percent of time levels of concern were exceeded at each sampling site around the Pico-Kenter storm drain. See text (Introduction) for description of levels of concern.

Figure 5. Percent of time levels of concern were exceeded at each sampling site around the Pico-Kenter storm drain in 1990 and 1991.

Figure 6 a-f. Plots of geometric means and 95% Confidence Intervals for each of the bacterial indicators measured in the surf zone at ankle and chest depths in 1990 and 1991.

Figure 1.

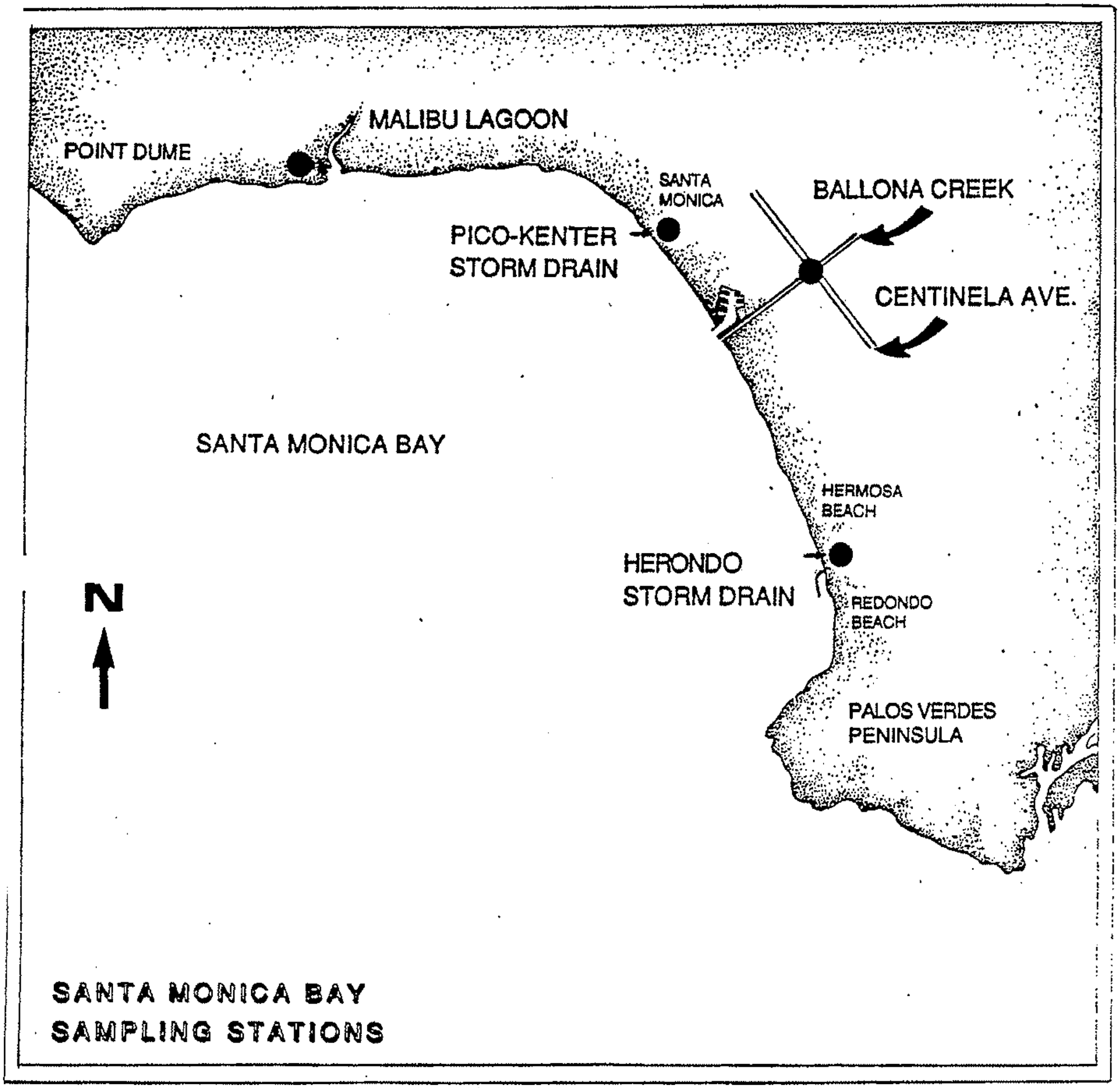
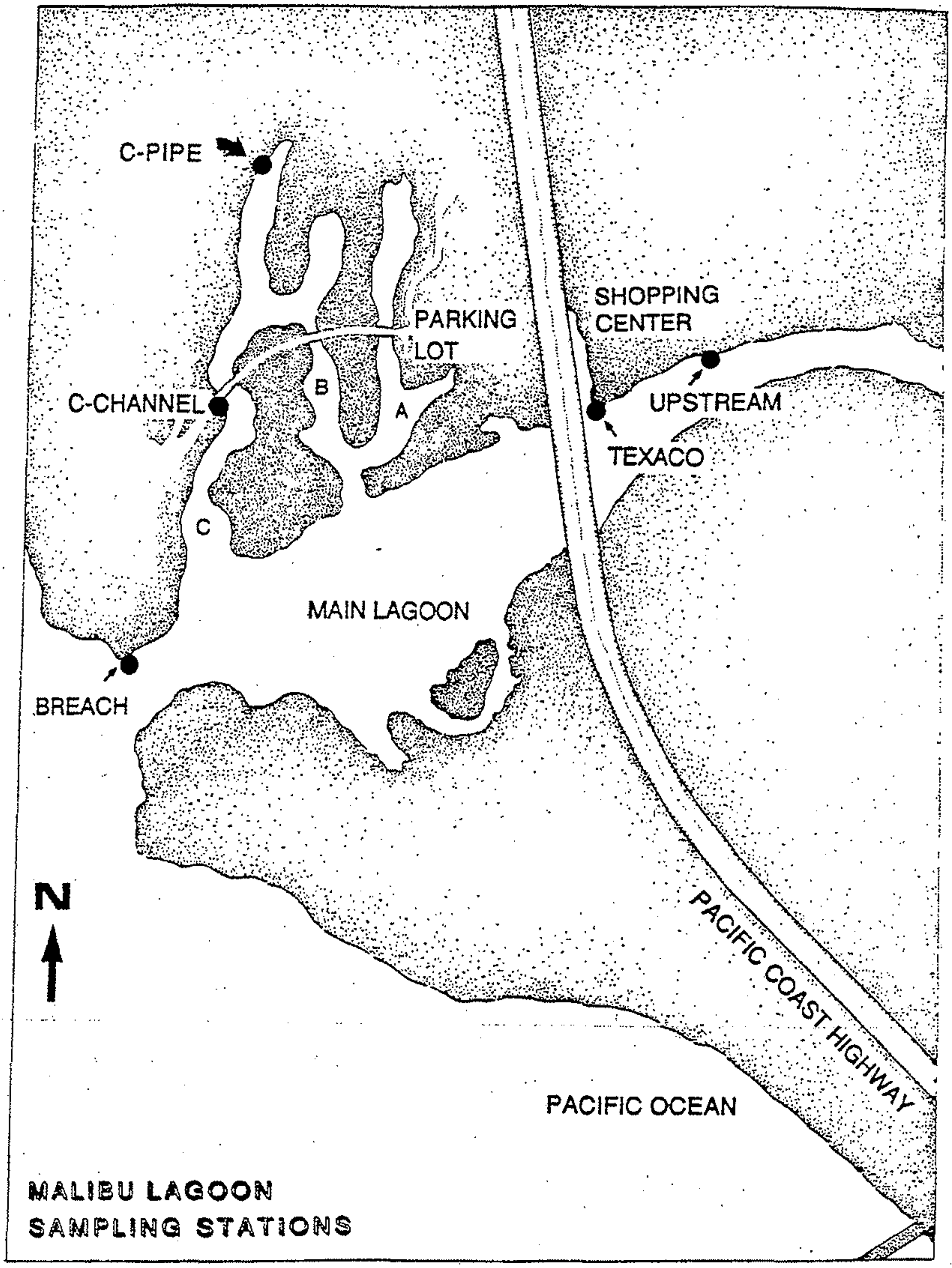

MALIBU LAGOON
POINT DUME
SANTA MONICA
BALLONA CREEK
PICO-KENTER STORM DRAIN
CENTINELA AVE.
SANTA MONICA BAY
HERMOSA BEACH
HERONDO STORM DRAIN
REDONDO BEACH
N
PALOS VERDES PENINSULA
SANTA MONICA BAY SAMPLING STATIONS

Figure 2.


C-PIPE
PARKING
LOT
SHOPPING
CENTER
UPSTREAM
C-CHANNEL
B
A
TEXACO
C
MAIN LAGOON
BREACH
N
PACIFIC COAST HIGHWAY
PACIFIC OCEAN
MALIBU LAGOON
SAMPLING STATIONS

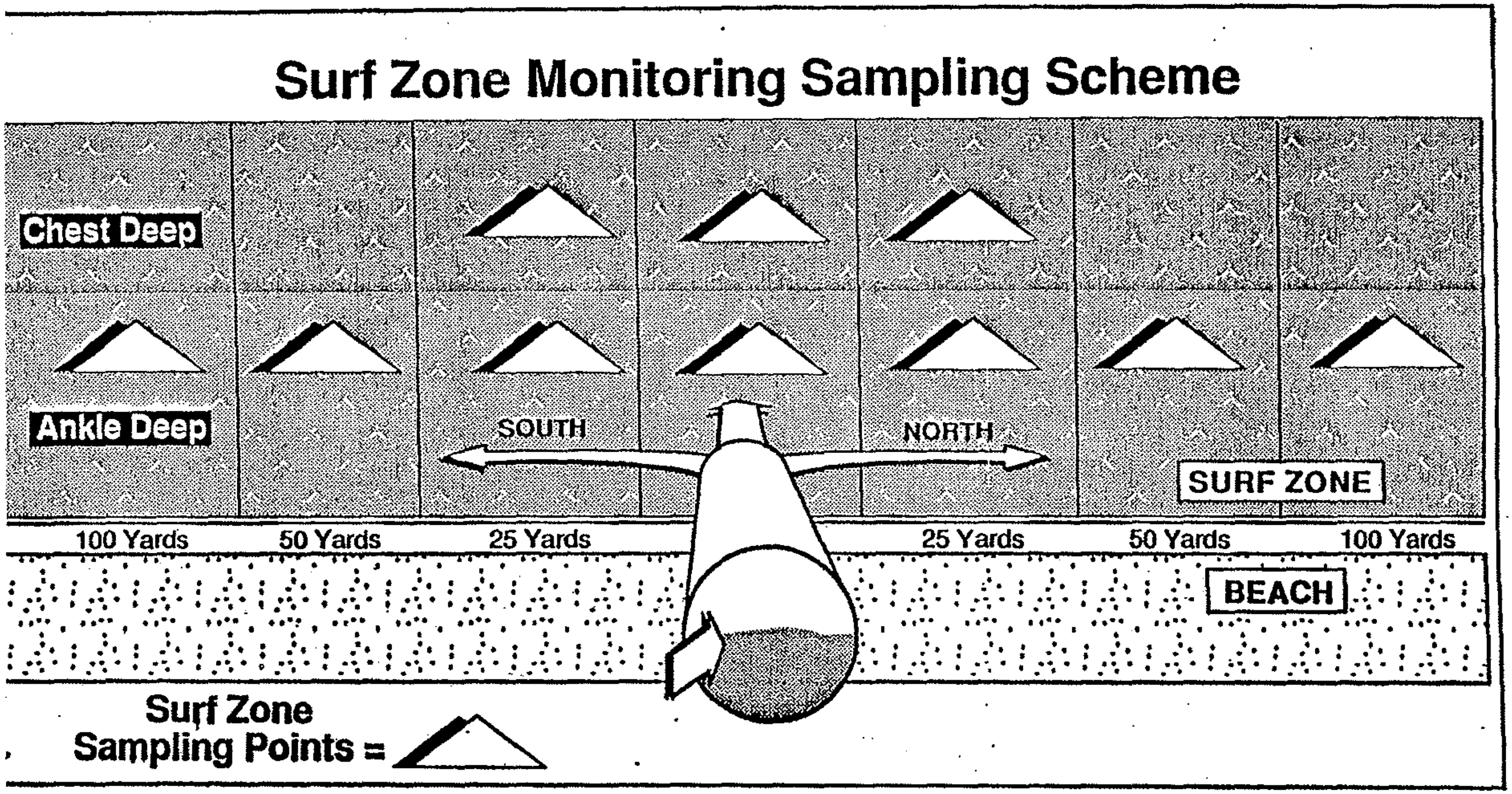
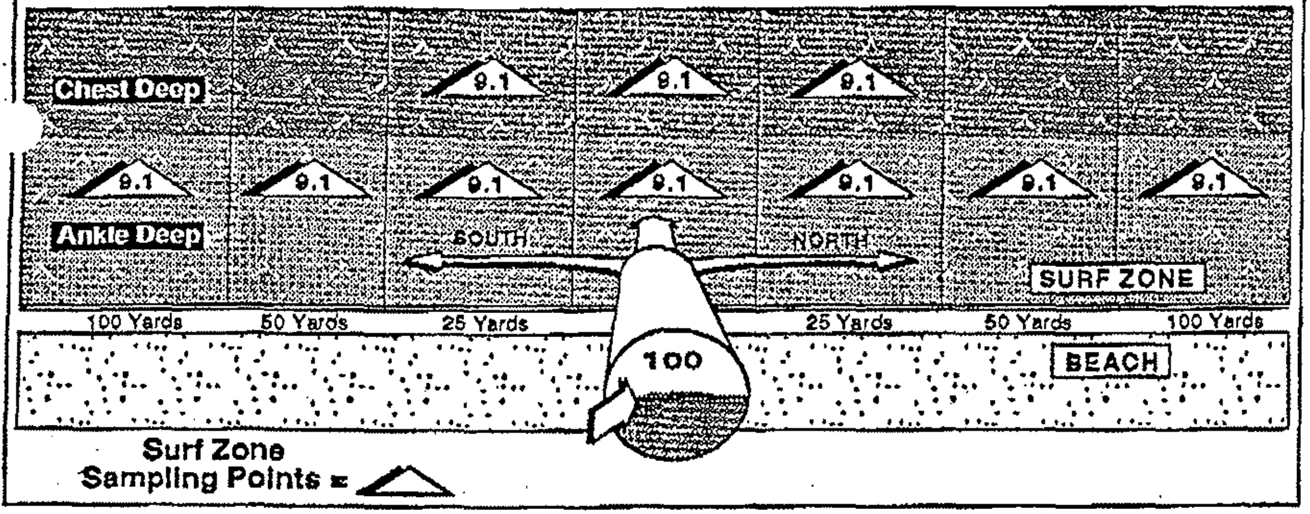
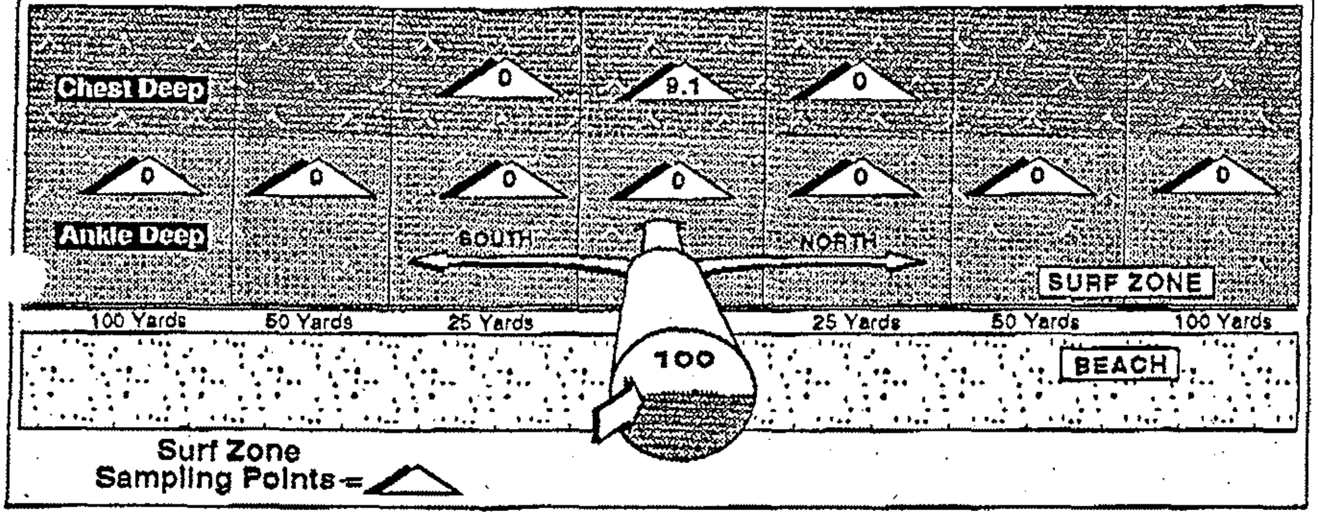

Surf Zone Monitoring Sampling Scheme
Chest Deep
Ankle Deep
SOUTH
NORTH
SURF ZONE
100 Yards
50 Yards
25 Yards
25 Yards
50 Yards
100 Yards
BEACH
Surf Zone
Sampling Points =

Figure 3.1

TOTAL COLIFORMS



FECAL COLIFORMS



ENTEROCOCCI

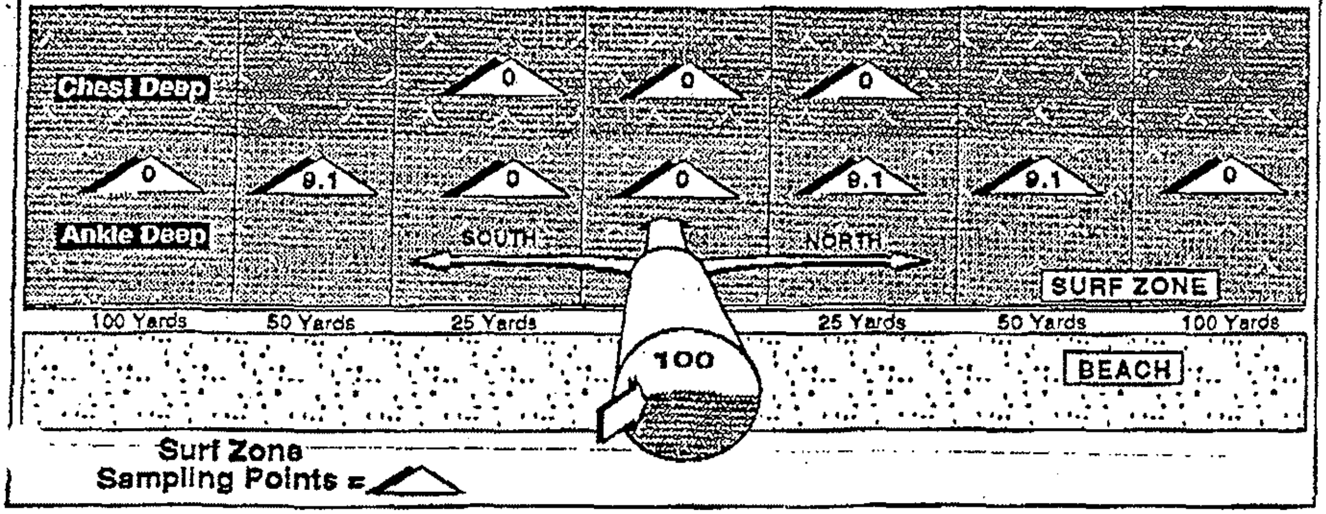


Figure 4.

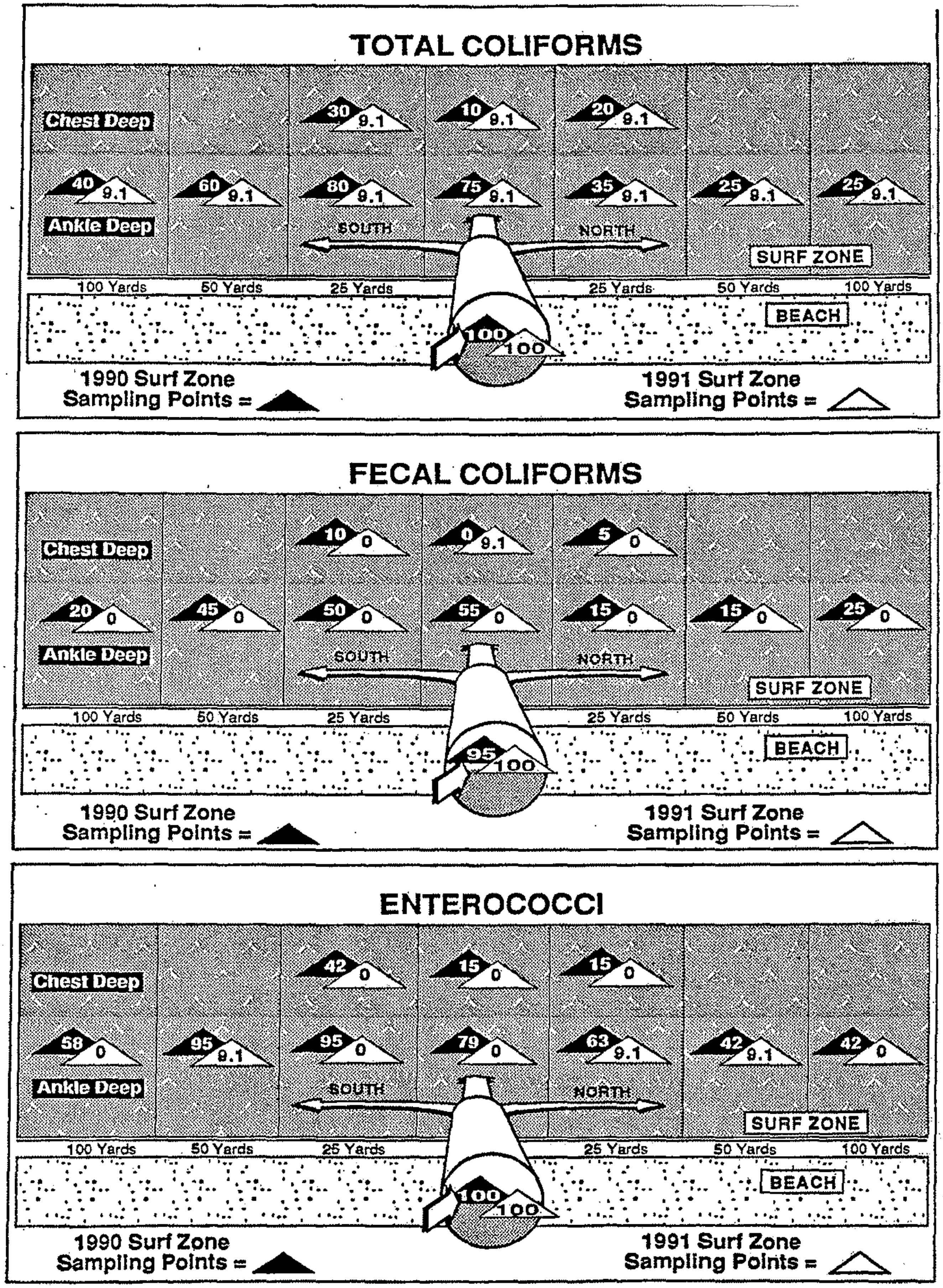

TOTAL COLIFORMS
Chest Deep
30
9.1
10
9.1
20
9.1
40
9.1
60
9.1
80
9.1
75
9.1
35
9.1
25
9.1
25
9.1
Ankle Deep
SOUTH
NORTH
SURF ZONE
100 Yards
50 Yards
25 Yards
25 Yards
50 Yards
100 Yards
BEACH
100
100
1990 Surf Zone Sampling Points = ▲
1991 Surf Zone Sampling Points = △
FECAL COLIFORMS
Chest Deep
10
0
0
9.1
5
0
20
0
45
0
50
0
55
0
15
0
15
0
25
0
Ankle Deep
SOUTH
NORTH
SURF ZONE
100 Yards
50 Yards
25 Yards
25 Yards
50 Yards
100 Yards
BEACH
95
100
1990 Surf Zone Sampling Points = ▲
1991 Surf Zone Sampling Points = △
ENTEROCOCCI
Chest Deep
42
0
15
0
15
0
58
0
95
9.1
95
0
79
0
63
9.1
42
9.1
42
0
Ankle Deep
SOUTH
NORTH
SURF ZONE
100 Yards
50 Yards
25 Yards
25 Yards
50 Yards
100 Yards
BEACH
100
100
1990 Surf Zone Sampling Points = ▲
1991 Surf Zone Sampling Points = △

Figure 5.

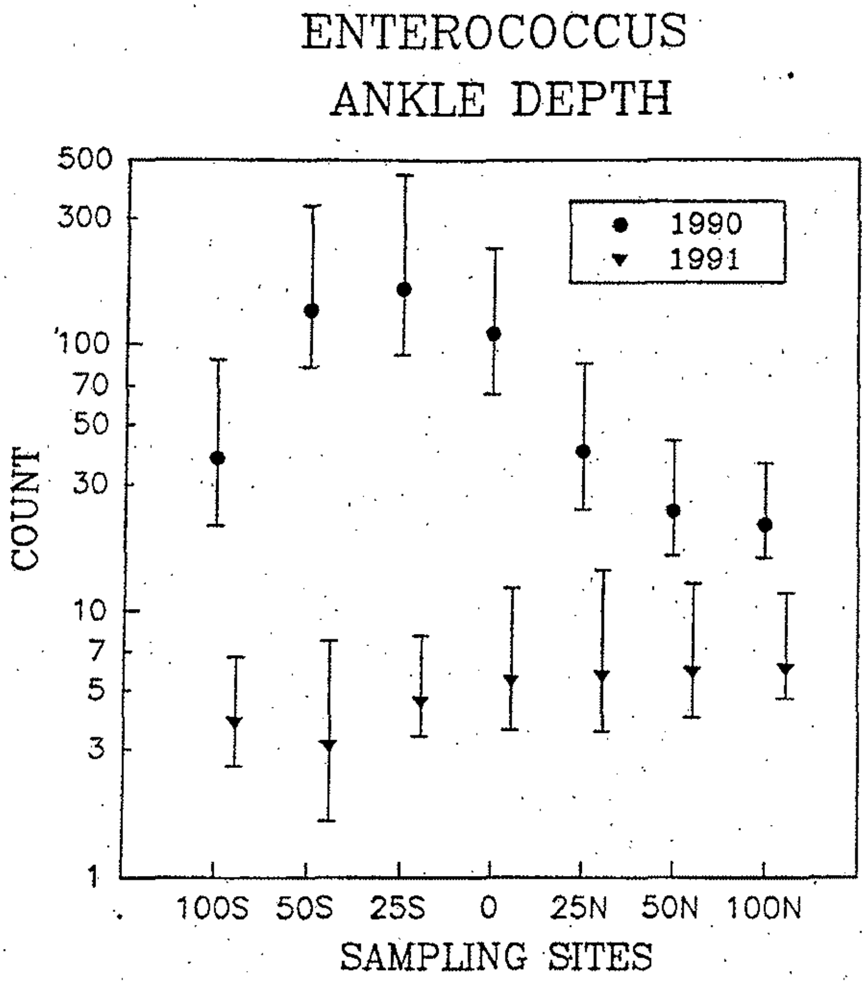

ENTEROCOCCUS
ANKLE DEPTH
500
300
100
70
50
30
10
7
5
3
1
COUNT
● 1990
▼ 1991
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6a.

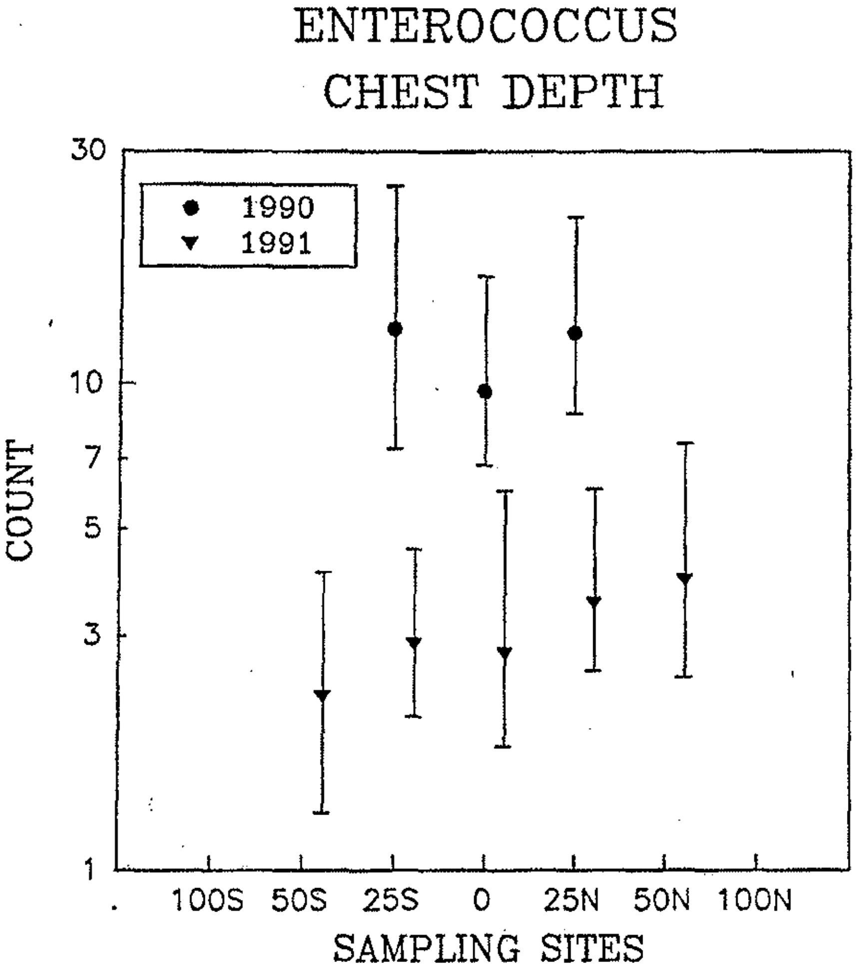

ENTEROCOCCUS
CHEST DEPTH
30
10
7
5
3
1
COUNT
● 1990
▼ 1991
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6b.

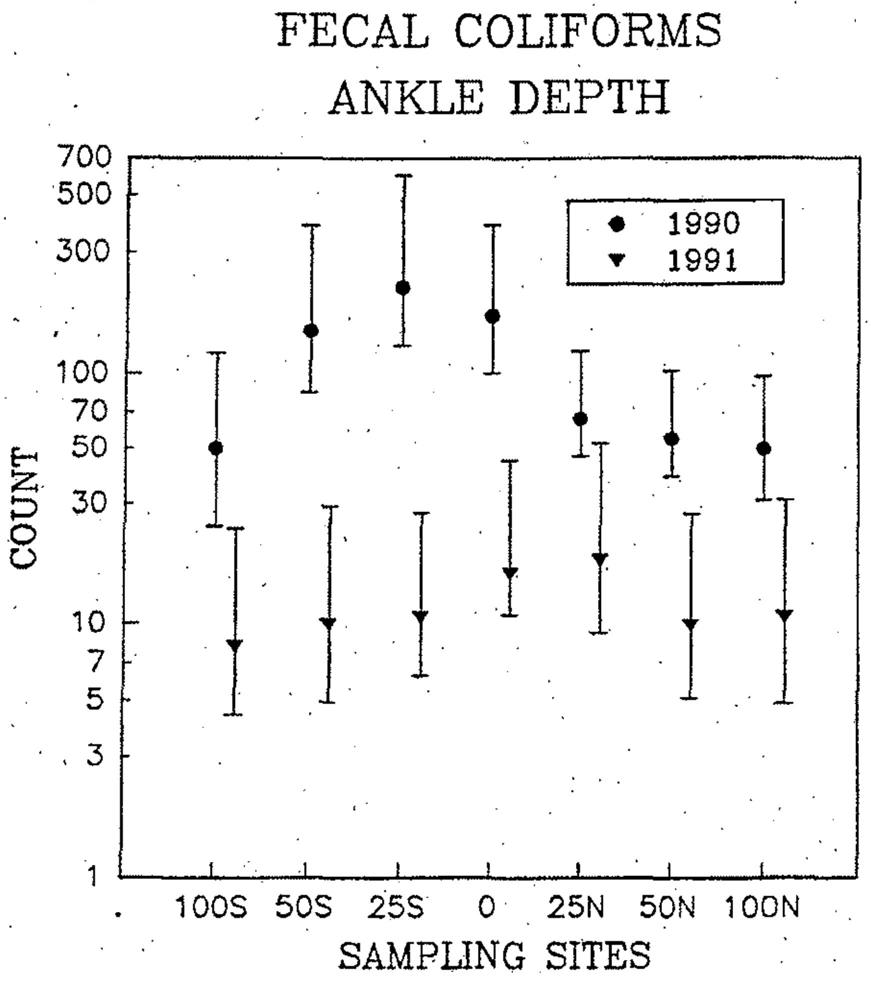

FECAL COLIFORMS
ANKLE DEPTH
● 1990
▼ 1991
COUNT
700
500
300
100
70
50
30
10
7
5
3
1
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6c

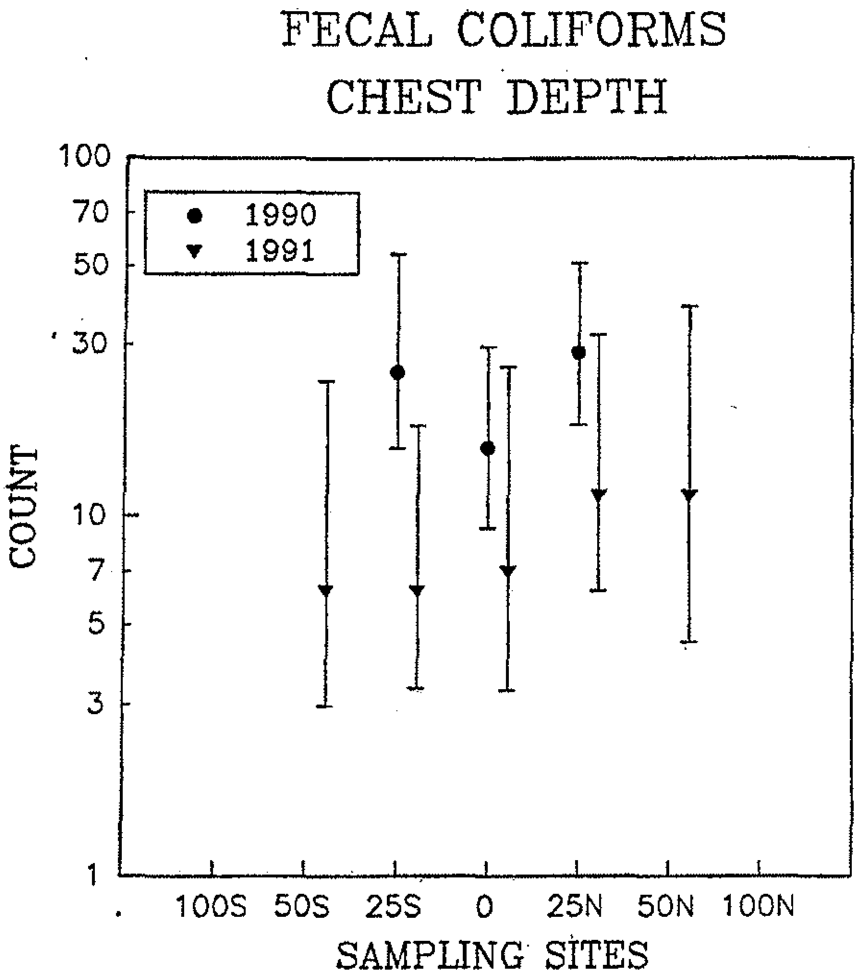

FECAL COLIFORMS
CHEST DEPTH
100
70
50
30
10
7
5
3
1
COUNT
● 1990
▼ 1991
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6d.

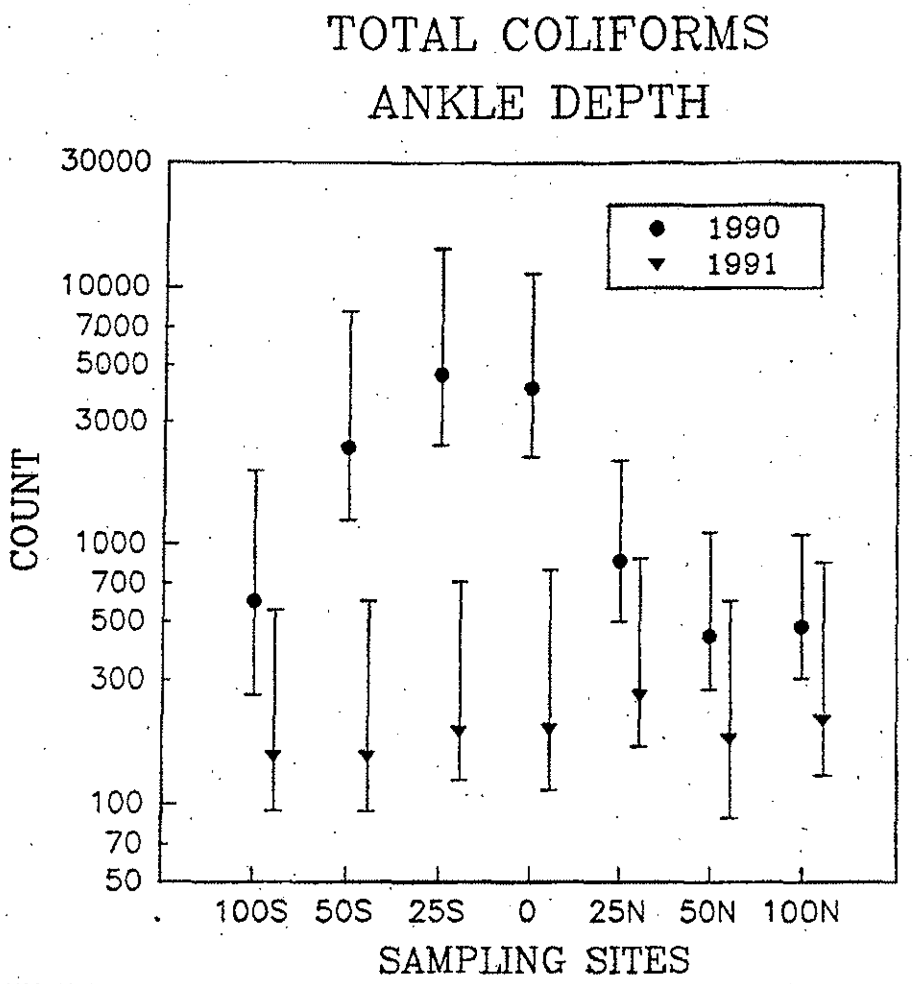

TOTAL COLIFORMS
ANKLE DEPTH
● 1990
▼ 1991
COUNT
30000
10000
7000
5000
3000
1000
700
500
300
100
70
50
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6 e.

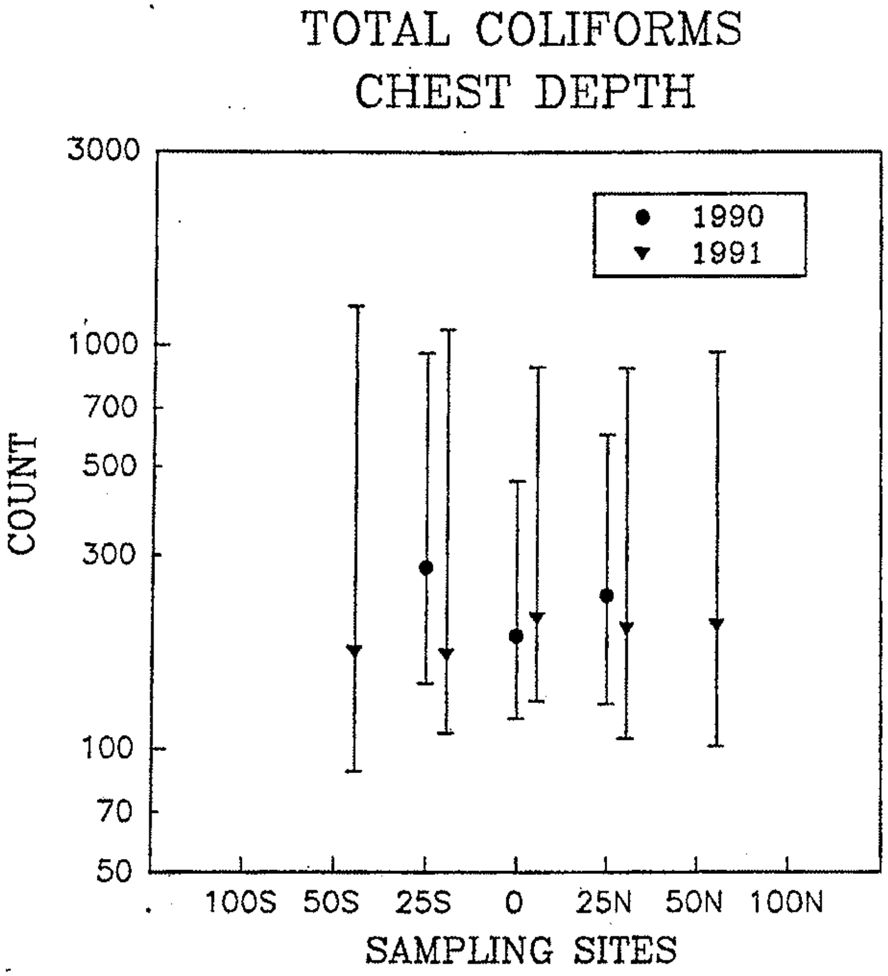

TOTAL COLIFORMS
CHEST DEPTH
● 1990
▼ 1991
COUNT
3000
1000
700
500
300
100
70
50
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6f.

All data included. Types are E = Enterococcus, F = Fecal coliforms, T = Total coliforms. Depths are A = ankle depth, C = Chest depth. DIST is distance up or down coast from storm drain, and DIR is the direction with N = north or upcoast, and S = south or downcoast.

PE	YEAR	DEPTH	DIR	DIST	GEOMETRIC MEAN	LOWER BOUND OF CI	UPPER BOUND OF CI
	1990	A	S	100	37.69	21.16	87.33
	1990	A	S	50	134.60	82.14	334.01
	1990	A	S	25	162.33	91.11	439.19
	1990	A		0	109.57	65.20	230.22
	1990	A	N	25	39.76	24.27	84.80
	1990	A	N	50	23.85	16.22	43.55
	1990	A	N	100	21.11	15.87	35.81
	1990	C	S	25	12.97	7.35	25.39
	1990	C		0	9.66	6.80	16.55
	1990	C	N	25	12.72	8.69	21.94
	1991	A	S	100	3.78	2.59	6.70
	1991	A	S	50	3.08	1.62	7.75
	1991	A	S	25	4.51	3.38	8.05
	1991	A		0	5.41	3.56	12.25
	1991	A	N	25	5.63	3.50	14.36
	1991	A	N	50	5.81	3.95	12.72
	1991	A	N	100	5.96	4.64	11.64
	1991	C	S	50	2.25	1.31	4.06
	1991	C	S	25	2.88	2.05	4.54
	1991	C		0	2.76	1.78	6.02
	1991	C	N	25	3.50	2.54	6.07
	1991	C	N	50	3.91	2.46	7.52
	1990	A	S	100	49.89	24.48	120.04
	1990	A	S	50	147.37	84.01	381.45
	1990	A	S	25	218.98	129.24	601.52
	1990	A		0	169.35	100.44	384.89
	1990	A	N	25	65.49	46.18	122.34
	1990	A	N	50	54.35	38.46	102.26
	1990	A	N	100	49.46	30.85	96.70
	1990	C	S	25	25.18	15.42	54.15
	1990	C		0	15.50	9.30	29.71
	1990	C	N	25	28.55	17.87	51.21
	1991	A	S	100	8.13	4.40	23.88
	1991	A	S	50	9.90	4.93	29.44
	1991	A	S	25	10.56	6.24	27.59
	1991	A		0	15.77	10.77	44.49
	1991	A	N	25	17.81	9.14	52.05
	1991	A	N	50	9.82	5.10	27.28
	1991	A	N	100	10.58	4.83	31.02
	1991	C	S	50	6.19	2.97	23.77
	1991	C	S	25	6.18	3.33	17.80
	1991	C		0	7.03	3.30	26.08
	1991	C	N	25	11.39	6.20	32.09
	1991	C	N	50	11.41	4.49	38.73
	1990	A	S	100	600.80	263.14	1932.16
	1990	A	S	50	2379.89	1237.72	8062.27
	1990	A	S	25	4592.05	2432.40	14028.80
	1990	A		0	4069.56	2183.80	11285.89
	1990	A	N	25	855.66	501.78	2117.67
	1990	A	N	50	439.53	275.65	1107.97
	1990	A	N	100	476.35	303.69	1086.63
	1990	C	S	25	280.37	145.02	958.43
	1990	C		0	190.15	118.77	460.76
	1990	C	N	25	238.47	128.52	601.76
	1991	A	S	100	152.20	94.32	553.72
	1991	A	S	50	152.27	93.45	598.14
	1991	A	S	25	189.36	123.85	710.35
	1991	A		0	193.32	112.67	792.04
	1991	A	N	25	260.16	167.12	874.34
	1991	A	N	50	177.77	88.18	599.98
	1991	A	N	100	209.82	128.15	841.93
	1991	C	S	50	173.79	88.45	1247.98
	1991	C	S	25	170.73	109.39	1095.52
	1991	C		0	209.70	130.73	886.37
	1991	C	N	25	197.56	105.85	878.16
	1991	C	N	50	201.65	101.72	965.23

E 2. Malibu bacterial data. Results of Tukey's studentized range test method; SAS 1990, Steel and Torrie 1960) comparing geometric means of the es. This method controls the experimentwise type-1 error at P <= .05. asterisk (*) indicates that the sites represented by the respective rows and s are significantly different.

ERO US

E	GEOMETRIC MEAN	LOWER BOUND 95% CI	UPPER BOUND 95% CI	CHANNEL	BREACH	UPSTREAM	TEXACO	HERONDO	PICO KENTER
EL	81.50	45.94	144.58			*	*	*	*
H	113.23	65.72	195.07			*	*	*	*
EAM	677.01	384.88	1190.85	*	*				*
ACO	804.38	484.29	1336.03	*	*				*
ONDO	1137.83	445.34	2907.09	*	*				
KENTER	6830.36	2653.48	17582.11	*	*	*	*		

AL COLIFORMS

E	GEOMETRIC MEAN	LOWER BOUND 95% CI	UPPER BOUND 95% CI	CHANNEL	BREACH	TEXACO	UPSTREAM	HERONDO	PICO KENTER
EL	277.65	158.07	487.68			*	*		*
H	564.12	318.75	998.40						*
ACO	948.28	422.56	2128.06	*					*
TREAM	1806.30	1157.32	2819.19	*					
DO	2511.40	695.69	9065.95						
KENTER	7528.32	3185.32	17792.76	*	*	*			

COLIFORMS

	GEOMETRIC MEAN	LOWER BOUND 95% CI	UPPER BOUND 95% CI	CHANNEL	BREACH	UPSTREAM	TEXACO	HERONDO	PICO KENTER
NNEL	1004.38	574.77	1755.10			*	*	*	*
ACH	1296.46	759.10	2214.22			*	*	*	*
EAM	5204.94	2887.55	9382.15	*	*			*	*
O	7573.30	3665.63	15646.64	*	*			*	*
NDO	174220.62	27913.02	1087407.37	*	*	*	*		
O KENTER	416451.75	147472.16	1176032.53	*	*	*	*		

Levels of concern were exceeded when:

a. total coliforms were greater than 1000 colony forming units per 100 ml of water (cfu/100 ml);

b. fecal coliforms were greater than 200 cfu/100 ml; and,

c. enterococci were greater than 24 cfu/100 ml.

Table 3. Seed Virus Recover Experiments.
Runoff was seeded with attenuated poliovirus.

Location	Virus Added	Virus Recovered	% Recovery
Pico-Kenter	3.4×10^6	2.7×10^5	7.9
Pico-Kenter	3.5×10^6	2.4×10^5	6.9
Pico-Kenter	3.8×10^6	2.7×10^5	7.1
Pico-Kenter	2.5×10^6	7.6×10^5	30.4
Herondo	6.3×10^5	1.4×10^4	2.2
Herondo	7.4×10^5	None	0
Herondo	2.7×10^6	1.3×10^6	48
Malibu Lagoon	2.9×10^6	8.4×10^4	2.9
Malibu Lagoon	2.6×10^6	2.0×10^5	7.7
Malibu Lagoon	2.9×10^6	4.9×10^5	16.9

TABLE 4. Geometric means and range of densities for F-male specific coliphage (coliphage/100 ml) collected simultaneously with virus samples at Pico-Kenter drain, Herondo drain and Malibu Lagoon.

SAMPLE SITE:	MEAN COLIPHAGE DENSITIES	RANGE OF COLIPHAGE DENSITIES
PICO-KENTER	1,040	25 TO 67,000
HERONDO	990	Below detection limit to 22,000
BREACH	100	Below detection limit to 1,700
C-CHANNEL	60	Below detection limit to 1,300
TEXACO	100	Below detection limit to 1,300
UPSTREAM	30	Below detection limit to 600

TABLE 5. Estimated densities of positive plaque assay (plaque forming units) and cytopathic effect (estimated infectious units) assays for human enteric viruses in the Pico-Kenter drain, Herondo drain, and Malibu Lagoon.

SAMPLE SITES:	DATE	PLAQUE ASSAY RESULTS	CYTOPATHIC EFFECT RESULTS
PICO-KENTER	8/13/91	1 pfu/57 gal	no CPE done
	9/25/91	1 pfu/2.8 gal	no CPE done
	10/2/91	none detected	≥1 Iu/23.2 gal
HERONDO	7/31/91	none detected	≥1 Iu/79.5 gal
	9/4/91	none detected	≥1 Iu/26.2 gal
BREACH	6/17/91	none detected	≥1 Iu/80 gal
C-CHANNEL	6/5/91	1 pfu/141 gal	no CPE done
	10/9/91	none detected	≥1 Iu/10.6 gal
UPSTREAM	9-3-91	none detected	≥1 Iu/28.7 gal
	9-30-91	none detected	≥1 Iu/63 gal

TABLE 6. The mean and range of values for conductivity (mmhos), pH and temperature (degrees Celsius) of samples collected simultaneously with virus samples at Pico-Kenter drain, Herondo drain and Malibu Lagoon.

SAMPLE SITES:	CONDUCTIVITY	pH	TEMPERATURE
PICO-KENTER	1.7 0.5 to 2.9	7.8 6.6 to 8.3	20.1 17.0 to 22.0
HERONDO	6.8 1.2 to 23.0	6.5 3.0 to 7.8	21.2 20.0 to 22.1
BREACH	22.9 1.6 to 51.5	8.1 6.5 to 9.2	20.3 17.5 to 24.5[1]
C-CHANNEL	23.7 3.0 to 50.4	8.2 7.0 to 9.4	20.3 17.5 to 24.5
TEXACO	6.9 2.1 to 28.1	7.7 6.0 to 8.5	20.3 17.5 to 24.5
UPSTREAM	12.5 0.5 to 48.0	7.8 5.9 to 8.9	20.3 17.5 to 24.5

---

[1] Temperatures were only measured at one sample site per day at Malibu Lagoon.

those in 1991. All data included. The symbols at each site indicate the results of the t test as follows:

*** P < .0001
** P < .001
* P < .005
. P < .05
blank P > .05
- no test

To control the experimentwise type-1 error to P <= .05, use P < .005 (*) as the critical level.

TEROCOCCUS

PTH	100S	50S	25S	0	25N	50N	100N
KLE	**	***	***	***	**	**	**
EST	-	-	**	*	**	-	-

CAL COLIFORMS

PTH	100S	50S	25S	0	25N	50N	100N
KLE	*	***	***	**	*	**	.
EST	-	-	.			-	-

COLIFORMS

PTH	100S	50S	25S	0	25N	50N	100N
KLE	.	**	***	***	.		
EST	-	-				-	-

APPENDIX 1. Pertinent Information on f2 Bacteriophage

The proprietary aspect to this methodology is the host strain of Escherichia coli which contains an F+ plasmid carrying resistances to ampicillin and streptomycin. The host used in this study was the V.J. Cabelli host. Media and procedures were also from Cabelli et. al. and they should be credited.

f2 Bacteriophage-Water Concentration Technique

1. Allow sample to warm to room temperature.
2. In a 250 ml centrifuge bottle add 1 g powdered tryptone (Difco Laboratories, Inc., Detroit, MI) and 1 g powdered beef extract (Scott Laboratories, Inc., Fiskville, RI).
3. Add 100 ml sample to centrifuge bottle, and mix.
4. Add 10 ml of host (F amp) culture grown 3-4 h in tryptone broth.
5. Incubate at 32 C on a rotary shaker set on low (very slow) for 50 minutes.
6. Centrifuge at 9000 x g for 15 min. at 3-4 C.
7. Aspirate all except 10 ml out of centrifuge bottle.
8. Resuspend pellet in the remaining 10 ml volume.
9. Assay these 10 ml by adding 2.5 ml volumes to 2.5 ml amounts of double strength top (soft) agar, tempered to 46-50 C, for each of 4 plates.
10. Mix immediately, pour top agar on bottom agar plates, and spread over entire plate surfaces by gentle swirling.
11. Incubate 18-24 h at 35 C.
12. Count plaques and calculate f2 per 100 ml.

Note: Do not shake host (F amp) culture vigorously. Vigorous mixing of host strain cells will remove pili and inhibit f2 infection.

The 3-4 hour culture added in step 4 can be established by adding a few milliliters of an overnight grown broth culture. The broth used for the overnight culture should contain ampicillin and streptomycin at the same concentration as bottom agar.

Note: Maintain the host strain on tryptone bottom agar slants containing 15 microgram per ml each ampicillin and streptomycin sulfate. Grow overnight at 35 C, then store in a refrigerator (2-6) for up to several weeks. Use this working stock culture to inoculate tryptone broths when assays are to be performed.

Stock cultures of the host strain must be maintained on antibiotic-containing media to insure the cells contain the F+ plasmid.

Media for f2 phage procedure

Tryptone Broth for 3-4 h host strain (F amp) cultures and for all dilutions involving f2 Bacteriophage.

Ingredient	Amount per liter
Tryptone	10 g
Dextrose	1 g
NaCl	5 g

Note: Tryptone broth is used for dilutions because it helps to disperse bacteriophage and minimizes clumping of f2.

Top Agar for f2 Bacteriophage Plaque Assay

Prepare double strength (2 x) as follows:

Ingredient	Amount per liter
Tryptone	20 g
Dextrose	2 g
NaCl	10 g
agar	14 g
1 ml of 1 M $CaCl_2$ solution	

Heat to dissolve and dispense 2.5 ml volumes per tube.
Store frozen.
Autoclave 15 min., 121 C and cool to 46-50 C before adding equal volume (2.5 ml) sample.

Bottom Agar for f2 Bacteriophage Plaque Assay and Stock Cultures of Host Strain (F amp).

Ingredient	Amount per liter
Tryptone	10 g
Dextrose	1 g
NaCl	5 g
agar	12 g

Add magnetic stirring bar to flask. Autoclave 15 min., 121 C.
Cool to 45-50 C.
Add antibiotics:

ampicillin	0.015 g
streptomycin sulfate	0.015 g

Magnetically mix gently, then pour plates.

APPENDIX 2 PHAGE ANALYSIS SUMMARY

Sample	Date	Phage/100 mL
Malibu Texaco	5/13/91	<5 pfu
Malibu Texaco	5/15/91	<5 pfu
Malibu Texaco	5/22/91	<5 pfu
Malibu Texaco	6/3/91	35 pfu
Malibu Texaco	7/3/91	15 pfu
Malibu Texaco	7/22/91	<5 pfu
Malibu Texaco	7/30/91	<13 pfu
Malibu Texaco	8/14/91	$8x10^2$ pfu
Malibu Texaco	8/20/91	$6x10^2$ pfu
Malibu Texaco	8/26/91	$1.3x10^3$ pfu
Malibu Texaco	9/3/91	15 pfu
Malibu Texaco	9/16/91	$6.0x10^2$ pfu
Malibu Texaco	9/18/91	$3.0x10^2$ pfu
Malibu Texaco	9/23/91	TNTC (>150/mL)
Malibu Texaco	9/30/91	70 pfu
Malibu Texaco	10/01/91	15 pfu
Malibu Texaco	10/08/91	50 pfu
Malibu Texaco	10/09/91	$3.0x10^2$ pfu
Malibu Texaco	10/15/91	$2.2x10^3$ pfu
Malibu Texaco	10/16/91	<5 pfu
Malibu Texaco	10/21/91	$1.1x10^2$ pfu
Malibu Texaco	10/22/91	$5.8x10^2$ pfu
Malibu Texaco	10/23/91	20 pfu
Malibu Texaco - 100 yards East	5/13/91	<5 pfu
Malibu Texaco - 100 yards East	5/15/91	<5 pfu
Malibu Texaco - 100 yards East	5/22/91	5 pfu
Malibu C Channel	5/13/91	<5 pfu
Malibu C Channel	5/15/91	<5 pfu
Malibu C Channel	5/22/91	<5 pfu
Malibu C Channel	6/3/91	5 pfu
Malibu C Channel	6/5/91	<5 pfu
Malibu C Channel	7/3/91	<5 pfu
Malibu C Channel	7/22/91	5 pfu
Malibu C Channel	7/30/91	<13 pfu

PHAGE ANALYSIS SUMMARY (cont.)

Malibu C Channel	8/14/91	<5 pfu
alibu C Channel	8/20/91	1.3×10^3 pfu
Malibu C Channel	8/26/91	9×10^2 pfu
alibu C Channel	9/3/91	2×10^2 pfu
Malibu C Channel	9/16/91	25 pfu
alibu C Channel	9/18/91	25 pfu
Malibu C Channel	9/23/91	1×10^2 pfu
alibu C Channel	9/30/91	55 pfu
Malibu C Channel	10/01/91	25 pfu
alibu C Channel	10/08/91	30 pfu
Malibu C Channel	10/09/91	4×10^2 pfu
alibu C Channel	10/15/91	50 pfu
Malibu C Channel	10/16/91	<5 pfu
Malibu C Channel	10/21/91	1.2×10^2 pfu
Malibu C Channel	10/22/91	45 pfu
Malibu C Channel	10/23/91	25 pfu
Malibu C Channel Pipe	5/13/91	<5 pfu
alibu C Channel Pipe	5/15/91	<5 pfu
Malibu C Channel Pipe	5/22/91	5 pfu
Malibu Breach	5/15/91	<5 pfu
Malibu Breach	5/22/91	<5 pfu
Malibu Breach	6/03/91	<5 pfu
Malibu Breach	6/17/91	not recorded
Malibu Breach	6/19/91	5 pfu
Malibu Breach	7/3/91	<5 pfu
Malibu Breach	7/22/91	135 pfu
Malibu Breach	7/30/91	<13 pfu
Malibu Breach	8/06/91	<5 pfu
Malibu Breach	8/14/91	<5 pfu
Malibu Breach	8/20/91	1.7×10^3 pfu
Malibu Breach	8/26/91	4×10^2 pfu
Malibu Breach	9/03/91	20 pfu
alibu Breach	9/16/91	40 pfu
Malibu Breach	9/18/91	25 pfu
Malibu Breach	9/23/91	2×10^2 pfu
Malibu Breach	9/30/91	60 pfu

PHAGE ANALYSIS SUMMARY (cont.)

Malibu Breach	10/01/91	15 pfu
Malibu Breach	10/08/91	40 pfu
Malibu Breach	10/09/91	$1x10^3$ pfu
Malibu Breach	10/15/91	$8x10^1$ pfu
Malibu Breach	10/16/91	<5 pfu
Malibu Breach	10/21/91	$6.4x10^2$ pfu
Malibu Breach	10/22/91	$2.9x10^2$ pfu
Malibu Breach	10/23/91	80 pfu
Malibu Station B	5/13/91	<5 pfu
Malibu Upstream	6/03/91	5 pfu
Malibu Upstream	6/10/91	<5 pfu
Malibu Upstream	6/12/91	<5 pfu
Malibu Upstream	7/03/91	<5 pfu
Malibu Upstream	7/17/91	<5 pfu
Malibu Upstream	7/22/91	<5 pfu
Malibu Upstream	7/30/91	<13 pfu
Malibu Upstream	8/05/91	<5 pfu
Malibu Upstream	8/14/91	<5 pfu
Malibu Upstream	8/20/91	<5 pfu
Malibu Upstream	9/03/91	10 pfu
Malibu Upstream	9/16/91	15 pfu
Malibu Upstream	9/18/91	55 pfu
Malibu Upstream	9/23/91	$6x10^2$ pfu
Malibu Upstream	9/30/91	$1x10^2$ pfu
Malibu Upstream	10/01/91	65 pfu
Malibu Upstream	10/08/91	50 pfu
Malibu Ustream	10/09/91	$4x10^2$ pfu
Malibu Upstream	10/15/91	20 pfu
Malibu Upstream	10/16/91	<5 pfu
Malibu Upstream	10/21/91	$1x10^2$ pfu
Malibu Upstream	10/22/91	10 pfu
Malibu Upstream	10/23/91	10 pfu
Herondo	5/15/91	1000 pfu
Herondo	7/1/91	$2.2x10^4$ pfu
Herondo	7/10/91	Not recorded
Herondo	7/31/91	$2x10^3$ pfu

PHAGE ANALYSIS SUMMARY (cont.)

Malibu Breach	10/01/91	15 pfu
Malibu Breach	10/08/91	40 pfu
Malibu Breach	10/09/91	1×10^3 pfu
M[illegible]u Breach	10/15/91	8×10^1 pfu
Malibu Breach	10/16/91	<5 pfu
Malibu Breach	10/21/91	6.4×10^2 pfu
Malibu Breach	10/22/91	2.9×10^2 pfu
Malibu Breach	10/23/91	80 pfu
Malibu Station B	5/13/91	<5 pfu
Malibu Upstream	6/03/91	5 pfu
Malibu Upstream	6/10/91	<5 pfu
Malibu Upstream	6/12/91	<5 pfu
Malibu Upstream	7/03/91	<5 pfu
Malibu Upstream	7/17/91	<5 pfu
Malibu Upstream	7/22/91	<5 pfu
Malibu Upstream	7/30/91	<13 pfu
Malibu Upstream	8/05/91	<5 pfu
[illegible]alibu Upstream	8/14/91	<5 pfu
M[illegible]u Upstream	8/20/91	<5 pfu
Malibu Upstream	9/03/91	10 pfu
Malibu Upstream	9/16/91	15 pfu
Malibu Upstream	9/18/91	55 pfu
Malibu Upstream	9/23/91	6×10^2 pfu
Malibu Upstream	9/30/91	1×10^2 pfu
Malibu Upstream	10/01/91	65 pfu
Malibu Upstream	10/08/91	50 pfu
Malibu Ustream	10/09/91	4×10^2 pfu
Malibu Upstream	10/15/91	20 pfu
Malibu Upstream	10/16/91	<5 pfu
Malibu Upstream	10/21/91	1×10^2 pfu
Malibu Upstream	10/22/91	10 pfu
Malibu Upstream	10/23/91	10 pfu
[illegible]erondo	5/15/91	1000 pfu
H[illegible]ndo	7/1/91	2.2×10^4 pfu
Herondo	7/10/91	Not recorded
Herondo	7/31/91	2×10^3 pfu

APPENDIX 2 PHAGE ANALYSIS SUMMARY

Sample	Date	Phage/100 mL
Malibu Texaco	5/13/91	<5 pfu
Malibu Texaco	5/15/91	<5 pfu
Malibu Texaco	5/22/91	<5 pfu
Malibu Texaco	6/3/91	35 pfu
Malibu Texaco	7/3/91	15 pfu
Malibu Texaco	7/22/91	<5 pfu
Malibu Texaco	7/30/91	<13 pfu
Malibu Texaco	8/14/91	8×10^2 pfu
Malibu Texaco	8/20/91	6×10^2 pfu
Malibu Texaco	8/26/91	1.3×10^3 pfu
Malibu Texaco	9/3/91	15 pfu
Malibu Texaco	9/16/91	6.0×10^2 pfu
Malibu Texaco	9/18/91	3.0×10^2 pfu
Malibu Texaco	9/23/91	TNTC (>150/mL)
Malibu Texaco	9/30/91	70 pfu
Malibu Texaco	10/01/91	15 pfu
Malibu Texaco	10/08/91	50 pfu
Malibu Texaco	10/09/91	3.0×10^2 pfu
Malibu Texaco	10/15/91	2.2×10^3 pfu
Malibu Texaco	10/16/91	<5 pfu
Malibu Texaco	10/21/91	1.1×10^2 pfu
Malibu Texaco	10/22/91	5.8×10^2 pfu
Malibu Texaco	10/23/91	20 pfu
Malibu Texaco - 100 yards East	5/13/91	<5 pfu
Malibu Texaco - 100 yards East	5/15/91	<5 pfu
Malibu Texaco - 100 yards East	5/22/91	5 pfu
Malibu C Channel	5/13/91	<5 pfu
Malibu C Channel	5/15/91	<5 pfu
Malibu C Channel	5/22/91	<5 pfu
Malibu C Channel	6/3/91	5 pfu
Malibu C Channel	6/5/91	<5 pfu
Malibu C Channel	7/3/91	<5 pfu
Malibu C Channel	7/22/91	5 pfu
Malibu C Channel	7/30/91	<13 pfu

Note: Maintain the host strain on tryptone bottom agar slants containing 15 microgram per ml each ampicillin and streptomycin sulfate. Grow overnight at 35 C, then store in a refrigerator (2-6) for up to several weeks. Use this working stock culture to inoculate tryptone broths when assays are to be performed.

Stock cultures of the host strain must be maintained on antibiotic-containing media to insure the cells contain the F+ plasmid.

```
those in 1991. All data included.  The symbols at each site indicate
the results of the t test as follows:

     *** P < .0001
      ** P < .001
       * P < .005
       . P < .05
   blank P > .05
       -   no test

To control the experimentwise type-1 error to P <= .05, use P < .005
(*) as the critical level.
```

```
ITEROCOCCUS

PTH    100S    50S     25S      0      25N     50N     100N
------------------------------------------------------------
KLE     **     ***     ***     ***      **      **      **
EST     -       -       **      *       **      -       -


CAL COLIFORMS

PTH    100S    50S     25S      0      25N     50N     100N
------------------------------------------------------------
KLE     *      ***     ***      **      *       **      .
EST     -       -       .                       -       -


   COLIFORMS

PTH    100S    50S     25S      0      25N     50N     100N
------------------------------------------------------------
KLE     .       **     ***     ***      .
EST     -       -                               -       -
```

TABLE 5. Estimated densities of positive plaque assay (plaque forming units) and cytopathic effect (estimated infectious units) assays for human enteric viruses in the Pico-Kenter drain, Herondo drain, and Malibu Lagoon.

SAMPLE SITES:	DATE	PLAQUE ASSAY RESULTS	CYTOPATHIC EFFECT RESULTS
PICO-KENTER	8/13/91	1 pfu/57 gal	no CPE done
	9/25/91	1 pfu/2.8 gal	no CPE done
	10/2/91	none detected	≥1 Iu/23.2 gal
HERONDO	7/31/91	none detected	≥1 Iu/79.5 gal
	9/4/91	none detected	≥1 Iu/26.2 gal
BREACH	6/17/91	none detected	≥1 Iu/80 gal
C-CHANNEL	6/5/91	1 pfu/141 gal	no CPE done
	10/9/91	none detected	≥1 Iu/10.6 gal
UPSTREAM	9-3-91	none detected	≥1 Iu/28.7 gal
	9-30-91	none detected	≥1 Iu/63 gal

Table 3. Seed Virus Recover Experiments.
Runoff was seeded with attenuated poliovirus.

Location	Virus Added	Virus Recovered	% Recovery
Pico-Kenter	3.4×10^6	2.7×10^5	7.9
Pico-Kenter	3.5×10^6	2.4×10^5	6.9
Pico-Kenter	3.8×10^6	2.7×10^5	7.1
Pico-Kenter	2.5×10^6	7.6×10^5	30.4
Herondo	6.3×10^5	1.4×10^4	2.2
Herondo	7.4×10^5	None	0
Herondo	2.7×10^6	1.3×10^6	48
Malibu Lagoon	2.9×10^6	8.4×10^4	2.9
Malibu Lagoon	2.6×10^6	2.0×10^5	7.7
Malibu Lagoon	2.9×10^6	4.9×10^5	16.9

[illegible]
All data included. Types are E = Enterococcus, F = Fecal Coliforms,
T = Total coliforms. Depths are A = ankle depth, C = Chest depth.
DIST is distance up or down coast from storm drain, and DIR is the
direction with N = north or upcoast, and S = south or downcoast.

YPE	YEAR	DEPTH	DIR	DIST	GEOMETRIC MEAN	LOWER BOUND OF CI	UPPER BOUND OF CI
E	1990	A	S	100	37.69	21.16	87.33
E	1990	A	S	50	134.60	82.14	334.01
E	1990	A	S	25	162.33	91.11	439.19
E	1990	A		0	109.57	65.20	230.22
E	1990	A	N	25	39.76	24.27	84.80
E	1990	A	N	50	23.85	16.22	43.55
E	1990	A	N	100	21.11	15.87	35.81
E	1990	C	S	25	12.97	7.35	25.39
E	1990	C		0	9.66	6.80	16.55
E	1990	C	N	25	12.72	8.69	21.94
E	1991	A	S	100	3.78	2.59	6.70
E	1991	A	S	50	3.08	1.62	7.75
E	1991	A	S	25	4.51	3.38	8.05
E	1991	A		0	5.41	3.56	12.25
E	1991	A	N	25	5.63	3.50	14.36
E	1991	A	N	50	5.81	3.95	12.72
E	1991	A	N	100	5.96	4.64	11.64
E	1991	C	S	50	2.25	1.31	4.06
E	1991	C	S	25	2.88	2.05	4.54
E	1991	C		0	2.76	1.78	6.02
E	1991	C	N	25	3.50	2.54	6.07
E	1991	C	N	50	3.91	2.46	7.52
F	1990	A	S	100	49.89	24.48	120.04
F	1990	A	S	50	147.37	84.01	381.45
F	1990	A	S	25	218.98	129.24	601.52
F	1990	A		0	169.35	100.44	384.89
F	1990	A	N	25	65.49	46.18	122.34
F	1990	A	N	50	54.35	38.46	102.26
F	1990	A	N	100	49.46	30.85	96.70
F	1990	C	S	25	25.18	15.42	54.15
F	1990	C		0	15.50	9.30	29.71
F	1990	C	N	25	28.55	17.87	51.21
F	1991	A	S	100	8.13	4.40	23.88
F	1991	A	S	50	9.90	4.93	29.44
F	1991	A	S	25	10.56	6.24	27.59
F	1991	A		0	15.77	10.77	44.49
F	1991	A	N	25	17.81	9.14	52.05
F	1991	A	N	50	9.82	5.10	27.28
F	1991	A	N	100	10.58	4.83	31.02
F	1991	C	S	50	6.19	2.97	23.77
F	1991	C	S	25	6.18	3.33	17.80
F	1991	C		0	7.03	3.30	26.08
F	1991	C	N	25	11.39	6.20	32.09
F	1991	C	N	50	11.41	4.49	38.73
T	1990	A	S	100	600.80	263.14	1932.16
T	1990	A	S	50	2379.89	1237.72	8062.27
T	1990	A	S	25	4592.05	2432.40	14028.80
T	1990	A		0	4069.56	2183.80	11285.89
T	1990	A	N	25	855.66	501.78	2117.67
T	1990	A	N	50	439.53	275.65	1107.97
T	1990	A	N	100	476.35	303.69	1086.63
T	1990	C	S	25	280.37	145.02	958.43
T	1990	C		0	190.15	118.77	460.76
T	1990	C	N	25	238.47	128.52	601.76
T	1991	A	S	100	152.20	94.32	553.72
T	1991	A	S	50	152.27	93.45	598.14
T	1991	A	S	25	189.36	123.85	710.35
T	1991	A		0	193.32	112.67	792.04
T	1991	A	N	25	260.16	167.12	874.34
	1991	A	N	50	177.77	88.18	599.98
	1991	A	N	100	209.82	128.15	841.93
	1991	C	S	50	173.79	88.45	1247.98
	1991	C	S	25	170.73	109.39	1095.52
	1991	C		0	209.70	130.73	886.37
	1991	C	N	25	197.56	105.85	878.16
	1991	C	N	50	201.65	101.72	965.23

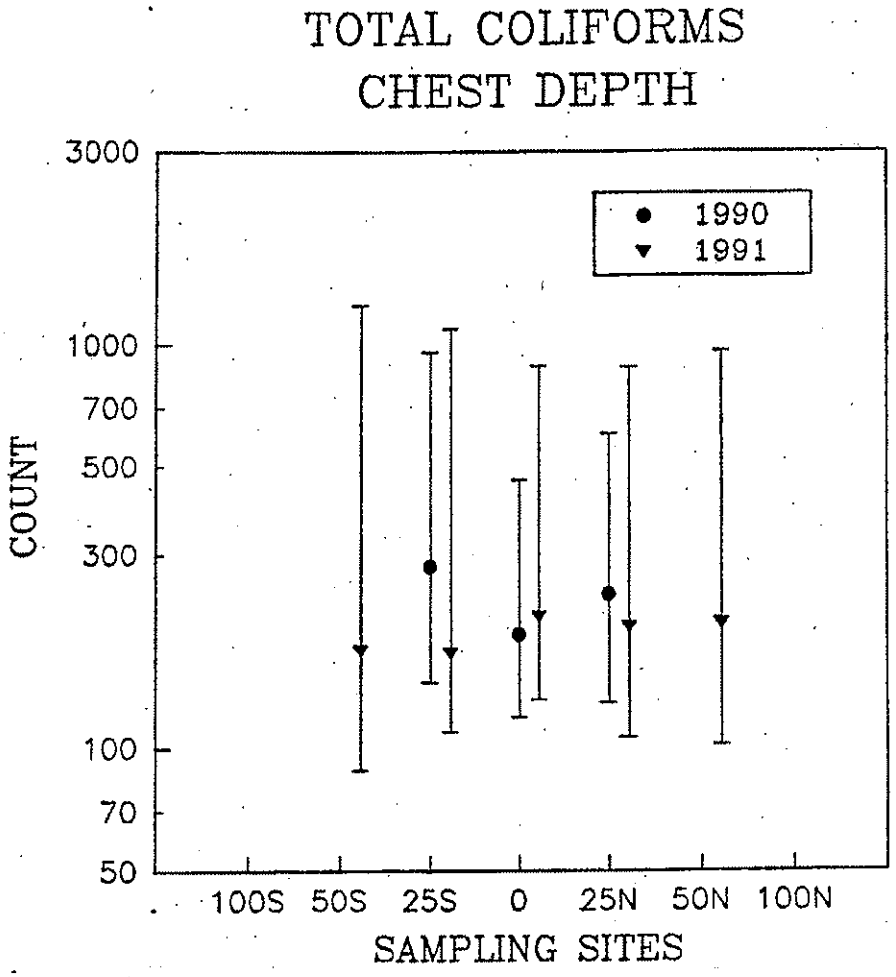

TOTAL COLIFORMS
CHEST DEPTH
● 1990
▼ 1991
3000
1000
700
500
300
100
70
50
COUNT
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6f.

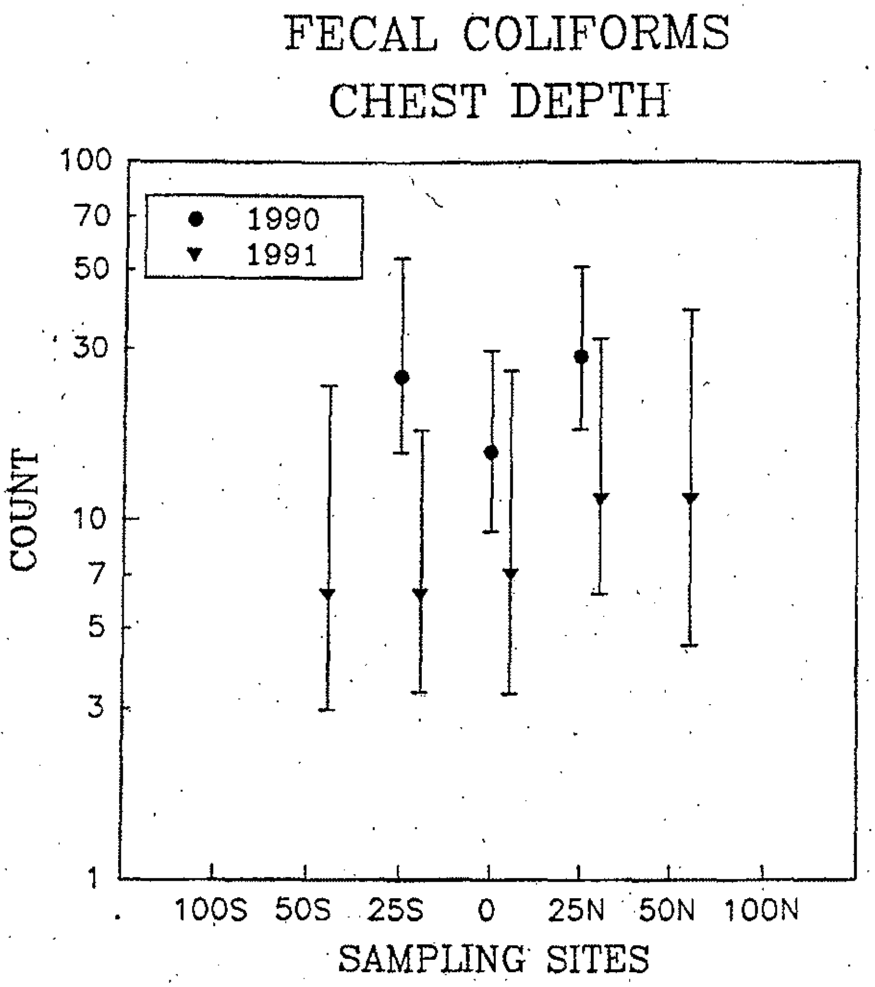

FECAL COLIFORMS
CHEST DEPTH
1990
1991
COUNT
100
70
50
30
10
7
5
3
1
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6d.

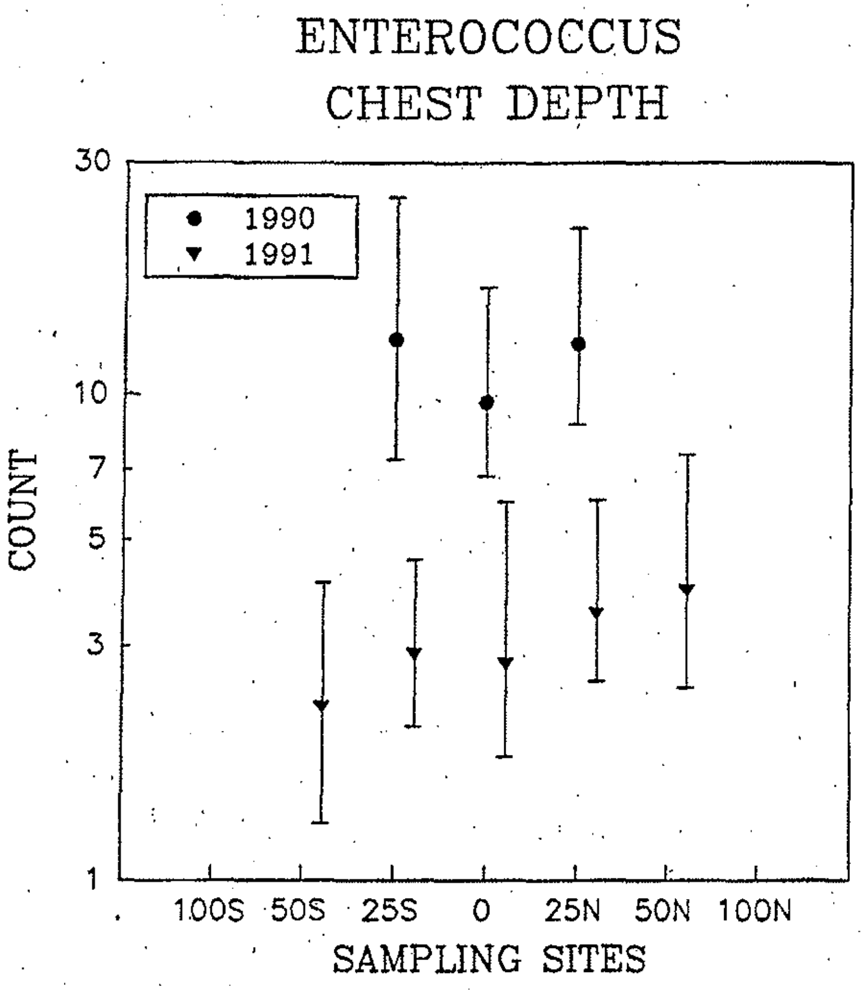

ENTEROCOCCUS
CHEST DEPTH
30
10
7
5
3
1
COUNT
● 1990
▼ 1991
100S
50S
25S
0
25N
50N
100N
SAMPLING SITES

Figure 6b.

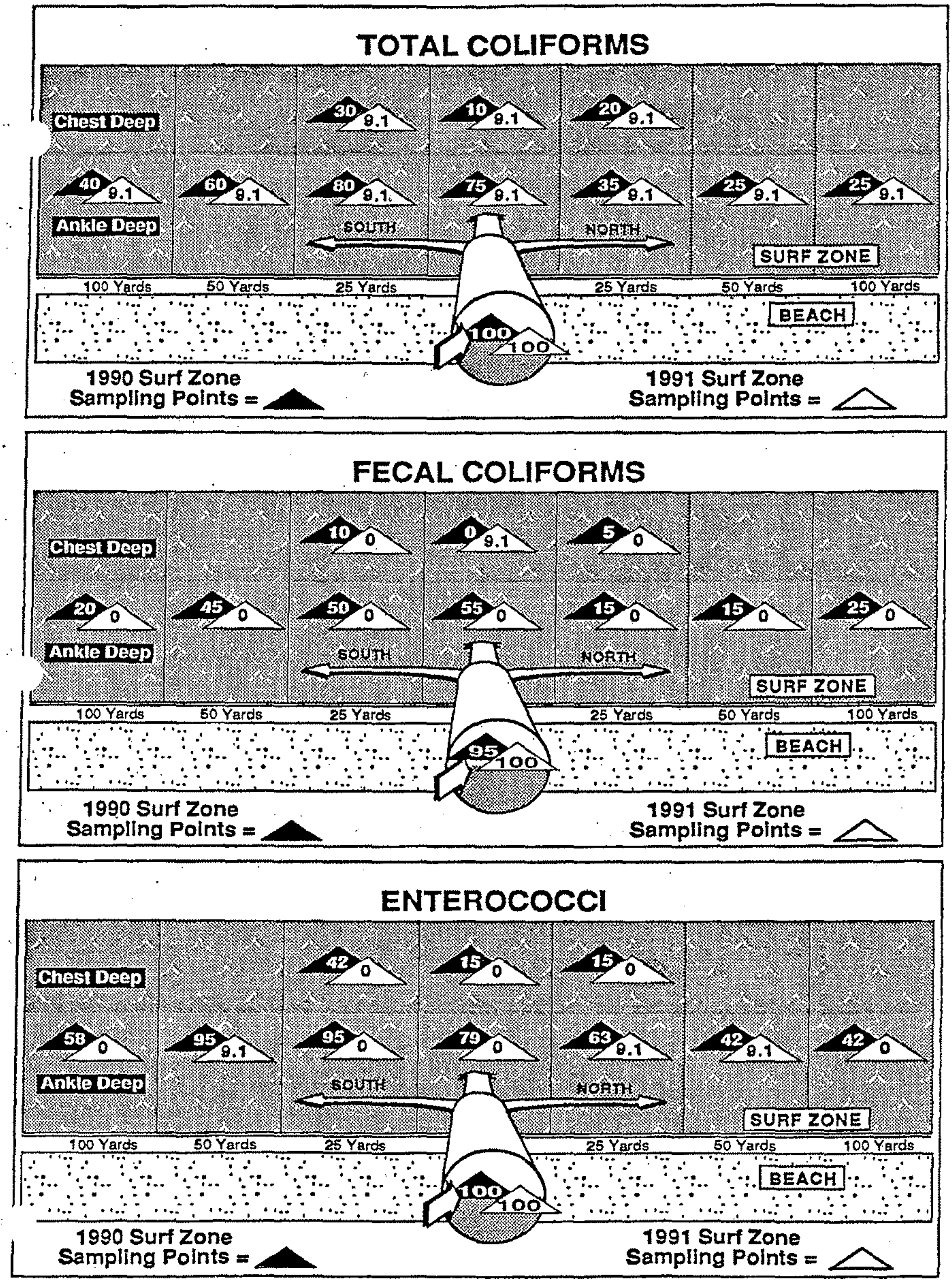
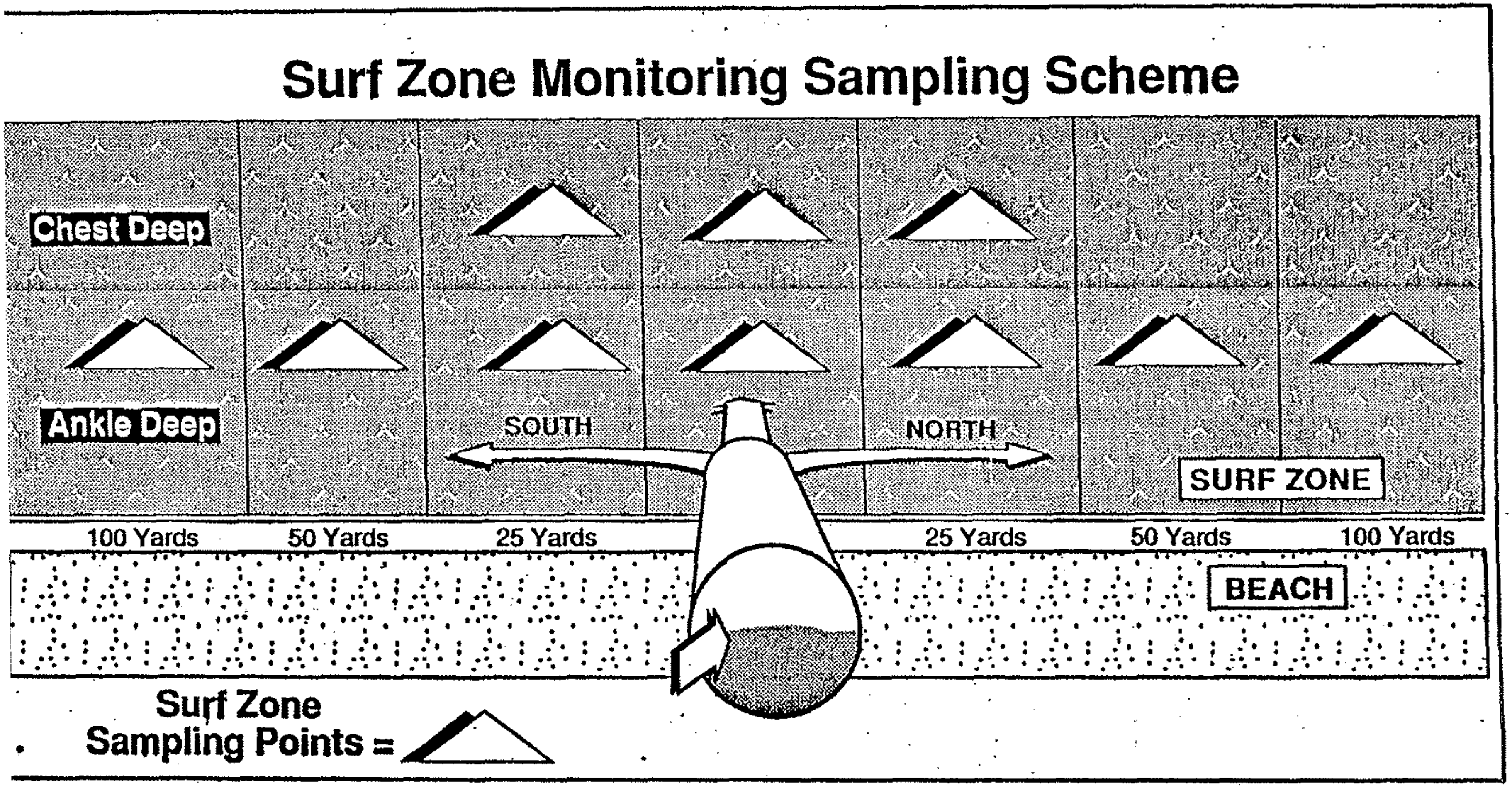

TOTAL COLIFORMS
Chest Deep
30 9.1
10 9.1
20 9.1
40 9.1
60 9.1
80 9.1
75 9.1
35 9.1
25 9.1
25 9.1
Ankle Deep
SOUTH
NORTH
SURF ZONE
100 Yards
50 Yards
25 Yards
25 Yards
50 Yards
100 Yards
BEACH
100
100
1990 Surf Zone Sampling Points =
1991 Surf Zone Sampling Points =
FECAL COLIFORMS
Chest Deep
10 0
0 9.1
5 0
20 0
45 0
50 0
55 0
15 0
15 0
25 0
Ankle Deep
SOUTH
NORTH
SURF ZONE
100 Yards
50 Yards
25 Yards
25 Yards
50 Yards
100 Yards
BEACH
95
100
1990 Surf Zone Sampling Points =
1991 Surf Zone Sampling Points =
ENTEROCOCCI
Chest Deep
42 0
15 0
15 0
58 0
95 9.1
95 0
79 0
63 9.1
42 9.1
42 0
Ankle Deep
SOUTH
NORTH
SURF ZONE
100 Yards
50 Yards
25 Yards
25 Yards
50 Yards
100 Yards
BEACH
100
100
1990 Surf Zone Sampling Points =
1991 Surf Zone Sampling Points =

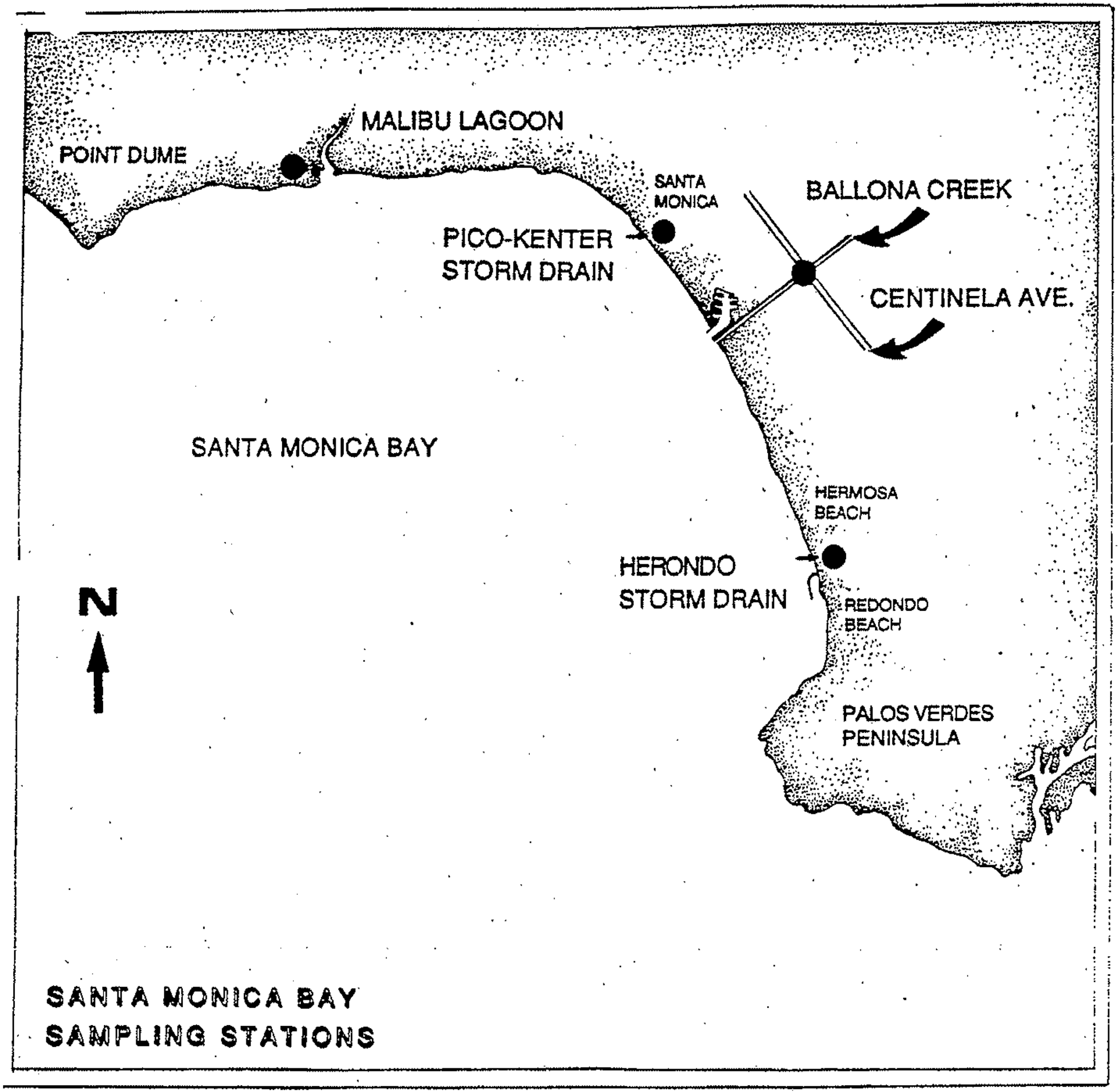
Figure 5.



Surf Zone Monitoring Sampling Scheme

Figure 3.

Figure 1.


MALIBU LAGOON
POINT DUME
SANTA MONICA
BALLONA CREEK
PICO-KENTER STORM DRAIN
CENTINELA AVE.
SANTA MONICA BAY
HERMOSA BEACH
HERONDO STORM DRAIN
REDONDO BEACH
N
PALOS VERDES PENINSULA
SANTA MONICA BAY SAMPLING STATIONS

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USEPA. See United States Environmental Protection Agency.

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one virus would result in infection (Bitton, 1980).

6. Other than gene probes, current techniques cannot detect some of the viruses (rotavirus and Norwalk viruses) which could cause swimming associated illnesses such as gastroenteritis.

VI. CONCLUSION

Historical monitoring data were used to identify surf zone sites where indicator bacteria densities frequently exceeded levels of concern. Human enteric virus samples were taken at the sites with high bacterial densities in order to assess whether there was human waste present. Because swimming associated illnesses are generally believed to be caused by human viruses, the presence of human waste at the sites would indicate a greater risk of swimming associated illnesses than at sites without human fecal inputs (Cabelli et al 1979, 1982). The original intent of the studies was to identify sites with high indicator bacteria densities caused by flowing storm drains with no sewage inputs. These sites were needed for an epidemiology study designed to determine the health risks from swimming in storm drain contaminated water.

Three years later, human enteric viruses were found at various locations in Malibu Lagoon, Herondo Drain, and three consecutive years at the Pico-Kenter storm drain. Human viruses were not found in limited sampling at Ballona Creek, the Santa Monica Canyon drain, and the Ashland drain, but that does not preclude them from occurring in those locations. In terms of the original study design, the enteric virus assessment has done what it was intended to do; to determine the presence or absence of human waste at the study sites. The study results can not be used to provide accurate information on potential sources of human viruses to the storm drains.

Human fecal contamination of storm drains is far more prevalent than originally assumed. Even though the sewer system in Los Angeles County is completely separate from the storm drain system, the storm drains are not free of human fecal inputs.

The results of the last three years of virus monitoring in storm drains support the following recommendations:

1) An investigation of the health risks associated with various levels of indicators of water quality or with swimming at various distances from flowing drains should move forward.
2) A human specific indicator that correlates with the densities and presence or absence of human pathogens is needed.

3) Tools need to be developed for the easy detection and quantification of specific human pathogens. Models could then be used to demonstrate health risks associated with the

Total:Fecal usually $\leq$10 (City of Los Angeles, Environmental Monitoring Division, unpublished data).

Indicator bacteria are useful for sanitary surveys when there is gross or undiluted sewage contamination (e.g. large sewage spill or leak) in the storm drains. In other situations, such as the conditions seen at the two drains and Malibu Lagoon, indicator densities provide little information on the source of virus contamination. Even at Malibu Lagoon, where the Total:Fecal coliform ratio was less than four at the virus sampling locations, indicator densities provided little information on the source(s) of bacterial and viral contamination. However, the intent of this study was only to determine if there was virus contamination, not to determine sources of viral contamination. Studies designed specifically to trace the source(s) of human fecal contamination to the drains are needed.

The only type of viruses detected were the Coxsackie B viruses which are often found in sewage contaminated waters. None of the positive isolates were poliovirus, so there was no cross contamination of the samples from the seed studies. In addition, any potential cross contamination was eliminated because all field samples were collected prior to performing the seed studies. Also, the virus concentrator used for collecting samples had never been used previously for seed studies.

Sources of the contamination to the storm drains are unknown. For the two drains, sources could include illegal connections to the sewage system, illegal discharges (e.g. from mobile homes or recreational vehicles), or leaks from the sewer system. The Malibu Lagoon also could be contaminated by these sources in addition to discharges to the watershed from septic tanks and diffuse sources such as campers and picnickers.

When the Lagoon was sampled, the Tapia Water Reclamation Plant was discharging tertiary treated water into Malibu Creek, about six miles upstream from the lagoon. According to the LVMWD, flows from Tapia during sampling periods in the lagoon were 1.04 MGD and ranged from 0 to 4.6 MGD (LVMWD, 1991). As stated earlier, levels of bacteria in the effluent were very low. A study of enteric virus in water produced by the Tapia plant was conducted in 1987 using similar virus sampling and analytical techniques. Only a single virus on one sampling day (90 gallon sample when the chlorination unit was not functioning) was detected out of 25 days of sampling (James M. Montgomery Engineers, Inc., 1990). However, Tapia does not have an enteric virus monitoring program, so the efficacy of the tertiary filters in virus removal has not been determined in over four years. Although Tapia can not be ruled out as a possible source of virus, it seems unlikely that this facility would represent a source of detectable virus in the Malibu Lagoon system unless the tertiary filters were not functioning properly.

The amount of viruses per volume of water presented in Table 5 must be considered as rough estimates given the following reasons:

1. At low densities, viruses are not normally distributed throughout the sample. It is not

within the Lagoon appeared to have little impact on bacterial densities in the Lagoon (Table 1 and bacterial density data). In general, bacterial densities were high all the time throughout the lagoon, regardless of the water level within the Lagoon. These results do not rule out septic systems within the watershed as a potential source of bacterial contamination, but they imply that bacterial densities are independent of the water level within the Lagoon in dry weather, when the water table is lower. These results support conclusions reached by Warshall et al.,(1992) on Malibu Lagoon water quality.

Possible other sources of indicator bacteria to the lagoon are warm blooded wild and domestic animals, vegetation and soil for total coliforms, numerous storm drains including three that discharge into the Lagoon and lower reaches of the watershed, and many other diffuse sources including campers, temporary residents, and picnickers within the watershed. Little information exists regarding potential sources of indicator bacterial contamination in this watershed. However, monthly monitoring reports on water quality in Malibu Creek are submitted to the Regional Water Quality Control Board (RWQCB) by the Las Virgenes Metropolitan Water District (LVMWD).

A review of one potential source of bacterial indicators, the LVMWD's Tapia Water Reclamation Facility, indicates that the plant could be discounted as a direct source because levels of bacteria in the effluent during the study were very low; in over 90% of the samples, total coliforms were not detected (≤ 1 Most Probable Number (MPN)/100ml (LVMWD, 1991). The highest reported value during the study period was 4 MPN/100 ml. However, the issue of bacterial regrowth in the watershed has never been investigated. In contrast to the Tapia plant's effluent, bacterial densities from Malibu Creek and its tributaries were well above levels of concern at the ten monitoring stations throughout the watershed in the Santa Monica Mountains. Total Coliform densities were high everywhere, while fecal coliform and enterococci densities were generally higher at lagoon stations than at upstream stations (LVMWD, 1991). It appears that many of the sources listed above, especially non-point sources, are contributing bacterial indicators to the lagoon.

B. Viruses

1. F-Male Specific Coliphage

Higher densities of male specific coliphage are expected in sewage contaminated waters than in waters without human fecal inputs (Cabelli, pers. comm., 1989). The trend predicted by Cabelli was not supported by these results. Enteric viruses were found at all five sampling locations despite the fact that mean coliphage densities were approximately an order of magnitude higher at the Pico-Kenter and Herondo drains than the Malibu Lagoon sites. Enteric virus was found at all three Malibu Lagoon sites, yet coliphage densities were always low in the lagoon. The coliphage densities on the days that virus was found were comparable to the mean densities. Also, virus was never found on days where coliphage densities were highest for the site.

the conductivity of runoff varied over a wide range from nearly fresh water to salt water.

The height of Malibu Lagoon above sea level and the tide height at the time of sampling at the Lagoon are also listed in Appendix 4. Lagoon height ranged from two feet (the lowest possible measurement with the measuring stick near the C-Channel station) to 5.4 feet above sea level. Of the 26 days where samples were collected and analyzed for enteric virus, 11 occurred when the lagoon was at its lowest at two feet and nine occurred when the lagoon elevation was over 3.5 feet. Tidal heights ranged from plus 1.2 to six feet during virus sampling at the lagoon.

In general, the conductivity of the samples was high when the lagoon mouth was open and/or when there was a very high tide. The sample conductivity was seldom below ten mmhos at the Breach, C-Channel and Upstream stations when the tide was higher than the lagoon height. The Texaco station was less susceptible to the impacts of the tide because samples collected there were predominantly treated discharge and runoff, and the sampling site was not directly in the lagoon.

IV. DISCUSSION

A. Bacterial Dispersion

1. After Storm Drain Pipe Extension

Only 11 of the 17 sampling dates were ideal for assessing the effectiveness of the drain extension for reducing indicator bacteria densities in the surf zone. The last six days of the sampling period produced different results because a nearby sewer replacement project contributed a large volume of water to the Pico-Kenter drain. The flow from dewatering was discharged into the Pico-Kenter drain where it then flowed across the beach and into the surf zone.

Bacterial levels of concern were always exceeded in the drain itself, but rarely if ever exceeded at ankle or chest depth in the surf zone on days when the pipe extension was operating as designed. Because of the logistical problems of sampling directly from the Pico-Kenter drain after the pipe extension, samples of drain effluent were only collected on days during enteric virus sampling. These dates were not usually the same days as the bacterial dispersion study. Based on the results of the bacterial dispersion study, the runoff plume either did not travel back to shore or the plume was diluted sufficiently to cause indicator densities to be low.

2. Comparison with Storm Drain Before Pipe Extension

The results of the 1991 dispersion study were drastically different from the results of the 1990 dispersion study. In 1990, bacterial levels of concern were frequently exceeded at ankle depth stations at distances of up to 100 yards from the drain (See figure 5). Chest depth samples also exceeded levels of concern 10%, 30% and 40% for fecal coliforms, total coliforms, and

Analysis of variance (ANOVA) was used to test for significant differences among means from stations at ankle and chest depths. Each station mean was calculated from all data gathered over the study period. The geometric means from the 1990 bacterial distribution study were compared to the 1991 data using separate t-tests.

ANOVA with posteriori multiple comparisons were run to test for site differences in geometric means. Tukey's studentized range test (HSD method; SAS, 1990, Steel and Torrie, 1960) was used for the geometric mean comparisons.

IV. RESULTS

A. Bacterial Indicators

1. Bacterial Distribution

Densities of indicator bacteria were low at all ankle and chest depth stations in the surf zone (Table 1) and extremely high in the Pico-Kenter effluent (Table 2). During each sampling period, indicator densities in drain effluent exceeded levels of concern for all three bacterial groups (Figure 4). In the surf zone, however, levels of concern were seldom exceeded at all ankle and chest depth stations for these indicators. The geometric means of the bacterial densities at all surf zone sites were not significantly different from each other. Ankle depth geometric means were not significantly different from chest depth means.

2. Bacterial Densities in Runoff

The geometric means and 95% Confidence Intervals of bacterial densities measured in runoff samples collected from the Malibu Lagoon, and the Pico-Kenter and Herondo storm drains are given in Table 2. Bacterial densities in the Pico-Kenter drain were approximately three to five times higher than the Herondo drain and one to two orders of magnitude higher than the densities at the four Malibu Lagoon sites. This trend held for all three bacterial indicators. In Malibu Lagoon, the densities were generally lowest to highest from C-channel to the breach to Upstream to Texaco.

The geometric mean densities at the Malibu Lagoon C-Channel and Breach stations were significantly different ($P \leq 0.5$) from the other four stations for total coliforms and enterococcus (Table 2). The geometric means for the Breach and C-Channel stations were not significantly different ($P \geq 0.5$) for all three indicators. The geometric means for the Pico-Kenter and Herondo drains were not significantly different from each other, but they were significantly higher than all other sampling sites for total coliforms and than the C-Channel and Breach sites for enterococcus.

B. Viruses

Two 35 gal containers were filled with storm drain effluent. The effluent was adjusted to pH 3.5, $AlCl_3$ was added to a final concentration of 0.005 M, and a known amount of attenuated poliovirus (vaccine strain) was added to each container. Triplicate grab samples were taken at the beginning and end of the filtration period. Each grab consisted of 1 ml of sample diluted with 9 ml of sterile Hank's Balanced Salt Solution (HBSS) to minimize any toxic effect to the virus by the drain effluent. The HBSS contained 2% fetal bovine serum which helps prevent virus inactivation when samples are frozen and thawed. Effluent in each container then was run through a concentrator (described below).

Grab samples before and after concentration were analyzed for densities of the poliovirus. Concentrations of virus in the first set of grab samples yielded baseline amounts. Any toxic effects of the effluent then could be assessed by comparing differences in viral concentrations between the first and second set of grab samples. The percent of recovery was measured by comparing virus levels in the final concentrate with baseline levels. The results of the seed study were used to give recovery estimates, not to adjust the densities of enteric viruses recovered from field samples.

3. Sampling and Analysis

a. F-Male Specific Coliphage

Grab samples were obtained on 14 days at the Pico-Kenter drain, five days at the Herondo drain, 25 days at the Malibu Lagoon breach site, 24 days at the C-channel site, 23 days at the Texaco site, and 26 days at the upstream site (from splits of the bacterial samples). Nearly all of the samples were collected on the same dates that virus sampling occurred. The samples were analyzed for F male-specific coliphage by the Orange County Sanitation Districts' (OCSD) virology laboratory. The F-male specific coliphage assay methods and E. coli host bacteria were obtained from Dr. V. Cabelli as described in Appendix I.

b. Enteric Viruses

Enteric viruses were sampled at the storm drain sites using a modified version of Standard Method 913-A (APHA, 1985) and "The USEPA Manual of Methods for Virology," 1984 (EPA-600/4-84-013. Adsorption conditions were pH 3.5 with an $AlCl_3$ molarity of 0.005. The eluent was 3% beef extract adjusted to pH 7.2. Approximately 50 to 140 gallons of effluent were filtered per sample. A detailed description of the enteric virus sampling protocol is in "The USEPA Manual of Methods for Virology" (1984).

Travel set up and the long field processing time (approximately 1.5 hours) required that sampling began in the morning and continued until noon. Only one sample was taken per day at each of the drains.

One-liter eluates from the field sample were delivered to the laboratory and reconcentrated in the afternoon using an organic flocculation procedure (Katzenelson et al.,

artificially by the California Department of Parks and Recreation (Breach);

2) At the bridge over the C-channel which is the western most channel of the lagoon, next to the Malibu Colony (C-channel);

3) In the soft bottomed rivulet (approximately 150,000 gallons per day) formed by the discharge of treated contaminated groundwater from the Texaco gas station treatment facility and runoff from the adjacent Cross Creek Road shopping center parking lot. The sampling point was three to five yards north of Malibu Creek, just east of the Pacific Coast Highway bridge (Texaco); and

4) At a point on the north bank of the creek, 100 yards upstream of the Texaco discharge (Upstream).

III. SAMPLING

A. Indicator Bacteria

1. Sampling Design and Frequency

The study was carried out over a six month period from mid- May to mid-November, 1991. Ideally, sampling would have occurred during weekends when the most people were using the beach. However, because of the logistical requirements of the microbiology laboratories, sampling was conducted during morning hours on weekdays. All bacterial samples were tested within six hours of sampling.

Sampling in the surf zone around the Pico-Kenter drain occurred on seventeen days over a five week period. Samples for bacterial analyses were collected at the same ten sites as the 1990 surf zone study (Figure 3):

1. Seven stations were positioned at ankle depth at 0, 25, 50, and 100 yard intervals; the "0" position was located directly west of the drain.

2. Three stations were positioned at chest depth at 0, and 25 yard intervals.

All samples were taken from the incoming breaking surf. The ankle depth samples were taken as the surf foam reached the sample bottle at the height of the sampler's ankle. The chest samples were taken where the breaking waves reached the chest height (approximately 3 to 4 ft) of a medium sized adult. Chest depth sampling usually occurred between 30 and 50 yards further away from the drain than ankle depth sampling.

Samples of drain effluent were only collected for bacterial analyses on each of the thirteen enteric virus sampling days. Because of logistical problems after the pipe was extended 600 feet, there was no sampling directly from the Pico-Kenter drain on days where surf zone

the release of the 1990 study, the City of Santa Monica has kept the beach closed, 100 yards north and south of the Pico-Kenter drain, and implemented measures to reduce or eliminate ponding of runoff on the beach.

A number of questions still need to be addressed prior to deciding whether or not to conduct a large scale epidemiology study. This study should be conducted on recreational bathers in waters with large variability in indicator bacteria densities adjacent to flowing storm drains. These conditions would provide information on the health risks from swimming in waters contaminated only by urban runoff.

In this report, results are presented from two studies performed during the third year of the project. They were designed to:

1. Further evaluate dispersion of indicator bacteria around the Pico-Kenter storm drain after the completion of the 600 foot pipe extension; and

2. Testing for the presence of human enteric virus in the Pico-Kenter and Herondo storm drains, and Malibu Lagoon.

Further work was done in the first study to refine our knowledge on the distribution of indicator bacteria around the Pico-Kenter drain. Gaining an understanding of indicator bacteria distribution in the surf zone is an essential component in deciding which portion of the beach and the swimming public is exposed to excessive indicator densities resulting from storm drain effluent. In this case, the Pico-Kenter drain was chosen because of the historically high levels of indicator bacteria measured in the surf adjacent to the drain. The drain was also chosen because it offered a unique opportunity to test the efficacy of storm drain extensions in reducing indicator bacteria densities in the surf zone.

In the second study, we continued to survey various storm drain effluents for the presence of human enteric viruses. The two storm drains and lagoon studied, like the sampling locations from previous studies, were chosen because they historically have been associated with high densities of indicator bacteria in the surf zone when the drains flow directly to the ocean. This year's virus study was the first time that such a large section of Santa Monica Bay was covered. The information from this study will be used to help determine the potential sites and need for an epidemiology study .

II. STUDY SITES

A. Pico-Kenter Storm Drain

The Pico-Kenter drain is located where Pico Boulevard meets the beach (See figure 1). The storm drain system drains a large area that includes much of Santa Monica and part of West L.A. and Brentwood. There are two drains that discharge to the beach: one is owned by Los