# TOMALES BAY SHELLFISH TECHNICAL ADVISORY COMMITTEE FINAL REPORT:

# INVESTIGATION OF NONPOINT POLLUTION SOURCES IMPACTING SHELLFISH GROWING AREAS IN TOMALES BAY, 1995-96

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# **EXECUTIVE SUMMARY**

Tomales Bay is an estuary located in western Marin County, California. The watershed drainage area receives intense rain during the winter months, with average annual rainfall ranging from 26 to 39 inches. The Bay has a long history of use for bivalve shellfish aquaculture, and currently supports seven certified shellfish companies. The major land uses in the watershed are livestock grazing, dairy farming, low-density residential, and parklands. Earlier studies found that high fecal coliform levels from rainfall runoff seriously affected the inner Bay south of Millerton Point and the outer Bay near the Walker Creek delta. From these studies, rural and livestock sources were determined to be the most likely cause of the high fecal coliform densities in the Bay.

In 1993, state legislation was passed (SB 417, Senator Marks) that enacted the Shellfish Protection Act of 1993. This bill requires the appropriate Regional Water Quality Control Board to form a technical advisory committee if a commercial shellfish growing area is determined to be threatened. Tomales Bay met the threatened designation, based on the number of days each year that the Bay is closed to shellfish harvesting. In the case of Tomales Bay, these closures are triggered by rainfall.

In 1994, the Tomales Bay Shellfish Technical Advisory Committee convened and determined that a study was necessary to investigate the sources of water quality degradation in the Bay. The study was conducted during the winter of 1995-1996, and consisted of 40 sampling stations throughout the Bay and watersheds. Samples were collected during two dry season periods and during four rainfall events. All samples were analyzed for four standard indicators of microbiological water quality: total coliform, fecal coliform, enterococcus, and *Escherichia coli* (*E. coli*). In addition, several sites were analyzed for coliphage and the anaerobic bacterium *Bacteriodes vulgatus*, indicators that are thought to be more specific for human fecal sources than the standard indicator organisms. A limited number of analyses were performed to detect the presence of pathogenic bacteria. *Salmonella typhirium* and *E. coli*:0157 were identified in separate watershed samples.

#### Watershed Water Quality

Bacterial densities usually exceeded the standards within the first one or two days of each rainfall event, then typically decreased to acceptable levels by the last day of sampling. Consistently high bacterial levels were detected during most of the study at sites within the Walker/Keyes/Chileno watershed and along the eastern shoreline watersheds. Slightly lower concentrations of fecal coliform were detected throughout the Lagunitas/Olema watershed. In contrast, bacterial levels at the western shoreline watershed stations were generally 10 to 100 times lower than those from all other watersheds.

The four commonly used indicator organisms described above were monitored during this study to see if any single indicator appeared to be more source-specific than others. *E. coli* levels tended to drop more slowly than the other indicators, and was often the only indicator exceeding its acceptable level by day 3 of the monitored rainfall events. Based on these data, enterococcus was the most sensitive and fecal coliform was the least sensitive indicator organism relative to their respective water quality objectives. Enterococcus was the only indicator organism exceeding water quality criteria at White Gulch, the freshwater control site. It may be that the enterococcus level used for this study was too low for fresh water use, or that enterococcus is identifying another source of contamination.

Fecal coliform loadings were calculated to estimate the amount of fecal coliform contributed by each watershed on a daily basis. The highest loadings occurred within the Walker/Keyes/Chileno Creek and the Lagunitas/Olema watersheds. The former region is primarily dairy and grazing with some residential dwellings, while the latter contains a mix of agriculture, commercial, and residential uses. Within the Walker/Keyes/Chileno Creek watershed, the highest fecal coliform loadings occurred in the Chileno Creek subwatershed. Within the eastern shoreline watershed, the highest fecal coliform loadings generally occurred in the watersheds represented by stations Milepost 40.35, Milepost 34.95, Millerton Creek, Milepost 32.12, Grand Canyon Creek, and Tomasini Creek. Within the Lagunitas/Olema watershed, Lagunitas Creek

contributed the largest share of the fecal load, followed by Olema Creek. The Bear Valley drainage contributed the lowest loadings for this watershed. Fecal coliform loadings from the western watershed were less than that those contributed by the other watersheds.

#### Bay Water Quality

Outer Bay sampling stations were adversely affected within the first two days following significant rainfall. Fecal coliform concentrations often remained elevated three days after the rainfall event, indicating either a long residence time in the outer Bay or a prolonged source of contamination. The highest fecal coliform concentrations were observed at station 34, which is in the direct influence of the branch of Walker/Keyes Creek that flows around Preston Point. Mid-Bay stations had fecal coliform levels that were generally lower than either the outer or inner Bay regions, although all Bay stations experienced elevated concentrations of fecal coliform immediately following rainfall. The inner Bay monitoring stations had levels of fecal contamination slightly greater than those of the mid-Bay, and did not always return to acceptable levels by the day shellfish growing waters were reopened for harvest (day X). During rainfall event 3, both inner Bay monitoring stations showed an obvious spike of fecal coliform on day X that greatly exceeded the concentrations detected within the first three days of rainfall. A possible explanation for this sharp increase would be a pulse of contamination from the watershed or nearshore area.

#### Shellfish Quality

The fecal coliform concentrations in oysters in the outer Bay reached extremely high levels following significant rainfall. In addition, these data suggest a pattern of increasing concentration throughout the winter, perhaps as a result of the continuous high fecal concentrations contributed by the watershed. In addition, lower water temperatures in winter may result in a reduced metabolic rate in the oysters, which in turn would lengthen the time necessary for satisfactory cleansing of contaminated shellfish. Consequently, oysters in the outer Bay do not always return to National Shellfish Sanitation Program (NSSP) market standard by the time the outer Bay is reopened for harvesting.

Within the outer Bay stations, samples were collected from sites representing two different culture techniques: top-culture (i.e., floating bags) and bottom culture (i.e., rack and bag). The top-culture station was significantly higher than the NSSP market standard during the first dry season sampling. It is likely that these elevated levels of fecal coliform are the result of localized contamination, possibly from birds roosting and defecating on the floating bags.

Oysters from the mid Bay were found to exceed the NSSP standard following significant rainfall but generally returned to acceptable levels for fecal coliform by day X. Oysters from the inner Bay typically exceed the NSSP market standard after significant rainfall, and the magnitude of contamination was generally equivalent to the observed levels in the outer Bay oysters.

#### Conclusions

The results of this study support the conclusions of earlier surveys, that the lands along the eastern watershed and the southern watershed drainages contribute significant fecal pollution during and immediately following significant rainfall. The primary land use in these eastern watersheds consists of dairies and cattle grazing land. Primary land uses in the southern watersheds include dairying, cattle grazing, public open space and watershed land, and residential.

Degradation of Bay water quality coincided with the pulses of fecal contamination from the watershed after rainfall.

This study evaluated general trends in water quality and contaminant sources on a watershed and subwatershed scale. As such, individual or localized sources of fecal coliform such as domestic sewage disposal systems or individual incidents of direct disposal of sewage (i.e., illegal dumping) into the Bay were not specifically evaluated. Fecal contamination can transmit viruses and diseases and poses a serious threat to both consumers of fish and shellfish and people swimming or having direct contact with Bay waters. A

subsequent human illness outbreak in May 1998, which was determined to be caused by a virus of human fecal origin reinforces the need to evaluate those sources of fecal contamination that were not adequately addressed in this study, including onsite sewage disposal systems and recreational and commercial boating and camping activities. The illness outbreak and follow-up actions are discussed in Appendix B of this report.

#### Recommendations

A number of recommendations developed by the TBSTAC are included in this report. These recommendations are based on the conclusions of this study and on other relevant information. They include further monitoring to identify pollutant sources in the watershed, development of pathogen source control measures, suggestions for policy development, and outreach and education programs.

The long-term health of Tomales Bay, and its ability to sustain the many beneficial uses that are now enjoyed, is dependent upon the success of the community and involved governmental agencies to address the sources of contamination that are threatening it. It is the hope of this committee that these groups will work cooperatively to ensure the continued use and appreciation of Tomales Bay by future generations.

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# INTRODUCTION

#### Legislation

On October 10, 1993, legislation was passed by the California legislature that enacted the Shellfish Protection Act of 1993 (SB 417, Marks). This bill requires the Regional Water Quality Control Board (RWQCB) to form a technical advisory committee for any commercial shellfish growing area that is determined to be threatened. One of the criteria for a "threatened" area is the number of days the area is closed to shellfish harvesting due to pollution threats. The Shellfish Protection Act states that a shellfish area shall be designated as threatened if it is closed to harvesting for more than thirty days in each of three consecutive calendar years. Based on the California Department of Health Services' (DHS) letter of January 5, 1994, notifying the RWQCB that Tomales Bay met the threatened designation, the RWQCB passed a resolution on January 19, 1994, authorizing formation of the TBSTAC and held its first meeting on February 15, 1994. According to the Shellfish Act, the purpose of the TBSTAC is to advise and assist the RWQCB in developing an investigation and remediation strategy to reduce pollution affecting the shellfish growing areas.

At the first TBSTAC meeting, the committee members determined that a study was necessary to investigate the sources of water quality degradation in Tomales Bay, and they appointed a study design subcommittee to develop a study plan. The study design subcommittee presented a plan to the TBSTAC in the summer of 1994. Originally scheduled for the winter of 1994-95, the study was postponed until the following winter (1995-96). During the winter of 1994-95, staff from the DHS's Environmental Management Branch conducted a preliminary survey of the watershed sampling sites identified in the study plan, with the RWQCB providing financial support to the DHS's Environmental Microbial Diseases Laboratory (EMDL) for all sample analyses. The results of this preliminary survey, together with recommended changes to the study design, were presented to the TBSTAC on July 26, 1995. The study design subcommittee distributed a final version of the study plan (Appendix A) on September 5, 1995, and the study was carried out in the winter of 1995-96.

The study was designed to address potential sources of fecal contamination from rainfall runoff to Tomales Bay from (i) non-point sources along the west and east shore of the Bay, and (ii) non-point sources originating from the predominantly agriculture-influenced watershed. In addition, the study investigated several potential indicator organisms in addition to the standard fecal coliform indicator group.

#### Site Description

Tomales Bay is an estuary located in western Marin County, California, approximately 50 km (40 miles) northwest of San Francisco (Figure 1). The Bay has an area of approximately 28 square kilometers (11 square miles). The mouth of Tomales Bay is at the southern end of Bodega Bay, and extends in a southeasterly direction along the line of the San Andreas fault. The Bay is about 12 miles in length with an average width of less than 1 mile. Tomales Bay is characterized by relatively shallow water, with the average depth being less than 20 feet (Figure 2). Hydrographic studies conducted from 1966-70 (Smith et al., 1971) indicated that the currents in the Bay are predominantly influenced by tidal cycles rather than wind-driven. They suggested that the Bay consists of three regimes, with significant flushing taking place in the outer Bay from the mouth to approximately Hog Island near the Walker Creek delta, the inner Bay (south of Double Point) taking much less part in the water exchange process, and only sluggish mixing in the mid-Bay (Pelican Point to Double Point) portion. These studies were done in the summer and fall periods so they do not reflect the influence of increased inflow from runoff.

The Tomales Bay watershed, consistent with the "Mediterranean" climate of the central coast of California, receives intense rain during the winter months (November through March), with 85% of the annual rain

usually falling during this period. Another 10% of the annual precipitation falls during October and April, with the remaining 5% during the other five months of the dry season. Average annual rainfall ranges from 26 inches per year in the north and east part of the watershed to 39 inches per year in the south. (Fischer et al., 1996).

#### Aquaculture

There was at least a minor fishery for native oysters (*Ostrea lurida*) from Tomales Bay as early as 1859 (Barrett, 1963). Although Eastern oysters (*Crassostrea virginica*) were initially transplanted to Tomales Bay near Millerton Station in 1875, these efforts were not entirely successful due to the abundant production of the San Francisco Bay oyster grounds, which were closer to the major markets in San Francisco. Non-native oysters were again introduced into Tomales Bay around 1907 in response to increased pollution of San Francisco Bay and the resultant failure of its oyster industry. The Tomales Bay Oyster Company was formed in 1913 and operated oyster beds near Millerton. About this same time Jensen's Oyster Company started operations near Hamlet, and the Consolidated Oyster Company began a short-lived operation at Blakes Landing.

The Tomales Bay Oyster Company was the first to introduce Pacific oysters (*Crassostrea gigas*) to Tomales Bay in 1929 following the earlier successful introduction of this species in Washington state oyster leases. This species now constitutes the majority of oysters currently produced in Tomales Bay.

There are currently seven certified commercial shellfish harvesters in Tomales Bay, with a combined aquaculture lease area of 483 acres (Table 1; Figure 3). With one exception, all commercial growers in Tomales Bay operate on eastern shoreline leases granted by the California Department of Fish and Game (DFG). The exception is the Frank Spenger Company, which operates on a Point Reyes National Seashore lease on the western shore.

Commercial shellfish production in Tomales Bay is primarily devoted to Pacific oysters (*C. gigas*) and bay mussels (*Mytilus edulis* and *M. galloprovincialis*). In addition, there is a small amount of commercial production of Eastern oysters (*Crassostrea virginica*), European oysters (*Ostrea edulis*), Kumomoto oysters (*Crassostrea gigas kumomoto*), and Manila clams (*Tapes semidecussata*).

# Watershed

The watershed drainage area for Tomales Bay is approximately 561 km<sup>2</sup> (216 square miles) with four major sources of input: (1) the immediate drainage from small tributaries along the west and east shores (73 km<sup>2</sup>; 28 mi<sup>2</sup>); (2) Lagunitas Creek (241 km<sup>2</sup>; 93 mi<sup>2</sup>) to the southeast; (3) Olema Creek (50 km<sup>2</sup>; 19 mi<sup>2</sup>), which flows into Lagunitas Creek close to the head of the Bay; and (4) Walker Creek (196 km<sup>2</sup>; 76 mi<sup>2</sup>) to the northeast (Table 2).

The U.S. Geological Survey maintains stream gauges on both Walker and Lagunitas creeks. These gauges measure only a portion of the runoff from their respective watersheds, as well as any water released from catchment reservoirs (Table 3). Fischer, et al. (1996) estimated that about two-thirds of the runoff into Tomales Bay comes through the Lagunitas-Olema Creek drainage even though this area only makes up about half of the watershed (Table 4, Table 2). The Walker Creek drainage, which includes Chileno, Arroyo Sausal, Salmon, and Keyes Creeks, makes up about 35% of the Tomales Bay watershed area, but produces about 25% of the annual runoff into the Bay (Fischer, et al. 1996). The remainder of the runoff into the Bay (approximately 10%) comes from the local bayshore drainages, which make up 13% of the total watershed area. It is estimated that sediment runoff from the major creeks and tributaries into Tomales Bay may be as high as 48,600 tons yearly. Approximately one third of the sediment is carried into the Bay from the Walker/Keyes Creek drainage. For the present study, the four sources of input to Tomales Bay listed above were further divided into smaller subwatersheds (Table 5; Figure 4) based on the location of sampling stations.

Marin Municipal Water District (MMWD) maintains five water catchment reservoirs in the Lagunitas watershed (four on Lagunitas Creek and one on Nicasio Creek) with a total capacity of approximately 69,000 acre feet. MMWD also has a reservoir on a tributary to Walker Creek, with a capacity of 10,572 acre feet.

# Land Use

The Tomales Bay watershed is a major recreational area and is used for hiking, boating, camping, picnicking, clamming, fishing, and birding. The Bay also supports the commercial cultivation and harvesting of shellfish, including oysters, mussels, and clams. Herring and halibut are also harvested commercially from wild populations, and there is a sport fishery for halibut in the Bay.

The major land uses in the watershed are livestock grazing, dairy farming, low-density residential, and parklands. Beef, sheep, and dairy farms have been an important part of the local economy since the mid-1800's, although numbers of dairies have been declining since the increase in competition from large Central Valley dairies. The relative pollutant contributions from these sources are discussed below under the Water Quality section.

There are nine small towns within the watershed, with limited commercial development and no industry. According to the 1990 census, the west side of Tomales Bay has a population of 1392, with a total of 650 households. The east side of the Bay, from Dillon Beach to Point Reves Station, has a population of 3217, with 1246 households. The population has probably increased since the last census due to some new residential development. All of the towns are served by onsite sewage disposal systems except the town of Tomales, which is served by a centralized wastewater treatment plant. There are seven other small sewage treatment systems within the watershed, and one facility that accepts septage waste. The Regional Water Ouality Control Board (RWOCB) prohibits direct discharge from treatment systems into Tomales Bay or the creeks within the watershed. A number of the sewage treatment systems are permitted to discharge to irrigation fields during the dry season (Table 6). The RWQCB has delegated authority for the regulation of individual on-site sewage disposal systems in Marin County to the County Health Officer, through Resolution 84-12, which waives Waste Discharge Requirements for individual systems. Under a county ordinance approved by the Board of Supervisors in August 1984, the Marin County Environmental Health Services has responsibility for overseeing individual on-site sewage disposal systems. This includes the responsibility for siting and design, installation and repair standards, and monitoring and inspection programs.

# Water Quality Indicators

Animal and human waste contains microorganisms that can cause disease in humans. Although animal waste is associated with a variety of bacterial pathogens, human waste can contain both bacterial and viral pathogens and is the greatest concern relative to human health impacts from contaminated water. Because it would be impossible to routinely monitor for all pathogenic organisms, indicator organisms are used to assess microbiological water quality. These organisms are not necessarily pathogenic, but are easily detected and are abundant in wastes from warm-blooded animals.

The four commonly used organisms are total coliform, fecal coliform, *Escherichia coli* (*E. coli*), and enterococcus. Total coliform are comprised of four genera of bacteria (Figure 5) that can exist on soil particles and plant surfaces as well as in fecal matter. Fecal coliform is a subset of total coliform and is specific to wastes from warm-blooded animals, but not necessarily to humans. *E. coli* is a subset of fecal coliform. Enterococcus is comprised of four species of bacteria, entirely separate from the coliform organisms. Coliform bacteria have historically been the indicator organism of choice, but have shortcomings. These organisms are not human-specific, and are inadequate to assess the health risk from human enteric viruses. Several epidemiological studies have suggested that enterococcus organisms are more highly correlated to human illnesses than the coliform organisms. Because of this, United States Environmental Protection Agency (EPA) strongly suggests that states adopt an enterococcus standard for marine waters.

All of these indicator organisms are measured and reported in the same way, as Most Probable Number per 100 milliliter of water (MPN/100 mL). This is not an exact count of the bacteria found in the water sample, but rather a statistical estimate of the number of bacteria in the sample.

#### Regulatory Agencies Involved in Protecting Water Quality

In California, water quality is regulated by one or more agencies, depending on the uses attributed to the particular water body. These uses, called beneficial uses, are activities that each water body can support, based on the existing water quality. Beneficial uses are protected by water quality objectives, standards, or criteria, depending on the regulating agency. Although they are technically different, for the purposes of this report we will consider objectives, criteria and standards to be the same: a defined value of a potential pollutant than cannot be exceeded. Since this study focused on bacterial levels of Tomales Bay and the surface waters feeding into the Bay, we will only discuss regulations and beneficial uses related to bacteria.

State water quality regulations require that beneficial uses of the waters within the State be defined and protected. The Porter-Cologne Water Quality Control Act requires that the State Water Resources Control Board (SWRCB) formulates and adopts policy to protect water quality. The SWRCB has developed a water quality control plan for ocean waters (Ocean Plan) and is in the process of developing policy for inland surface waters, bays, and estuaries. Of these plans and policies, only the Ocean Plan contains bacterial standards. Using guidance provided by the SWRCB documents, each of the nine RWQCBs develop Basin Plans that will ensure the reasonable protection of the beneficial uses for waterbodies under their jurisdiction.

The RWQCB has designated the following beneficial uses for Tomales Bay: shellfish harvesting, water contact recreation, and non-contact water recreation (Table 7). The RWQCBs also are required to develop lists of impaired waterbodies for each region, along with the causes of impairment. The RWQCB has listed Tomales Bay as an impaired water body for pathogen, sediments, metals, and nutrients. The beneficial uses and the related water quality objectives for Tomales Bay are discussed below.

#### Shellfish growing waters

One of the beneficial uses attributed to Tomales Bay is for use as a shellfish growing area. The California Department of Health Services (DHS) and the SWRCB both have regulations regarding shellfish growing waters. The SWRCB regulations are included in the Ocean Plan, and apply to all shellfish-growing areas. The Ocean Plan standard requires that, at all areas where shellfish may be harvested for human consumption, the median total coliform density must not exceed 70 MPN/ 100 mL, with no more than 10 percent of the samples exceeding 230 MPN/100 mL. For water bodies used for commercial shellfish growing, DHS standards must be met. These standards follow the criteria developed by the National Shellfish Sanitation Program (NSSP), which is administered by the U.S. Food and Drug Administration (FDA). The NSSP standards are based on acceptable levels of fecal coliform in shellfish and shellfish growing waters. An "Approved" growing area must meet both of the following criteria: (1) the geometric mean of samples collected at a particular site must be less than 14 MPN per 100 mL; and (2) less than 10 percent of all samples from a particular site can exceed an MPN of 43. For the Tomales Bay commercial shellfish growing areas, DHS regulations supersede those contained in the Ocean Plan.

Because Tomales Bay is subject to intermittent microbiological pollution from various sources, all of the current certified shellfish growing areas are classified as "Conditionally Approved" according to the criteria developed by the NSSP. In order to allow the proper control over commercial harvesting from a conditionally approved growing area, the NSSP requires that an intermittent pollution event affecting the area be predictable and manageable.

#### **Recreational water uses**

Another beneficial use of Tomales Bay and the surface waters feeding the Bay is for recreational use. Recreational water uses are divided into two categories: water contact activities (i.e. swimming or wading) and non-contact recreational activities. Non-contact recreation is considered to be any activity in or near the water where a person normally wouldn't expect to get their entire body wet, such as fishing or boating. The standards for these uses are summarized in Table 7, and fall under the primary jurisdiction of the RWQCB.

#### Standards Used in this Study

<u>Bay Water Samples</u>. To compare fecal coliform study results, we will use NSSP's fecal coliform geometric mean of 14 MPN/100 mL. This standard was selected over the recreational water values because it is a more stringent standard. Because there are no state standards for enterococcus  $\alpha$  *E. coli*, we will use the recommended criteria of the EPA for water contact recreation: 104 and 235 MPN/100 mL, respectively.

<u>Fresh Water Stream Samples</u>. To compare fecal coliform study results, we will use the RWQCB's Basin Plan (San Francisco Bay RWQCB, 1995) value for non-contact water recreation (mean <2000 MPN/100 mL) and EPA's criteria for enterococcus (104 MPN/100 mL) and E. coli (406 MPN/100 mL).

#### **Potential Sources of Bacterial Contaminants**

Potential sources of bacteriological contaminants into Tomales Bay included (1) the approximately 20 dairy ranches in the watershed, which contribute animal waste from confined animal areas and grazing areas into the tributaries draining to the Bay; (2) cattle and sheep ranches, with runoff from grazing lands; (3) other domestic animals, including dogs and cats; (4) domestic wastes from small centralized sewage treatment systems; (5) domestic wastes from onsite sewage disposal systems along the Bay; (6) discharge of waste from boaters (e.g., fishing, kayaking) and campers who frequent the western shoreline beaches; (7) terrestrial and aquatic wildlife, including perennial inhabitants such as sea gulls and those migratory birds that over-winter on the Bay.

Table 9 shows approximate numbers of livestock in different areas of the watershed and estimates of potential manure production. This information was derived from rough estimates made by the University of California Cooperative Extension in 1990. The number of dairies has decreased since this time, so that numbers of dairy cows may be lower. Attempts were made to get current numbers of dairy and beef cattle in the watershed by contacting the University of California Cooperative Extension, the Marin County Agricultural Commissioner, and the Sonoma County Department of Health Services, but we were told that these numbers are not available. Table 10 shows the manure characteristics of different livestock in terms of pounds per day of total waste (feces and urine) produced by a typical animal.

Shoreline surveys and sanitary surveys of Tomales Bay and its watershed have periodically been conducted by the DHS, often with technical support from the FDA's Northeast Technical Services Unit. Each of these surveys has identified rainfall-related non-point source pollution as the principal pollution event impacting the shellfish growing areas of Tomales Bay. The following is a brief summary of each of these studies.

#### Past Studies

Several previous bacteriological surveys have been carried out in Tomales Bay: (1) a shellfish and water quality study was conducted in 1974 by the DHS (Sharpe, 1974); (2) a shoreline and watershed water quality survey was carried out in 1976-77 and 1977-78 by the RWQCB (Jarvis et al., 1978); and (3) a sanitary survey was conducted by the Department of Health and Human Services of FDA (Musselman, 1980). The current study was also preceded by a pilot study in the winter of 1994-95 to test sampling methods and locations. The sampling locations, methods, and results of these studies are discussed briefly below.

#### <u> 1974 Study – California Department of Health Services</u>

The 1974 study by the DHS (Sharpe, 1974) was designed to determine the water quality of Tomales Bay and tributary streams during wet weather conditions and relate the results to the bacteriological quality of the shellfish grown in the Bay. The study also included a sanitary survey for potential pollutant sources, with a detailed description of the potential of contamination from land uses and recreational uses in and along Tomales Bay. Water samples were collected at 17 Bay sampling stations, 19 shoreline stations and 49 tributary stream stations for 12 days in December, following a three-day rain event totaling 1.98 inches. Samples were analyzed for total and fecal coliform. Shellfish from six locations were also sampled for coliform and heavy metals.

Results from the Bay samples generally showed that the Bay waters did not exceed the median standard of 14 MPN/100 mL for shellfish waters, but some stations did exceed the requirement that no more than 10% of samples may exceed 43 MPN/100mL. Shoreline samples showed elevated total and fecal coliform levels at numerous stations, which were attributed to the possibility of shoreline drainage, tributary streams entering the Bay, and possible failing septic systems. Shellfish samples were also elevated in most instances. In spite of fairly low runoff because of dry conditions in the watershed, results from tributary samples showed high total and fecal coliform counts. The streams were considered the major source of pollutants to the Bay. The study concluded that the high coliform counts were due to contribution of wastes by upstream dairies and, in lower Keyes Creek, from raw sewage discharges from the town of Tomales. This study predates the adoption of RWQCB requirements to improve handling of animal wastes on dairy farms and the construction of the Tomales sewage treatment plant.

#### <u>1976-78 Study – Regional Water Quality Control Board</u>

The San Francisco Bay RWQCB conducted a shoreline and tributary sampling survey during the winters of 1976-77 and 1977-78 (Jarvis et al., 1978), with the purpose of evaluating the effectiveness of the RWQCB's recent requirements for dairy waste practices. The RWQCB adopted "Minimum Guidelines for Protection of Water Quality from Animal Wastes" in 1973 and required dairies to be in compliance with manure handling practices by September 1, 1976. Samples were taken from 20 stream stations and six shoreline stations (not every station was sampled during each survey nor during both years). Samples were analyzed for total and fecal coliform, total organic carbon, and ammonia. Samples were only taken during the rainy season (from November through March in 1976-77 and November through January in 1977-78).

Results indicated improvement in stream conditions in areas where dairies had come into compliance with the minimum guidelines, although none of the shoreline or stream stations sampled met coliform objectives for water contact and non-contact recreation following periods of rainfall. The 1976-77 season had very light rainfall and the January 3, 1977, sampling event was the first major rain (approx. 2 inches in three days). The January 14, 1978 sampling event followed a 2.5 inch rain event in three days; however, there was significant rainfall in November and December, so that the runoff from the watershed was greater than the previous year's. There were much higher coliform levels along the shoreline in the 1977-78 season as compared with the previous year; this was attributed to greater freshwater inflows into the Bay during 1977-78. Stream stations showed decreases in coliform between 1976-77 and 1977-78 following implementation of the Minimum Guidelines. The report also concluded that sewering of the town of Tomales in June 1977 resulted in decreased levels of coliform in Keyes Creek below the town.

#### <u>1980 Study – U.S. Food and Drug Administration</u>

The 1980 sanitary survey was conducted from February 24 through March 12 by the FDA to determine the degree of pollution and recovery rate of the Bay during periods of rainfall. Samples were taken from 45

stations in the Bay and on tributary stations close to the Bay. A total of 393 samples were collected and analyzed for total and fecal coliform and fecal streptococci. Shellfish samples were taken from two sites in the Bay and analyzed for total and fecal coliform.

Results showed that the shellfish market standard of 230 MPN fecal coliform was exceeded in all Bay water quality stations during wet periods. The dry period samples met the standard, with the exception of stations at the head of the Bay and near the mouth of Walker Creek. Seven out of eight shellfish samples exceeded the market standard of 230 MPN. Tributary samples ranged from low fecal coliform densities during the dry periods to high densities during rainfall events. In order to quantify the numbers of bacteria entering the Bay, daily estimates of stream flow were made on major streams (Walker, Keyes, Lagunitas, Olema, and Bear Valley Creeks) and several eastshore tributaries to the Bay (Millerton Gulch, Tomasini Creek, Grand Canyon Creek, and Cypress Grove). It was determined that the fecal coliform densities in the streams during dry weather were equal to sewage from about 150 to 200 people. During wet weather, fecal coliform densities increased to the equivalent of sewage from 1500 to 2000 people or 500 to 700 cows. The highest loadings following rains revealed a bacterial equivalent of 40,000 to 50,000 people or 15,000 to 20,000 cows.

The 1980 study concluded that the portions of the Bay most seriously affected by pollution from rainfall and runoff were the head of the Bay (Millerton Point south) and the Walker Creek delta. Rural and livestock sources of non-point pollution were considered to be the most likely cause of high fecal coliform densities in the Bay.

# <u>1994-95 Pilot Study – Department of Health Services</u>

The pilot study conducted by the DHS in the winter of 1994-95 (California Department of Health Services, 1996) was a prelude to the study detailed in this report and was designed to evaluate indicator species, test sampling methods and laboratory analyses, and finalize site selection of watershed sampling stations for the 1995-96 study. A total of 352 samples were collected from 12 stations in the Bay and from 35 watershed stations on nine different sampling dates during both closed and open harvesting periods. Samples were analyzed for total and fecal coliform, *Enterococci*, anaerobic bacterial indicators, and Methylene Blue-Active Substances (MBAS), which are common surfactants in detergent. A total of 26 shellfish samples were collected for total and fecal coliform analysis.

Results showed the impact of rainfall on the water quality of the tributaries entering Tomales Bay and on the water quality of the Bay itself following runoff events. The data supported the study's theory that the major source of fecal contamination to the Bay is rainfall-related runoff from the tributaries. Two seasonal patterns of fecal coliform concentrations were observed: (1) sites that showed declining fecal coliform densities throughout the winter, suggesting a nonrenewable source, and (2) sites that exhibited high fecal coliform densities throughout the season, suggesting a constant source. The results of this pilot study were used to determine what types of analyses would be used for the full-scale study during the 1995-96 winter season and which stations should be added or deleted from the sampling design.

#### Comparisons of Fecal Coliform Results Among Studies

In order to try to assess trends in fecal coliform numbers over time, data from the past studies were compared for selected Bay and watershed stations. Sampling locations were chosen that were common to all or the majority of the studies. Since there were few overlaps in sampling stations on the south and west sides of the Bay, stations were chosen along the east shore where the sampling record was more consistent. The rainy seasons were variable from study to study and not all studies included the complete rainy season. None of the earlier studies sampled during the dry season. The 1974 study sampled the first significant rainfall of the season (December) and therefore the results reflect a low runoff from tributary streams. The 1976-77 and 1977-78 studies reflect a lower than average and moderately heavy rainfall year, respectively. The 1980

samples were taken beginning in late February following several months of moderate to heavy rainfall. Sampling dates of February 29th and March 3rd were included in the comparison since both followed periods of moderate rainfall (1.37 inches on February 28th and 0.78 inch cumulative rainfall on the 3rd). Both the 1994-95 and 1995-96 samples were taken over a complete rainy season, with overall moderate rainfall, including several major rain events.

Since the data sampling schedules were so variable, the studies were compared using the highest, lowest, and median fecal coliform values over the course of each study. Pre- and post-wet season samples from the 1995-96 study were not included. Data were compared for four watershed stations (Walker Creek, Millerton Creek, Grand Canyon Creek, and Olema Creek at Bear Valley Road) and four Bay stations (Walker Creek delta, Marconi Cove, Blake's Landing, and Tomales Bay Oyster Company) (Table 11-12, respectively). Figures 6a and 6b are graphs of the data for the Bay and watershed stations, respectively.

Lack of data on other environmental variables related to sampling (e.g., stream flow and precipitation) and variability in rainfall, stream flow, and soil saturation make it difficult to come to any clear conclusion about fecal coliform trends over the years from 1974 to 1996. In general, results for Bay stations showed that the coliform levels were lowest during the low rainfall years (1974 and 1976-77). The lowest levels have remained essentially the same over the years, with some increases in 1977-78 (as noted, this was a higher rainfall year than either of the previous years). Median values also increased in 1977-78 and 1980 and returned to earlier levels in 1995. In general, levels of fecal coliform have stayed high during moderate to high rainfall periods over the past twenty years, particularly at the Walker Creek and TBOC stations.

Results for the watershed stations showed a somewhat different pattern, with highest fecal coliform levels remaining elevated in all studies. Low and median values consistently remained higher than in Bay stations, with watershed stations in many cases an order of magnitude higher than Bay stations. There were no clear overall trends of increasing or decreasing fecal coliform levels in the watershed stations except for Millerton Creek, which showed an increase in high coliform levels over the course of the studies. Highest numbers overall were at Olema Creek in the 1974 study and Grand Canyon Creek in the 1995-96 study.

# <u> 1995-96 Study – Tomales Bay Shellfish Technical Advisory Committee</u>

The context of the current study has been discussed above in the Legislation and Purpose of Current Study section. Following completion of the study, the results were presented to the TBSTAC in February 1997, with follow-up meetings in May and June to discuss the results and proposed report contents. A draft report was prepared by staff from the DHS, SWRCB, and RWQCB for presentation to the TAC in December 1998. TBSTAC members had requested the report and recommendations prior to initiating remediation actions in the watershed. Following a review and comment period, including several follow-up meetings in the spring through fall of 1999, the report was revised and reissued as a final report by the TBSTAC.

# MATERIALS AND METHODS

# Study Design

The complete study design is provided in Appendix A. Exceptions to the study design are noted in the appropriate section in the presentation of results. In summary, the study consisted of 6 sampling periods (2 dry events, 4 rainfall events) and 40 primary sampling stations throughout the Bay and watershed. The two dry season sampling events each consisted of a single day and occurred before and after the rainfall season. Each of the four rainfall sampling events consisted of four sampling days. Samples were collected from all Bay and watershed sampling stations on the first three days following the start of a rainfall harvest closure (0.5 inch of rain within a 24-hour period). The fourth sampling day, referred to as day X, occurred on the day the Bay reopened for harvesting.

#### Sample Collection

Samples were collected in 100 milliliter (mL) and 500 mL sterile, screw-cap polypropylene bottles. All bottles were immediately placed in an insulated ice chest containing sufficient ice packs ("blue ice") to maintain a temperature between 4° and 10° C. Samples were transported to the EMDL in Berkeley, where all analyses were performed within the holding times required under the NSSP.

#### Analytical Methods

#### Total Coliform, Fecal Coliform, E. coli

All water samples were analyzed for total coliform (TC), fecal coliform (FC), and *Escherichia coli* (*E. coli*) using the most probable numbers (MPN) estimate of bacterial density in a multiple tube fermentation test (Standard Methods, 18th ed., Part 9221, 1992). The reporting units for this method are most probable number per 100 milliliters (MPN/100 mL) for water and MPN/100 grams for shellfish or sediment samples. For the sake of simplicity, the reporting units for all data discussed in this report are abbreviated to MPN (e.g., 43 MPN means 43 MPN/100 mL or 43 MPN/100 g).

Shellfish samples were analyzed for TC, FC, and *E. coli* using a most probable numbers estimate of bacterial density in a multiple tube fermentation test. Total bacterial counts were made via heterotrophic plate count (HPC) by a pour plate method (American Public Health Association, 1970; Standard Methods, 18th ed., Part 9215 A and B, 1992).

#### **Enterococcus**

Shellfish samples were tested for *Enterococci* using a multiple tube MPN technique in azide-dextrose broth (Standard Methods, Part 9230 B, 1992). Presumptively positive MPN cultures were confirmed on bile esculin agar and in brain-heart infusion broth at elevated temperature and with 6.5% NaCl (Standard Methods, 18th ed., Part 9230 C, 1992). The confirmed enterococcus MPN values were reported.

Water samples were analyzed for *Enterococcus spp.* by two different methods. The first method provided a MPN estimation of *Enterococcus spp.* density on 1600-square hydrophobic grid membrane filters grown on mE enterococcus agar and confirmed on bile esculin agar and in brain-heart infusion broth at elevated temperature and with 6.5% NaCl (Standard Methods, 18th ed., Part 9230 C, 1992). A new rapid method for determining *Enterococcus spp.* was also employed on the water samples. This method provided a rapid MPN estimation of *Enterococcus spp.* density grown in "Enterolert" defined substrate medium in "quantitrays" (IDEXX Laboratories, Inc. Westbrook, ME).

#### Sth Toxin Gene

Selected water samples were analyzed for the Sth toxin gene, which encodes for the production of a heat stable toxin in *E. coli* that causes diarrhea in humans. Samples were filtered, the filter membrane was placed in a sterile tube and vortexed in the presence of a buffer for one minute. The buffer was removed and any deoxyribonucleic acid (DNA) present in the solution was amplified using polymerase chain reaction techniques.

#### <u>E.coli 0157:H7</u>

Water samples were tested for pathogenic *E. coli* 0157:H7, by initial culture in selective enrichment medium. The cultures were then screened for the incriminating 0157 antigen using a commercial enzyme immuno assay kit. Positive cultures were further tested on selective media and individual suspicious colonies were screened with a latex-agglutination slide test to detect the 0157 antigen associated with pathogenicity of some strains of this organism. Positive reacting strains were then confirmed with biochemical tests and for their

ability to produce Shiga-like toxins responsible for pathogenicity. Results are reported as "Presence/Absence".

#### **Coliphage**

*E. coli*-specific bacteriophage analyses were conducted on samples from the designated indicator stations using the male-specific coliphage plaque assay (Fout, 1996). The host *E. coli* strain used is designated "F+amp male." This assay was not capable of separating coliphage on the basis of source (e.g., animal versus human). Results are reported as plaque-forming units per 100 mL (pfu/100 mL).

#### <u>Bacteroides</u>

Total anaerobic bacteria were analyzed via plate counts grown on brucella blood agar within an anaerobic chamber. The *Bacteroides fragilis* group of anaerobes was analyzed by plate counts grown on *Bacteroides* Bile Esculin Agar (BBE) within an anaerobic chamber. *Bacteroides vulgatus* analyses were made by plate counts grown on *Bacteroides vulgatus* selective agar within an anaerobic chamber (Wadford et al., 1995; Straub, 1997). Results are reported as colony-forming units per 100 mL (cfu/100 mL).

#### Quality Control

Two types of quality control (QC) samples were collected on each sampling day: (1) Field duplicates were collected at 10% of all sampling stations to provide a measure of variability associated with sample collection, and (2) Laboratory duplicates were collected at 10% of all sampling stations to provide a measure of variability associated with sample analysis. The sampling stations for field and laboratory duplicates were randomly selected each day.

#### Data Analysis

Because microbes grow logarithmically and not linearly, measurements of microbial growth must be transformed prior to calculating descriptive statistics or performing graphical or statistical analyses on the data. In addition, microbial density data often is unsuitable for use with normal parametric statistics as several of the assumptions of parametric statistics cannot be met (e.g., the data are not normally distributed, the variance may vary with the mean).

All microbiological data in this study were converted to  $base_{10}$  logarithms prior to analysis. Geometric means are calculated by taking the antilog of the mean of the log values. For the sake of clarity all graphical analyses are performed with the nontransformed data on logarithmic scales.

#### **RESULTS AND DISCUSSION**

#### Rainfall and Stream Flow

One theory that has been proposed relative to water quality impacts in watersheds is that of a "first flush" effect. In this scenario the first significant rainfall would result in the washing of contamination that has accumulated over the preceding dry season into creeks and the Bay. Subsequent rainfall events would result in lesser and lesser amounts of contamination being introduced downstream. To determine if this pattern existed in any of the watersheds sampled during this study, records were kept of both precipitation and stream flow for later comparison with the bacteriological data.

All rainfall measurements were taken from the remote weather station located at the end of Tomasini Point near the southern extent of Tomales Bay. Data from this gauge is transmitted to the California-Nevada River Forecast Center, where it is posted for retrieval via an electronic bulletin board. The DHS closely monitored

rainfall throughout each day of the week. Study participants from the RWQCB and the SWRCB were contacted by the DHS for concurrence prior to initiating a sampling event.

Small amounts of precipitation provided a total of 0.5 inch of rain from June through November 1995. The first significant rainfall that exceeded the closure threshold of 0.5 inch within 24 hours did not occur until the first week of December. The heaviest periods of rain occurred in mid-December and again in mid-January (Figure 7). The January storms persisted for several weeks.

The first wet-weather sampling event began on December 4, 1995 following 0.52 inch of rain in 24 hours (Figure 8). The long dry period preceding this rainfall event, together with the relatively small amount of rainfall, resulted in no measurable increases in flow at the stream gauge stations. The first rainfall resulting in measurable runoff (i.e., the "first flush") began on December 10. This rainfall episode was not sampled due to the proximity to the preceding sampling event. The severity of this storm (3.74 inches in one day, 5.12 inches in three days) resulted in localized flooding, which would have interfered with sampling efforts.

The second wet-weather sampling event began on January 16, 1996 (Figure 9). A subsequent storm on January 18 produced another 0.99 inches of precipitation, therefore this "day 3" sampling event may be viewed as a "day 1" event. Sporadic rainfall continued throughout the month of January, resulting in continued closure of the growing areas. The active study participants from the RWQCB, the SWRCB, and the DHS concurred that an additional day of sampling was needed to determine the impacts on water quality from the extended rainfall. This sampling event took place on January 31 and was referred to as "Day A". The series of storms ended in the first week of February, and the Bay opened for harvesting (i.e., "day X") on February 11.

The third wet-weather sampling event began on March 11, 1996 (Figure 10). Additional rain fell on March 12 (Day 2), totaling 1.69 inches over three days. Several days of dry weather followed, and the Bay reopened for harvesting on March 18.

The fourth wet-weather sampling event began on April 1, 1996 (Figure 11). Total precipitation amounted to 1.81 inches, followed by several days of dry weather. The Bay reopened for harvesting on April 8.

The response of the two gauged watersheds, Walker and Lagunitas, was noticeably different throughout the course of the study (Figure 12). In general, while each watershed responded immediately to significant precipitation, the flow volume was consistently greater in the Lagunitas watershed (Table 13). These observations are consistent with the relative flow contribution of each watershed discussed above and reported by Fischer (1996).

# Watershed Water Quality

The results of the quality control samples are presented in the following section, along with background information on the accuracy and precision of the methods used to estimate the most probable numbers of bacteria. Following the discussion of the QC data are several sections that describe the response of the various watersheds (Figure 4) to rainfall with respect to levels of bacterial contamination at each of the tributary sampling stations.

# Quality Control

Paired data were randomly collected for field duplicates and lab duplicates throughout the study. Each pair of replicates represents a unique set of conditions, both temporally and spatially. Preliminary statistical analyses were performed on each set of quality control data (field duplicates, lab duplicates) to determine if these data met the assumptions of parametric statistics. A Wilk-Shapiro test was used to test for normality in each set of paired data. The p-value for each paired data set was less than 0.05, therefore neither the field replicates or lab replicates were normally distributed. For each data set the difference between pairs was plotted against the mean for the pair to determine if the discrepancy between pairs increased or decreased as a function of the

level of contamination. The plotted data for both quality control data sets was highly scattered with greater variability towards the lower concentrations, indicating that the variance was not independent of the mean. Because these two tests indicated that the quality control data did not meet the assumptions inherent in parametric statistics, it was decided to analyze these data with a nonparametric test.

The paired data for the field and laboratory duplicates are presented in Table 20. The Wilcoxin Matched Pairs Signed Rank Test (Sokol and Rolf, 1969) was used to determine if there was a significant difference between pairs for each data set. For the paired field replicate data the calculated  $t_s$  was -0.03, indicating that there was no significant difference in the paired field replicate data (P = 0.05,  $t = \pm 2.01$ ). For the paired laboratory replicate data the calculated  $t_s$  was -0.24, indicating that there was no significant difference in the paired field replicate data (P = 0.05,  $t = \pm 2.01$ ).

# Walker/Keyes/Chileno Watershed

Table 14 contains the results of the Walker/Keyes/Chileno Creeks watershed sampling. Comparisons of total coliform versus *E. coli*, fecal coliform versus *E. coli*, and fecal coliform versus enterococcus are summarized in Figures 13, 18, and 23.

Bacterial levels for the seven sampling sites were high for all indicator organisms. With the exception of the third rainfall event, levels of all indicators typically decreased each day during each rainfall event. There was no evidence to support the first-flush theory for this watershed. With progressive rain events day 1 samples from each station were either within the same range or higher.

There were several instances when fecal coliform levels did not drop below 2000 MPN/100 mL (i.e., the water quality objective for non-contact recreation) by day X. During rainfall events 2 and 3, fecal levels from Chileno Creek remained elevated (4600 and 2200 MPN, respectively). Chileno Creek samples remained above the 406 MPN value for *E. coli* throughout rainfall events 2-4 (the creek was not sampled during event 1 due to insufficient flow).

Chileno Creek had consistently elevated levels for all three indicator organisms during most of the study and also contained the highest levels of indicator organisms measured within the watershed.

**Dry Weather Samples: Pre-Season.** Samples were not collected for four of the seven sites in this watershed because of insufficient water flow. Fecal coliform levels were low at the three sites where samples were collected, ranging from 18 to 49 MPN. *E. coli* values never exceeded the 406 MPN benchmark, ranging from 18 to 49 MPN. Enterococcus values were very low, ranging from 1 to 25 MPN.

**Rainfall Event 1.** Flow in Chileno Creek (at Milepost 3.66) was not sufficient to take samples during this rainfall event. For all other sites, fecal coliform sample results were comparatively low. Day 1 fecal coliform values were 240 MPN or less, with the exception of Keyes Creek, Irvin Road. Water sampled at this location had 9200 MPN fecal coliform present; this level dropped to 490 MPN by the next day's sample. Day X sample levels were all low, ranging from 5.7 to 80 MPN.

Day 1 *E. coli* levels were also fairly low, with only one sample (Keyes Creek, Irvin Road, with a value of 9200 MPN) above 406 MPN. This location stayed elevated the next day of sampling, but dropped to 26 MPN by day 3. Day X samples were very low for all sites, ranging from 3.3 to 65 MPN.

Several sites had enterococcus levels above 108 MPN. Samples from Keyes Creek, Irvin Road were elevated throughout the entire first rainfall sampling period, dropping only to 570 MPN by day X. Samples from Keyes Creek (below the City of Tomales Wastewater Treatment Plant) were elevated on days 2 and 3 of sampling (no day 1 sample was collected). Walker Creek Ranch had slightly elevated levels over the first two days of sampling, dropping on day 3, and rising to 140 MPN by day X, a level just above the 108 MPN benchmark. The remaining three sample locations had low day X sample results, ranging from 14 to 74 MPN enterococcus levels.

**Rainfall Event 2.** Sample levels were greatly elevated for all indicator organisms as compared with the first rainfall event. Day 1 fecal coliform levels ranged from 1977 to 54,000 MPN, with only one site (Keyes Creek, Milepost 3.66) below the standard of 2000 MPN. All sites were elevated for at least two days of this sampling event. With the exception of Chileno Creek and Keyes Creek at Irvin Road, all sites dropped below 2000 MPN by day X. Chileno Creek had a day X value of 4600 MPN; Keyes Creek at Irvin Road had a day X value of 5030 MPN.

With only three exceptions during the entire rainfall event, all samples exceeded the 406 MPN for *E. coli*. Day 1 values ranged from 2300 to 54,000 MPN. Although sample levels decreased throughout the event, only Keyes Creek (below the City of Tomales wastewater treatment plant) and Walker Creek had levels below 406 MPN (no day X sample was taken from Walker Creek Ranch).

All enterococcus day 1 samples exceeded the 108 MPN value, with results ranging from 186 MPN at Keyes Creek Milepost 3.66 to 1600 at Walker Creek. Day X samples were not analyzed for enterococcus.

**Rainfall Event 3.** During this sampling period, additional rain fell on day 2, bringing the rainfall total to 1.69 inches over three days. This resulted in a sharp increase in day 2 fecal coliform and *E. coli* levels for all stations except Walker/Keyes Creek. Day 2 enterococcus levels were also elevated (for all stations except Walker Creek) in comparison to day 1, but not as notably as with the other two indicator organisms.

Day 1 fecal coliform samples ranged from 230 to 35,000 MPN, exceeding the 2000 MPN fecal coliform level at all stations except Keyes Creek at Irvin Road, Keyes Creek at Milepost 3.66, and Walker Creek Ranch. Day 2 samples ranged from 4900 to 854,000 MPN (at Chileno Creek). By day X, all sites except Chileno Creek had levels well below 2000 MPN.

*E. coli* day 1 samples ranged from 230 to 35,000 MPN, with only Walker Creek Ranch falling below the 2000 MPN value. By the following day, all sites exceeded the acceptable value for *E. coli*, with samples ranging from 2800 to 54,000 MPN. Excluding Chileno Creek (with a day X sample of 2200 MPN) all day X sample values ranged from 2 to 330 MPN.

Day 1 enterococcus samples ranged from 10 to 1030 MPN, with Chileno Creek, Walker Creek, and Walker/Keyes Creek having levels above 108 MPN. Samples from the next day were higher than those from day 1 at all sites except Walker Creek. By day X, sample values ranged from 10 to 180 MPN, with only Chileno Creek exceeding 108 MPN.

**Rainfall Event 4.** All stations exceeded the 2000 MPN fecal coliform value on day 1 of this monitoring period, with sample results ranging from 2200 to 160,000 MPN. Fecal coliform levels remained elevated during at least the first two days of sampling for all stations except Walker Creek Ranch. By day X, samples ranged from 170 to 3600 MPN, with only Keyes Creek below the City of Tomales wastewater treatment plant above the 2000 MPN value.

Day 1 *E. coli* samples were all above the 406 MPN comparative value, ranging from 1400 to 160,000 MPN. With the exception of the Walker Creek Ranch site, all samples remained above 406 MPN through the first three days of sampling. Day X sample values ranged from 170 to 3600 MPN, with Chileno Creek, Keyes Creek at Irvin Road, and Keyes Creek below the City of Tomales wastewater treatment plant still exceeding 406 MPN. These three stations never dropped below 406 MPN during rainfall event 4 sampling.

Day 1 samples from all sites exceeded the 108 MPN value for enterococcus, with values ranging from 158 to 3570 MPN. Samples remained elevated over the first two days of sampling, with two sites (Keyes Creek at Milepost 3.66 and Keyes Creek below the City of Tomales wastewater treatment plant) still above 108 MPN by day X. Day X sample values ranged from 30 to 330 MPN.

**Dry Weather Samples: Post-Season.** Two creeks were not sampled because of insufficient flow. The remaining five sites had fecal coliform levels ranging from 20 to 1700 MPN. Two of the five sampled sites had *E. coli* levels exceeding 406 MPN. Chileno Creek had a level of 790 MPN, and Keyes Creek (below the

City of Tomales wastewater treatment plant) sample result was 1700 MPN. For all other sites, results ranged from 20 to 170 MPN. Enterococcus levels ranged from 10 to 90 MPN.

#### East Shoreline Watershed

Table 15 contains the results of the eastern watershed sampling. Comparisons of total coliform vs. *E. coli*, fecal coliform vs. *E. coli*, and fecal coliform vs. enterococcus are summarized in Figures 14, 19, and 24.

Bacterial levels from the eight sampling sites in the eastern watershed were in a similar range to those of Walker/Keyes/Chileno Creeks. Several of the creeks remained elevated during most of the study; samples from Milepost 32.12 never dropped below the benchmark values for any of the indicator organisms. Samples from Milepost 34.95 and Milepost 40.35 also remained elevated for most of the study.

As was noted for the Walker/Keyes/Chileno watershed, there was no apparent "first flush" effect. Day 1 sample results were either within the same range or higher with each progressive rain event.

**Dry Weather Samples: Pre-Season.** Samples were not collected at four sites because of insufficient water flow. Fecal coliform levels at the remaining five sites were low, ranging from 18 to 511 MPN. *E. coli* values ranged from 18 to 511 MPN, with only Milepost 36.17 exceeding the 406 MPN benchmark. Enterococcus values ranged from 18 to 128 MPN, with Milepost 36.17 and Milepost 38.54 exceeding 108 MPN.

**Rainfall Event 1.** Only Milepost 36.17, Milepost 38.54, and Millerton Creek had sufficient stream flow to sample during this storm. Millerton Creek had very low levels of all indicator organisms during this storm event. For those sites that could be sampled, day 1 fecal coliform levels ranged from 64 to 2200 MPN, with only Milepost 36.17 exceeding the 2000 MPN fecal value. All day X samples were 230 MPN or less.

Day 1 *E. coli* values exceeded 406 MPN at two (Milepost 36.17 and Milepost 38.54) of the three sites, with levels ranging from 64 to 2200 MPN. By day X, all samples were at or below 11 MPN.

Day 1 enterococcus levels ranged from 30 to 650 MPN, with only the Millerton Creek sample below 108 MPN. Day X samples were all below 108 MPN, ranging from 10 to 90 MPN.

**Rainfall Event 2.** Fecal coliform levels measured on day 1 ranged from 490 to 43,000 MPN, with only one site (Milepost 38.54) below 2000 MPN. Sample sites at Milepost 34.95 and 40.35 remained elevated during the three days of sampling, dropping below 2000 MPN by day X. The Milepost 32.12 site never dropped below 2000 MPN for the remainder of the study, with a day X value of 13,000 MPN. Excluding Mile post 32.12, day X samples ranged from 18 to 790 MPN.

*E. coli* levels were well above 406 MPN for most of the monitored days of this rainfall event. Day 1 sample values ranged from 490 to 43,000 MPN. Sample levels from three sites (Grand Canyon Creek, Mile post 34.95, Milepost 32.12,) did not drop below 406 MPN during this event, with day X levels at 490, 490, and 13,000 MPN respectively. Day X samples from the remaining five sites ranged from 18 to 330 MPN.

Enterococcus levels stayed elevated at most of the sites throughout the first four days of sampling. Day 1 samples were all above 108 MPN, ranging from 300 to 3100 MPN. Day X samples were not analyzed for enterococcus during this rainfall event.

**Rainfall Event 3.** Day 1 sample values ranged from 78 to >160,000 MPN, exceeding the 2000 MPN fecal coliform level at five of the eight sites. By day X all sites, with the exception of Milepost 32.12 (6222 MPN) and Milepost 36.17 (2200 MPN), had dropped below 2000 MPN. The day X value from Milepost 32.12 was that site's lowest fecal coliform value of this study.

Day 1 samples exceeded the 406 MPN level for *E. coli* at all but two sites (Milepost 36.17, with a sample value of 130 MPN, and Milepost 38.54, with a sample value of 78 MPN). Excluding these sites, sample values ranged from 460 to >160,000 MPN. Milepost 32.12 and Milepost 36.17 still exceeded 406 MPN by day X, with levels of 6222 and 2200 MPN, respectively, for *E. coli* concentrations. Sample values from the remaining six sites ranged from 18 to 330 MPN by day X.

**Rainfall Event 4.** Day 1 fecal coliform levels exceeded 2000 MPN at all sampling stations, with values ranging from 8400 to 160,000 MPN. Three of the sites, Milepost 32.12, Milepost 34.95, and Milepost 40.35 remained elevated throughout this sampling event. By day X, samples from the remaining five sites ranged from 130 to 1300 MPN.

All day 1 *E. coli* samples exceeded 406 MPN, ranging from 7436 to 160,000 MPN. Six of the eight sites remained elevated on day X, with only Milepost 36.17 and lower Tomasini Creek dropping below 406 MPN. Day X results from these six sites ranged from 711 to 160,000 MPN.

Six of the eight day 1 enterococcus samples exceeded 108 MPN, with values ranging from 420 to 1900 MPN. The two exceptions were Milepost 34.95 (with a sample level of 20 MPN), and Milepost 40.35 (with a sample level of 10 MPN). Day X samples remained elevated for five of the eight sites, with results ranging from 130 to 1580 MPN. The exceptions were Grand Canyon Creek, Milepost 36.17, and Millerton Creek.

**Dry Weather Samples: Post-Season.** Samples were not collected from Milepost 32.12 and Milepost 34.95 stations because of insufficient flow. Five of the remaining sites had fecal coliform levels below 2000 MPN, with results ranging from 20 to 230 MPN. The sixth site, Milepost 38.54, had a fecal coliform level of 3300 MPN. Only one sample (from Milepost 38.54, with a post-season sample value of 2300 MPN, exceeded the 406 MPN *E. coli* value. For all other sites, results ranged from 20 to 230 MPN. Only Milepost 36.17, with a value of 1700 MPN, exceeded the 108 MPN enterococcus level. Samples from the remaining sites ranged from 10 to 90 MPN.

#### Lagunitas/Olema Watershed

Table 16 contains the results of the Lagunitas/Olema watershed sampling. Comparisons of total coliform vs. *E. coli*, fecal coliform vs. *E. coli*, and fecal coliform vs. enterococcus are summarized in Figures 15, 20, and 25.

Bacterial levels from the five sites sampled in the Lagunitas/Olema watershed were lower than those of the Walker/Keyes/Chileno and eastern shoreline watersheds but higher than those from the western watershed. With the exception of the second rainfall event, levels of all indicators typically decreased each day during each storm event. Due to an additional 0.99 inches of rain on day 3 of the second rainfall event, all day 3 samples showed a sharp increase in indicator organism levels. In all cases, values dropped by the next day. Fecal coliform levels often exceeded 2000 MPN/mL during the first sampling day of each rain event, but were well below this level by day X. Enterococcus and *E. coli* results also followed the same patterns, with levels typically highest on the first day for all rainfall events except the second.

There was no evidence of a "first flush" effect within the Lagunitas/Olema watershed. In most cases, the day 1 sample from storm event 4 was higher than day 1 sample values from all other storm events.

**Dry Weather Samples: Pre -Season.** Samples analyzed for fecal coliform ranged from 130 to 1700 MPN. Only Bear Valley Creek, with an *E. coli* level of 1700 MPN, exceeded the 406 MPN benchmark. All other sites had levels ranging from 78 to 402 MPN. Three of the five sites exceeded an enterococcus level of 108 MPN. These sites were Bear Valley Creek (343 MPN), upper Olema Creek (155 MPN), and Whitehouse Pool (244 MPN).

**Rainfall Event 1.** Olema Creek flows were inadequate for sampling. Fecal coliform concentrations from Bear Valley Creek were much higher during this rainfall event than samples from all other locations in this watershed. The day 1 fecal coliform value was 16,000 MPN, but dropped to 130 MPN by day X. Day 1 fecal coliform levels from all other sampling locations were below 500 MPN during this rainfall event, dropping to a range of 17 to 130 by day X.

*E. coli* samples from Bear Valley Creek were also elevated over those from the other stations; the day 1 sample result was 9200 MPN, dropping to 130 by day X. *E. coli* values from all other stations ranged from 27 to 330 MPN, with day X values ranging from 17 to 110 MPN.

All day 1 samples exceeded the 108 MPN value. Excluding Bear Valley Creek, levels ranged from 210 to 690 MPN. The enterococcus value from Bear Valley Creek was 28,470 MPN on day 1, and remained high throughout the first storm event. All day X samples exceeded the 108 MPN value for enterococcus, ranging from 120 to 1280 MPN.

**Rainfall Event 2.** By the second rainfall event, fecal coliform concentrations for all creek samples were within the same range. Day 1 fecal coliform concentrations ranged from 700 to 17,000 MPN. Only two sites, Bear Valley Creek and Lagunitas Creek, were below 2000 MPN. Day X samples ranged from 93 to 310 MPN.

All day 1 samples exceeded the 406 MPN level for *E. coli*, with values ranging from 700 to 17,000 MPN. Whitehouse Pool had the high value of 17,000 MPN. Sample results dropped below the level of concern at all sites by day X, with values ranging from 68 to 191 MPN.

All enterococcus day 1 samples exceeded 108 MPN, with values ranging from 190 to 880 MPN. Day X samples were not analyzed for enterococcus.

**Rainfall Event 3.** Indicator organism levels during this rainfall were typically lower than during the previous sampling event. Fecal coliform levels for day 1 samples ranged from 330 to 3300, with only Lagunitas Creek exceeding 2000 MPN. By day X, these levels had decreased to a range of 51 to 490 MPN.

With the exception of Bear Valley Creek, all day 1 *E. coli* samples exceeded 406 MPN. Values ranged from 330 to 3300 MPN, with Lagunitas Creek again having the highest level. By day X, sample levels ranged from 51 to 490 MPN, with only one sample (Olema Creek) above the 406 *E. coli* level.

Day 1 enterococcus ranged from 30 to 70 MPN. Only two samples were above the 108 enterococcus level during this rainfall event; Olema Creek on day 2 (340 MPN) and Whitehouse Pool on day 2 (110 MPN). By day X, sample results were 20 MPN or less.

**Rainfall Event 4.** Day 1 sample results for fecal coliform ranged from 790 to 17,000 MPN, with only one sample (Bear Valley Creek) below 7000 MPN. All of these samples dropped sharply by day 2 and by day X samples ranged from 20 to 749 MPN.

All day 1 *E. coli* levels were elevated, with samples ranging from 790 to 17,000 MPN. By day X, all stations, with the exception of Whitehouse Pool, were below 406 MPN. Whitehouse Pool, at 470 MPN, was slightly above the *E. coli* benchmark value of 406 MPN.

With the exception of upper Olema Creek, day 1 samples were above 108 MPN enterococcus value, with results ranging from 23 to 1150 MPN. Day X values were all low, ranging from 10 to 60 MPN.

**Dry Weather Samples: Post-Season.** Fecal coliform concentrations were low at all sites, ranging from 20 to 490 MPN. *E. coli* sample results ranged from 20 to 490 MPN, with Whitehouse Pool exceeding the 406 MPN value. All sites were below the 108 MPN benchmark value for enterococcus.

#### West Shoreline Watershed

Table 17 contains the results of the western watershed sampling. Comparisons of total coliform versus *E. coli*, fecal coliform vs. *E. coli*, and fecal coliform vs. enterococcus are summarized in Figures 16, 21, and 26.

Bacterial levels from the four sites in the western shoreline watershed were generally 10 to 100 times lower than levels detected from all other watersheds. By day X, all sites were below the levels of concern for fecal coliform and *E. coli*. Although sample levels were generally low throughout the study, there can be seen a tendency toward a first-flush effect within this watershed, especially at Milepost 25.86 and Milepost 28.29.

**Dry Weather Samples: Pre-Season.** Fecal coliform results were low, ranging from 2 to 790. Milepost 28.29 exceeded the 406 MPN *E. coli* limit, with a value of 790 MPN. All other *E. coli* samples ranged from 2 to 252 MPN. Three of the four sites within the watershed exceeded the enterococcus value of 108 MPN.

These sites were Milepost 28.29 (530 MPN), Milepost 28.86 (276 MPN), and White Gulch (490 MPN). White Gulch was the fresh water control site.

**Rainfall Event 1.** Although samples were collected from each of the creeks during this rainfall event, each creek's flow was minimal. Day 1 fecal coliform samples collected during this rainfall event ranged from 70 to 490 MPN, and by day X ranged from 2 to 63.

Day 1 *E. coli* levels ranged from 70 to 460 MPN, with only one sample (Milepost 25.86) exceeding the 406 MPN value. By Day X, all sample values were below 406 MPN, ranging from 2 to 63 MPN.

Day 1 enterococcus levels ranged from 110 to 850 MPN, and exceeded the 108 MPN level almost every day during the first rainfall event sampling, with the exception of day 2 and day X at Milepost 25.86 and day X at Teachers Beach.

**Rainfall Event 2.** Due to an additional 0.99 inches of rain on day 3 of this rainfall event, all day 3 samples showed a sharp increase in indicator organism levels. In all cases, samples dropped by the next day. Fecal coliform levels ranged from 110 to 3300 MPN on day 1, and from 18 to 276 MPN by day X. Samples exceeded the 2000 MPN fecal coliform level only twice during this rainfall event, on days 1 and 3 at Milepost 28.86.

Day 1 *E. coli* results ranged from 68 to 1300 MPN, exceeding the 406 benchmark value at Milepost 28.29 and Milepost 28.86. All stations exceeded this value for day 3. Samples from Milepost 28.86 also exceeded 406 MPN on days 2 and 3 of this rainfall event, but dropped to an acceptable level by day X. By day X all stations were below 406 MPN, with samples ranging from 18 to 276 MPN.

Day 1 enterococcus levels ranged from 90 to 2018 MPN. The 106 MPN benchmark was exceeded on days 1 and 3 at Milepost 28.29, Milepost 28.86 and Teacher's Beach, and on day 3 at Milepost 25.86. Day X samples were not analyzed for enterococcus.

**Rainfall Event 3.** Samples from this rainfall event were lowest of the four monitored during the study. The fecal coliform standard of 2000 MPN was never exceeded during this rainfall event. Day 1 fecal coliform counts ranged from 6 to 460 MPN, with day X samples ranging from 18 to 173 MPN.

*E. coli* level ranged from 6 to 460 MPN on day 1, only once exceeding the 406 MPN benchmark (at Milepost 28.86). Day X samples ranged from 18 to 173 MPN.

Day 1 enterococcus levels ranged from 10 to 50 MPN. Only days 2 and 3 samples from Milepost 28.86 exceeded 108 MPN. By day X, all samples taken were less than 20 MPN.

**Rainfall Event 4.** All samples were below the 2000 MPN fecal coliform standard during this rainfall event. Fecal coliform levels ranged from 78 to 1200 MPN on day 1, dropping to a range of 18 to 44 MPN by day X.

Day 1 *E. coli* values ranged from 78 to 1200 MPN, with only one sample (Milepost 28.86) exceeding the 406 MPN *E. coli* value. By day X, all samples were below 20 MPN.

Enterococcus levels ranged from 80 to 310 MPN on day 1, with two samples (Milepost 25.86 and Milepost 28.86) exceeding the 108 MPN enterococcus benchmark. Day X samples ranged from 1 to 50 MPN.

**Dry Weather Samples: Post-Season.** Fecal coliform sample results were low at all sites, ranging from 20 to 220 MPN. *E. coli* results were also low, ranging from 68 to 230 MPN. Only Milepost 28.86 (420 MPN) and White Gulch (155 MPN) exceeded the 108 MPN enterococcus level.

#### Relationships Between Common Indicator Organisms

This study was designed to monitor four commonly used indicator organisms at all sample sites within the watershed and in the Bay. The intention was to see if any organism appeared to be more source-specific than the others. If, for example, enterococcus levels were found to be highest just below a sewage outflow, we might conclude that this organism is more human-specific than the other indicator organisms.

Paired comparisons were made for all water samples from the tributary stations throughout the watershed (Figure 28). Only fecal coliform and *E. coli* appear to have a linear relationship, with a correlation coefficient of 0.99 (Table 18). The ratio of fecal coliform to *E. coli* was approximately 1:1, i.e. all of the fecal coliform detected is comprised of *E. coli*. This in not unexpected, since *E. coli* is one of the three bacteria that comprise the fecal coliform group. We can conclude that this high correlation is the result of fecal contamination as opposed to interfering organisms.

The correlation between enterococcus and all other indicators was poor (Table 18). It may be that enterococcus and the coliform organisms are indicating different sources of contamination. In order to investigate this, a comparison was done of the number of days fecal coliform, *E. coli*, and enterococcus organisms exceeded their comparative values. Total coliform results were not included in this analysis. Only days with results for all three indicators were used for these comparisons.

Of the 720 data sets that contained results for all three indicators, there were 110 instances when all three indicators exceeded their limits. The majority (97) of these instances occurred within the eastern watersheds. Within the Walker/Keyes/Chileno watershed, Walker Creek Ranch had only three days when all three indicators were above their respective levels of concern. For all other sites, especially Chileno Creek and the Keyes Creek sites, there were many days when all three indicators exceeded their limit values.

In 35 cases, *E. coli* was the only indicator exceeding a water quality limit. Sample locations exceeding the limits were evenly distributed among the Lagunitas/Olema watershed stations, eastern shoreline tributary stations, and the Walker/Keyes/Chileno Creek watershed stations. All three indicators typically exceeded their limits during the first two days of each rainfall event. However, *E. coli* levels tended to drop more slowly than the other two indicators. By day 3, *E. coli* was often the only indicator organism exceeding a water quality limit. The only exception within these three watershed areas occurred at Bear Valley Creek, where *E. coli* was never the only indicator organism exceeding a water quality limit.

In 53 cases, enterococcus was the only indicator to exceed a water quality level of concern. These incidents occurred primarily during the first two rainfall events and within two watersheds, the west shore tributary stations and the Lagunitas/Olema stations.

Fecal coliform was never the only indicator organism exceeding the water quality standard. However, there were 25 instances when the fecal coliform and *E. coli* samples both exceeded their respective limits, while the enterococcus sample was below the 108 MPN limit. These events occurred in all watersheds except the west shore. Within the Walker/Keyes/Chileno Creek watershed and the eastern shoreline tributary stations, these high fecal coliform and *E. coli* values typically occurred on days 2 and 3 of rainfall events. This observation supports the suggestion that *E. coli* levels drop more slowly than other indicators. However, this trend did not hold true for samples within the Lagunitas/Olema Creek watershed; all *E. coli* values that exceeded 406 MPN occurred the first two days of the rainfall event.

The relations between commonly used indicator organisms were further evaluated by standardizing these data against their respective water quality objectives. For each complete set of data points, the logarithmic value of each point was calculated. Logarithmic values were also calculated for each objective (2000 MPN for fecal coliform, 406 MPN for *E. coli*, and 108 MPN for enterococcus). The logarithmically transformed sample values were then normalized by subtracting the logarithmic value of the appropriate objective (i.e. the logarithm of 406 is subtracted from each transformed *E. coli* value) to obtain the points that were plotted in Figure 29. These graphs can be interpreted by looking at the two axes framing each quadrant. If both axes are negative, all points within that quadrant are below the levels of concern for both indicator organisms. Conversely, if both axes are positive, all points within that quadrant are above the levels of concern for both indicator organisms. If one axis is positive and one is negative, the points within that quadrant exceed the level of concern for one indicator, but not for the other.

There were 86 incidents where samples were below the fecal coliform standard, but exceeded the 108 MPN enterococcus level. There were also 53 incidents where samples were below the *E. coli* level of concern, but

exceeded the 108 MPN enterococcus value. As discussed above, most of these incidents occurred during the first two rainfall events and within two watersheds, the western shoreline tributary stations and the Lagunitas/Olema creek stations. These two watersheds had lower indicator organism levels than the other watersheds sampled during the study. Conversely, there were 25 incidents when *E. coli* was the only indicator organism exceeding a level of concern. These incidences occurred primarily in the Walker/Keyes/Chileno Creek watershed and in the eastern shoreline watershed, where all indicator levels tended to be high. Based on these data, enterococcus was the most sensitive indicator of impairment, and fecal coliform was the least sensitive, relative to their respective water quality objectives. It may be that the enterococcus level recommended by EPA for regulating non-contact recreational use is too low for fresh water use, or that enterococcus is identifying a different source of contamination than that indicated by fecal coliform and *E. coli*. It is interesting to note that enterococcus was the only indicator organism to be exceeded at White Gulch, the freshwater control site.

# Special Indicator Studies

Results are presented below for the additional potential indicator organism assays that were run on samples from selected sampling stations. Results from the special indicator portion of the study were inconclusive due to the small number of samples assayed and because of limitations experienced with specific methods, as described below.

**Sth Tox Gene.** The *E. coli* gene responsible for human-specific toxin production was not detected during this study. Prior to the sample analyses, the researchers conducting this analysis believed that their method was sufficiently sensitive to detect the Sth toxin gene deoxyribonucleic acid (DNA) without concentrating the water sample. This was not the case. The researchers found that *E. coli* in the natural environment carry far fewer copies of the toxin gene DNA than that of the laboratory model DNA. Thus, even though all samples were below the laboratory detection limit, it cannot be concluded that the samples were free from human waste sources.

*E. coli* 0157:H7. There were 42 watershed samples analyzed for the presence of *E. coli* 0157. One of these samples, collected from Milepost 32.12 on April 2, 1996, contained this pathogenic organism. Another sample, collected on December 5, 1995 at the Walker/Keyes Creek station, was found to contain another pathogenic organism, *Salmonella typhirium*. There are no current water quality standards for either *E. coli* 0157 or *Salmonella typhirium* However, the identification of two pathogenic bacteria in a relatively small number of samples reinforces the public health concerns that are associated with the high levels of fecal contamination of the Bay from watershed sources.

**Coliphage.** Coliphage is a virus that infects and replicates within *E. coli*. It can be found whenever total and fecal coliform organisms are present. Some researchers believe that, because coliphages are viruses, they might be better indicators of pathogenic human viruses. However, this is not a widely accepted view.

There were 44 samples analyzed for coliphage, of which 27 were positive (Table 21). There are no current or recommended water quality standards for coliphage, therefore interpretation of these data relative to the significance of absolute concentrations is not possible. In general, coliphage was detected at each of the five special indicators stations.

The highest coliphage concentration was detected on Keyes Creek below the wastewater treatment plant on September 12, 1995 (310 pfu), the first dry season sampling date. There was no flow in the creek at this time and the sampling location consisted of a small pool of water frequented by a number of cows. Lower amounts of coliphage were detected at the same site (42 pfu) and at the creek at White Gulch (40 pfu), the freshwater control site, on July 9, 1996, the second dry season sampling date. There was no flow into the Keyes Creek station and the White Gulch site was very shallow with little flow. Coliphage concentrations between 10 and 26 pfu's were detected under wet weather conditions at all sites except the seawater control station. The seawater control site contained the lowest amounts of coliphage throughout the study, ranging

from <2 to 4 pfu. There was no direct correlation between the coliphage densities and the concentration of fecal coliform.

**Bacteroides.** Of the 35 water samples analyzed for anaerobic bacteria (23 creek samples, 12 Bay samples), none were found to contain either *Bacteroides fragilis* or *B. vulgatus* (Straub, 1997). Total anaerobic counts varied over the course of the study and were not related to runoff or time of year (Table 22).

#### Tomales Bay Water Quality

#### Fecal Coliform and Salinity

Overall there was a poor correlation between salinity and fecal coliform concentration in water samples from the Bay sampling stations. Bay salinities less than 15 parts per thousand (ppt) were associated with fecal coliform concentrations that exceeded acceptable water quality standards for shellfish growing waters (Figure 30). However the relationship between salinity and fecal coliform concentration is less clear at higher salinities. For example, despite the fact that the first rainfall event had no impact on stream flows or Bay salinities, fecal coliform concentrations were elevated at several Bay stations. Possible explanations for these observations include: (i) significant local fecal contamination (e.g., creek beds, shoreline, roadsides and parking lots) exists that is washed into the Bay by the first rain; and (ii) significant fecal contamination can be contributed to the growing areas by the watershed even when the soil is not saturated.

#### Relationships Between Common Indicator Organisms

Paired comparisons of the major bacteriological indicator groups (i.e., total coliform, fecal coliform, *E. coli*, and enterococcus) were made for all water samples from the Bay stations throughout the watershed (Figure 31). The patterns and relationships among the indicator groups described previously for watershed stations also hold true for the data from the Bay stations. Briefly, the only clear relationship between indicator groups was that between fecal coliform and *E. coli* (correlation coefficient equals 0.99; Table 19; Figures 32-34). The ratio of fecal coliform to *E. coli* was approximately 1:1, i.e. all of the fecal coliform detected is comprised of *E. coli*, and is the result of fecal contamination as opposed to interfering organisms. The correlation between pairs of indicators for water sample data from Tomales Bay was slightly higher than that for tributary water samples (Table 19 and Table 18, respectively). A comparison of fecal coliform and enterococcus concentration is provided in Figures 35-37 and Table 23.

Because water quality standards for fecal coliform are employed in the shellfish growing areas, the following discussion of bacteriological data will focus on this indicator organism. The following sections summarize the levels and types of bacterial contamination observed in the water quality sampling stations located throughout the Bay (Figure 4).

#### <u>Outer Bay – Fecal Coliform</u>

The outer Bay stations ranged from Lawson's Landing near the mouth of Tomales Bay southward to Blakes Landing.

Despite the apparent lack of increased flow at the Walker Creek gauging station after the first significant rainfall of the year (as discussed earlier), outer Bay stations 42, 1, 34, 32 and 39 contained fecal coliform concentrations above the growing water standards (Figure 32). It therefore appears that the stream gauge data is not a good indicator of the potential for fecal contamination in the outer Bay.

The highest concentrations of fecal coliform detected were equal to or greater than 10,000 MPN (stations 32, 34, 1, 39, 43). The highest fecal coliform concentrations were observed at station 34, which is in the direct influence of the branch of Walker/Keyes creek that flows around Preston Point. All of the outer Bay stations exhibited high levels of fecal coliform contamination following rainfall (Figure 32). The maximum impact to water quality in the outer Bay usually occurred within the first two days following significant rainfall. This

suggests a quick transport time of contamination from the watershed or nearshore sources to the outer Bay. Fecal coliform concentrations often remained elevated three days after the rainfall event, indicating either a long residence time in the outer Bay or a prolonged source of contamination. Several of the outer Bay stations did not always return to acceptable levels of fecal coliform by day X.

The first-flush scenario does not seem to apply to outer Tomales Bay. There was no clear pattern of declining fecal coliform levels over the course of the study for outer Bay stations. Many stations experienced equivalent levels of contamination throughout the season, suggesting a renewable resource of fecal contamination.

# <u>Mid Bay – Fecal Coliform</u>

The mid-Bay stations ranged from aquaculture Lease M-430-14, located just north of Cypress Point, southward to an area that includes leases M-430-12, M-430-13, and M-430-19, just north of Tomasini Point on the eastern shoreline. The mid-Bay region includes one sampling station on the west shoreline at Indian Beach.

Fecal coliform concentrations within the mid-Bay region were generally lower than observed in the outer Bay and seldom exceeded 1000 MPN (Figure 33). All stations did experience elevated concentrations of fecal coliform following rainfall, however. The pattern and timing of maximum water quality impact to the mid-Bay following rainfall was not consistent among mid-Bay stations. In addition, the mid-Bay stations did not always return to acceptable levels of fecal coliform by day X.

The west shore station near Indian Beach (46) generally exhibited the lowest levels of fecal contamination of any mid-Bay site. This station did, however, exceed acceptable levels of fecal coliform on several occasions.

#### <u>Inner Bay – Fecal Coliform</u>

The inner Bay is comprised of Lease M-430-05, located south of Tomasini Point, and includes the southernmost Bay station on the west shore near Inverness.

The two inner Bay stations exhibited similar patterns of contamination to the mid-Bay station just north of Tomasini Point (Figure 34). The magnitude of contamination was slightly greater in the inner Bay, however. The inner Bay stations did not always return to acceptable levels of fecal coliform by day X. On one occasion (Event 3, March 18) both stations showed an obvious spike of fecal coliform on day X that greatly exceeded the concentrations detected within the first three days of rainfall. One possible explanation for this sharp increase in fecal coliform would be a pulse of contamination from the watershed (e.g., a breached pond, accidental dumping) or nearshore area. The highest fecal coliform concentrations in the inner Bay occurred towards the end of the rainfall season in April.

# Relationship of Watershed Water Quality to Bay Water Quality

In order to investigate the effect of high levels of fecal coliform in watershed samples to Bay water quality samples, results from Bay water quality samples were compared with samples collected from the watershed site determined to be of the closest proximity (Table 24). Although no firm conclusions can be drawn from these comparison, some interesting trends were found. The fecal coliform standard was exceeded far more often than either the enterococcus or E. coli comparative values. In the majority of cases (20 out of 28), when the fecal coliform standard was exceeded in the watershed sample, the Bay's sample also exceeded its respective standard. This occurred most notably during the second rainfall event, when the matched Baywatershed stations exceeded the respective fecal coliform standards each day. Conversely, there were 32 cases when the fecal coliform standard was exceeded in the Bay, but not in the watershed samples. No trends appeared when comparing the enterococcus or E. coli monitoring data.

#### Tomales Bay Shellfish Quality

Consistent with the observations for the Bay water quality data, the shellfish tissue data revealed a 1:1 relationship between fecal coliform and *E. coli*. The NSSP Manual of Operations does not contain recommendations for acceptable coliform concentrations in shellfish that are grown and harvested from certified areas. The Manual of Operations does contain a reference to a post-harvest standard of 230 MPN for fecal coliform with respect to lot-testing of shellstock suspected of being temperature-abused. This application depends upon replicate sampling and not upon the analysis of single samples. This market standard of 230 MPN will be used as a reference point in the following discussion. For practical purposes it should be kept in mind that the 95% confidence interval for a population mean of 230 MPN ranges from 70 to 700 MPN for fecal coliform. The following sections summarize the levels of fecal coliform contamination observed in the shellfish sampling stations located throughout the Bay (Figure 4 and Table 25).

#### Outer Bay

The outer Bay shellfish stations were established inside of Tom's Point (M-430-15; bottom culture) and on the outer margin of the Walker Creek Delta (M-430-02). The latter location had two sampling sites representing two different culture techniques: top-culture (i.e., floating bags) and bottom culture (i.e., rack and bag). All stations were sampled for oysters on each dry season event and on day 2 and day X of each rainfall event.

The two bottom-culture stations slightly exceeded the NSSP market standard for fecal coliform in shellfish during the first dry season sampling on September 12, 1995 (Figure 38). The concentration of fecal coliform in oysters from the top-culture station (1700 MPN) was significantly higher than the NSSP standard. It is likely that these elevated levels of fecal coliform are the result of localized contamination. The DHS has previously documented the potential for fecal contamination of shellfish in floating bags from birds that roost and defecate on them.

The first rainfall event resulted in elevated fecal coliform levels (4900 MPN) in oysters from M-430-15 and M-430-02 (top-cultured). By day X these two stations had returned to acceptable levels, however oysters from M-430-02 (bottom-cultured) experienced an increase in fecal coliform (3100 MPN).

The second rainfall event also resulted in elevated fecal coliform levels in oysters from M-430-15 (1400 MPN), M-430-02 (top-cultured; 2300 MPN) and M-430-02 (bottom-cultured; 4900 MPN) on day 2. Fecal coliform concentrations remained extremely high by day X of this event for oysters from M-430-15 (7900 MPN) and M-430-02 (top-cultured; 23000 MPN).

The third rainfall event on March 11, 1996 resulted in extremely high fecal coliform concentrations (i.e., greater than or equal to 24000 MPN) for all outer Bay shellfish stations. The fecal coliform levels at these stations decreased markedly, however the top-cultured oysters on M-430-02 remained 10 times higher (2300 MPN) than the NSSP market standard.

The fourth rainfall event on April 1 also resulted in extremely high fecal concentrations at all three outer Bay stations: 7900 MPN at M-430-15, 46000 MPN at M-430-02 (top-cultured), and 31000 MPN at M-430-02 (bottom-cultured). Despite these high levels, all sites had returned to acceptable levels by day X.

The fecal coliform contamination of oysters in the outer Bay reached extremely high levels following significant rainfall. In addition, these data suggest a pattern of increasing contamination over time, perhaps as a result of the continuous high fecal concentrations contributed by the watershed, as discussed earlier. In addition, the lower water temperatures in winter may result in lower metabolic rates in the oysters, which in turn would lengthen the time necessary for satisfactory cleansing of contamination. Consequently, oysters in the outer Bay do not always return to NSSP market standards by the time this area has been reopened for harvesting.

#### Mid Bay

The mid-Bay station was established at lease M-430-06, located in Marconi Cove. Bottom bags of oysters were sampled at this site.

Oysters at this location remained within acceptable levels for fecal coliform through the first rainfall event. By the second rainfall event (January 16) an impact was observed to oysters at this site, although the concentration of fecal coliform (700 MPN) was lower than observed in the outer Bay stations. Fecal coliform concentrations remained slightly elevated at day X (430 MPN).

The third rainfall event resulted in the highest observed fecal coliform concentrations in oysters at this site (1700 MPN). By day X the levels were well within acceptable levels. The fourth rainfall event did not have a significant impact on fecal coliform concentrations at this site.

#### Inner Bay

The inner Bay is comprised of Lease M-430-05, located south of Tomasini Point. Bottom bags of oysters were sampled at this site.

Oysters at this location remained within acceptable levels for fecal coliform through the first rainfall event. By the second rainfall event an impact was observed to oysters at this site, and the concentration of fecal coliform (2200 MPN) was similar to the concentrations observed in the outer Bay stations. Fecal coliform concentrations remained elevated at day X (3300 MPN).

Interestingly, the third rainfall event did not result in a significant increase in fecal contamination, in contrast to the pattern observed in the outer and mid-Bay stations. The fourth rainfall event resulted in the highest fecal coliform concentrations in oysters at this site (13000 MPN). By day X the fecal coliform concentration had returned to an acceptable level.

#### Fecal Coliform Loadings: Watershed

The known flows of the gauged portions of Walker Creek and Lagunitas Creek, together with the fecal coliform density data reported above, were used to calculate fecal coliform loadings. This loading value reflects the amount of fecal coliform contributed by each watershed on a daily basis (FC/Day).

To estimate the FC loadings, it was first necessary to derive flow information for the ungauged portions of the watershed. This was accomplished by establishing a ratio of flow to drainage area for the gauged portions of the watershed. This ratio was based on the daily average flow recorded at the stream gauges on Walker Creek and Lagunitas Creek for each day that sampling occurred (Table 13). The Walker Creek gauge data was used to estimate flow for the ungauged portions of the Keyes Creek, Chileno Creek, and Walker Creek watersheds, as well as for the east and west shore drainages. The Lagunitas gauge data was used to estimate flow for the Lagunitas Creek, Olema Creek, and Bear Valley Creek watersheds. Although the Walker Creek flow data was used for the west shore watersheds, calculations were also performed using the Lagunitas flow data. No significant difference existed between either estimate.

The flow ratio was used in conjunction with the fecal coliform concentration to calculate a FC loading value for each watershed on each day of sampling. This method allows a more direct comparison of the watersheds based on their FC loading values. The following sections will discuss the FC loadings for each watershed.

#### Walker/Keyes/Chileno Watershed.

Of the six sampling stations in this watershed, the highest fecal coliform loadings were detected at the Chileno Creek site (Figure 39). From the second rainfall event through the fourth, FC loadings often exceeded 1 x  $10^{12}$  per day (i.e., 1,000,000,000 fecal coliform in 24 hours).

Fecal loading for the Walker Creek watershed was  $>10^{13}$  FC/day for most of the samples taken during the study, beginning with the day X sample of the first rainfall event. The levels dropped slightly ( $10^{11}$  FC /day) on day 2 of rainfall event three, and remained at this level for the remainder of sample days within this rainfall event. Levels again rose to  $> 10^{13}$  on day 1 of rainfall event four, but dropped during the remainder of the sample days. Samples from the Walker Creek Ranch watershed showed a different pattern. Fecal loading was typically an order of magnitude lower than that of the lower Walker Creek watershed, the only exceptions being on day X of rainfall event three, and days 3 and X of rainfall event four.

#### East Shoreline Watershed

Fecal loading from the sites along the eastern shoreline of Tomales Bay ranged from  $<10^7$  to  $10^9$  during the first rainfall event (Figure 39). Levels began to increase during rainfall event 2. Millerton Creek and Tomasini Creek watersheds contributed  $>10^{13}$  FC /day during days 1 and 3 of this rainfall event. By day 3, all eastern shore sample stations were calculated as contributing  $>10^{13}$  fecal coliform to the Bay, with the exception of Mileposts 36.17 and 38.54. Fecal loading from Milepost 36.17 watershed never varied from  $<10^7$  per day during the entire study. Rainfall events 3 - 4 showed the same fecal coliform loading pattern for all eastern shore stations: higher loading levels for all stations (except Milepost 36.17) on day 1, with decreasing levels throughout the subsequent sampling.

The highest fecal coliform loadings generally occurred in the watersheds represented by stations Milepost 40.35, Milepost 34.95, Millerton Creek, Milepost 32.12, Grand Canyon Creek, and Tomasini Creek. Each of these watersheds frequently exceeded daily fecal coliform loadings of  $1 \times 10^{12}$ .

#### Lagunitas/Olema Watershed

Fecal coliform loading from this watershed was consistently higher than that of the western watershed (Figure 40). During the first rainfall event, the Bear Valley watershed contributed the greatest share of the fecal load,  $10^{10}$  FC /day. By rainfall event two, this pattern had changed, with Lagunitas contributing the largest share of fecal coliform (>10<sup>13</sup> per day), Olema contributing the next largest load, and Bear Valley contributing the least. This pattern was consistent over rainfall events 2 to 4.

#### West Shoreline Watershed

Fecal coliform loading from this watershed was relatively low, ranging from less than  $10^7$  to  $10^9$  FC/day for most of the rain event days sampled (Figure 40). The only exceptions occurred during the second rainfall event; on days 1 and 3 of this sampling series, fecal loading was  $10^{10}$  FC/day. However, even on these two days, fecal loading from the western watershed was less than that contributed by the other watersheds.

#### Entire Tomales Bay Watershed

The relationship between the various subwatersheds relative to fecal coliform loadings is displayed in a series of grey-shaded maps (Figures 41 through 59) and is discussed in the following sections.

**Rainfall Event 1**. The first significant rainfall of the year resulted in fecal coliform loadings that were slightly higher than observed during the baseline dry season event (Figures 42 and 41, respectively). The highest observed loadings occurred on day 1 in the Keyes Creek, Walker Creek, and Bear Valley watersheds  $(10^{11} \text{ FC /day})$ . Inexplicably, the loading rates increased in the Walker Creek watershed on day X.

**Rainfall Event 2**. Significant fecal coliform loadings can be seen in several subwatersheds on January 16 (Figure 46), with the highest loadings in the Chileno Creek watershed  $(10^{14})$ . Loadings decreased by approximately one log factor on day 2 in several areas. On day 3, following renewed rainfall, several areas increased, notably Milepost 34.95, Grand Canyon Creek, Tomasini Creek, and Lagunitas Creek (Figure 48). In general, the day 3 loadings were the highest observed during the study, with four watersheds at  $10^{14}$  and 10

watersheds at  $10^{13}$ . By day X there had been a noticeable decrease in fecal coliform loading throughout the watershed, with the exception of the Walker Creek subwatershed, which remained at  $10^{13}$ .

**Rainfall Event 3**. The third rainfall event resulted in the highest fecal coliform loadings (Figure 51) in the Chileno, Walker Creek, and Lagunitas Creek watersheds  $(10^{13})$ . On day 2 the Chileno and Lagunitas Creek watersheds remained at this concentration (Figure 52). By day 3 there was an overall decline in loadings, although four watersheds remained at  $10^{12}$  (Figure 53). On day X the Keyes Creek watershed sampled at Irvin Road exhibited an increase to  $10^{13}$  (Figure 54).

**Rainfall Event 4**. The fourth rainfall event again produced the highest fecal coliform loadings (Figure 55) in the Chileno Creek watershed  $(10^{14})$ . High loadings were also observed for the Walker Creek, Grand Canyon Creek, and Lagunitas watersheds  $(10^{13})$ . By day 3 there was a noticeable decrease in fecal coliform loadings, with only the Chileno Creek and Lagunitas Creek watersheds contributing  $10^{12}$  FC /day and 11 additional watersheds contributing  $10^{11}$  FC /day. This downward trend continued on day X (Figure 58), with only the Chileno Creek and Milepost 40.35 watersheds contributing  $10^{11}$  FC /day and 12 additional watersheds contributing  $10^{10}$  FC /day.

The highest fecal coliform loadings were contributed by the Chileno Creek watershed, followed by the Walker Creek and Lagunitas Creek watersheds. Of interest is the fact that several very small watersheds to the east of Tomales Bay contributed relatively high loadings of fecal coliform.

Overall, the calculated fecal coliform loadings for the major portions of the watershed (i.e., Walker/Keyes/Chileno and Lagunitas/Olema) were equivalent throughout the course of the study. Although the fecal coliform loadings from the Walker/Keyes/Chileno watershed was slightly greater than that for the Lagunitas/Olema watershed, the difference was usually not significant (i.e., less than a log factor).

The watersheds to the west of Tomales Bay contributed the least amount of fecal coliform, as did the watershed of Milepost 36.17 on the eastern shore near Marconi Cove.

# CONCLUSIONS

#### General

1. The data from this study demonstrate the effect of rainfall-related runoff on water quality in Tomales Bay, as measured by increases in total and fecal coliform levels in the tributaries entering the Bay and by elevated levels of fecal bacteria in shellfish growing waters and shellfish tissue.

2. There was a rapid increase in coliform levels during and immediately following significant rainfall throughout the watershed and Bay. Maximum fecal coliform concentrations usually occurred within 24 to 48 hours of the rainfall event. This rapid response is consistent with the flow data from the Walker Creek and Lagunitas Creek gauging stations, which show a rapid, short-term increase in flow immediately after a rainstorm. Coliform densities at stations throughout the Bay were low in the absence of recent rainfall.

3. There was no discernible "first flush" phenomenon during the period of this study, with the possible exception of the western shoreline watershed. There was a pattern of sustained or increasing fecal coliform concentrations and loadings throughout the winter in all other watersheds. This would indicate the presence of a renewable source, or the introduction of new sources, of fecal contamination throughout portions of the watershed. Potential new or renewable sources could include discharges from overflowing waste ponds or failing onsite sewage disposal systems, or runoff from manure pastures, including runoff from spray irrigation during winter months.

4. The decrease in fecal coliform loadings over time in the western shoreline watershed suggests that there are fewer new or renewable sources of fecal contamination to Tomales Bay in that area. The difference in soil type and vegetation cover in the western watershed, compared to that of the eastern watershed, may also be a factor in the observed lower fecal coliform loadings as the more porous soils and greater vegetation cover may provide more filtering and less runoff.

5. Fecal coliform in the creeks and Bay was comprised almost entirely of *E. coli*. There is no evidence of interfering organisms that could result in false positives.

6. From limited analysis of samples for bacterial pathogens during the course of this study, two pathogens, *Salmonella* and *E. coli*:0157, were identified. The presence of these pathogens indicates the potential risk to the humans consuming shellfish as a result of fecal inputs from the watershed.

7. Several previous studies were reviewed for comparison with this study's results. Although it was difficult to compare studies due to lack of consistency in sampling stations and methods, in general it appears that levels of fecal coliform in Bay stations have stayed high during moderate to high rainfall periods over the past twenty years. There were no clear overall trends of increasing or decreasing fecal coliform levels in watershed stations, although some improvement was noted following implementation of dairy waste guidelines and sewering of the town of Tomales in the mid-1970's.

8. This study evaluated general trends in water quality and contaminant sources on a watershed and subwatershed scale. As such, individual or localized anthropogenic sources of fecal coliform, such as domestic onsite sewage disposal systems or individual incidents of direct disposal of sewage from sources not associated with rainfall, such as recreational boating and camping, were not specifically evaluated. Although samples were taken along the shoreline at several locations, individual systems were not targeted for sampling,

9. The TBSTAC contacted the Marin County Environmental Health Department at the start of this study to develop a program for targeted sampling of onsite sewage disposal systems or to get a commitment of future investigations, but the County did not want to commit to such a study. The current status of on-site investigations, along with other work on localized pollution sources, is discussed in the appendix to this report on the human illness outbreak.

#### Watershed Stations

1. The highest fecal coliform loadings occurred in the Chileno Creek watershed, followed by the watersheds of Keyes Creek, Walker Creek, Lagunitas Creek, and portions of the eastern shoreline watershed. Fecal coliform loadings were consistently high immediately following significant rainfall; however the Chileno watershed contributed high fecal coliform loadings for a longer period of time than the other watersheds. Several previous studies (cited in the introduction above) have indicated that the primary source of fecal coliform to Tomales Bay was from dairies and livestock grazing land. This is consistent with the present study findings of high loadings in the Chileno/Keyes/Walker Creek watersheds, but does not explain the loadings for Lagunitas Creek, which is primarily surrounded by low-density urban residential areas and parklands. It should be noted (see Conclusion #7 below, that Lagunitas Creek has lower concentrations of coliform but much higher flows than the other creeks in the study).

2. Chileno Creek contained the highest concentrations of indicators observed in this study. Fecal coliform concentrations at this site exceeded the water quality objective for non-contact water recreation (mean < 2000 MPN) for all but 2 days of the study.

3. Fecal coliform concentrations in Chileno, Keyes, and Walker creeks reached levels that were 10 to 100 times higher than the water quality standards set by the State Water Resources Control Board.

4. High concentrations of fecal coliform were observed at almost all of the eastern shoreline watershed stations during the study, with highest levels occurring at Milepost 40.35 and Milepost 32.12. The concentrations of fecal indicator bacteria in these tributaries generally exceeded all water quality objectives after significant rainfall.

5. Comparatively low concentrations of fecal coliform were detected in the eastern shoreline drainages at Milepost 36.17 and Milepost 38.54, which enter the Bay at Marconi Cove and the Hog Island Oyster Company wet storage facility, respectively.

6. Portions of the eastern shoreline watershed of Tomales Bay contributed relatively high loadings of fecal coliform despite their small drainage area.

7. The concentrations of fecal coliform detected in Lagunitas Creek were generally lower than those observed in the Walker/Keyes/Chileno watershed stations. However, because the flow in Lagunitas was at least twice as high as the flow in Walker Creek, the fecal coliform loadings from these two watersheds were equivalent. The concentrations of fecal indicator bacteria in Lagunitas creek generally exceeded all water quality objectives after significant rainfall.

8. Fecal coliform concentrations and loadings for Olema and Bear Valley Creeks were generally equivalent to those of Lagunitas Creek.

9. The western shoreline tributaries generally showed the smallest increases in fecal coliform concentration following significant rainfall of all the Tomales Bay subwatersheds sampled during this study. Fecal coliform water quality objectives were exceeded only twice during the course of this study, and both of these occurrences were at Milepost 28.86. In addition, the fecal coliform loadings from these tributaries along the western shoreline were lower than the loadings for the other subwatersheds studied.

10. White Gulch, the control station at the northwest end of Tomales Bay, had consistently low fecal coliform levels and low loadings relative to the other sample stations. None of the samples collected at this site exceeded the water quality objectives for fecal coliform or *E. coli*. Since this is an area with a sizeable elk population and minimal human access, it was considered to be a good representative site for determining the amount of coliform present from wildlife and other natural sources in the watershed.

11. Fecal coliform loadings at the White Gulch freshwater control station were 10 to 100,000 times lower than the loadings from the watersheds of Walker/Keyes/Chileno and Lagunitas/Olema creeks.

12. The correlation between enterococcus and all other indicators was poor. Enterococcus was the most sensitive indicator, and fecal coliform was the least sensitive, relative to the number of times these indicators exceeded their respective water quality objectives. This suggests the possibility that the enterococcus level recommended by EPA for regulating non-contact recreational use is too low for fresh water use. It is also possible that enterococcus is identifying a different source of contamination, which may be comprised of a greater percentage of enterococcus than fecal coliform or *E. coli*.

# **Bay Stations and Shellfish Stations**

1. All areas in Tomales Bay experienced significant increases in fecal coliform within 24 to 48 hours of a rainfall event.

2. Fecal coliform concentrations were highest in the outer Bay stations near the Walker Creek delta and in the inner Bay south of Tomasini Point.

3. Fecal coliform concentrations in Bay waters did not always return to an acceptable level by the time the Bay was reopened for harvesting.

4. Fecal coliform concentrations in oyster tissues did not always return to an acceptable level by the time the growing areas were reopened for harvesting.

5. The lowest levels of fecal coliform in oysters were observed in the mid-Bay station at Marconi Cove. The creek at Milepost 36.17, which enters the Bay at Marconi Cove, was also observed to have unusually low fecal coliform concentrations and loadings.

6. Fecal coliform concentrations at sampling sites throughout the Bay frequently exceed the ocean water contact standard for one or more days following significant rainfall. In most cases the fecal coliform concentration met this standard by day X.

## RECOMMENDATIONS

The RWQCB will continue to work with the TBSTAC in developing a remediation strategy to reduce pollution affecting the commercial shellfish growing areas, as directed by the Shellfish Protection Act of 1993. As noted in the Act, the RWQCB will work with other agencies, the shellfish growers, and the agricultural community representatives to "develop and implement specific remediation strategies". The shellfish study pointed out the impacts to the Bay from the eastern watershed streams that primarily flow through dairy and grazing lands. In addition to these sources of contamination, the RWQCB and the DHS are working with the National Park Service, California State Parks, and the County of Marin to address the pollutant impacts from on-site domestic and commercial sewage systems and from recreational activities such as boating and camping.

Remediation strategies in response to the findings of this study and other information was developed by the TBSTAC, whose membership includes the responsible regulatory agencies, park service staff, agricultural representatives, shellfish growers, local environmental groups, and community members. Although many recommendations identify the TBSTAC as the lead, the responsibility for follow-up will likely be delegated to the responsible agency or agencies, a subcommittee of the TBSTAC, or by other represented groups participating in this effort.

The report has been accepted with the understanding that more study and research are needed to clarify the movement and longevity of human pathogens in Tomales Bay.

## Pathogen Source Control Measures

#### <u>Agriculture</u>

1. The Tomales Bay Agricultural Group (TBAG) should develop a performance-based program for each dairy in the watershed, which will include custom-based farm plans that will be designed to prevent water quality violations. These plans will include nutrient budgets; pond capacity; stream protection; and manure management, including spreading and irrigation, erosion control, animal housing, and recordkeeping of application of animal wastes. It will also include a self-monitoring third-party testing program to reduce fecal coliform. The entire program will be developed with the help of the U.C. Cooperative Extension and other experts. Progress reports and results of pilot projects should be included as part of the program, along with details on the strategy and approach. Inclusion of other agricultural producers should be included as the situation warrants.

2. The TAC should develop a priority list of pilot watershed projects to address impacts of animal waste facilities on water quality. For example, projects designed to make improvements in animal confinement areas, use of innovative animal waste treatment technologies, improvements in manure disposal strategies, and riparian corridor protection projects, could have a major beneficial impact to water quality in Tomales Bay and its watershed. If deemed a priority, livestock numbers in the watershed, both existing and historical, should be quantified.

#### Domestic and Municipal Wastewater/Sewage Systems

1. The TAC should encourage and support efforts to develop community consensus. For example, the East Shore Planning Group has adopted the following programs:

Formation of a subcommittee to explore the viability of establishing a separate local non-profit entity comprised of property owners on the east shore of Tomales Bay. This proposed organization can

tentatively be called the Marshall Water Quality Association (MWQA). This ESPG subcommittee will seek property owners' consensus to identify the most appropriate measures that would continue to maintain Tomales Bay water quality.

The ESPG subcommittee will explore and evaluate through MWQA the coordination and possible cost sharing of the following septic management properties:

a. Evaluation b. Water quality monitoring	e. Repairs f. Education
c. Pumping	g. Water conservation
d. Maintenance	

MWQA would explore options for specific individual or neighborhood septic management programs, which fit each of the area's geographic conditions and respective complex site conditions. MWQA will enlist expertise and material support from the County, State and federal agencies, which have the responsibility for Tomales Bay Water Quality.

2. All on-site systems need to be addressed by regular evaluation. Communities should be encouraged to develop their own plans for on-site system evaluation and monitoring. The County and Regional Board should provide support for these community efforts.

#### **Recreational Activities**

1. The National Park Service, as lead agency, working with State and County parks departments, should develop a needs assessment and management plan, with a timeline for implementation, to improve boater facilities and procedures for day use and overnight camping waste collection, including a) pollution controls (including packout provisions) on unimproved beaches, b) increased public education and signs at points of entry, and c) enhanced and coordinated enforcement.

#### Education and Outreach Activities

1. The TBSTAC should identify educational and outreach needs to reach ranchers, media, local communities and visitors, to educate them about the resource values of Tomales Bay, the impacts to the Bay from different pollutant sources, and the remediation efforts that are currently underway or that need to be addressed.

2. The TBSTAC should develop local library repositories of information on Tomales Bay, with links to similar efforts in other areas.

3. The TBSTAC should evaluate existing data and the need to identify ways to publicise advisories to users that water quality may not meet the standards for water contact and recreational shellfish harvesting following significant rainfall events.

#### Policy Development

1. Designate the shoreline region of Tomales Bay as a sensitive zone relative to potential impacts to beneficial uses. Activities within this designated area would require greater oversight by all users and the responsible agencies to ensure protection of all beneficial uses and public health. As part of this effort, the RWQCB should investigate the requirements and desirability of having the Environmental Protection Agency designate Tomales Bay as a no-discharge area for vessel sewage wastes. This would include an

investigation of the existing regulations in the National Marine Sanctuary regarding vessel wastes and mooring regulations.

2. Obtain support from the Marin County Board of Supervisors and the Marin County Environmental Health Services to assign a high priority to the protection of the beneficial uses of Tomales Bay.

## Monitoring and Assessment

1. Compare land use practices and water sources in the watersheds represented by the sampling stations at Mileposts 36.17 and 38.54, which had low coliform counts, with the remaining eastern shoreline watersheds that exhibited high fecal coliform loadings.

2. In those areas that experience high levels of fecal contamination but where obvious sources of fecal contamination cannot be identified, a monitoring strategy should be developed for impacted waterbodies and pollutant sources, based on the results of the current shellfish study. The purpose of the monitoring would be to identify specific point sources of fecal coliform in order to be able to develop specific remediation activities. This effort should be coordinated with the Tomales Bay Agricultural Group actions where appropriate.

3. Pursue the identification of sources of fecal contamination in the watershed, with a priority on the watersheds for Chileno and Lagunitas creeks. This may involve the development of a priority list and plan of action for surveying the areas of concern. This effort should be coordinated with the Tomales Bay Agricultural Group actions where appropriate.

4. Investigate the use of DNA fingerprinting techniques and other indicator studies as to usefulness of determining specific sources of coliform.

5. Promote studies aimed at improving our understanding of pathogen transport processes.

6. Develop a monitoring program to track the environmental fate of pathogens, aimed at evaluating spatial and temporal pathogen concentration trends and loadings, and the effectiveness of source control efforts.

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**TABLES 1 - 25** 

COMPANY	REG. NO.	DFG LEASE	NO. ACRES	PRODUCTS
Bay Bottom Beds, Inc.	00256	M-430-02	5	Pacific Oysters, Manila Clams
		M-430-04	62	
		M-430-19	25	
Cove Mussel Co.	00311	M-430-06	10	Bay Mussels, Pacific Oysters
Hog Island Oyster Co. Inc.	00265	M-430-10	5	Pacific Oysters, Eastern Oysters, European Oysters, Manila Clams, Bay Mussels
		M-430-11	5	
		M-430-15	98	
		Intake	n/a	
Intertidal Aquafarms, Inc.	00364	M-430-12	25	Pacific Oysters, Eastern Oysters, European Oysters, Kumamoto Oysters, Bay
_				Mussels
The Marshall Store	00333	Intake point	N/a	Pacific Oysters, Bay Mussels, Eastern Oysters, European Oysters
Point Reyes Oyster Co.	00416	M-430-13	25	Pacific Oysters, European Oysters, Kumamoto Oysters, Bay Mussels
		M-430-14	5	
		M-430-17	62	
Frank Spenger Co.	00280	None:	1	Pacific Oysters
		PRNS Parcel		
Tomales Bay Shellfish Farms, Inc.	00330	M-430-05	156	Pacific Oysters, Bay Mussels, Manila Clams, European Flat Oysters
		Intake		

## Table 1. Commercial shellfish growers and wet storage operators in Tomales Bay.

WATERSHED	AREA (km <sup>2</sup> )	AREA (%)
Walker	196.35	35
Lagunitas	241.72	43
Olema	50.0	9
Remainder	72.93	13
TOTALS	561	100%

Table 2. Tomales Bay watershed area estimates, including reservoirs (adapted from Fischer, 1996).

Table 3. Area estimates for the gauged portions of the Tomales watershed, including release and spill from catchment reservoirs and unimpaired flow from the watershed below the reservoirs (Fischer, 1996).

WATERSHED	AREA $(km^2)$	AREA (%)
Walker	78.54	14
Lagunitas	213.18	38
Remainder	269.28	48
TOTALS	561	100%

Table 4. Estimates of watershed contributions to runoff into Tomales Bay (Fischer, 1996).

WATERSHED	% of TOTAL
Walker	25
Lagunitas	66
Remainder	9
TOTALS	100%

SUBWATERSHED	AREA $(km^2)$
Walker/Keyes/Chileno:	
Chileno Creek, mid	26.65
Keyes Creek, Irvin Rd	4.922
Keyes Creek, WWTP	5.658
Walker Creek, Ranch	23.73
Walker Creek (Walker + Chileno)	102.1
East Shore Tributaries:	
Milepost 40.35	0.936
Milepost 38.54	3.139
Milepost 36.17	1.458
Milepost 34.95	4.348
Millerton Creek	8.917
Milepost 32.12	0.834
Grand Canyon Ck.	6.852
Tomasini Ck lower	8.754
Lagunitas/Olema:	
Lagunitas Creek	213.2
Olema Creek Upper	13.710
Olema Creek	19.120
Bear Valley Creek	7.340
West Shore Tributaries:	
Milepost 25.86	2.483
Milepost 28.29	2.166
Milepost 28.86	1.620
Teachers Beach Ck	1.755
White Gulch	0.256

Table 5. Tomales Bay subwatershed designations and their respective areas.

Table 6. Permitted sewage treatment systems in the Tomales Bay watershed that are regulated under Waste Discharge Requirements from the San Francisco Bay Regional Water Quality Control Board.

NAME	LOCATION	WASTE (GPD <sup>1</sup> )	WASTE SOURCE	TREATMENT TYPE	DISPOSAL	OPERATOR
Tomales Wastewater Treatment Plant	3 miles from Bay along Keyes Creek	38,000 (design) 11,000 (average)	Tomales (89 homes & school dist.)	Aerated storage ponds	Spray Irrigation April to November	North Marin Water District
Marconi Conference Center	Highway 1 at Marconi Cove	25,000 (design) 13,500 (actual)	Conference facilities	Package plant secondary treatment	Leaching trench w/backup irrigation	California State Parks
Borello Sewage Ponds	NE of Millerton Point above Millerton Creek	3400 (average)	Domestic and commercial septage	Holding ponds	Spray irrigation April- October	Owner operated
Skywalker Ranch	Lucas Valley Road, upper Nicasio Creek	8975 (maximum)	250 daytime users	Three septic tanks	Dual leachfields	Skywalker Ranch
Olema Campground	3.5 miles SW of Tomales Bay along Olema Creek	18,000 daily maximum	238 unit Campground	Septic tanks, holding tank, storage ponds	Spray irrigation, April – October	Campground owner
Samuel P. Taylor Park	10 miles SE of Bay along Lagunitas Creek	80,000 (design) 45,000 (actual)	Campground, park	Digestor, primary clarifier, trickling filter	Leachfields, spray disposal if necessary	California State Parks
Blue Mountain	2 miles E of Tomales on Keyes Creek	4000 (actual)	50 residents, day use	Septic tanks, holding tank, 2 evaporation ponds	Discharge to leachfields	Blue Mountain Center
Spirit Rock	Sir Francis Drake Blvd. in Woodacre	9000 (design) 4875 (actual)	Residents, classes	2 Septic, one convential, one sand filter	Leach fields	Insight Meditation Center
Walker Creek Ranch	11 miles from Bay, on Petaluma-Pt. Reyes Road	20,000 (design) 14,000 (actual)	100-220 overnighters, 230 day use	Package plant, activated sludge	Holding pond, pasture irrigation May – Sept.	Marin County Office of Education

<sup>1</sup> GPD = Gallons per Day

Table 7. Water quality objectives for coliform bacteria<sup>2</sup>. (From San Francisco Bay Regional Water Quality Control Plan [Basin Plan], 1995).

Beneficial Use	Fecal Coliform	Total Coliform
Water Contact Recreation <sup>3</sup>	log mean < 200	median < 240
	90 <sup>th</sup> percentile < 400	no sample > 10,000
Shellfish Harvesting <sup>4</sup>	Geometric Mean < 14	Geometric Mean < 70
	90 <sup>th</sup> Percentile < 43	90 <sup>th</sup> Percentile < 230 <sup>5</sup>
Non-Contact Water <sup>6,7</sup>	Mean < 2000	
	90 <sup>th</sup> Percentile < 4000	
Municipal Supply:		
surface water <sup>8</sup>	Log Mean < 20	Log Mean < 100
ground water		< 1.19

<sup>&</sup>lt;sup>2</sup> Based on a minimum of five consecutive samples equally spaced over a 30-day period.

<sup>&</sup>lt;sup>3</sup> Freshwater and ocean water. Freshwater values are based on DHS recommended values.

<sup>&</sup>lt;sup>4</sup> Source: National Shellfish Sanitation Program.

<sup>&</sup>lt;sup>5</sup> Based on a five-tube decimal dilution test. Use 300 MPN/100 mL when a three-tube decimal dilution test is used.

<sup>&</sup>lt;sup>6</sup> Source: Report of the Committee on Water Quality Criteria, National Technical Advisory Committee, 1968.

<sup>&</sup>lt;sup>7</sup> Freshwater

<sup>&</sup>lt;sup>8</sup> Source: DHS recommendation.

<sup>&</sup>lt;sup>9</sup> Based on multiple tube fermentation technique; equivalent test results based on other analytical techniques, as specified in the National Primary Drinking Water Regulation, 40 CFR, Part 141.21(f), revised June 10, 1992, are acceptable.

Table 8.	Recommended	standards for	enterococcus	(EPA).
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LOCATION	SALT WATER ENTEROCOCCI (MPN)	E.COLI (MPN)	FRESH WATER ENTEROCOCCI (MPN)
designated beach	104	235	61
moderate use area	124	298	89
light use area	276	406	108
infrequent use area	500	576	151

DRAINAGE	DAIRY (Cows and Heifers)	MANURE Lbs/Day	BEEF	MANURE Lbs/Day	SHIEEP	MANURE Lbs/Day	TOTAL HEAD	TOTAL MANURE
Chileno Creek	2592	231,693	230	12,834			2563	244,527
Keyes Creek	786	70,151					786	70,151
Walker Creek	1182	105,553	540	30,132	1000	7200	2722	142,885
Marshall to Pt. Reyes Station	3847	343,553	550	30,690			4397	374,243
Lagunitas/Nicasio Reservoir	2563	229,135	230	12,834			2793	241,969
Totals	10,970	980,085	1550	86,490	1000	7200	11,254	1,073,775

Table 9. Estimated numbers of livestock<sup>10</sup> and manure production in Tomales Bay watershed (totals/watershed/day)<sup>11</sup>.

 <sup>&</sup>lt;sup>10</sup> Approximate numbers based on rough estimates by the University of California Cooperative Extension
 <sup>11</sup> Table adapted from R. Bennett and S. Larson, *Preventing Animal Wastes from Degrading Water Quality: The Case for Tomales Bay, California, 1990.*

Table 10. Fresh manure production and characteristics<sup>12</sup>.

PARAMETER	DAIRY 1400 LB	BEEF 800 LB	SHEEP 60 LB	HORSE 1000 LB	DUCKS (3 LB)
Total Manure (lb/day)	120.4	46.4	2.4	51	0.33
Urine (lb/day)	36.4	14.4	0.009	10	NA <sup>13</sup>
Total Nitrogen (lb/day))	0.63	0.27	0.025	0.30	0.005
Ammonia (lb/day)	0.11	0.07	NA	NA	NA
Total Coliform (#colonies)	700	23	54	220	NA
Fecal Coliform (#colonies) <sup>14</sup>	10.1	10.4	12	0.042	0.24

<sup>&</sup>lt;sup>12</sup> Numbers are based on manure produced per 1000 lb live animal unit, per day. Data are adapted from the Agricultural Sanitation and Waste Management Committee, Manure Production and Characteristics, 1989. Numbers have been adjusted to conform to the average sized animal, as noted under each animal type. <sup>13</sup> data not available.

 $<sup>^{14}</sup>$  Mean bacteria colonies per average animal mass multiplied by 10  $^{10}$ .

		Walker C	reek		Blake's La	anding		Marconi (	Cove		TBOC	
Dates	High	Low	Median	High	Low	Median	High	Low	Median	High	Low	Median
1974	79	2	5	49	2	2	130	2	2	170	2	2
1976-77	230	2	60	130	3	27				2300	2	20
1977-78	4600	20	1045	13000	348	2300	2300	50	251	3300	50	790
1980	>16000	20	790	5400	20	130	16000	20	310	16000	20	350
1995-96	>16000	2	200	2800	2	75	2100	2	45	16000	2	33

Table 11. Comparison of Tomales Bay fecal coliform concentrations for several watershed stations, sampled between 1974 and 1996.

Table 12. Comparison of Tomales Bay fecal coliform concentrations for several Bay stations, sampled between 1974 and 1996.

		WALKEI	R CREEK		MILLER CREEK	TON			CANYON EEK		OLEMA	CREEK
DATES	HIGH	LOW	MEDIAN	HIGH	LOW	MEDIAN	HIGH	LOW	MEDIAN	HIGH	LOW	MEDIAN
1974	110000	40	430	1500	30	51	24000	30	965	240000	90	230
1976-77	24000	4	2525							9200	1100	1300
1977-78	13000	3300	4900	13000	790	1100	7900	460	4180	3300	80	2400
1980	35000	330	3300	24000	790	4100	13000	230	700	1300	220	490
1994-95	>16000	70	1585	24000	17	2800				3500		
1995-96	>16000	220	2200	35000	2	2000	160000	346	4900	35000	110	790

SAMPLING	WALKER CREEK FLOW	LAGUNITAS FLOW
DATE	(cubic feet per second)	(cubic feet per second)
9/12/95	4.9	6
12/4/95	11	48
12/5/95	10	36
12/6/95	10	35
12/9/95	9.6	30
1/16/96	162	877
1/17/96	72	540
1/18/96	321	899
1/31/96	435	1430
2/11/96	51	238
3/11/96	96	254
3/12/96	170	391
3/13/96	128	381
3/18/96	43	146
4/1/96	86	361
4/2/96	75	609
4/3/96	45	331
4/8/96	18	80
7/9/96	5.1	9.8

Table 13. Daily average flow recorded at the stream gauges on Walker Creek and Lagunitas Creek for each day that sampling occurred.

Sample Site	Date Sampled	Total Coliform	Fecal Coliform	E. coli	Enterococcus
Chileno Creek	9/12/95				
	12/4/95				
	12/5/95				
	12/6/95				
	12/9/95				
	1/16/96	160000	24000	*24000	*1260
	1/17/96	92000	*9539	*7000	*500
	1/18/96	14000	*2300	*2300	*160
	1/31/96	24000	*7900	*7900	*130
	2/11/96	22000	*4600	*4600	
	3/11/96	54000	*35000	*3100	*310
	3/12/96	54000	*54000	*54000	*850
	3/13/96	13000	*3300	*3300	70
	3/18/96	4900	*2200	*2200	*180
	4/1/96	>160000	*160000	*160000	*158
	4/2/96	160000	*35000	*35000	*780
	4/3/96	24000	*4990	*4900	*160
	4/8/96	2300	1300	*1300	90
	7/9/96	790	790	*790	50
Keyes Creek, Irvin Rd	9/12/95				
	12/4/95	>16000	*9200	*9200	*13530
	12/5/95	5400	490	*490	*3820
	12/6/95	95	70	26	*950
	12/9/95	790	33	33	*570
	1/16/96	54000	*7900	*2300	*560
	1/17/96	14000	*14000	*11000	*280
	1/18/96	35000	*24000	*24000	*1030
	1/31/96	13000	*4900	*4900	*270
	2/11/96	14457	*5030	*939	
	3/11/96	24000	1700	*1700	60
	3/12/96	92000	*35000	*35000	*260
	3/13/96	7000	*7000	*7000	40
	3/18/96	1700	490	330	30
	4/1/96	50596	*29093	*17076	*2695
	4/2/96	54000	*7900	*7900	*990
	4/3/96	7900	*3300	*3300	90
	4/8/96	3100	790	*790	60
	7/9/96				

Table 14. Bacteriological monitoring results<sup>15</sup> for stations in the Walker/Keyes/Chileno watershed.

 $<sup>^{15}</sup>$  A value preceded by an "\*" exceeds the respective water quality standard: Fecal Coliform = 2000 MPN; E. coli = 406 MPN; Enterococcus = 108 MPN.

Keyes Creek, Milepost 3.66	9/12/95				
	12/4/95				
	12/5/95				
	12/6/95				
	12/9/95				
	1/16/96	70484	1977	*1977	*186
	1/17/96	160000	*7900	*7900	*110
	1/18/96	35000	*3300	*1700	100
	1/31/96	54000	790	*790	80
	2/11/96	28000	1700	*700	
	3/11/96	17000	700	*700	30
	3/12/96	35000	*7900	*2800	100
	3/13/96	13000	330	330	20
	3/18/96	4900	330	330	<10
	4/1/96	>160000 92000	*24000 *7000	*24000 *7000	*1050 *300
	4/3/96	17000	*7000	*7000	70
	4/8/96	3300	330	330	*330
	7/9/96	5500	550	550	550
Keyes Creek, WWTP	9/12/95	130	49	49	25
c ,	12/5/95	350	240	240	*1880
	12/6/95	70	13	13	*320
	12/9/95	16000	49	33	40
	1/16/96	70484	*12410	*10909	*626
	1/17/96	>16000	*9200	*5400	30
	1/18/96	39547	*3450	*3450	*1200
	1/31/96	22000	*17000	*17000	*390
	2/11/96	2200	460	170	
	3/11/96	22000	*7000	*4600	80
	3/12/96	>16000	*>16000	*16000	*590
	3/13/96	17000	1700	*1700	50
	3/18/96	33	2	2	
	4/1/96	>160000	*160000 *>16000	*160000 *>16000	*2140 *710
	4/2/90	20199	*4900	*4900	*214
	4/8/96	7048	*3600	*4900	*140
	7/9/96	3500	1700	*1700	90
		2200	1700	1700	
Walker Creek Ranch	9/12/95				
	12/4/95	5400	230	230	*310
	12/5/95	62	1.9	1.8	*124
	12/6/95	13	13	13	40
	12/9/95	11	11	11	*140
	1/16/96	7900	*3300	*3300	
	1/17/96	>1300	1300	*1300	80
	1/18/96	4900	*2300	*2300	20

	1/31/96	3300	330	330	4
	2/11/96				
	3/11/96	1400	230	230	
	3/12/96	13000	*4900	*4900	
	3/13/96	11000	230	230	4
	3/18/96	790	20	20	<1
	4/1/96	92000	*7900	*4900	
	4/2/96	4900	700	*700	*11
	4/3/96	3300	130	130	
	4/8/96	790	330	330	
	7/9/96	790	170	170	<1
Walker Creek	9/12/95	70	21	21	
	12/4/95	5400	130	130	ç
	12/5/95	170	70	49	4
	12/9/95	336	80	65	1
	1/16/96	>160000	*28000	*28000	*160
	1/17/96	16000	*3500	*3500	1
	1/18/96	35000	*3300	*3300	8
	1/31/96	13000	1700	*1700	*48
	2/11/96	1700	700	220	
	3/11/96	7900	*7900	*2300	*103
	3/12/96	>16000	*>16000	*16000	*53
	3/13/96	6028	390	310	4
	3/18/96	1700	220	220	1
	4/1/96	4900	*2200	*1400	*20
	4/2/96	>16000	*>16000	*>16000	*68
	4/3/96	14866	1794	*1400	
	4/8/96	490	170	170	e
	7/9/96	150	150	120	1
	0/10/05	20	.10	10	
Walker/Keyes Creek	9/12/95	20	<18	<18	
	12/4/95 12/5/95	790 16	49 2.8	1.8	3
	12/6/95	20	13	7.8	
	12/9/95	16	5.9	3.3	7
	1/16/96	>160000	*54000	*54000	*104
	1/17/96	21331	*12880	*21000	*22
	1/18/96	14000	*3100	*1700	
	1/31/96	92000	*9500	*9500	*49
	2/11/96	17000	490	*490	
	3/11/96	70484	*35000	*35000	*16
	3/12/96	38884	*16248	*24000	*59
	3/13/96	4900	790	*490	2
	3/18/96	1300	330	330	<1
	4/1/96	>160000	*>160000	*>160000	*92
	4/2/96	54000	*13000	*13000	*79

4/3/96	17000	*4900	*4900	100
4/8/96	2300	490	330	40
7/9/96	20	20	20	<10

Sample Site	Date Sampled	Total Coliform	Fecal Coliform	E. coli	Enterococcus
Grand Canyon Ck.	9/12/95				
-	12/4/95				
	12/5/95				
	12/6/95				
	12/9/95				
	1/16/96	22000	*7000	*7000	*1180
	1/17/96	7000	490	*490	90
	1/18/96	>160000	*>160000	*>160000	*7380
	1/31/96	22000	1800	*1800	*260
	2/11/96	1700	490	*490	
	3/11/96	54000	*4900	*4900	*940
	3/12/96	7900	*4900	*1400	*380
	3/13/96	3300	1100	*1100	10
	3/18/96	1241	346	284	10
	4/1/96	160000	*160000	*70484	*455
	4/2/96	54000	*54000	*54000	*720
	4/3/96	1100	790	*790	90
	4/8/96	1530	1111	*711	65
	7/9/96	490	230	230	90
Milepost 32.12	9/12/95				
•	12/4/95				
	12/5/95				
	12/6/95				
	12/9/95				
	1/16/96	>160000	*43000	*43000	*3100
	1/17/96	160000	*160000	*92952	*1540
	1/18/96	>160000	*>160000	*>160000	*30090
	1/31/96	92000	*17000	*17000	*490
	2/11/96	24000	*13000	*13000	
	3/11/96	>160000	*>160000	*>160000	*1400
	3/12/96	160000	*160000	*82946	*815
	3/13/96	54000	*35000	*24000	*1660
	3/18/96	10844	*6222	*6222	*160
	4/1/96	>16000	*>16000	*>16000	*990
	4/2/96	160000	*121326	*121326	*1012
	4/3/96	160000	*22000	*22000	*1760
	4/8/96	17000	*17000	*17000	*1580
	7/9/96				

 $<sup>^{16}</sup>$  A value preceded by an "\*" exceeds the respective water quality standard: Fecal Coliform = 2000 MPN; E. coli = 406 MPN; Enterococcus = 108 MPN.

Milepost 34.95	9/12/95				
	12/4/95				
	12/5/95				
	12/6/95				
	12/9/95				
	1/16/96	35000	*13000	*13000	*1540
	1/17/96	35000	*2800	*2800	<10
	1/18/96	>160000	*>160000	*>160000	*720
	1/31/96	35000	*4900	*3300	*360
	2/11/96	4600	790	*490	
	3/11/96	54000	*7000	*3300	*160
	3/12/96	11000	*3300	*3300	60
	3/13/96	35000	*17000	*3500	70
	3/18/96	1700	110	110	10
	4/1/96	>35000	*35000	*35000	20
	4/2/96	5674	*4674	*4674	*194
	4/3/96	3100	*2300	*2300	*320
	4/8/96	7000	*3300	*3300	*380
	7/9/96				
Milen est 26 17	0/12/05	724	511	*511	*100
Milepost 36.17	9/12/95	734 2800	511 *2200	*511 *2200	*128 *650
	12/4/93	64	33	33	*150
	12/6/95	33	17	17	*120
	12/0/95	460	230	<1.8	90
	1/16/96	92000	*3300	*3300	*440
	1/17/96	3300	790	*790	30
	1/18/96	>160000	*4600	*4600	*980
	1/31/96	2800	330	330	80
	2/11/96	3357	120	96	
	3/11/96	2300	490	130	10
	3/12/96	7900	*3704	*3704	*174
	3/13/96	3300	330	110	20
	3/18/96	4900	*2200	*2200	22
	4/1/96	92000	*30298	*11000	*420
	4/2/96	4300	790	*790	*150
	4/3/96	4600	490	*490	50
	4/8/96	4900	1100	260	<10
	7/9/96	490	45	45	*1700
Milepost 38.54	9/12/95	270	78	78	*117
	12/4/95	3500	1663	*790	*455
	12/5/95	330	79	79	*150
	12/6/95	33	33	33	60
	12/9/95	26	11	11	30
	1/16/96	22000	490	*490	*300

	1/17/96	2900	110	110	70
	1/18/96	160000	*2300	*2300	*270
	1/31/96	7900	110	110	40
	2/11/96	1300	<18	<18	
	3/11/96	1700	78	78	<10
	3/12/96	24000	1300	*1300	50
	3/13/96	1700	78	78	<1
	3/18/96	540	20	20	<1
	4/1/96	>160000	*8400	*8400	*1900
	4/2/96	13000	790	*790	*14
	4/3/96	3357	55	55	3
	4/8/96	1700	1300	*1300	*13
	7/9/96	3300	*3300	*2300	70
Milepost 40.35	9/12/95				
· · ···	12/4/95				*58
	12/5/95				
	12/6/95	6.8	<1.8	<1.8	2
	12/9/95	6.8	<1.8	<1.8	2
	1/16/96	>160000	*22000	*22000	*176
	1/17/96	92000	*4900	*4900	*23
	1/18/96	>160000	*>160000	*>160000	*345
	1/31/96	17000	*2100	*1400	*12
	2/11/96	3589	115	70	
	3/11/96	22000	*3300	*2300	*16
	3/12/96	54000	*35000	*11000	*67
	3/13/96	39547	*6222	*4900	*48
	3/18/96	11000	330	330	5
	4/1/96	160000	*160000	*160000	1
	4/2/96	>160000	*54000	*54000	*37
	4/3/96	54000	*54000	*36000	*51
	4/8/96	160000	*160000	*160000	*47
	7/9/96	78	45	45	4
Millerton Creek	9/12/95	20	<18	<18	1
	12/4/95	490	<18 64	<18 64	3
	12/4/95	490	<1.8	<1.8	
	12/5/95	13	<1.8	<1.8	2
	12/0/93	7.8	<1.8	<1.8	<1
	1/16/96	17000	*7900	*7900	*44
	1/10/96	11000	**7900 790	*7900 *790	
	1/17/90	>160000	*7900	*7900	*47
	1/31/96	3300	1100	*1100	*24
	2/11/96	2300	45	45	• 24
	3/11/96	17664	*10134	*6222	*16
	3/11/96	11004	*10134	*0222 *2200	*10
					· [ ]

	3/18/96	490	130	130	20
	4/1/96	>160000	*35000	*13000	*540
	4/2/96	35000	*2300	*2300	*470
	4/3/96	7000	1700	*1700	50
	4/8/96	4900	1100	*1100	90
	7/9/96	1062	222	222	10
Tomasini Ck lower	9/12/95	490	220	130	81
	12/4/95				
	12/5/95				
	12/6/95				
	12/9/95				
	1/16/96	92000	*7000	*7000	*2520
	1/17/96	13000	490	*490	*140
	1/18/96	>160000	*35000	*35000	*4120
	1/31/96	11000	1200	*840	*370
	2/11/96	4900	330	330	
	3/11/96	2800	460	*460	50
	3/12/96	7900	1300	*1300	*230
	3/13/96	4900	330	330	20
	3/18/96	490	78	78	<10
	4/1/96	50596	*9899	*7436	*1177
	4/2/96	13000	950	*950	*380
	4/3/96	3300	490	230	70
	4/8/96	230	130	130	*130
	7/9/96	330	20	20	<10

Location	Date Sampled	Total Coliform	Fecal Coliform	E. coli	Enterococcus
Bear Valley Ck.	9/12/95	1700	1700	*1700	*343
	12/4/95	>16000	*16000	*9200	
	12/5/95	1100	700	*700	*5720
	12/6/95	490	490	140	*1410
	12/9/95	170	130	130	*1280
	1/16/96	4900	700	*700	*860
	1/17/96	10507	734	*734	*455
	1/18/96	22000	*3300	*3300	*540
	1/31/96	110	78	78	50
	2/11/96	1550	167	167	
	3/11/96	1300	330	330	30
	3/12/96	1591	101	101	71
	3/13/96	1100	45	45	10
	3/18/96	237	51	51	14
	4/1/96	13000	790	*790	*410
	4/2/96	3300	1300	*790	*510
	4/3/96	2200	78	78	50
	4/8/96	230	45	45	10
	7/9/96	2300	68	68	40
Lagunitas Ck.	9/12/95	1300	230	130	29
	12/4/95	3500		27	*210
	12/5/95	280	6.8	6.8	70
	12/6/95	156	4	4	32
	12/9/95	1200	33	33	*130
	1/16/96	24000	790	*790	*880
	1/17/96	2200	170	130	*110
	1/18/96	92000	*17000	*17000	*810
	1/31/96	2300	330	330	*190
	2/11/96	7900	93	93	
	3/11/96	7900	*3300	*3300	50
	3/12/96	4600	1300	*490	100
	3/13/96	1159	69	69	14
	3/18/96	490	78	78	<10
	4/1/96	92000	*7000	*7000	*1150
	4/2/96	1300	20	20	<10
	4/3/96	4900	45	45	20
	4/8/96	1300	20	20	<10
	7/9/96	130	20	20	<10

Table 16. Bacteriological monitoring results<sup>17</sup> for stations in the Lagunitas/Olema watershed.

 $<sup>^{17}</sup>$  A value preceded by an "\*" exceeds the respective water quality standard: Fecal Coliform = 2000 MPN; E. coli = 406 MPN; Enterococcus = 108 MPN.

Olema Creek	9/12/95	330	130	78	16
	12/4/95				
	12/5/95				
	12/6/95				
	12/9/95				
	1/16/96	28000	*4900	*4900	*350
	1/17/96	13000	*3300	*3300	30
	1/18/96	160000	*35000	*35000	*1023
	1/31/96	490	330	330	<10
	2/11/96	1149	191	191	
	3/11/96	7000	790	*790	70
	3/12/96	92000	*4900	*3300	*340
	3/13/96	2300	330	330	10
	3/18/96	17000	490	*490	20
	4/1/96	54000	*7900	*7900	*480
	4/2/96	7900	1300	*1300	*110
	4/3/96	4600	700	*700	40
	4/8/96	622	402	252	60
	7/9/96	490	220	220	20
Olema Creek,Upper	9/12/95	1300	490	402	*155
	12/4/95	16000	230	230	*690
	12/5/95	490	70	46	*370
	12/6/95	110	23	23	*120
	12/9/95	790	110	110	*460
	1/16/96	7900	*2200	*2200	*190
	1/17/96	10383	*2392	*2392	85
	1/18/96	>160000	*24000	*24000	*1160
	1/31/96	3300	460	*460	<10
	2/11/96	2200	130	130	(0)
	3/11/96	7000	912	*912	60
	3/12/96	11000	*2300	*1300	100
	3/13/96 3/18/96	1300 4600	170 170	170 170	<10
		92000	*14000	*14000	<10
	4/1/96	92000 15556			23
	4/2/96	2755	364 481	364 *481	105
	4/3/96	330	330	330	<u> </u>
	7/9/96	840	330	330	30
			510	510	50
Whitehouse Pool	9/12/95	330	230	230	*244
	12/4/95	3500	490	330	*410
	12/5/95	330	49	49	*520
	12/6/95	32	13	13	*134
	12/9/95	230	13	17	*120
	1/16/96	54000	*17000	*17000	*820
	1/17/96	4900	130	130	100

1/18/96	92000	*4900	*4900	*570
1/31/96	3300	1700	*1700	*180
2/11/96	4900	310	68	
3/11/96	1967	602	*602	60
3/12/96	3300	1700	*1700	*110
3/13/96	773	137	137	28
3/18/96	2200	130	130	<10
4/1/96	160000	*17000	*17000	*510
4/2/96	1700	490	*490	*120
4/3/96	2200	1100	*1100	30
4/8/96	1044	749	*470	39
7/9/96	1300	490	*490	20

Location	Date Sampled	Total Coliform	Fecal Coliform	E. coli	Enterococcus
Milepost 25.86	9/12/95	193	28	28	64
	12/4/95	16000	460	*460	*110
	12/5/95	170	7.8	7.8	10
	12/6/95	24	<1.8	<1.8	*130
	12/9/95	330	4	4	60
	1/16/96	2300	110	68	90
	1/17/96	4900	20	20	80
	1/18/96	54000	490	*479	*380
	1/31/96	130	20	20	<10
	2/11/96	1700	<18	<18	
	3/11/96	390	81	19	14
	3/12/96	1100	330	170	30
	3/13/96	170	<18	<18	<10
	3/18/96	700	68	68	<10
	4/1/96	4900	130	130	*150
	4/2/96	210	<18	<18	<10
	4/3/96	640	18	18	10
	4/8/96	490	20	20	10
	7/9/96	490	156	156	25
Milepost 28.29	9/12/95	2400	790	*790	*530
	12/4/95	9200	330	330	*770
	12/5/95	490	49	33	*770
	12/6/95	79	33	33	*500
	12/9/95	220	5.5	5.5	*126
	1/16/96	7000	1300	*1300	*250
	1/17/96	1400	20	20	30
	1/18/96	5400	1300	*1300	*660
	1/31/96	790	20	20	<10
	2/11/96	790	130	130	
	3/11/96	200	45	45	10
	3/12/96	490	45	45	<10
	3/13/96	340	<18	<18	10
	3/18/96	1300	40	40	<10
	4/1/96	220	78	78	90
	4/2/96	260	<18	<18	<10
	4/3/96	260	45	45	10
	4/8/96	700	<18	<18	50
	7/9/96	790	68	68	80

Table 17. Bacteriological monitoring results<sup>18</sup> for stations in the western shoreline watersheds.

 $<sup>^{18}</sup>$  A value preceded by an "\*" exceeds the respective water quality standard: Fecal Coliform = 2000 MPN; E. coli = 406 MPN; Enterococcus = 108 MPN.

Milepost 28.86	9/12/95	1196	252	252	*276
	12/4/95	16000	490	330	*850
	12/5/95	>230	79	79	*230
	12/6/95	330	67	46	*198
	12/9/95	216	63	63	*230
	1/16/96	13183	*3300	*832	*2018
	1/17/96	3300	790	*490	30
	1/18/96	14000	*4900	*4900	*4870
	1/31/96	330	130	130	40
	2/11/96	481	276	276	
	3/11/96	1700	460	*460	50
	3/12/96	6400	140	110	*35(
	3/13/96	790	40	40	*150
	3/18/96	1487	173	173	17
	4/1/96	11000	1200	*1200	*310
	4/2/96	790	78	78	50
	4/3/96	4900	<18	18	60
	4/8/96	330	110	<18	20
	7/9/96	1348	156	156	*420
Teachers Beach Ck	9/12/95	93	2	2	54
	12/4/95	9200	70	70	*120
	12/5/95		6.8	6.8	*16
	12/6/95	170	6.8	6.8	*180
	12/9/95	460	2	2	10
	1/16/96	4900	330	330	*140
	1/17/96	790	<18	<18	20
	1/18/96	7900	700	*700	*430
	1/31/96	40	18	18	<10
	2/11/96	1300	20	20	
	3/11/96	639	6	6	10
	3/12/96	1615	198	173	14
	3/13/96	790	<18	<18	<10
	3/18/96	230	<18	<18	10
	4/1/96	1300	110	110	80
	4/2/96	2800	130	130	90
	4/3/96	2800	<18	<18	<10
	4/8/96	1100	<18	<18	<
	7/9/96	330	230	230	30
White Gulch	9/12/95	170	170	170	*490
	12/5/95	16000	700	220	*490
	12/3/93				*140
		3500	46	46	
	1/17/96 2/11/96	9200 2200	33	33	10
	3/11/96	12133	33	33	1/
	3/11/96	12133	230	230	10

3/13/96	2214	34	34	10
3/18/96	9200	13	13	<10
4/1/96	>16000	490	230	*760
4/2/96	1400	130	130	70
4/3/96	16000	79	79	20
4/8/96	38367	43	43	85
7/9/96	2400	230	230	*155

Table 18. Correlation coefficients for paired comparisons of the log-transformed concentrations of total coliform, fecal coliform, *E. coli*, and *enterococcus* for tributary water samples.

	Total Coliform	Fecal Coliform	E. coli	Enterococcus
Total Coliform	1	0.86	0.86	0.53
Fecal Coliform	0.86	1	0.99	0.60
E. coli	0.86	0.99	1	0.59
Enterococcus	0.53	0.60	0.59	1

Table 19. Correlation coefficients for paired comparisons of the log-transformed concentrations for total coliform, fecal coliform, *E. coli*, and *enterococcus* from Bay water samples.

	Total Coliform	Fecal Coliform	E. coli	Enterococcus
Total Coliform	1	0.90	0.89	0.63
Fecal Coliform	0.90	1	0.99	0.68
E. coli	0.89	0.99	1	0.66
Enterococcus	0.63	0.68	0.66	1

Date Sampled	Sample Site	Duplicate #1 (FC MPN)	Duplicate #2 (FC MPN)
Field Duplicates:			
09/12/95	MP 28.86	490	130
09/12/95	Seawater Control	1.8	1.8
09/12/95	WQ #1	1.8	11
09/12/95	WQ#45	1.8	1.8
12/4/95	WQ#45	18	18
12/4/95	WQ#6	20	20
12/5/95	Walker Ck. Ranch	1.8	2
12/5/95	WQ#39	26	49
12/5/95	WQ#4	1.8	2
12/5/95	WQ#7	1.8	2
12/6/95	MP 28.86	41	110
12/6/95	WQ#12	4.5	13
12/6/95	WQ#32	24	33
12/6/95	WQ#43	1.8	17
12/9/95	MP 28.29	17	1.8
12/9/95	MP 28.86	31	130
12/9/95	WQ#42	110	95
1/16/96	Keyes Creek, WWTP	7000	22000
1/16/96	WQ#1	16000	16000
1/16/96	WQ#6	70	130
1/17/96	MP 32.12	160000	160000
1/17/96	WQ#30	490	2200
1/17/96	WQ#4	460	330
1/17/96	WQ#46	1700	1200
1/18/96	Olema Creek	35000	35000
1/18/96	WQ#44	230	1100
2/11/96	Keyes Creek, Irvin Road	2300	11000
2/11/96	MP 28.86	330	230
2/11/96	MP 36.17	130	110
2/11/96	MP 40.35	170	78
2/11/96	Olema Creek	330	110
3/11/96	MP 25.86	330	20

Table 20. Paired replicate fecal coliform data for field duplicates and laboratory duplicates.

3/11/96	Teacher's Beach Creek	18	2
3/11/96	Walker/Keyes Creek	35000	35000
3/12/96	MP 32.12	160000	160000
3/12/96	WQ#6	49	33
3/12/96	WQ#7	49	130
3/12/96	WQ#9	490	130
3/13/96	Lagunitas Creek	78	61
3/13/96	MP 40.35	7900	4900
3/13/96	White Gulch	49	23
3/13/96	Whitehouse Pool	170	110
3/18/96	Bear Valley Creek	20	130
3/18/96	Grand Canyon Creek	260	460
3/18/96	MP 28.86	130	230
3/18/96	MP 36.17	2200	2200
4/1/96	MP 36.17	54000	17000
4/1/96	MP 40.35	160000	160000
4/1/96	Tomasini Creek lower	7000	14000
4/1/96	WQ#44	330	1400
4/2/96	MP 32.12	160000	92000
4/2/96	MP 34.95	9500	2300
4/2/96	WQ#30	5400	16000
4/2/96	WQ#43	3500	9200
4/3/96	MP 40.35	54000	54000
4/3/96	Walker Creek	1400	2300
4/3/96	WQ#4	23	79
4/3/96	WQ#45	14	11
4/8/96	MP 25.86	20	18
4/8/96	Olema Ck	490	330
4/8/96	Whitehouse Pool	170	3300
4/8/96	WQ#32	130	23
7/9/96	MP 25.86	220	11(
7/9/96	Millerton Creek	1100	45
7/9/96	WQ#30	1.8	1.8
7/9/96	WQ#4	1.8	1.8

Table 21. Coliphage results<sup>19</sup> for special indicator stations in Tomales Bay.

Sample Site	Date Sampled	Coliphage
Keyes Creek, WWTP	09/12/95	310
	12/05/95	22
	12/09/95	10
	02/11/96	2
	03/12/96	12
	03/18/96	12
	04/02/96	6
	04/08/96	<2
	07/09/96	42
Walker Creek	09/12/95	42 25 <2
	12/09/95	<2
	12/09/95	6
	02/11/96	<2
	03/12/96	26
	03/18/96	16
	04/02/96	8
	04/08/96	6
	07/09/96	10
WQ Station #44	09/12/95	<2
	12/05/95	<2
	12/09/95	<2
	02/11/96	2 2 2 2 2 2
	03/12/96	<2
	03/18/96	12
	04/02/96	2
	04/08/96	
	07/09/96	<2
White Gulch	09/12/95	40
	12/05/95	2 2 40 2 2
	12/09/95	<2
	02/11/96	4
	03/12/96	10
	03/18/96	10

<sup>&</sup>lt;sup>19</sup> Plaque-forming units per 100 mL.

	04/02/96	<2
	04/08/96	<2
	07/09/96	8
Seawater Control	09/12/95	5
	12/05/95	2
	02/11/96	<2
	03/12/96	<2
	03/18/96	4
	04/02/96	<2
	04/08/96	<2
	07/09/96	<2

Sample Site	Date	Anaerobes	Bacteroides	Bacteroides
	Sampled	000	species	vulgatus
Keyes Creek, WWTP	09/12/95	800	0	0
	12/05/95	1900	200	0
	12/09/95	710	0	0
	02/11/96	1500	0	0
	03/12/96	670	0	0
	03/18/96	1500	20	0
	04/02/96	320	30	0
	04/08/96	210	0	0
	07/09/96	787	105	0
Walker Creek	09/12/95	300	0	0
	12/09/95	270	20	0
	12/09/95	2000	180	0
	02/11/96	1000	20	0
	03/12/96	430	30	0
	03/18/96	1300	120	0
	04/02/96	560	0	0
	04/08/96	90	0	0
	07/09/96	650	0	0
WQ Station #44	09/12/95	0	0	0
	12/05/95	400	10	0
	12/09/95	0	0	0
	02/11/96	1300	0	0
	03/12/96	500	0	0
	03/18/96	500	150	0
	04/02/96	230	0	0
	04/08/96	200	0	0
	07/09/96	210	0	0
White Gulch	09/12/95	1700	0	0
	12/05/95	2000	0	0
	12/09/95	540	0	0
	02/11/96	1000	0	0
	03/12/96	1600	0	0
	03/12/90	4300	0	0
	05/10/20	4300	0	0

Table 22. Anaerobic bacteria results<sup>20</sup> for special indicator stations in Tomales Bay.

<sup>&</sup>lt;sup>20</sup> Colony-forming units per 100 mL.

	04/02/96	450	0	0
	04/08/96	70	0	0
	07/09/96	340	0	0
Seawater Control	09/12/95	1200	0	0
	12/05/95	100	0	0
	02/11/96	1600	0	0
	03/12/96	1400	0	0
	03/18/96	300	0	0
	04/02/96	100	0	0
	04/08/96	250	0	0
	07/09/96	65	0	0

Sample Site	Date Sampled	Total Coliform	Fecal Coliform	E. coli	Enterococcus
WQ Station #1	9/12/95	5	5	5	7
	12/4/95	45	*45	45	<10
	12/5/95	17	*17	17	20
	12/6/95	33	*33	33	<10
	12/9/95	13	5	4.5	10
	1/16/96	16000	* <sup>1</sup> 16000	16000	<sup>eb</sup> 890
	1/17/96	2400	* <sup>f</sup> 330	330	70
	1/18/96				
	1/31/96	95	*70	70	40
	2/11/96	49	*22	17	
	3/11/96	3500	* <sup>f</sup> 3500	1800	<10
	3/12/96	5400	* <sup>f</sup> 5400	5400	<sup>b</sup> 200
	3/13/96	170	*79	79	<10
	3/18/96	9200	2	2	<10
	4/1/96	9500	* <sup>†</sup> 490	330	10
	4/2/96	2400	* <sup>f</sup> 790	790	10
	4/3/96	790	*79	70	<10
	4/8/96	7.8	4.5	4.5	<10
	7/9/96	2	<1.8	<1.8	<10
WQ Station #12	9/12/95	2.7	1.9	1.9	<1
	12/4/95	710	*73	55	79
	12/5/95	8	2	2	10
	12/6/95	12	8	7.6	10
	12/9/95	8	1.8	2	<10
	1/16/96	16000	* <sup>f</sup> 2800	1800	50
	1/17/96	2200	** 280	280	60
	1/18/96	16000	* <sup>1</sup> 1300	790	ь 170
	1/31/96	1300	*79	79	50
	2/11/96	330	*49	49	
	3/11/96	49	*17	17	<10
	3/12/96	1700	*110	110	20
	3/13/96	460	*79		<10
	3/18/96	6.1	2.7	1.7	10
	4/1/96	140	*22	17	40
	4/2/96	>16000	* <sup>f</sup> 2200	700	90
	4/3/96	270	*92	91	10
	4/8/96	3.7	<1.8	<1.8	<10
	7/9/96	2	<1.8	<1.8	<10
WQ Station #30	9/12/95	7.8	7.8	7.8	5
-	12/4/95	790	*170	130	<sup>b</sup> 200

Table 23. Bacteriological monitoring results<sup>21</sup> for water quality stations in Tomales Bay.

 <sup>&</sup>lt;sup>21</sup> A value preceded by an "\*" exceeds the shellfish growing area water quality standard: Fecal Coliform = 14 MPN.
 <sup>f</sup> This fecal coliform value exceeds the ocean water contact standard of 200 MPN.
 <sup>e</sup> This Enterococcus value exceeds the ocean water light contact standard of 276 MPN.

<sup>&</sup>lt;sup>b</sup> This Enterococcus value exceeds the ocean water designated beach standard of 104 MPN.

	12/5/95	79	*23	23	30
	12/3/93	26	*23	16.9	
	12/0/95	33	*23	33	14
	1/16/96		- 33		10
	1/17/96	16000	* <sup>f</sup> 1040	1000	68
	1/18/96	10000	1040	1000	00
	1/31/96	2200	* <sup>†</sup> 790	490	<sup>b</sup> 210
	2/11/96	490	* 790	490 110	210
	3/11/96	490 880	*110		10
	3/11/96	3500	*170	160 490	<u> </u>
	3/12/90		* 700 * <sup>†</sup> 790		
	3/13/96	790 116	·· /90 9.4	490 7.3	<u> </u>
	4/1/96		9.4 * <sup>1</sup> >16000		
	4/1/96	>16000	* >16000 * <sup>†</sup> 9295	>16000	070
		16000	* 9293 * <sup>1</sup> 1400	5900	<sup>b</sup> 240
	4/3/96	2800		1400	<10
	4/8/96	170	*49	33	<10
WO St. A #22	7/9/96 9/12/95	1.8	1.8	1.8	10
WQ Station #32		4	1.8 *20	1.8	1
	12/4/95	45	*20 *46	20 21	80
	12/5/95	490			40
	12/6/95	36	*28	20	35
	12/9/95	3	1.8 * <sup>†</sup> 9200	1.8	10 eb 280
	1/16/96	>16000		9200	<sup>eb</sup> 380
	1/17/96	700	*110	49	10
	1/18/96 1/31/96	700	* <sup>†</sup> 790	220	00
	2/11/96	790 790	* 790 * <sup>1</sup> 330	220 230	90
	3/11/96	460	* 460	310	<10
	3/11/96	3500	* 400 * 1700	1100	<10
			* 1700		<10 b 120
	3/13/96 3/18/96	3500 29		790 <1.8	b 120 <10
		460	<1.8 *33		
	4/1/96 4/2/96	3500	*33 * <sup>1</sup> 950	33 950	<10 40
	4/2/90	130	* 930 *79	930 79	
		73	*79	32	<10 10
	4/8/96 7/9/96		4.5		<10
WQ Station #34	9/12/95	6.8 7.8	4.5	4.5 7.8	<10
WQ Station #34	12/4/95	130	*78	45	10
	12/4/93	36	*29	43	24
	12/3/93	330	*110		24 50
	12/9/95 1/16/96	13 >16000	13 * <sup>†</sup> >16000	13 >16000	<10 eb 1900
	1/17/96	>16000	* <sup>1</sup> >16000	>16000	100
	1/18/96	1100	* <sup>†</sup> 220	120	00
	1/31/96	1100	* 220 * <sup>†</sup> 1100	130	80
	2/11/96	1700		1100	40
	3/11/96	>16000	* <sup>t</sup> >16000	>16000	40 eb 1080
	3/12/96	>16000	* <sup>t</sup> >16000	>16000	1000
	3/13/96	1600	* <sup>f</sup> 1200	930	53

	3/18/96	140	*17	17	<10
	3/18/96	5400	*17 *1700	17 1300	
	4/1/96	>16000	* 1/00 * <sup>†</sup> >16000	>16000	<u> </u>
	4/2/90	16000	* >10000 *79	>10000 79	
	4/3/96	1300	*79 *1790	79	<10 10
	7/9/96	1.8	1.8	1.8	10
WQ Station #39	9/12/95	<1.8	<1.8 *20	<1.8	l
	12/4/95	45		20	<10
	12/5/95 12/6/95	40	*36	23 4.5	54 <10
	12/0/93	25	8	4.5	
	1/16/96	>16000	* <sup>1</sup> 9200	9200	<10 <sup>b</sup> 140
	1/17/96	2800	* 9200 * <sup>1</sup> 950	9200	
	1/17/96	2800	* 950	950	40
	1/18/96	5400	*23	13	20
	2/11/96	22	*23	15	20
	3/11/96	>16000	*1>16000	11	<10
	3/11/90	16000	* <sup>†</sup> 9200	9200	<10 <sup>b</sup> 130
	3/12/90	790	* 9200	9200	<10
	3/13/90	517	1.8	1.8	<10 10
	4/1/96	2200	*170	1.8	40
	4/1/90	9200	* <sup>1</sup> 1400	1400	28
	4/3/96	1300	*170	1400	<10
	4/3/90	7.8		<1.8	<10
	7/9/96	2	2	<1.8	<10
WQ Station #4	9/12/95	<1.8	<1.8	<1.8	10
WQ Station #4	12/4/95	40	<1.0	1.8	<10
	12/5/95	1.8	1.8	1.9	10
	12/6/95	1.8	1.8	2	<10
	12/9/95	8	8	7.8	<10
	1/16/96	6700	* <sup>f</sup> 2200	1800	100
	1/17/96	12000	* <sup>f</sup> 390	380	24
	1/18/96	12000	570	500	21
	1/31/96	79	11	11	50
	2/11/96	33	7	6.8	
	3/11/96	33	1.8	2	<10
	3/12/96	33	5	4.5	<10
	3/13/96	2200	* <sup>†</sup> 330	330	<10
	3/18/96	6.8	<1.8	<1.8	<10
	4/1/96	>16000	* <sup>†</sup> 3500	3500	60
	4/2/96	16000	* <sup>f</sup> 2200	2100	63
	4/3/96	73	*43	43	10
	4/8/96	<1.8	<1.8	<1.8	<10
	7/9/96	1.8	1.8	1.8	10
WQ Station #42	9/12/95	17	*17	17	6
	12/4/95	<18	*<18	<18	
	12/5/95	170	*70	26	ь 120
	12/6/95	330	*79	79	90
	12/9/95	160	*100	83.7	<sup>b</sup> 210

	1/16/96				
	1/17/96	>16000	* <sup>f</sup> 310	310	50
	1/18/96	>10000	510	510	50
	1/31/96	330	* <sup>f</sup> 330	330	<sup>b</sup> 130
	2/11/96	110	*110	70	150
	3/11/96	33	8	7.8	<10
	3/12/96	3500	* <sup>f</sup> 2400	2400	<10
	3/13/96	230	*79	49	10
	3/18/96	4	2	2	<10
	4/1/96	1700	** 230	230	20
	4/2/96	700	* <sup>f</sup> >220	>140	10
	4/3/96	280	* <sup>f</sup> 280	280	<10
	4/8/96	16	3	3	10
	7/9/96	46	*23	23	<10
WQ Station #43	9/12/95	<1.8	<1.8	<1.8	<1
WQ Station #45	12/4/95	<18	<1.0	<1.0	20
	12/5/95	17	*17	11	<10
	12/6/95	6	5	5.5	10
	12/9/95	5	5	4.5	20
	1/16/96	>16000	* <sup>f</sup> 16000	5400	<sup>eb</sup> 520
	1/17/96	1400	* <sup>f</sup> 330	330	60
	1/18/96	1400	550	550	00
	1/31/96	790	*130	130	20
	2/11/96	49	130	130	20
	3/11/96	700	** 330	170	<10
	3/12/96	3500	* <sup>f</sup> 3500	3500	20
	3/13/96	330	*130	130	10
	3/18/96	17	<1.8	<1.8	<10
	4/1/96	>16000	** 1800	1800	<sup>b</sup> 260
	4/2/96	16000	* <sup>†</sup> 5700	3900	10
	4/3/96	280	*79	49	<10
	4/8/96	2	2	2	<10
	7/9/96	1.8	<1.8	<1.8	<10
WQ Station #44	9/12/95	7	.5	5	
	12/5/95	4	<1.8	<1.8	10
	12/9/95	170	7	6.8	<10
	1/16/96	1800	*79	79	40
	1/17/96	3500	* <sup>f</sup> 330	330	12
	1/18/96	3900	* <sup>†</sup> 500	500	<sup>b</sup> 140
	1/31/96	130	13	13	10
	2/11/96	33	*23	13	-
	3/11/96	26	8	7.8	<10
	3/12/96	27	13	7.8	<10
	3/13/96	840	* <sup>f</sup> 200	160	10
	3/18/96	5400	*110	110	<10
	4/1/96	16000	** 680	680	<sup>eb</sup> 350
	4/2/96	1700	* <sup>f</sup> 1100	230	60
	4/3/96	5400	*170	110	<10
	4/8/96	1.8	<1.8	<1.8	<10

	7/9/96	2	<1.8	<1.8	<10
WQ Station #45	9/12/95	3	1.8	<1.8	<1
	12/4/95	28	*18	<18	10
	12/5/95	22	14	14	20
	12/6/95	1.8	1.8	2	<10
	12/9/95	1.8	<1.8	<1.8	<10
	1/16/96	2200	*22	22	30
	1/17/96	1100	*79	79	40
	1/18/96				
	1/31/96	230	*22	22	20
	2/11/96	213	*56	56	
	3/11/96	33	13	13	<10
	3/12/96	15	12	11	10
	3/13/96	330	*170	170	<10
	3/18/96	2	2	2	<10
	4/1/96	3500	*17	17	10
	4/2/96	>16000	* <sup>f</sup> 3500	3500	10
	4/3/96	148	12.4	11	14
	4/8/96	2	2	2	<10
	7/9/96	<1.8	<1.8	<1.8	<10
WQ Station #46	9/12/95	1.8	1.8	2	<1
	12/4/95	45	*<18	<18	20
	12/5/95	1.8	1.8	1.9	10
	12/6/95	5	1.8	<1.8	<10
	12/9/95	5	1.8	2	10
	1/16/96				
	1/17/96	12000	* <sup>f</sup> 1400	1500	<sup>eb</sup> 380
	1/18/96	2800	* <sup>f</sup> 220	220	50
	1/31/96	23	*23	23	30
	2/11/96	<1.8	<1.8	<1.8	
	3/11/96	2	<1.8	<1.8	<10
	3/12/96	220	* <sup>f</sup> 200	<1.8	100
	3/13/96	196	*51	51	10
	3/18/96	17	<1.8	<1.8	
	4/1/96	2200	<1.8	<1.8	<10
	4/2/96	41	4.5	4.5	
	4/3/96	350	*130	130	<10
	4/8/96	2	<1.8	<1.8	<10
	7/9/96	<1.8	<1.8	<1.8	<10
WQ Station #6	9/12/95	<1.8	<1.8	<1.8	<1
	12/4/95	30	*20	20	10
	12/5/95	33	2	<1.8	<10
	12/6/95	<1.8	<1.8	<1.8	<10
	12/9/95	<1.8	<1.8	<1.8	<10
	1/16/96	170	*95	95	eb 1200
	1/17/96	>16000	* <sup>f</sup> 2100	2100	<sup>eb</sup> 1200
	1/18/96			10	
	1/31/96	79	*49	13	30
	2/11/96	170	*33	33	

	2/11/06	100	* ( 1	(1	10
	3/11/96	180	*64	64	10
	3/12/96	62	*40	29	10
	3/13/96	700	* <sup>f</sup> 460	460	10
	3/18/96	34	4 *1210	4	<10
	4/1/96	950	* <sup>f</sup> 310	310	40
	4/2/96	16000	* <sup>f</sup> 790	790	10
	4/3/96	40	*21	21	<10
	4/8/96	4	<1.8	<1.8	<10
	7/9/96	<1.8	<1.8	<1.8	
WQ Station #7	9/12/95	<1.8	<1.8	<1.8	<1
	12/4/95	<18	*<18	1.0	<10
	12/5/95	3	2	1.9	10
	12/6/95	1.8	1.8	1.8	<10
	12/9/95	<1.8	<1.8	<1.8	<10
	1/16/96	1 1000	. f a <b>a</b> a a		
	1/17/96	>16000	* <sup>†</sup> 9200	9200	97
	1/18/96	1000	, f e e e	100	h 100
	1/31/96	1300	* <sup>f</sup> 230	130	<sup>ь</sup> 190
	2/11/96	79	11	11	
	3/11/96	330	* <sup>f</sup> 230	230	10
	3/12/96	100	*80	62	38
	3/13/96	3500	* <sup>f</sup> 1100	280	<10
	3/18/96	4.5	<1.8	<1.8	<10
	4/1/96	>16000	* <sup>f</sup> 920	920	80
	4/2/96	1700	* <sup>f</sup> 700	700	<10
	4/3/96	1700	*170	110	<10
	4/8/96	6.8	4.5	4.5	<10
	7/9/96	<1.8	<1.8	<1.8	<10
WQ Station #9	9/12/95	1.8	1.8	2	1
	12/4/95	51	*18	18	20
	12/5/95	4	2	2	20
	12/6/95	<1.8	<1.8	<1.8	20
	12/9/95	<1.8	<1.8	<1.8	10
	1/16/96				
	1/17/96	>16000	* <sup>f</sup> 3500	3500	<sup>eb</sup> 1300
	1/18/96		-		
	1/31/96	5400	* <sup>f</sup> 2200	470	210
	2/11/96	800	*170	170	
	3/11/96	1300	* <sup>f</sup> 330	330	20
	3/12/96	740	* <sup>f</sup> 260	26	20
	3/13/96	2400	* <sup>f</sup> 1300	350	20
	3/18/96	<1.8	<1.8	<1.8	<10
	4/1/96	16000	* <sup>1</sup> 16000	16000	<sup>eb</sup> 410
	4/2/96	16000	* <sup>1</sup> 3500	3400	ев 55000
	4/3/96	170	*79	79	<10
	4/8/96	130	14	9.3	10
	7/9/96	<1.8	<1.8	<1.8	<10
Control	12/9/95				
	1/17/96	170	14	9.2	<10

2/11/96	1.8	1.8	2	
3/12/96	5	1.8	<1.8	<10
3/18/96	<1.8	<1.8	<1.8	<10
4/2/96	<1.8	<1.8	<1.8	<10
4/8/96	2	<1.8	<1.8	<10
7/9/96	23	7.8	7.8	<10

Sample Site	Date Sampled	Fecal C	oliform	Entero	Enterococcus		E. coli	
		Creek	Bay	Creek	Bay	Creek	Bay	
Milepost 34.95 &	9/12/95		1.8		1		<1.8	
Bay Station #7	12/4/95		*18		10			
	12/5/95		2		10		1.9	
	12/6/95		1.8		10		1.8	
	12/9/95		1.8		10		<1.8	
	1/16/96	*13000		*1540		*13000		
	1/17/96	*2800	*9200	10	97	*2800	9200	
	1/18/96	*160000		*720		*160000		
	1/31/96	*4900	*230	*360	*190	*3300	130	
	2/11/96	790	11			*490	11	
	3/11/96	*7000	*230	*160	10	*3300	230	
	3/12/96	*3300	*80	60	38	*3300	62	
	3/13/96	*17000	*1100	70	10	*3500	280	
	3/18/96	110	1.8	10	10	110	<1.8	
	4/1/96	*35000	*920	20	80	*35000	920	
	4/2/96	*9500	*700	*180	10	*9500	700	
	4/2/96	*2300	*170	*210	10	*2300	110	
	4/3/96	*2300	4.5	*320	10	*2300	4.5	
	4/8/96	*3300	1.8	*380	10	*3300	<1.8	
	7/9/96							
Milepost 36.17 &	9/12/95	511	1.8	*128	1	*511	<1.8	
Bay Station #6	12/4/95	*2200	*20	*650	10	*2200	20	
	12/5/95	33	2	*150	10	33	<1.8	
	12/6/95	17	1.8	*120	10	17	<1.8	
	12/9/95	230	1.8	90	10	1.8	<1.8	
	1/16/96	*3300	*95	*440	53	*3300	95	
	1/17/96	790	*2100	30	*1200	*790	2100	
	1/18/96	*4600		*980		*4600		
	1/31/96	330	*49	80	30	330	13	
	2/11/96	120	*33			96	33	
	3/11/96	490	*64	10	10	130	64	
	3/12/96	*3704	*40	*174	10	*3704	29	
	3/13/96	330	*460	20	10	110	460	
	3/18/96	*2200	4	22	10	*2200	4	
	4/1/96	*30298	*310	*420	40	*11000	310	
	4/2/96	790	*790	*150	10	*790	790	
	4/3/96	490	*21	50	10	*490	21	
	4/8/96	1100	1.8	10	10	260	<1.8	
	7/9/96	45	1.8	*1700	10	45	<1.8	
Milepost 38.54 &	9/12/95	78	1.9	*117	1	78	1.9	
Bay Station #12	12/4/95	1663	*73	*455	79	*790	55	

Table 24. Comparison of Bay and watershed bacteriological monitoring results<sup>22</sup>.

<sup>22</sup> \* indicates samples that exceeded water quality standards/criteria

	12/5/95	79	2	*150	10	79	2
	12/6/95	33	8	60	10	33	7.6
	12/9/95	11	1.8	30	10	11	2
	1/16/96	490	*2800	*300	50	*490	1800
	1/17/96	110	*280	70	60	110	280
	1/18/96	*2300	*1300	*270	*170	*2300	790
	1/31/96	110	*79	40	50	110	79
	2/11/96	110	*49	0	50	110	49
	3/11/96	78	*17	10	10	78	17
	3/12/96	1300	*110	50	20	*1300	110
	3/13/96	78	*79	10	10	78	79
	3/18/96	20	*15	10	10	20	1.7
	4/1/96	*8400	*22	*1900	40	*8400	17
	4/2/96	790	*2200	*140	90	*790	700
	4/3/96	55	*92	37	10	55	91
	4/8/96	1300	1.8	*130	10	*1300	<1.8
	7/9/96	*3300	1.8	70	10	*2300	<1.8
Milepost 40.35 &	9/12/95		1.8		1		<1.8
Bay Station #4	12/4/95		18	*580	10		18
	12/5/95		1.8		10		1.9
	12/6/95	1.8	1.8	20	10	1.8	2
	12/9/95	1.8	8	20	10	1.8	7.8
	1/16/96	*22000	*2100	*1760	100	*22000	1800
	1/17/96	*4900	*380	*230	24	*4900	380
	1/18/96	*160000		*3450		*160000	
	1/31/96	*2100	11	*120	50	*1400	11
	2/11/96	115	7			70	6.8
	3/11/96	*3300	1.8	*160	10	*2300	2
	3/12/96	*35000	5	*670	10	*11000	4.5
	3/13/96	*6222	*330	*484	10	*4900	330
	3/18/96	330	1.8	50	10	330	<1.8
	4/1/96	*160000	*3500	10	60	*160000	3500
	4/2/96	*54000	*2100	*370	63	*54000	2100
	4/3/96	*54000	*43	*515	10	*36000	43
	4/8/96	*160000	1.8	*470	10	*160000	<1.8
	7/9/96	45	1.8	40	10	45	1.8
Milepost 28.86 &	9/12/95	252	7.8	*276	5	28	252
Bay Station #30	12/4/95	490	*170	*850	*200		330
	12/5/95	79	*23	*230	30	7.8	79
	12/6/95	67	*23	*198	14	1.8	46
	12/9/95	63	*33	*230	10	4	63
	1/16/96	*3300		*2018		68	*832
	1/17/96	790	*1100	30	68	20	*490
	1/18/96	*4900		*4870		*479	*4900
	1/31/96	130	*790	40	*210	20	130
	2/11/96	276	*110			18	276
	3/11/96	460	*170	50	10	19	*460
	3/12/96	140	*700	*350		170	110
	3/13/96	40	*790	*150	31	18	40

3/18/96	173	11	17	10	68	173
4/1/96	1200	*16000	*310	*670	130	*1200
4/2/96	78	*9200	50	*240	18	78
4/3/96	18	*1400	60	10	18	18
4/8/96	44	*49	26	10	20	18
7/9/96	156	1.8	*420	10	156	156

Sample Site	Date Sampled	Total Coliform	Fecal Coliform	E. coli	Enterococcus
#M430-02	9/12/95	16000	<sup>o24</sup> 1700	>16000	700
	12/5/95	7900	<sup>c25</sup> 4900	2800	4900
	12/9/95	23000	330	210	330
	1/17/96	70000	°2300	2800	230
	1/31/96	14000	°1700		1700
	2/11/96	46000	°23000	22000	23000
	3/12/96	46000	°31000	2200	31000
	3/18/96	540000	°2300	13000	2300
	4/2/96	70000	°46000	3300	
	4/8/96	1100	330	40	17000
	7/9/96	23000	78		78
#M430-02-B	9/12/95	>16000	220	220	170
	12/5/95	7000	220	790	220
	12/9/95	4300	°3100	35000	3100
	1/17/96	130000	°4900	1800	3300
	1/31/96	4600	°1300		1300
	2/11/96	13000	490	790	2200
	3/12/96	35000	°24000	340	24000
	3/18/96	540000	330	790	330
	4/2/96	64000	°31000	3300	
	4/8/96	3300	45	<18	8900
	7/9/96	790	<18		<18
#M430-05	9/12/95	2200	<18	<18	<18
	12/5/95	230	45	45	2
	12/9/95	330	45	20	18
	1/17/96	23000	°2200	320	2200
	1/31/96	7900	°1700		1700
	2/11/96	7000	°3300	1300	1300
	3/12/96	3300	230	78	230
	3/18/96	79000	330	130	170

Table 25. Bacteriological monitoring results<sup>23</sup> for shellfish stations in Tomales Bay.

The National Shellfish Sanitation Program does not recommend a coliform concentration standard for shellfish in a certified growing area. <sup>24</sup> Sample exceeds approximate 95% confidence interval (70 to 700 MPN) for NSSP market standard (230 MPN)

during "Open" harvest status. <sup>25</sup> Sample exceeds approximate 95% confidence interval for NSSP market standard during "Closed" harvest status.

	490	°13000	70000	4/2/96	
10700	<18	93	2300	4/8/96	
<18		<18	2300	7/9/96	
20	20	20	9200	9/12/95	# <b>M430-06</b>
<1.8	20	<1.8	79	12/5/95	
20	68	20	23000	12/9/95	
700	490	700	4900	1/17/96	
790		790	3300	1/31/96	
210	210	430	3500	2/11/96	
1700	130	°1700	4900	3/12/96	
<18	<18	<18	22000	3/18/96	
	460	170	17000	4/2/96	
6700	20	20	330	4/8/96	
<18		<18	23000	7/9/96	
230	170	330	>16000	9/12/95	#M430-15
4900	130	°4900	4900	12/5/95	
230	170	230	700	12/9/95	
950	1700	°1400	11000	1/17/96	
3300		°3300	7900	1/31/96	
2800	1800	°7900	31000	2/11/96	
33000	78	°33000	33000	3/12/96	
490	45	490	4600	3/18/96	
	1300	°7900	13000	4/2/96	
6600	18	130	330	4/8/96	
230		230	230	7/9/96	

**FIGURES 1 - 59** 

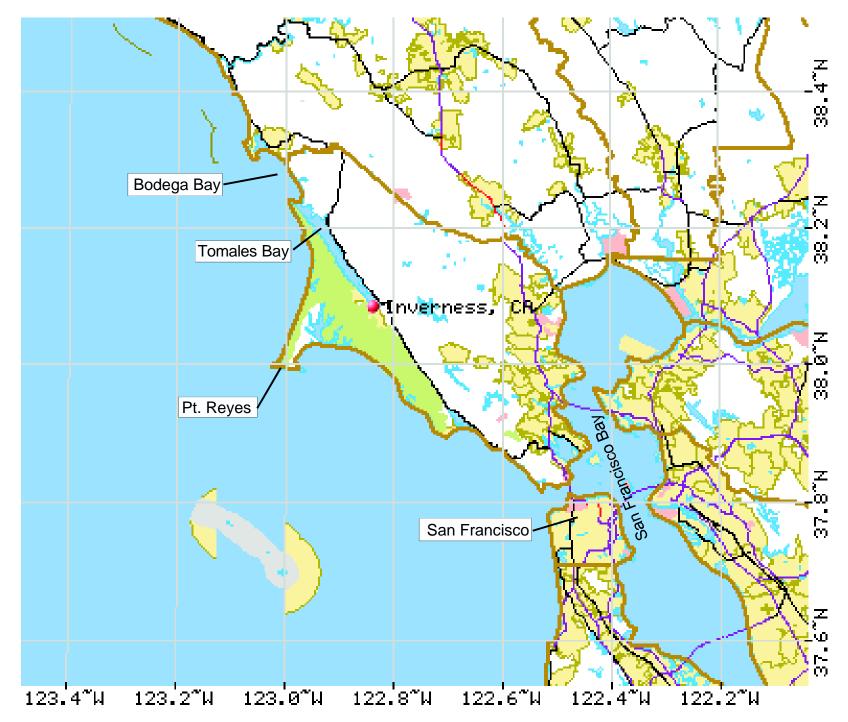


Figure 1. Location of Tomales Bay, Marin County, California (U.S. Census Tiger Map).

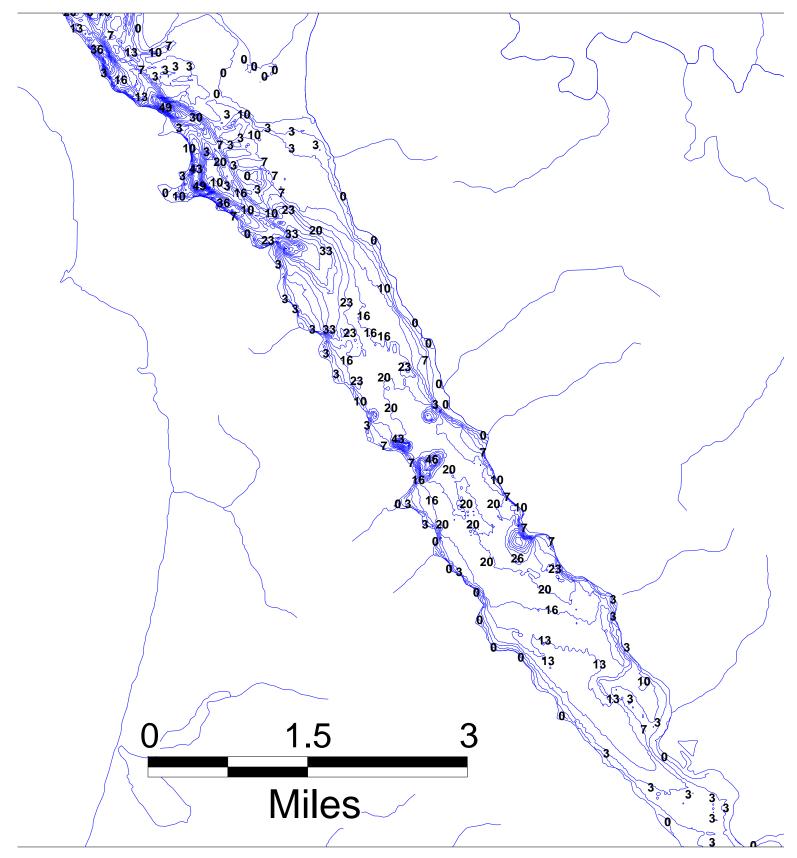


Figure 2. Bathymetry of Tomales Bay, California (NOAA, 1993).

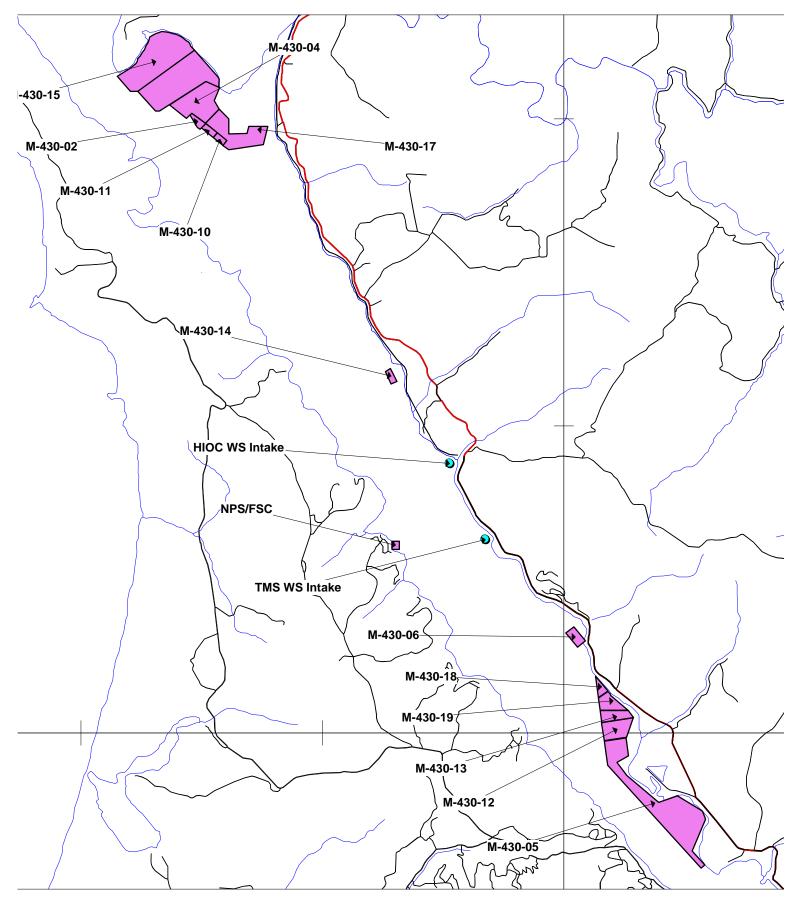


Figure 3. General location of commercial shellfish growing area leases in Tomales Bay, California.

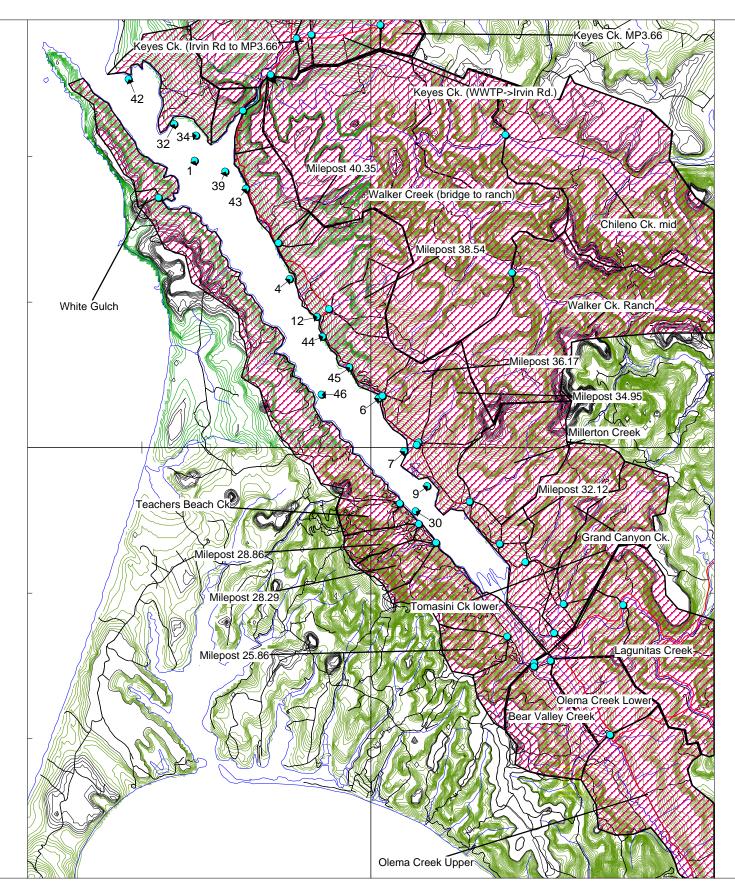


Figure 4. Location of Tomales Bay sampling stations, and tributary stations with their respective subwatersheds. (Scale: 1 inch = 1.9 miles)

## COLIFORMS

## TOTAL

Escherichia Citrobacter Klebsiella Enterobacter **FECAL** 

Escherichia coli Citrobacter freundii Klebsiella pneumoniae

## Streptococci CATEGORIES

## ENTEROCOCCUS GROUP

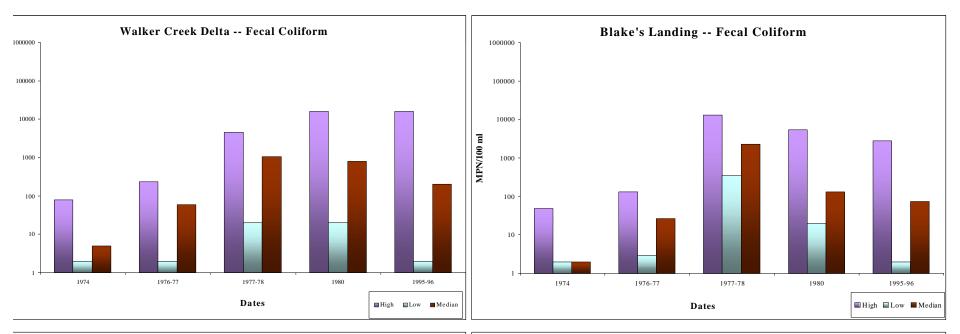
S. bovis S. equinus

S. faecalis S. faecium

S. gallinarum

S. avium

Figure 5. Relations of indicator bacteria.



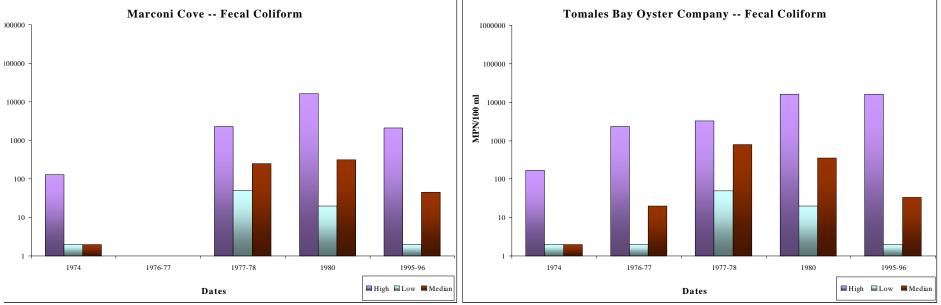
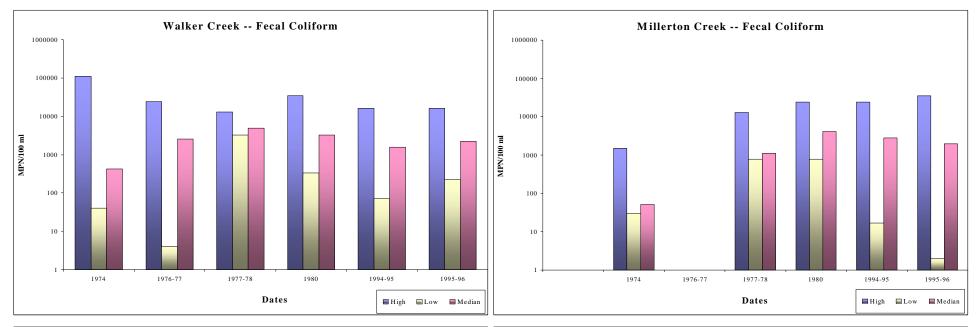


Figure 6a. Comparison of fecal coliform data for common bay stations over several past studies.



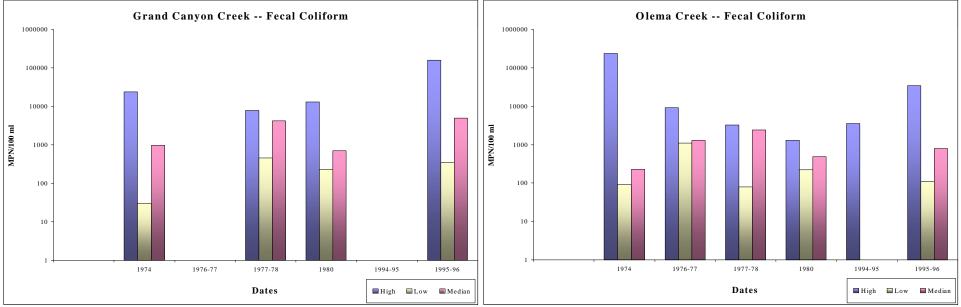


Figure 6b. Comparison of fecal coliform data for common creek stations over several past studies.

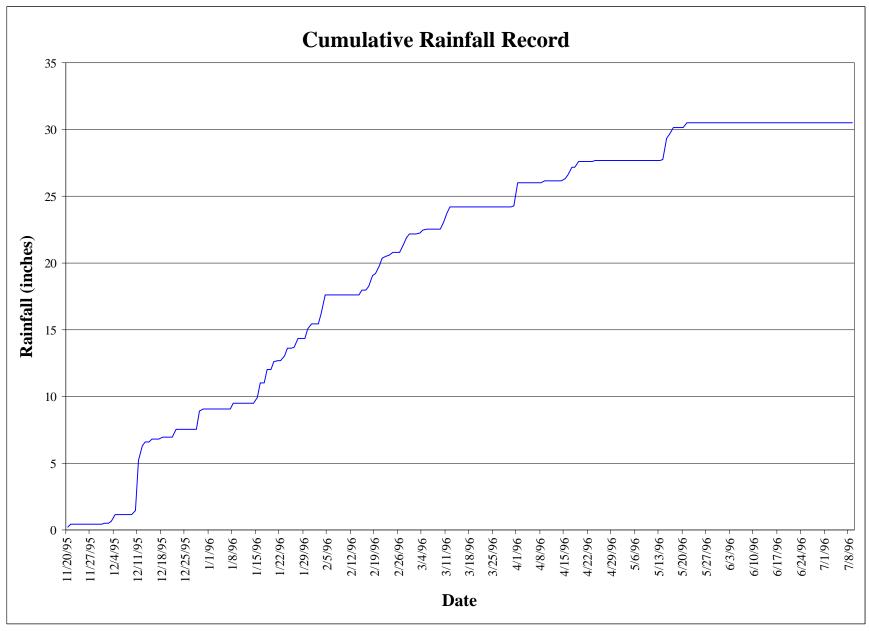
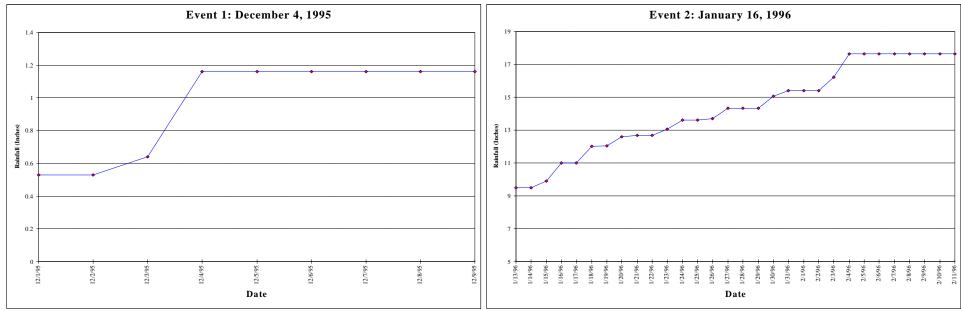
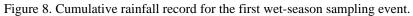
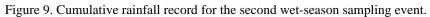


Figure 7. Cumulative rainfall record for the study period, obtained from the California Department of Health Services remote weather station at Tomasini Point, Tomales Bay.







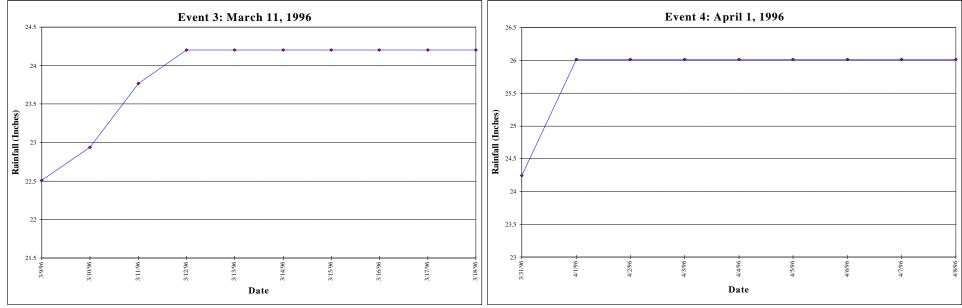


Figure 10. Cumulative rainfall record for the third wet-season sampling event.

Figure 11. Cumulative rainfall record for the fourth wet-season sampling event.

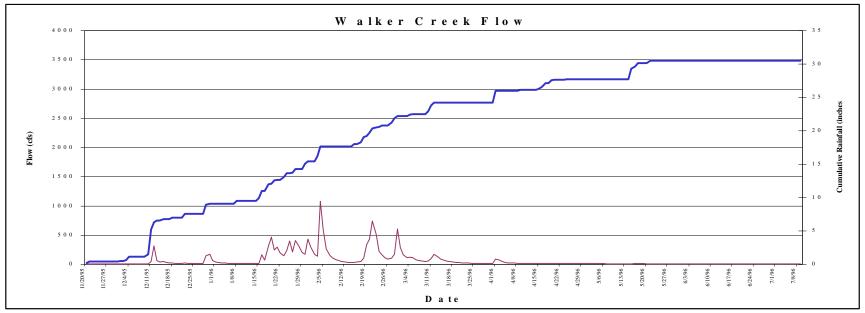


Figure 12a. Daily average stream gauge flow (cubic feet per second) for Walker Creek during the study period.

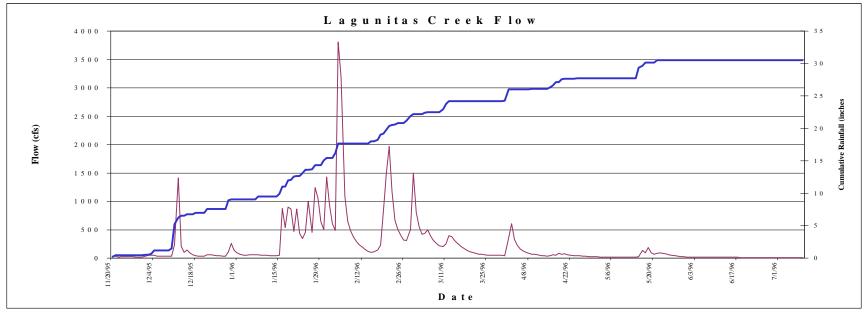
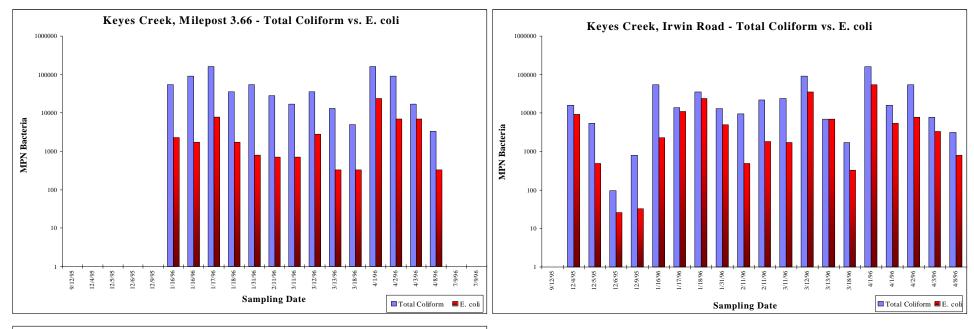
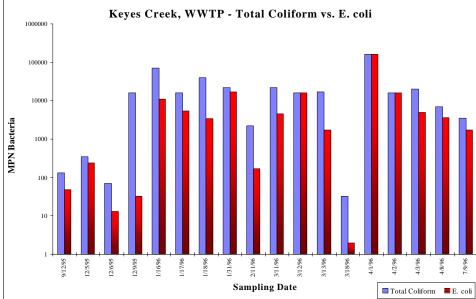


Figure 12b. Daily average stream gauge flow (cubic feet per second) for Lagunitas Creek during the study period.







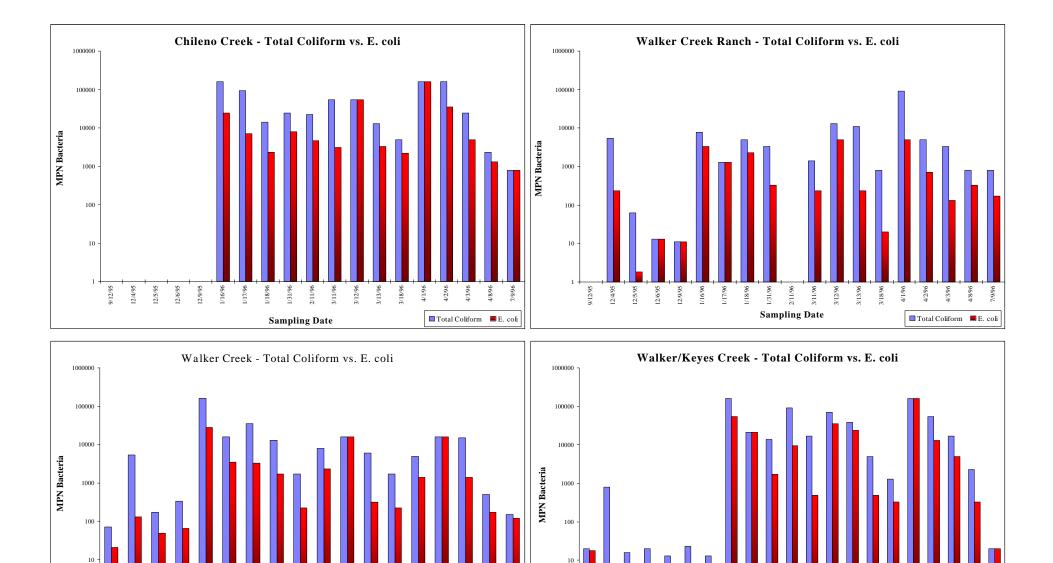


Figure 13b. Total coliform and Escherichia coli concentrations in water samples from the Chileno and Walker Creek stations.

4/2/96

4/3/96

Total Coliform E. coli

4/8/96

2/9/96

9/12/95 12/4/95 12/5/95 12/6/95

12/9/95

12/9/95 . 12/9/95 1/16/96

1/17/96

1/18/96

1/31/96

Sampling Date

2/11/96 3/11/96 3/12/96 3/13/96 3/18/96 4/1/96 4/2/96 4/3/96 4/8/96

Total Coliform E. coli

7/9/96

3/13/96

3/18/96

2/11/96

Sampling Date

12/5/95

12/4/95

9/12/95

1/16/96 1/17/96 1/18/96 1/31/96

12/9/95

3/11/96

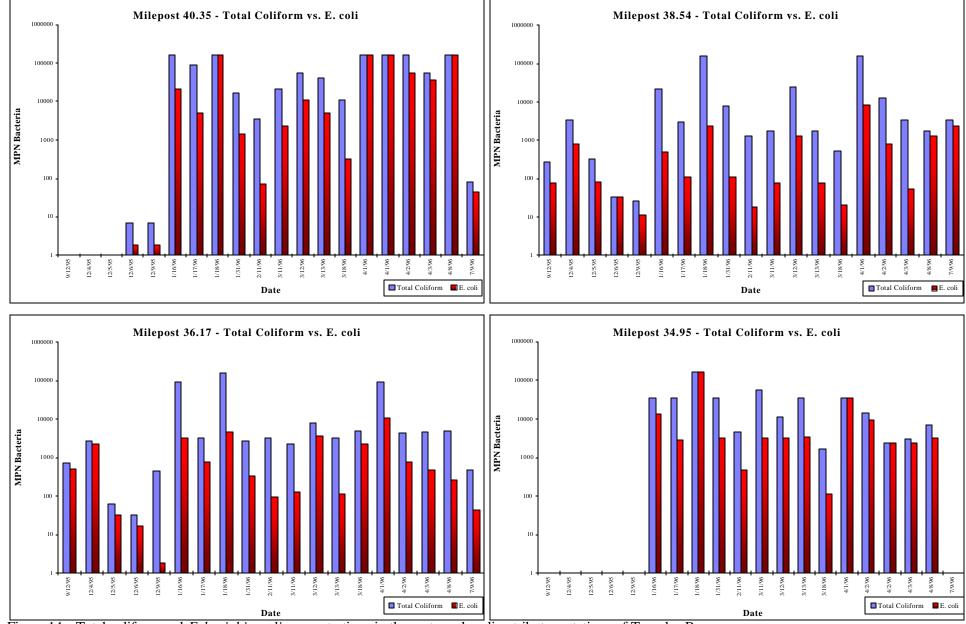


Figure 14a. Total coliform and *Esherichia coli* concentrations in the eastern shoreline tributary stations of Tomales Bay.

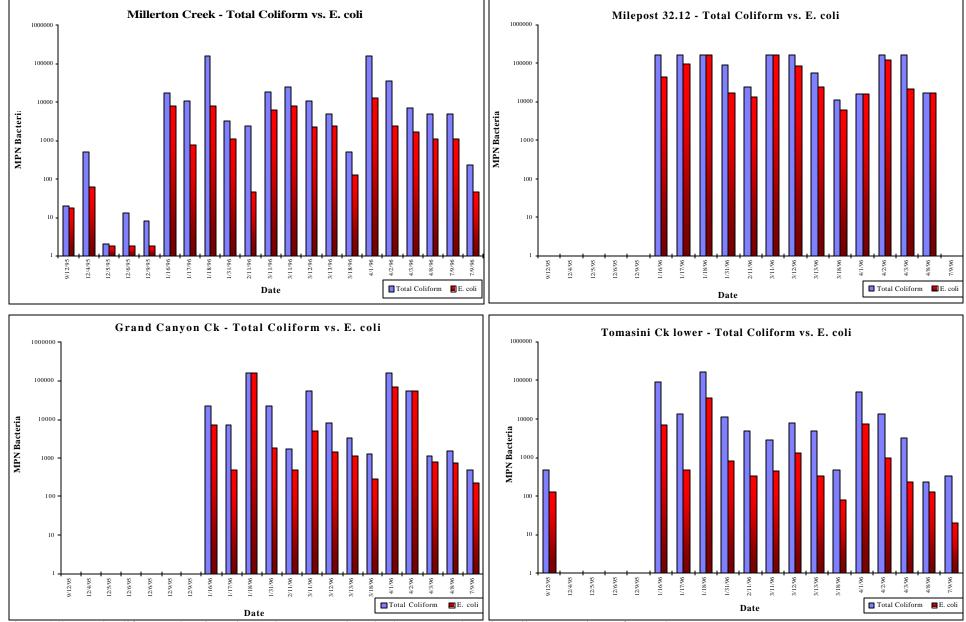


Figure 14b. Total coliform and *Esherichia coli* concentrations in the eastern shoreline tributary stations of Tomales Bay.

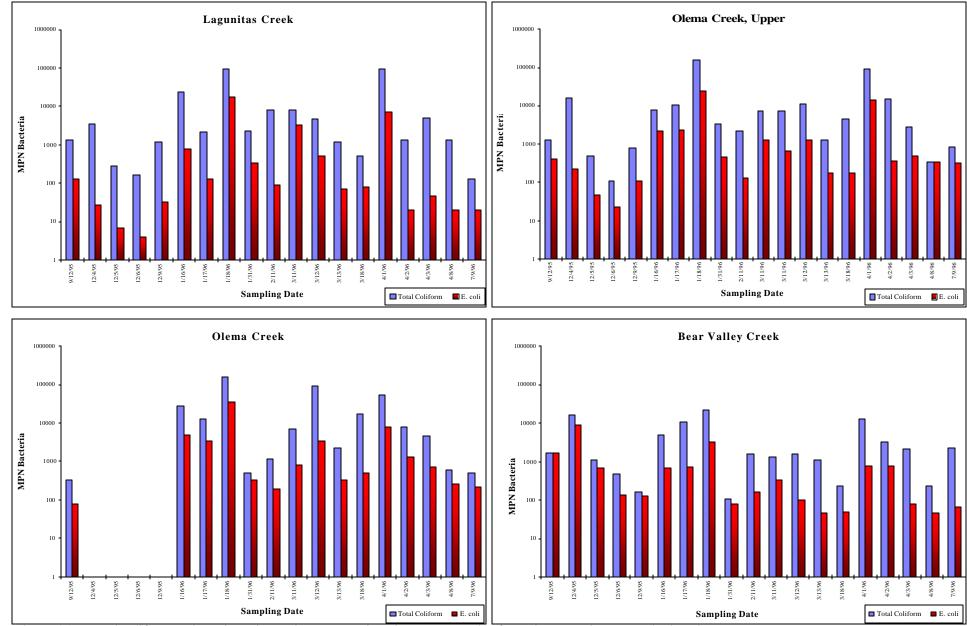


Figure 15a. Total coliform and Escherichia coli concentrations in water samples from the Lagunitas watershed stations.

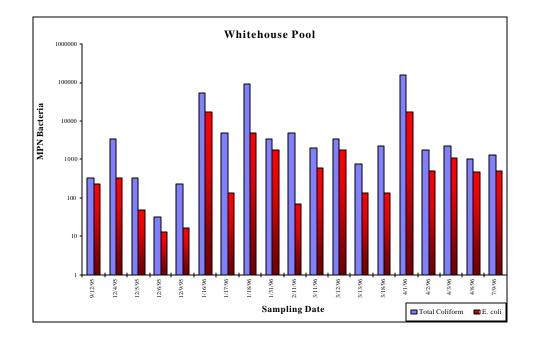
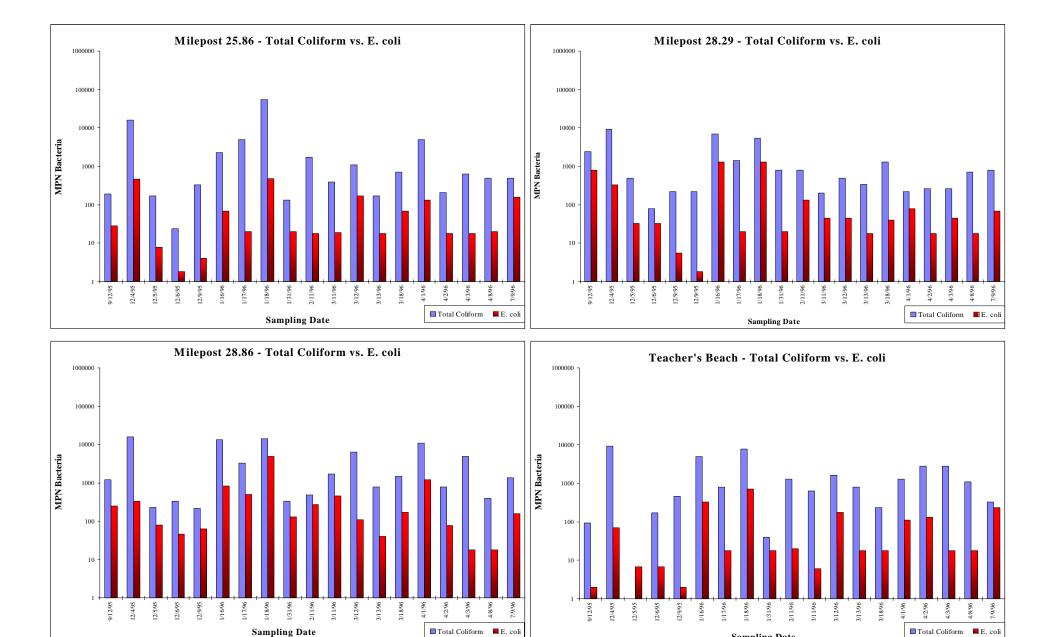


Figure 15b. Total coliform and *Escherichia coli* concentrations in water samples from the Lagunitas watershed stations.



Sampling Date

Figure 16. Total coliform and *Esherichia coli* concentrations in the west shore tributary stations of Tomales Bay.

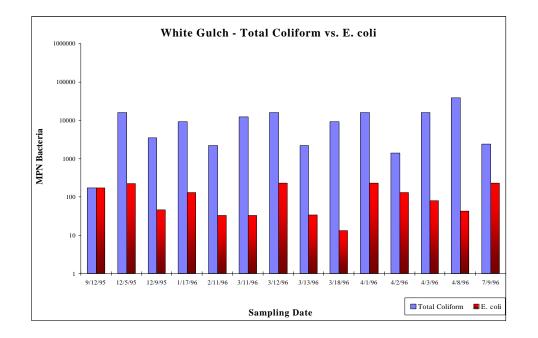
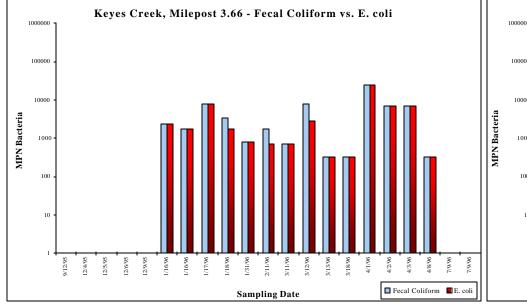
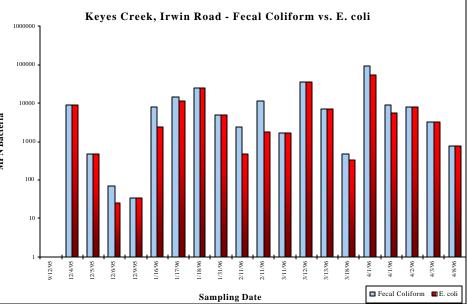


Figure 17. Total coliform and *Esherichia coli* concentrations in the freshwater control station.





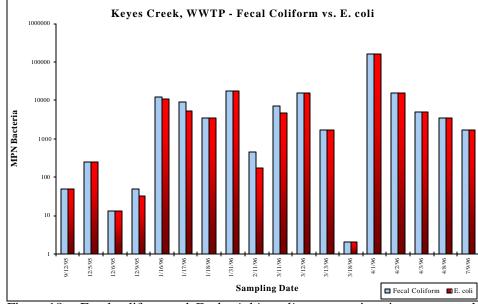


Figure 18a. Fecal coliform and Escherichia coli concentrations in water samples from the Keyes Creek stations.

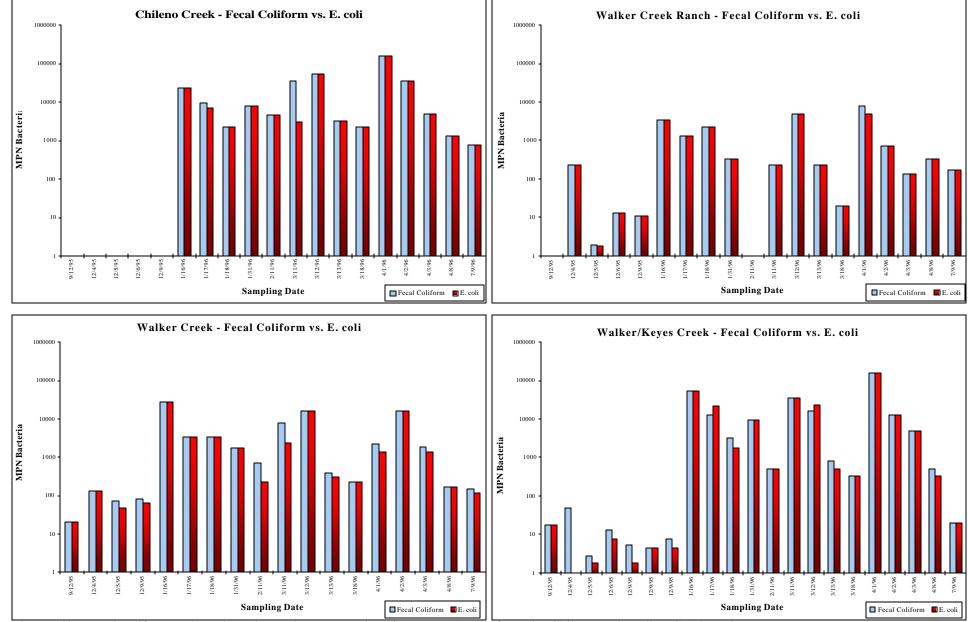


Figure 18b. Fecal coliform and Escherichia coli concentrations in water samples from the Chileno and Walker Creek stations.

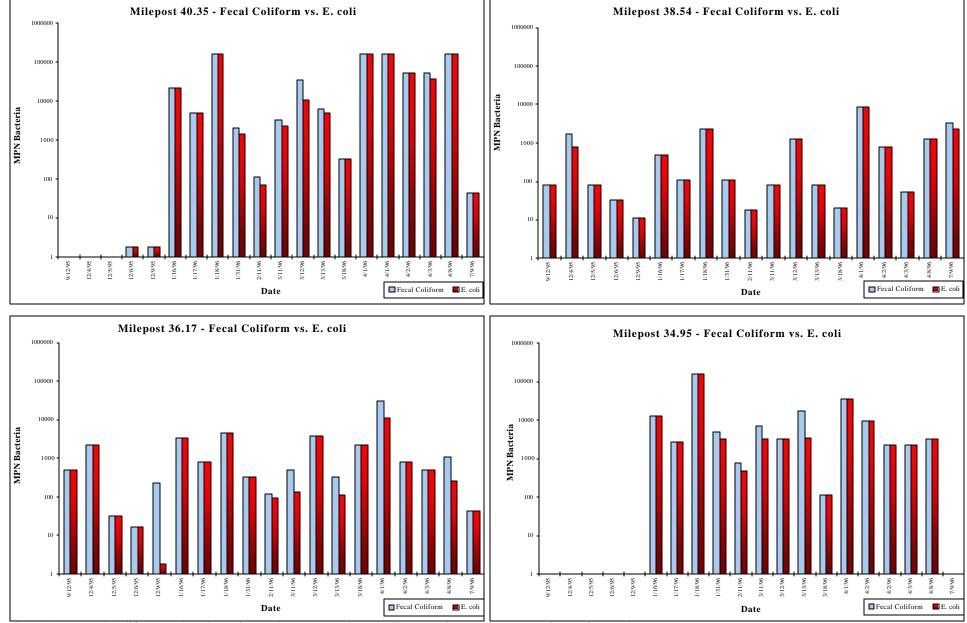


Figure 19a. Fecal coliform and *Esherichia coli* concentrations in the east shore tributary stations of Tomales Bay.

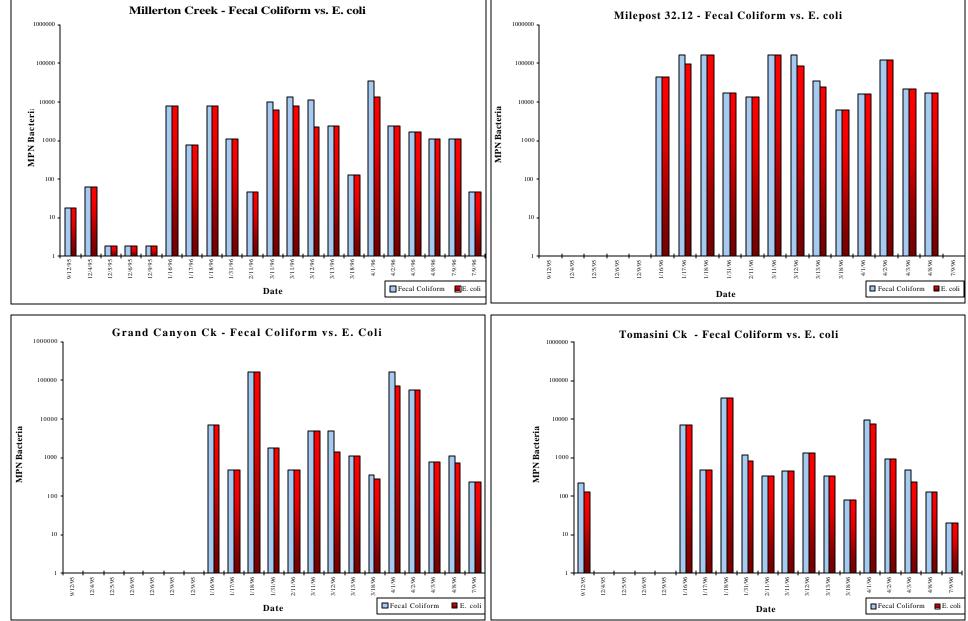
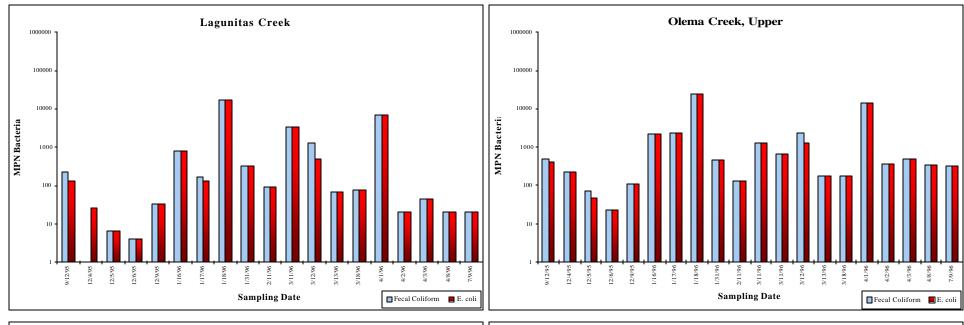


Figure 19b. Fecal coliform and *Esherichia coli* concentrations in the east shore tributary stations of Tomales Bay.



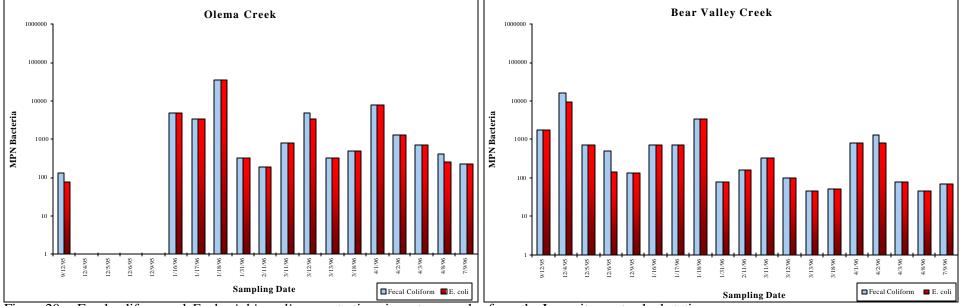


Figure 20a. Fecal coliform and Escherichia coli concentrations in water samples from the Lagunitas watershed stations.

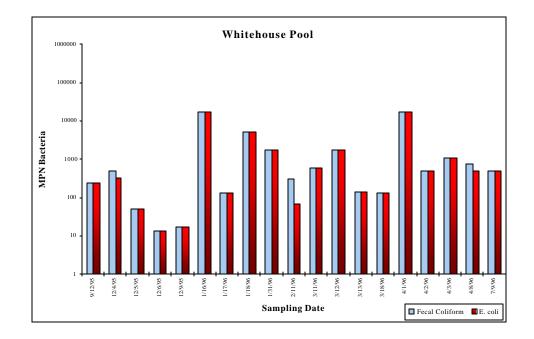
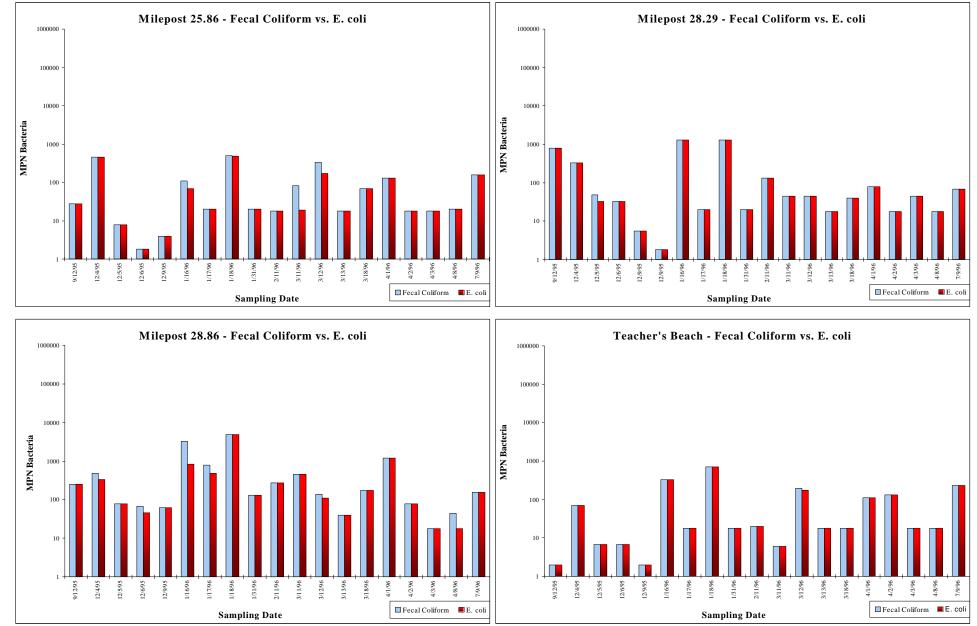
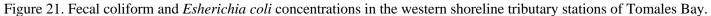


Figure 20b. Fecal coliform and *Escherichia coli* concentrations in water samples from the Lagunitas watershed stations.





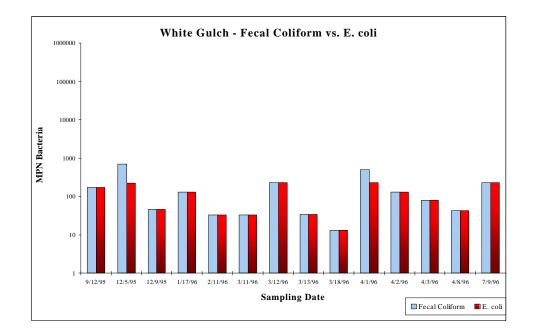
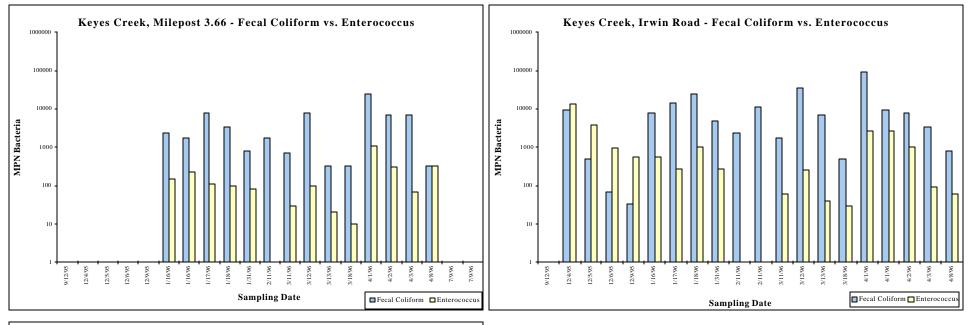
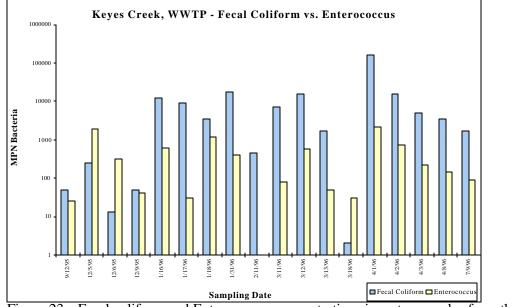
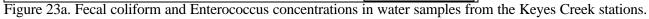


Figure 22. Fecal coliform and Esherichia coli concentrations in the freshwater control station.







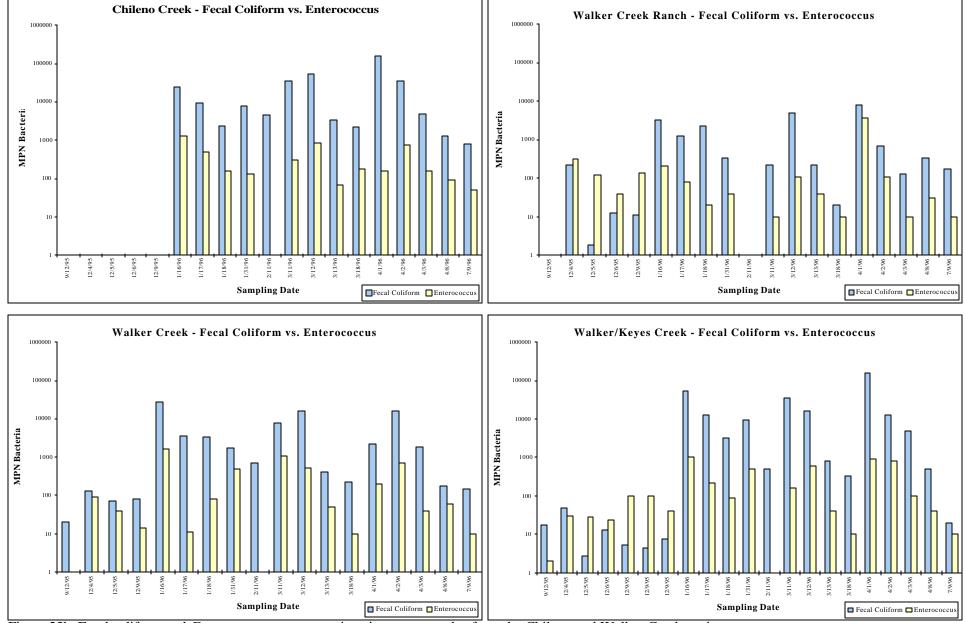


Figure 23b. Fecal coliform and Enterococcus concentrations in water samples from the Chileno and Walker Creek stations.

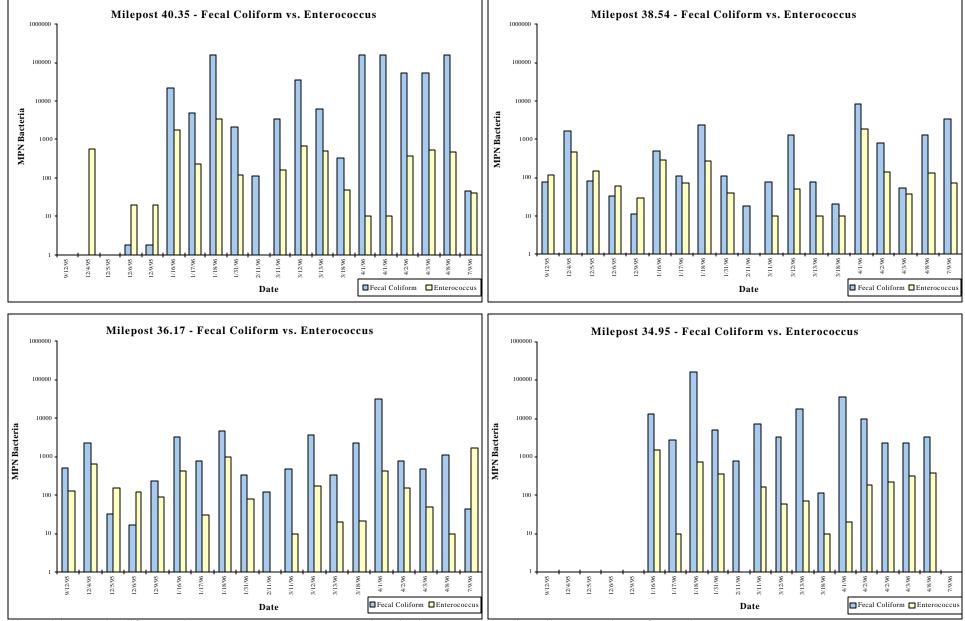


Figure 24a. Fecal coliform and *Enterococcus spp.* concentrations in the eastern shoreline tributary stations of Tomales Bay.

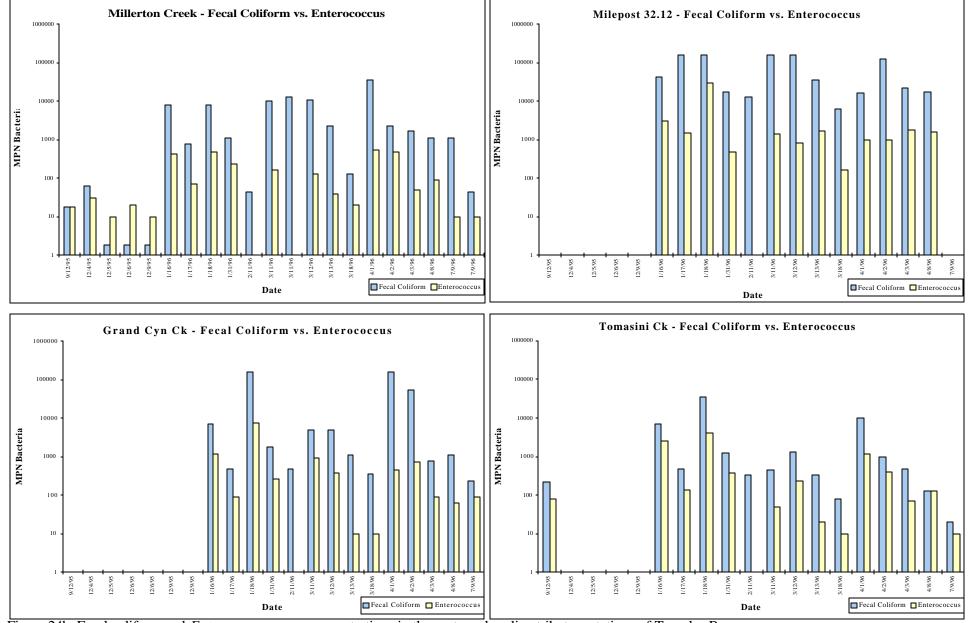


Figure 24b. Fecal coliform and Enterococcus spp. concentrations in the eastern shoreline tributary stations of Tomales Bay.

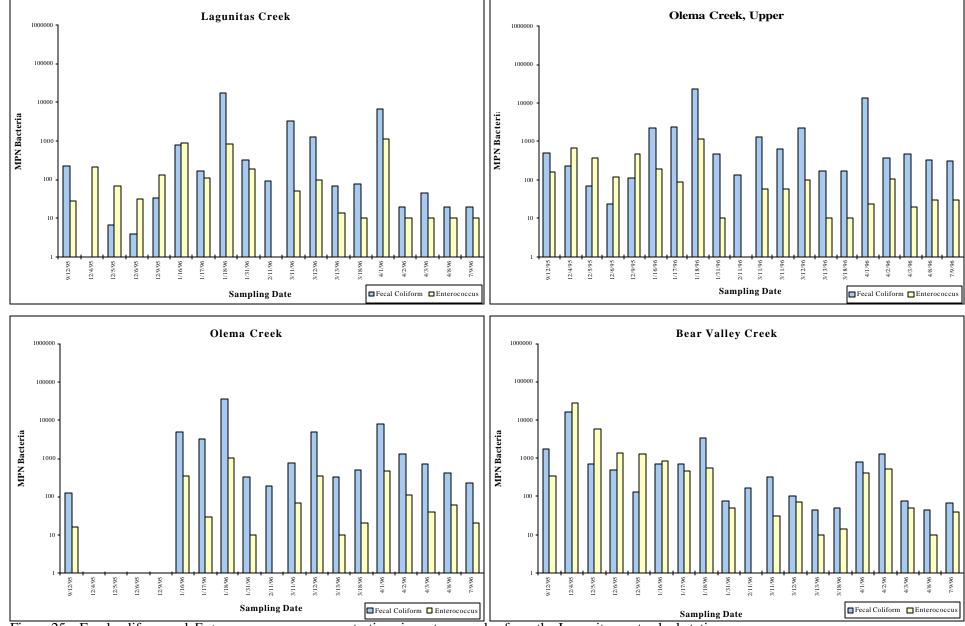


Figure 25a. Fecal coliform and *Enterococcus spp*. concentrations in water samples from the Lagunitas watershed stations.

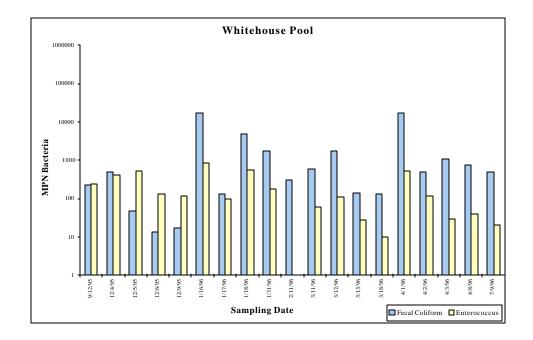


Figure 25b. Fecal coliform and *Enterococcus spp*. concentrations in water samples from the Lagunitas watershed stations.

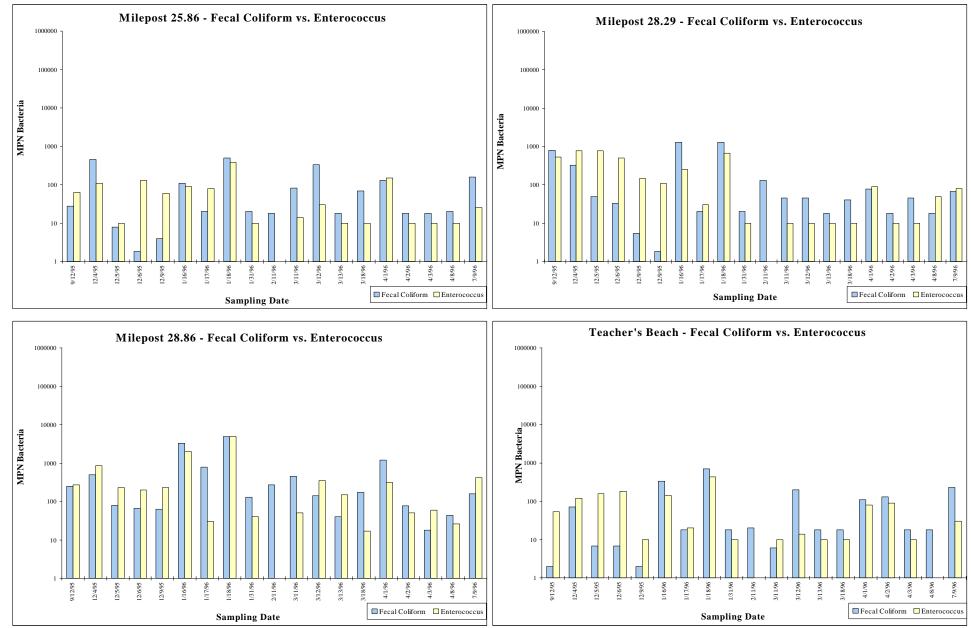


Figure 26. Fecal coliform and *Enterococcus spp.* concentrations in the western shoreline tributary stations of Tomales Bay.

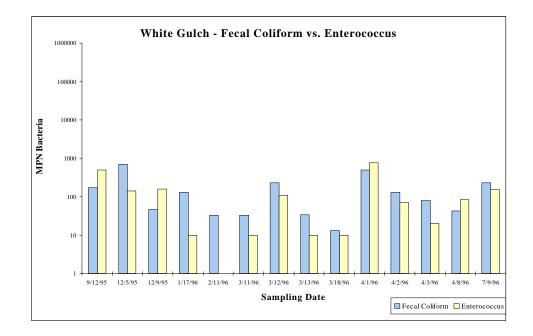


Figure 27. Fecal coliform and *Enterococcus spp.* concentrations in the freshwater control tributary station.

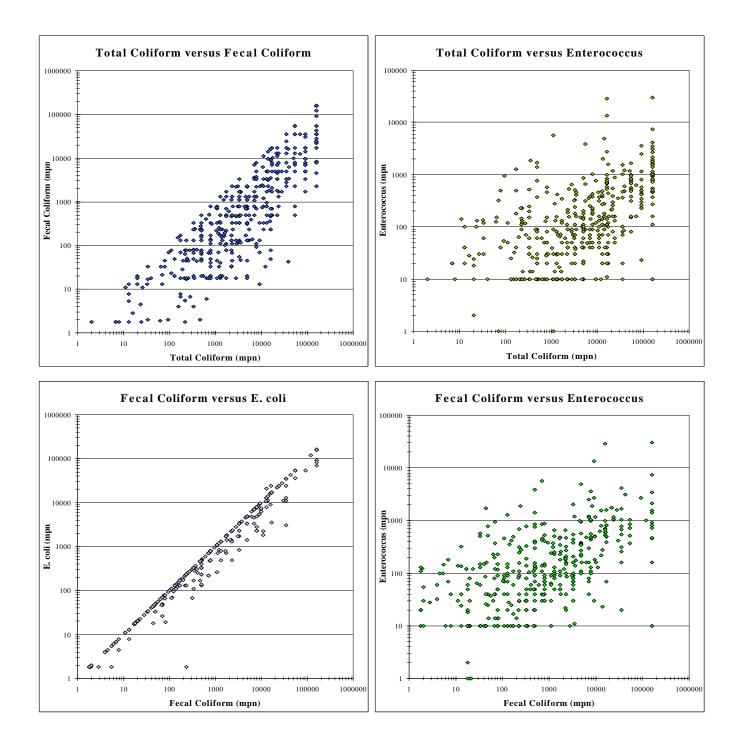


Figure 28. Relationships between concentrations of total coliform, fecal coliform, *E. coli* and enterococcus for data from watershed sampling stations.

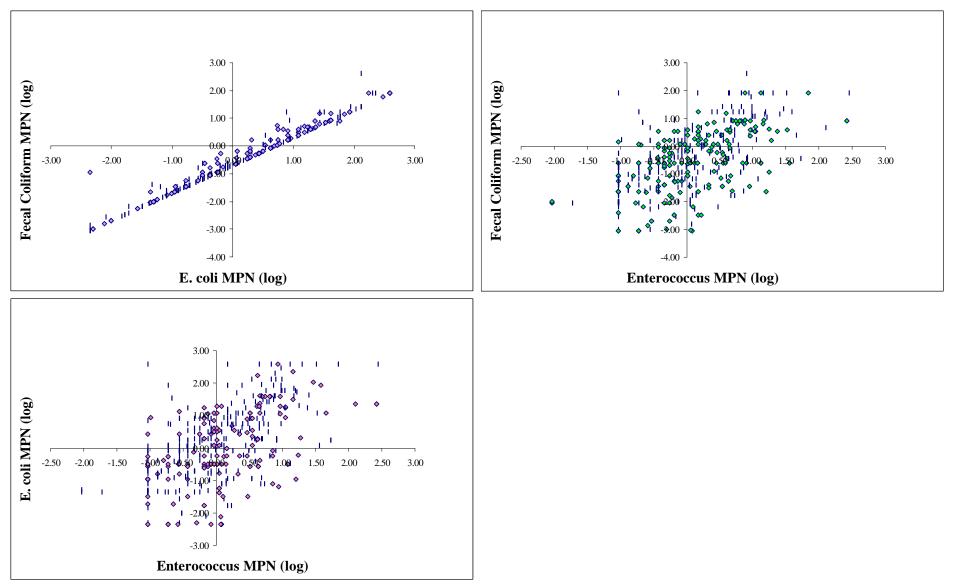


Figure 29. Comparison of indicator data for watershed stations, standardized against their respective water quality objectives (Table 7-8).

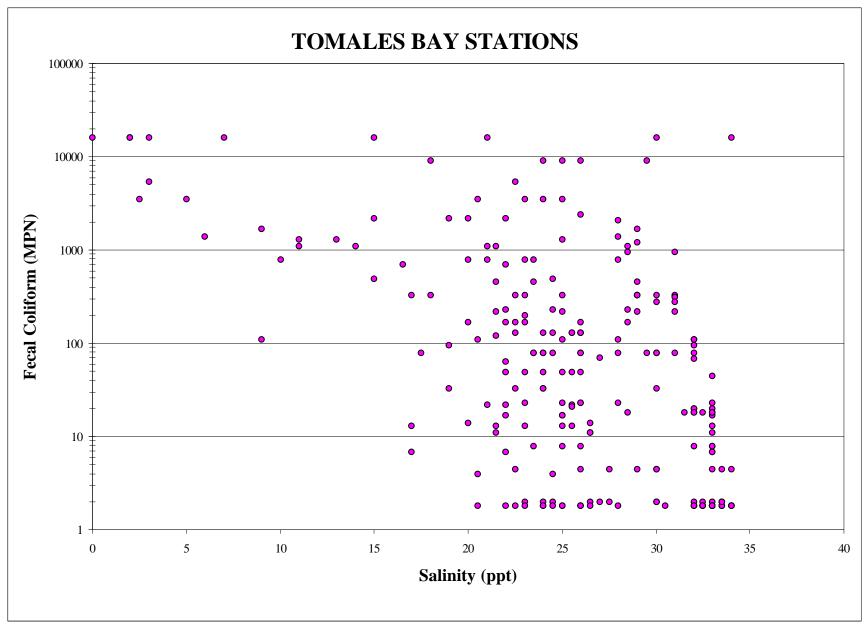


Figure 30. Relation between fecal coliform concentration and salinity for all Bay sampling stations.

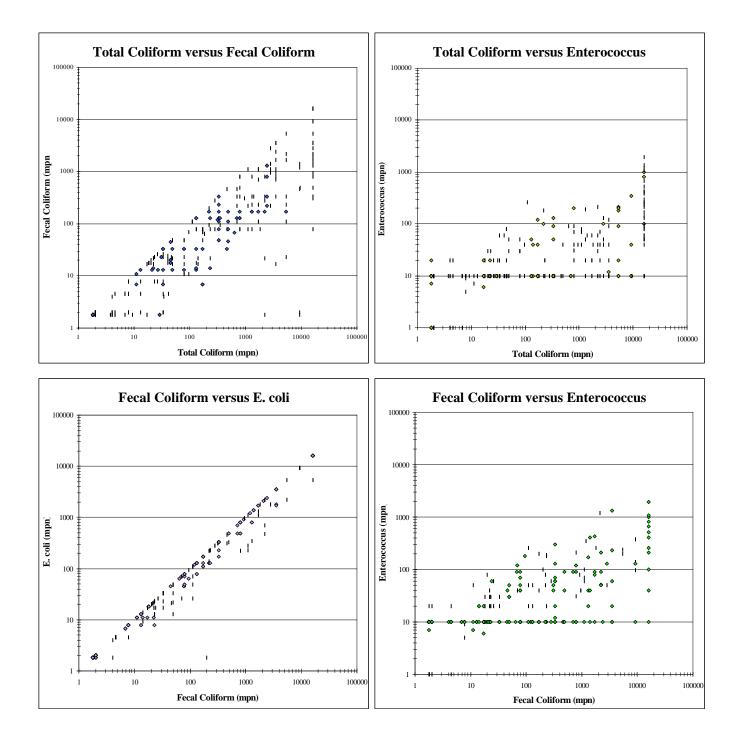
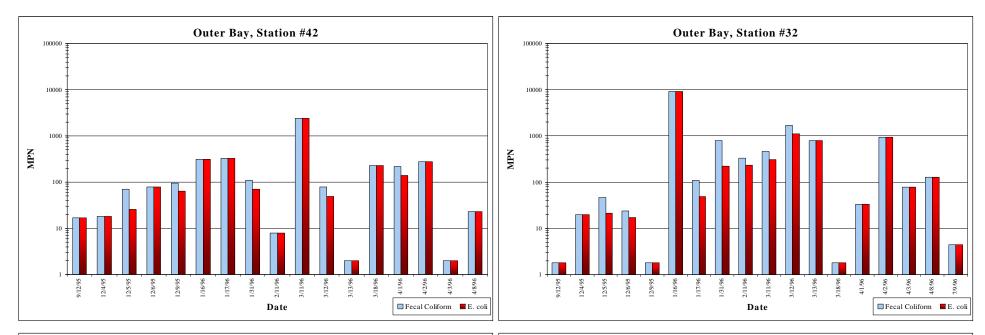


Figure 31. Relations between concentrations of total coliform, fecal coliform, *E. coli* and enterococcus for data from Bay sampling stations.



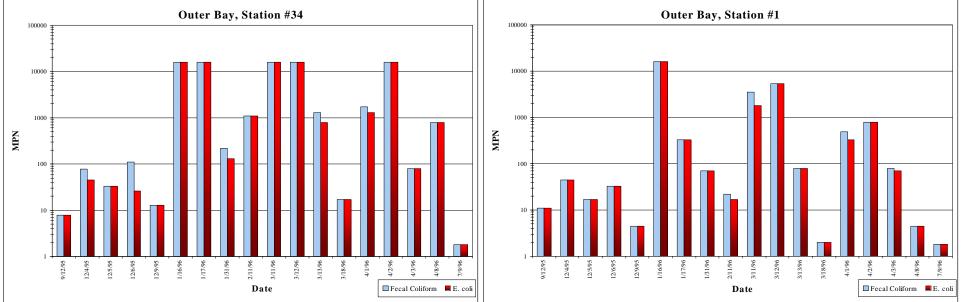


Figure 32a. Fecal coliform and *Esherichia coli* concentrations in water samples from outer Tomales Bay stations.

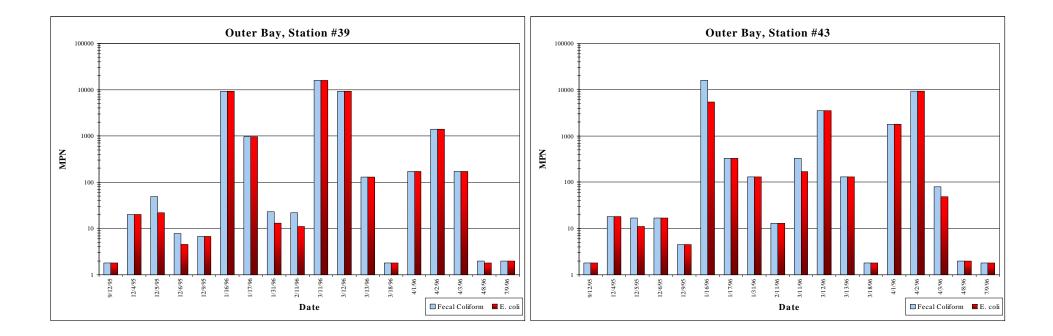
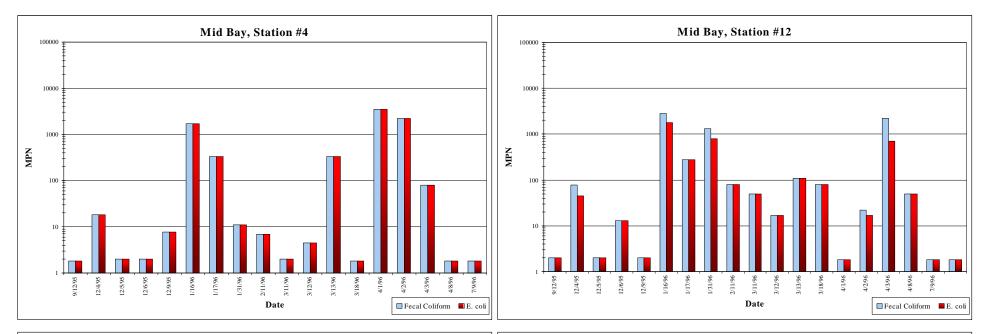


Figure 32b. Fecal coliform and *Esherichia coli* concentrations in water samples from outer Tomales Bay stations.



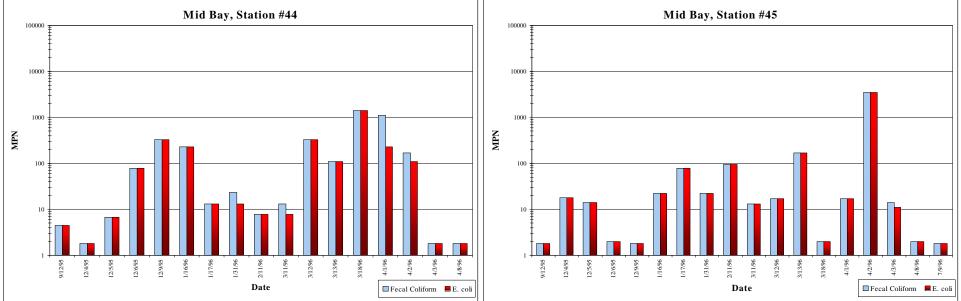
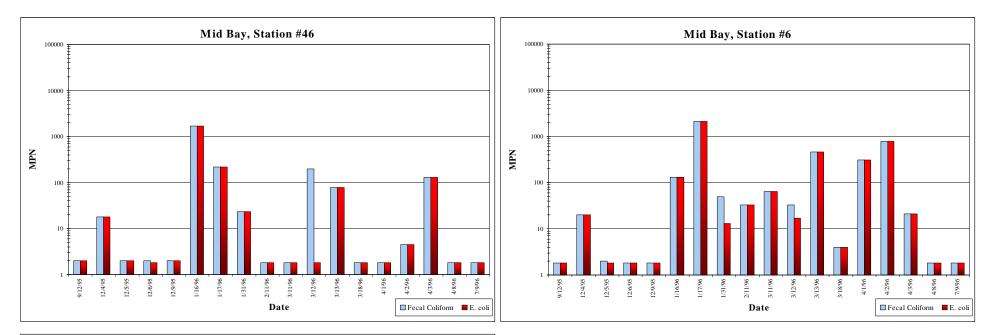


Figure 33a. Fecal coliform and *Esherichia coli* concentrations in water samples from mid Tomales Bay stations.



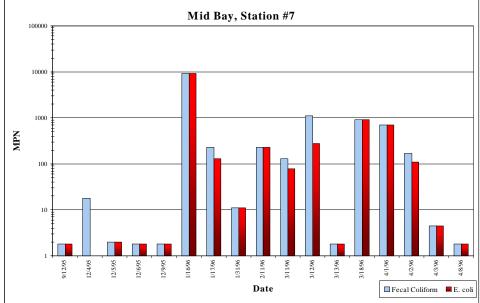


Figure 33b. Fecal coliform and *Esherichia coli* concentrations in water samples from mid Tomales Bay stations.

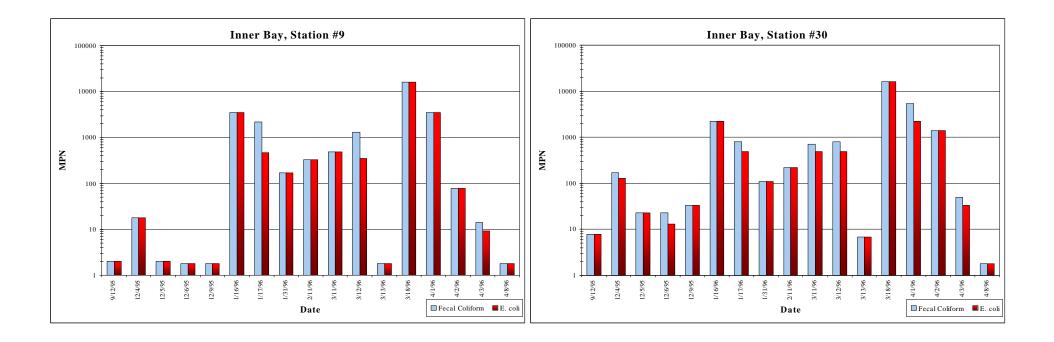


Figure 34. Fecal coliform and *Esherichia coli* concentrations in water samples from inner Tomales Bay stations

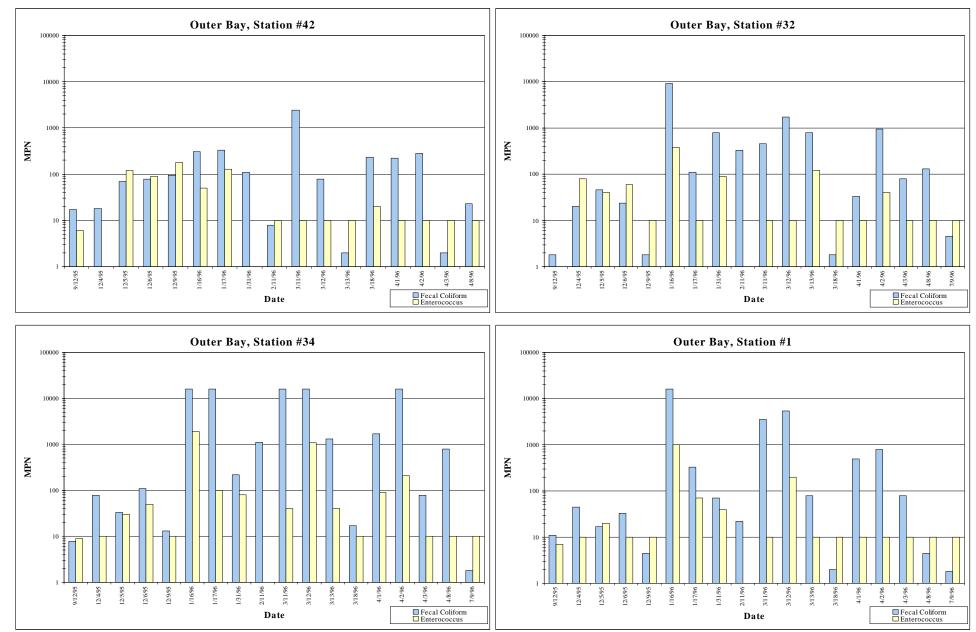


Figure 35a. Fecal coliform and enterococcus concentrations in water samples from outer Tomales Bay stations.

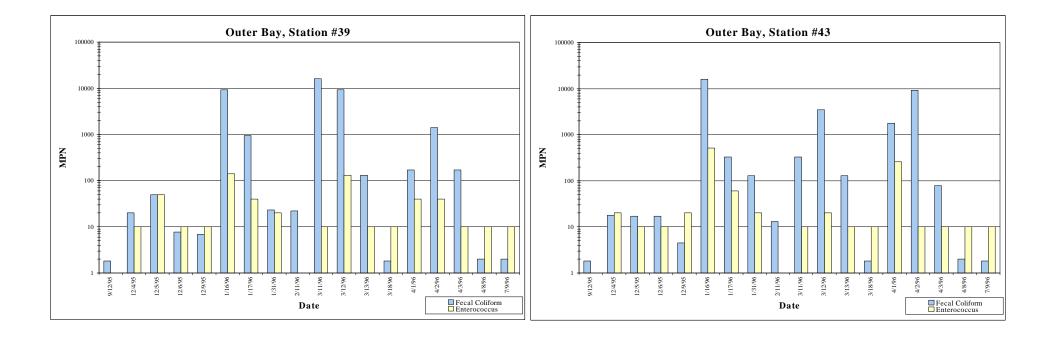


Figure 35b. Fecal coliform and enterococcus concentrations in water samples from outer Tomales Bay stations.

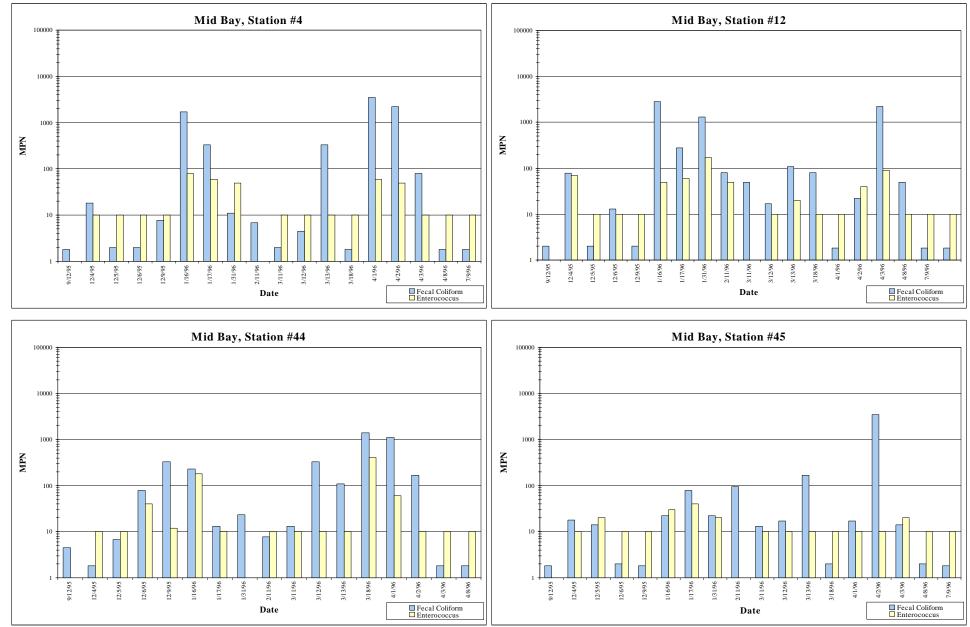
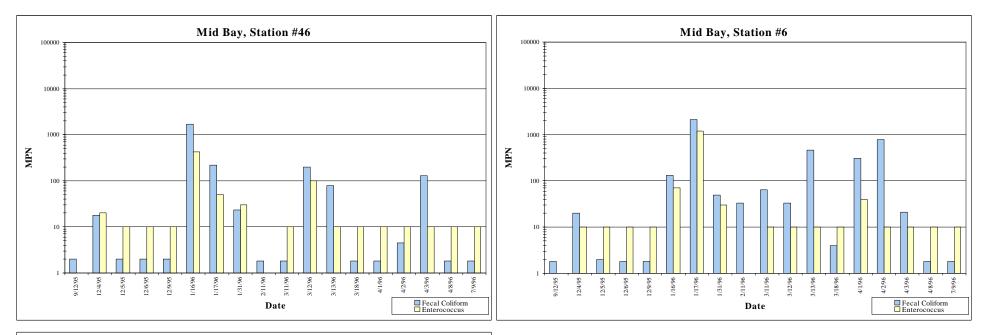


Figure 36a. Fecal coliform and enterococcus concentrations in water samples from mid Tomales Bay stations.



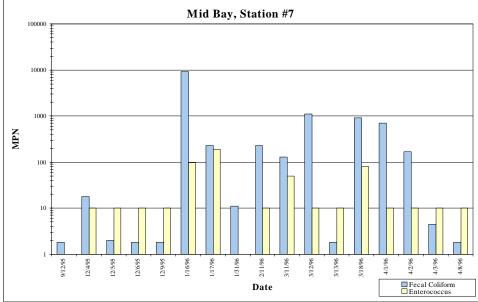


Figure 36b. Fecal coliform and enterococcus concentrations in water samples from mid Tomales Bay stations.

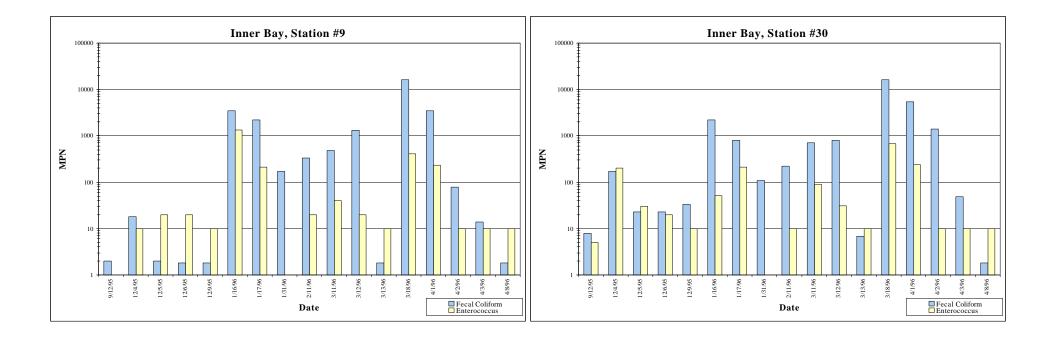


Figure 37. Fecal coliform and enterococcus concentrations in water samples from inner Tomales Bay stations.

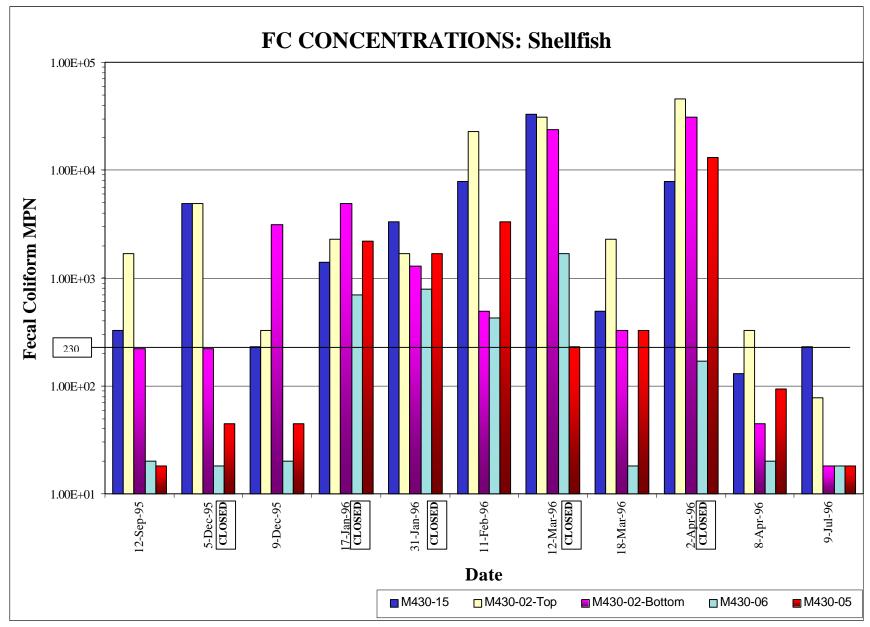
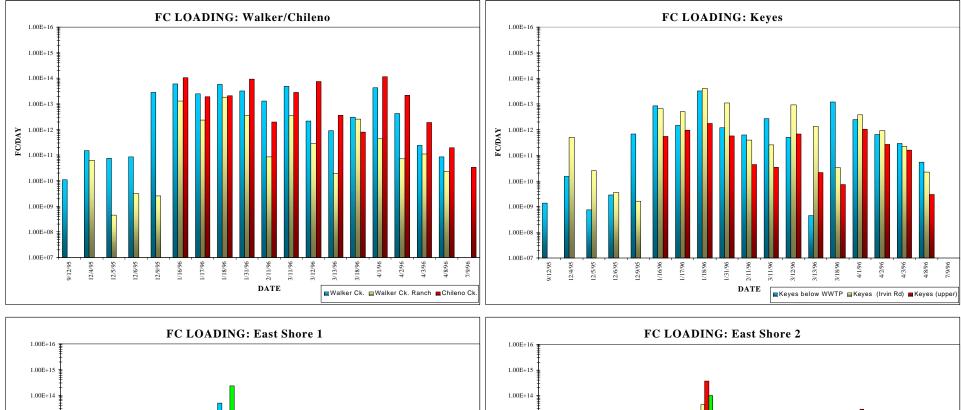


Figure 38. Fecal coliform concentrations for all shellfish sampling stations, with closed harvest days noted.



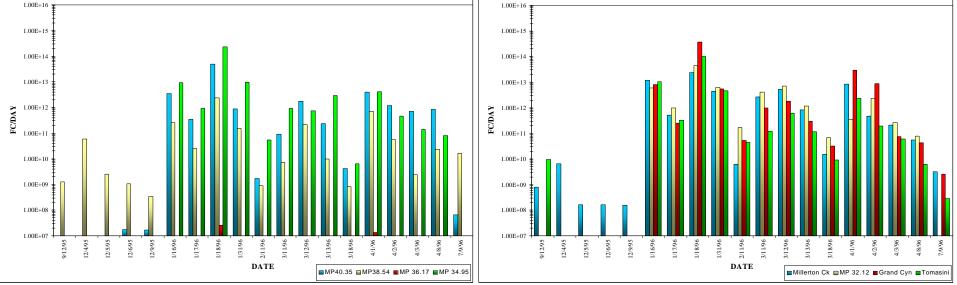
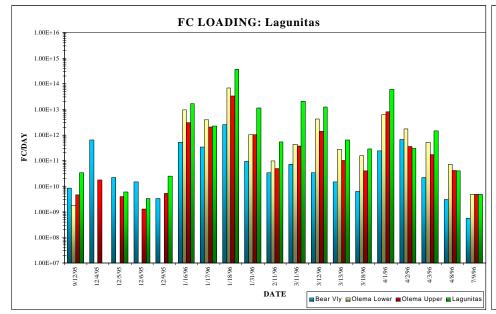
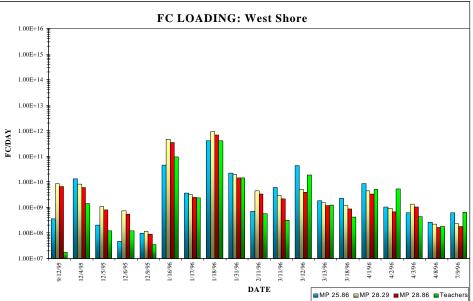


Figure 39. Fecal coliform loadings (FC/day) for the Walker/Chileno, Keyes, and eastern shoreline subwatershed drainages.





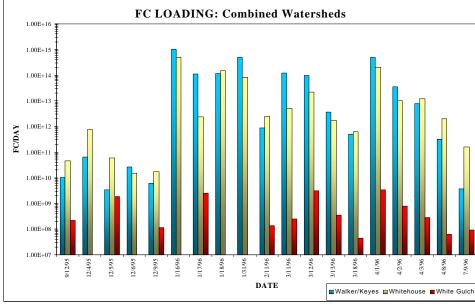


Figure 40. Fecal coliform loadings (FC/day) for the Lagunitas and western shoreline subwatershed drainages. The lower plot presents the FC loadings for each of the two major subwatersheds and for the freshwater control watershed.

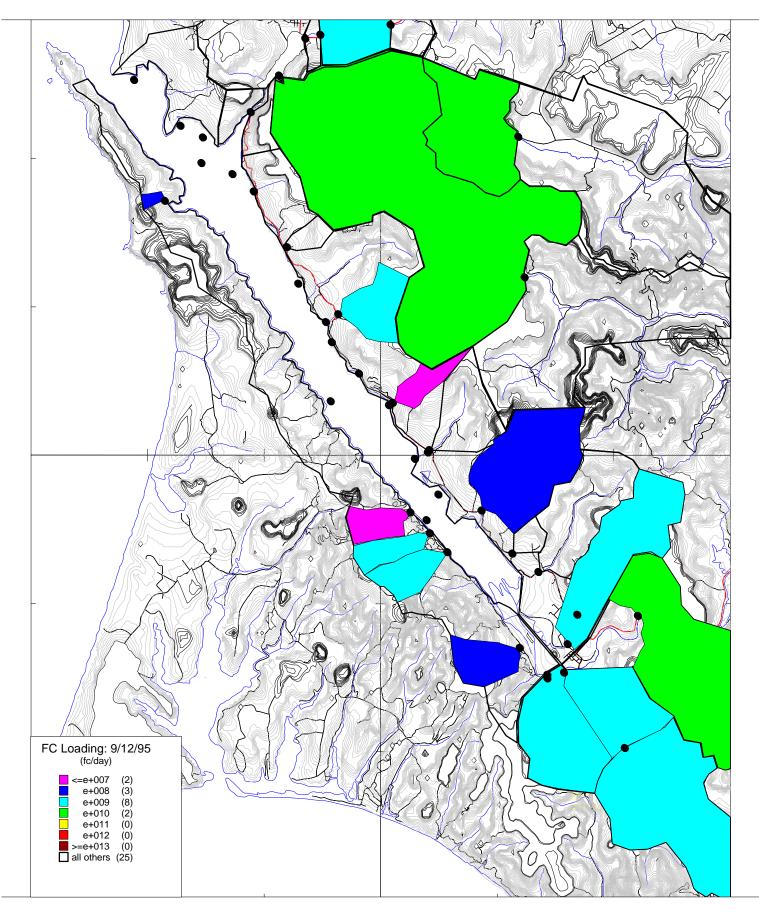


Figure 41. Fecal coliform loadings in the Tomales Bay watershed on 9/12/95 (dry season event 1).

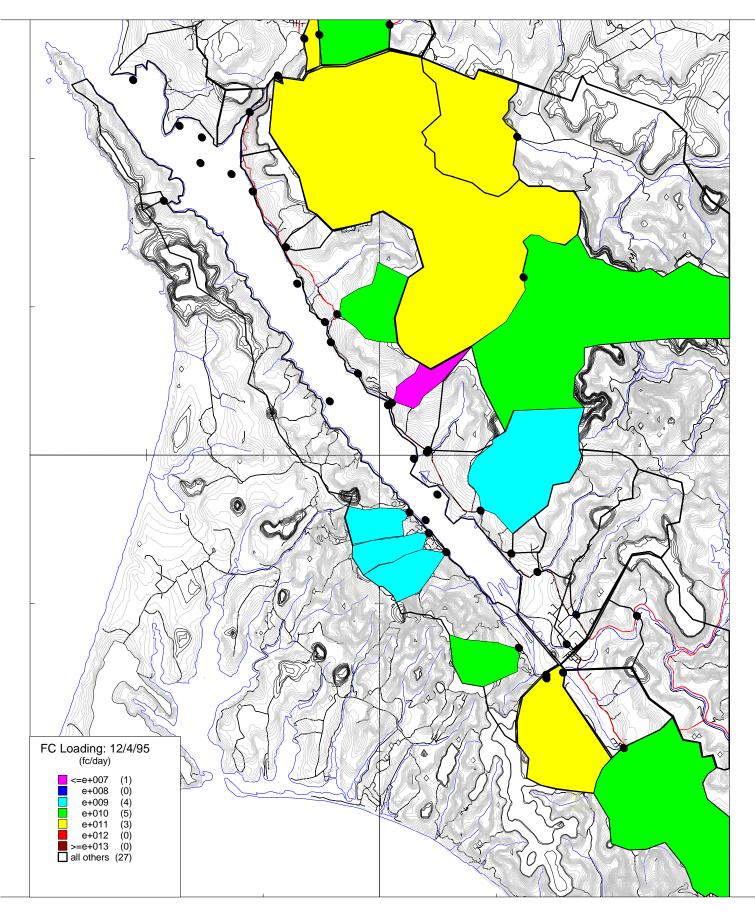


Figure 42. Fecal coliform loadings in the Tomales Bay watershed on 12/4/95 (event 1, day 1).

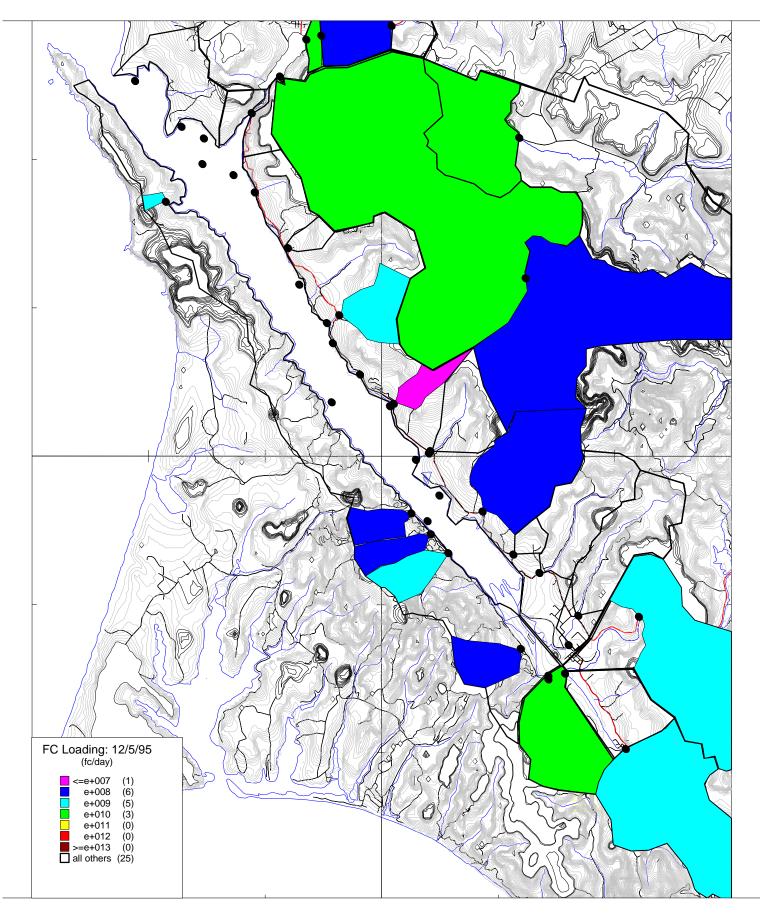


Figure 43. Fecal coliform loadings in the Tomales Bay watershed on 12/5/95 (event 1, day 2).

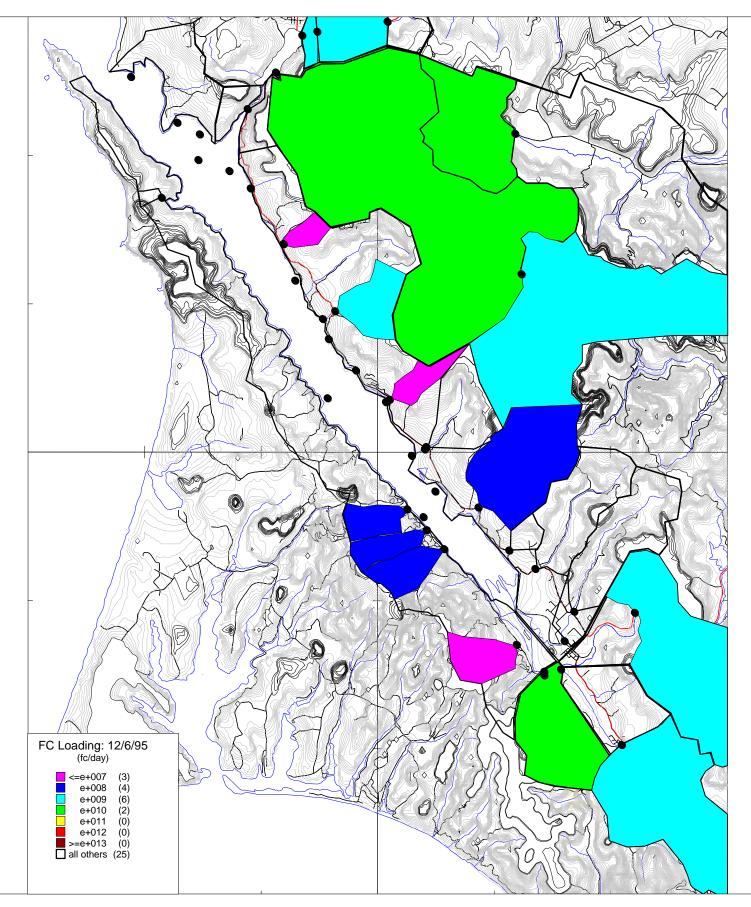


Figure 44. Fecal coliform loadings in the Tomales Bay watershed on 12/6/95 (event 1, day 3).

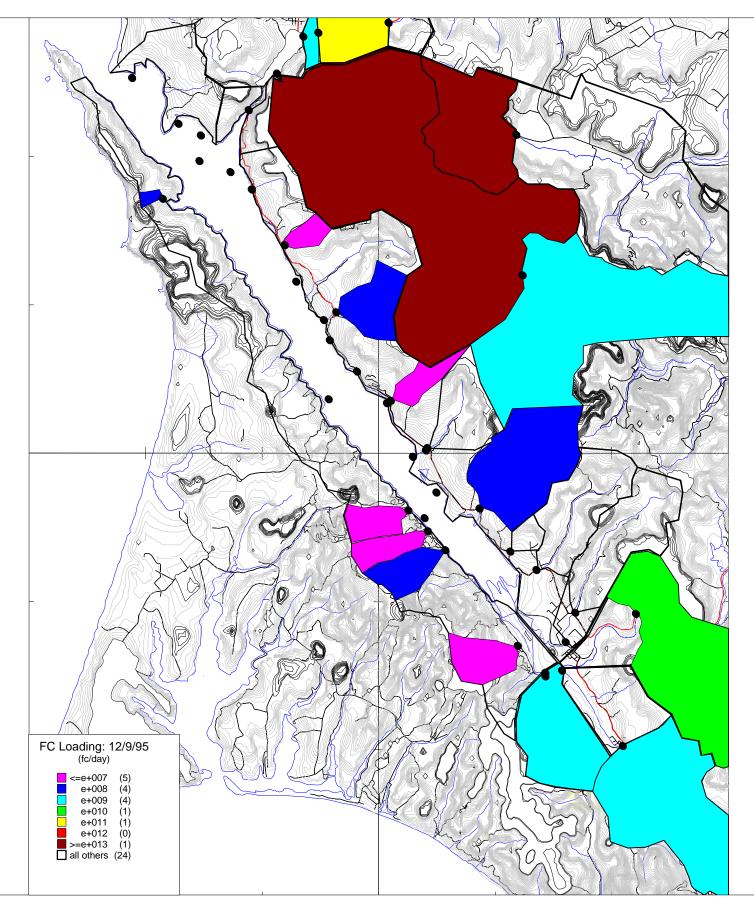


Figure 45. Fecal coliform loadings in the Tomales Bay watershed on 12/9/95 (event 1, day x).

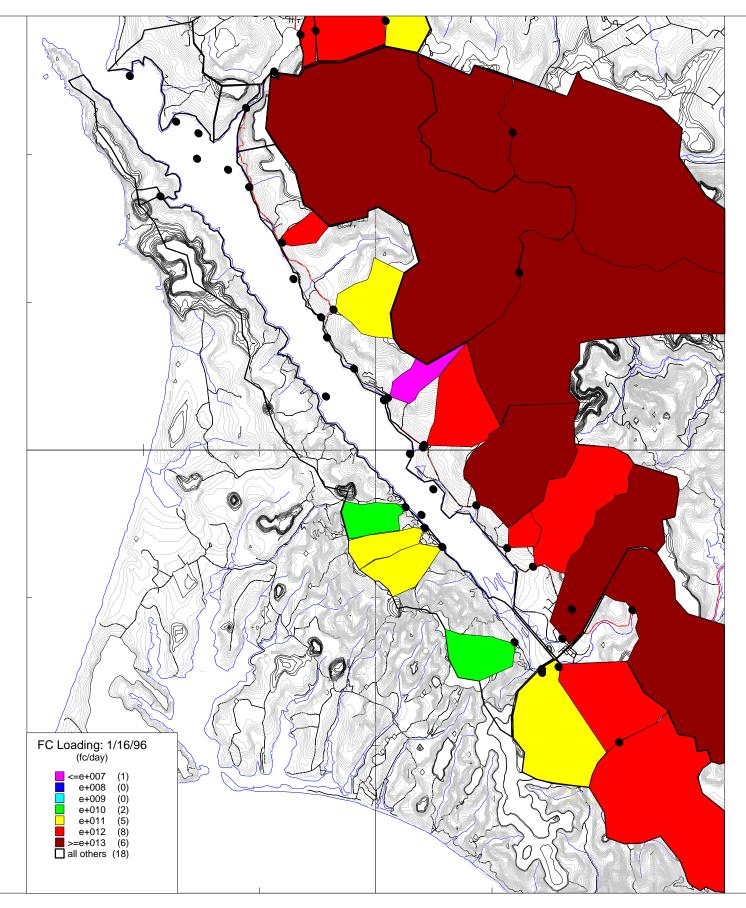


Figure 46. Fecal coliform loadings in Tomales Bay watershed on 1/16/96 (event 2, day 1).

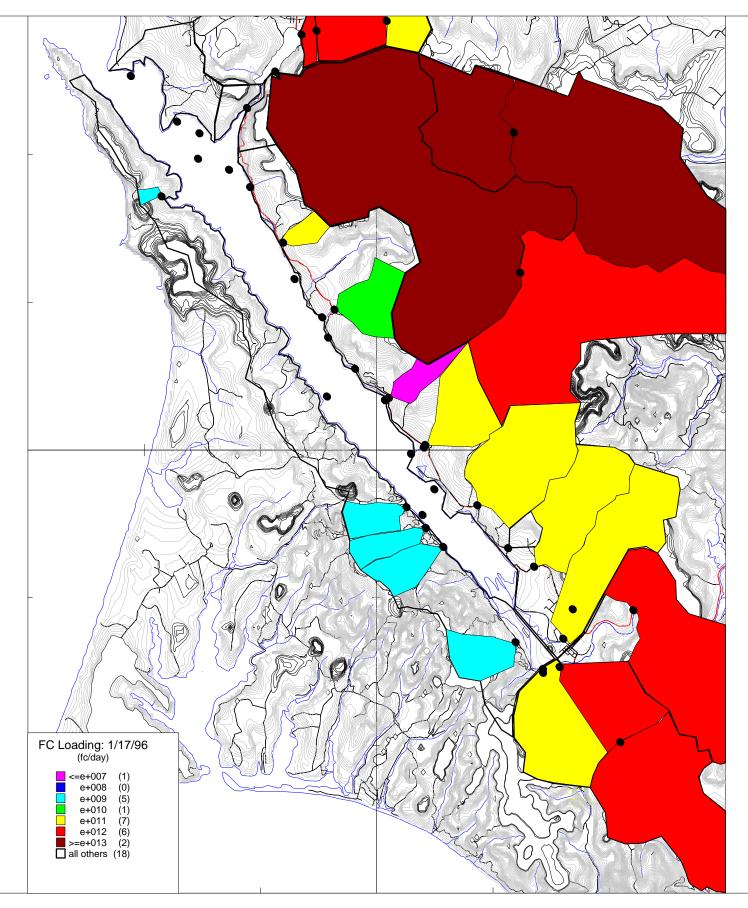


Figure 47. Fecal coliform loadings in Tomales Bay watershed on 1/17/96 (event 2, day 2).

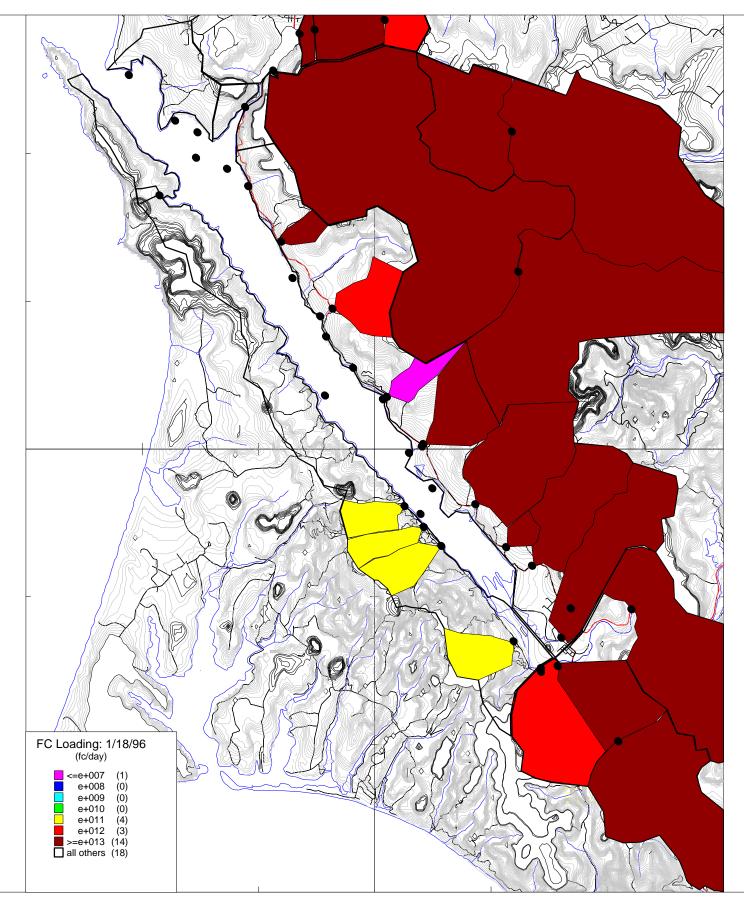


Figure 48. Fecal coliform loadings in Tomales Bay watershed on 1/18/96 (event 2, day 3).

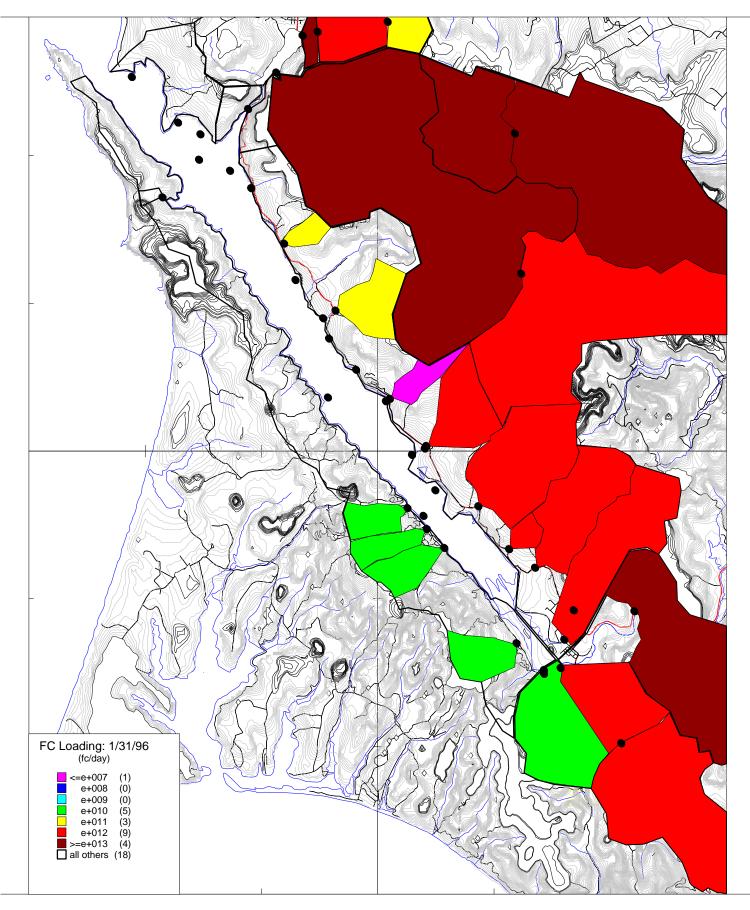


Figure 49. Fecal coliform loadings in Tomales Bay watershed on 1/31/96 (event 2, day A).

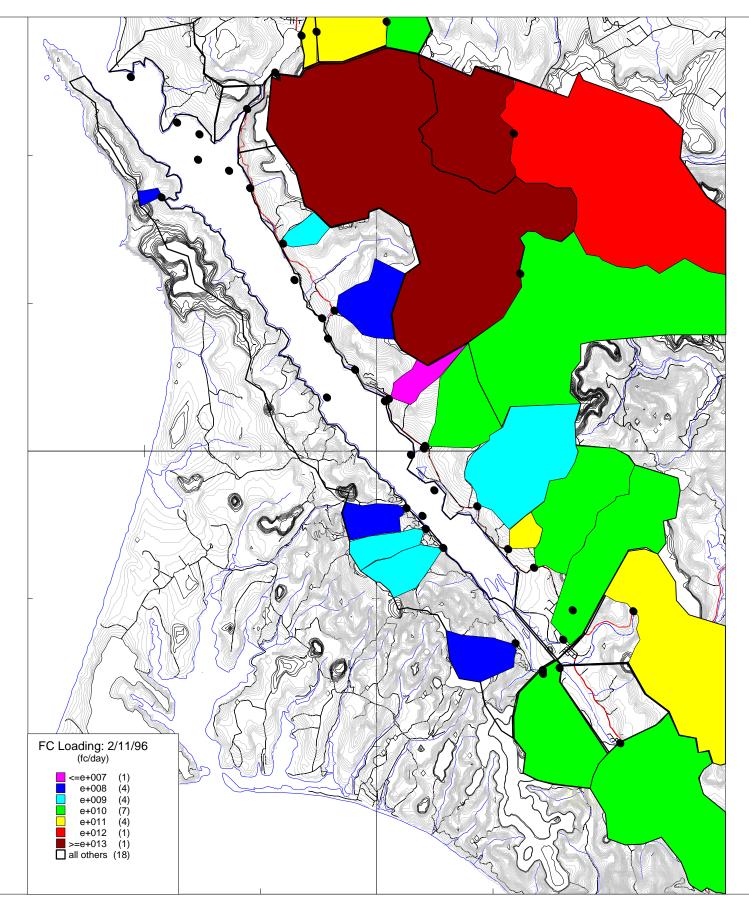


Figure 50. Fecal coliform loadings in Tomales Bay watershed on 2/11/96 (event 2, day x).

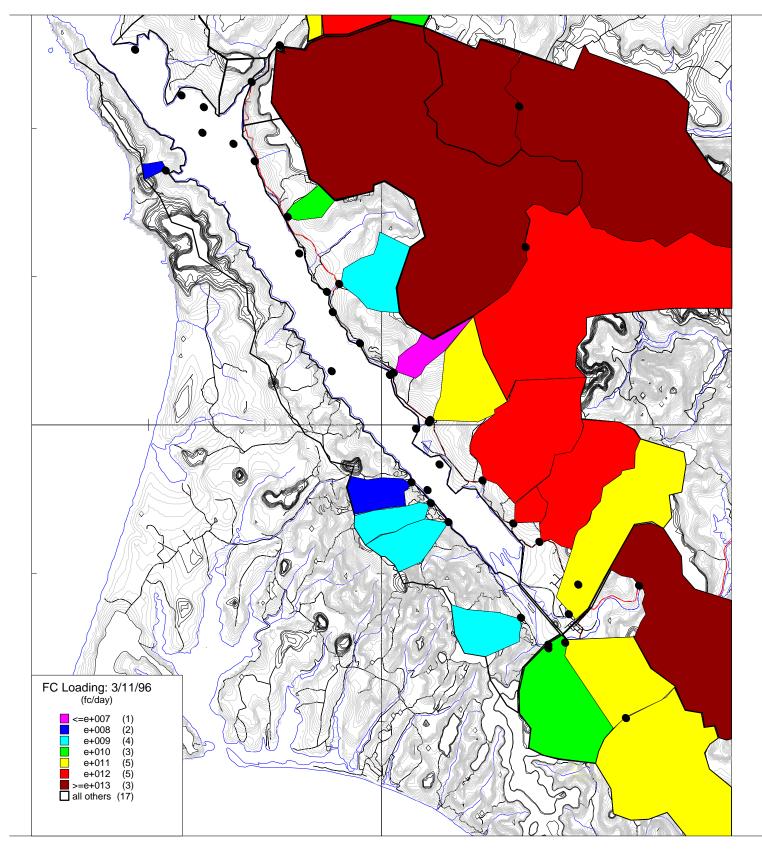


Figure 51. Fecal coliform loadings in the Tomales watershed on 3/11/96 (event 3, day 1).

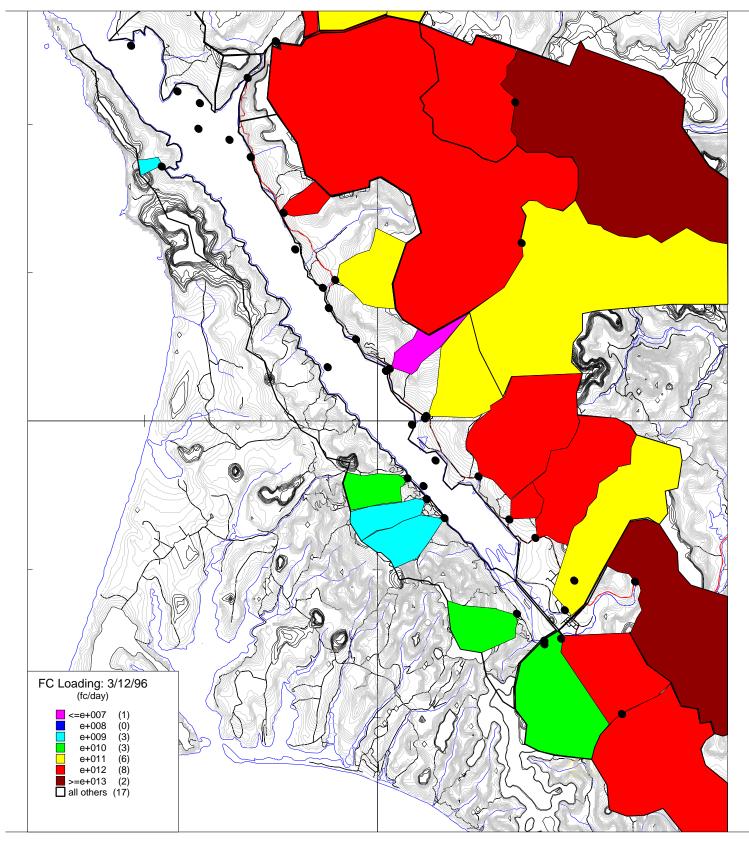


Figure 52. Fecal coliform loadings in the Tomales watershed on 3/12/96 (event 3, day 2).

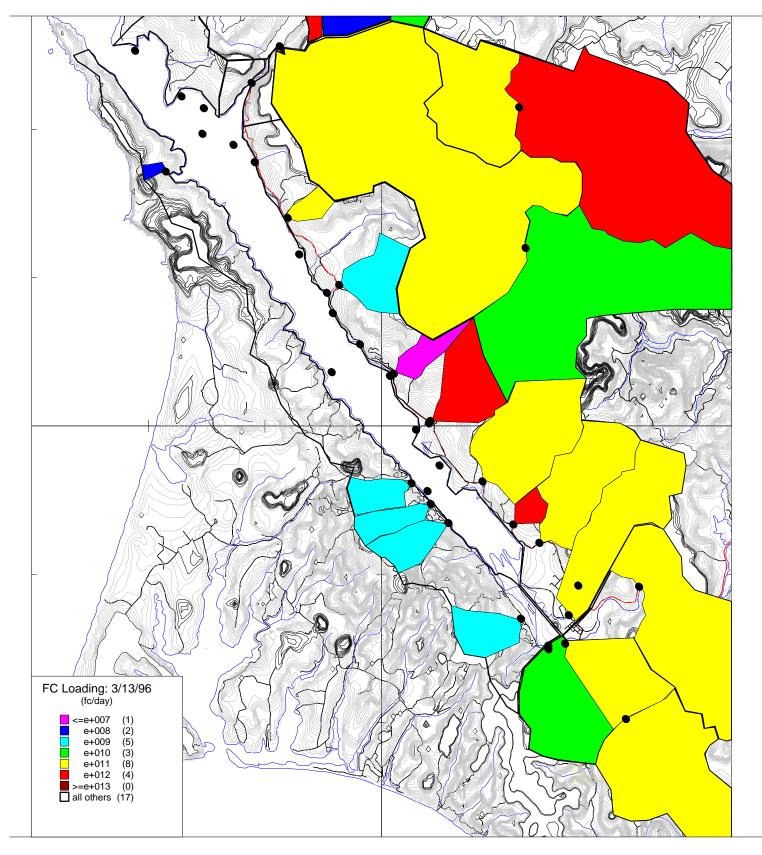


Figure 53. Fecal coliform loadings in the Tomales watershed on 3/13/96 (event 3, day 3).

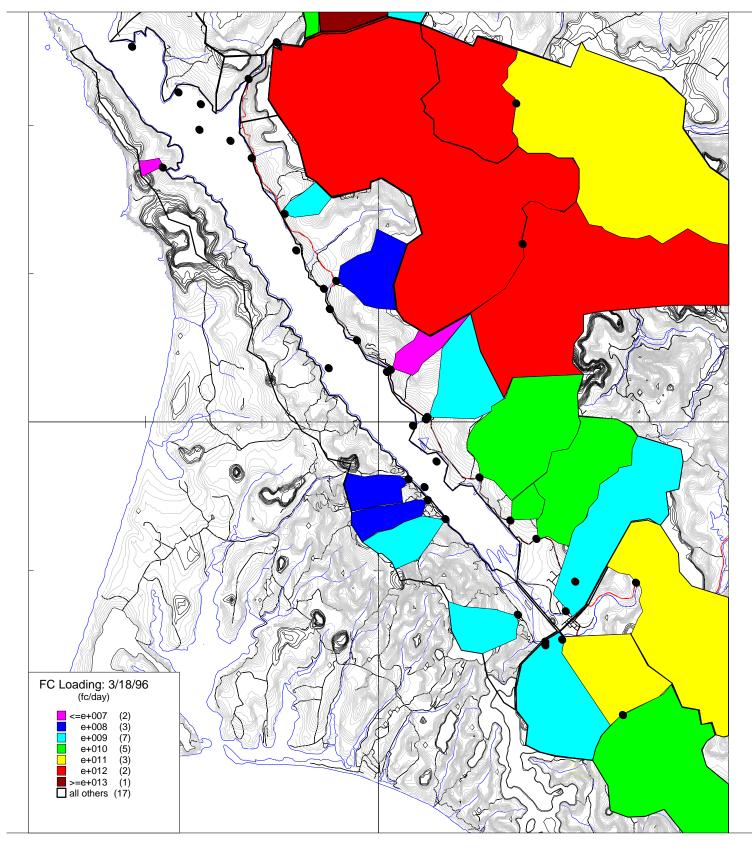


Figure 54. Fecal coliform loadings in the Tomales watershed on 3/18/96 (event 3, day x).

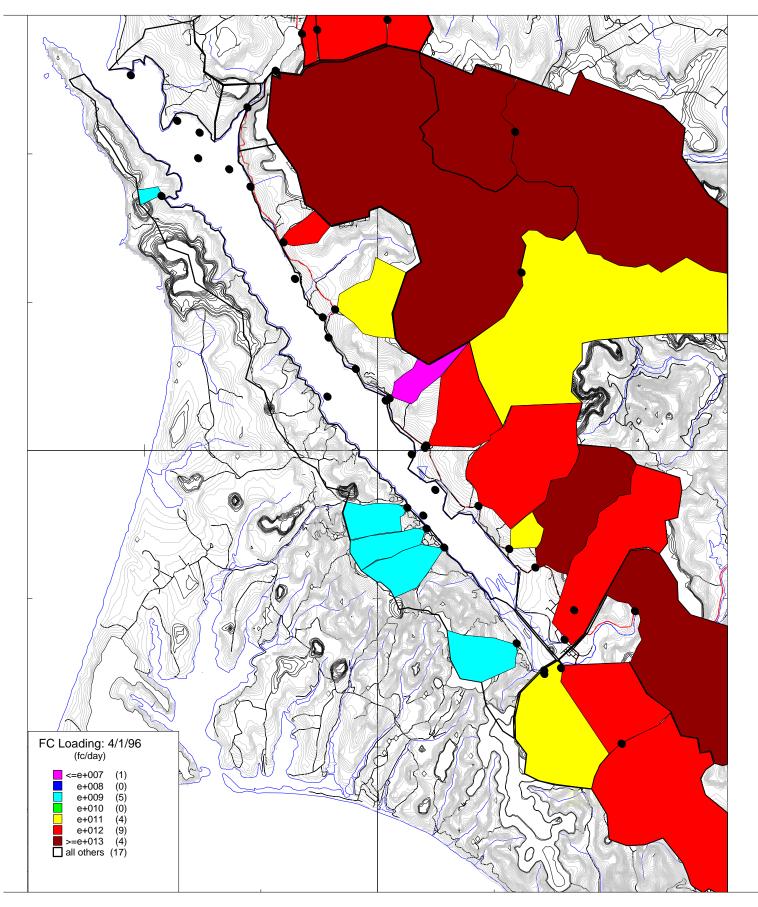


Figure 55. Fecal coliform loadings in the Tomales Bay watershed on 4/1/96 (event 4, day 1).

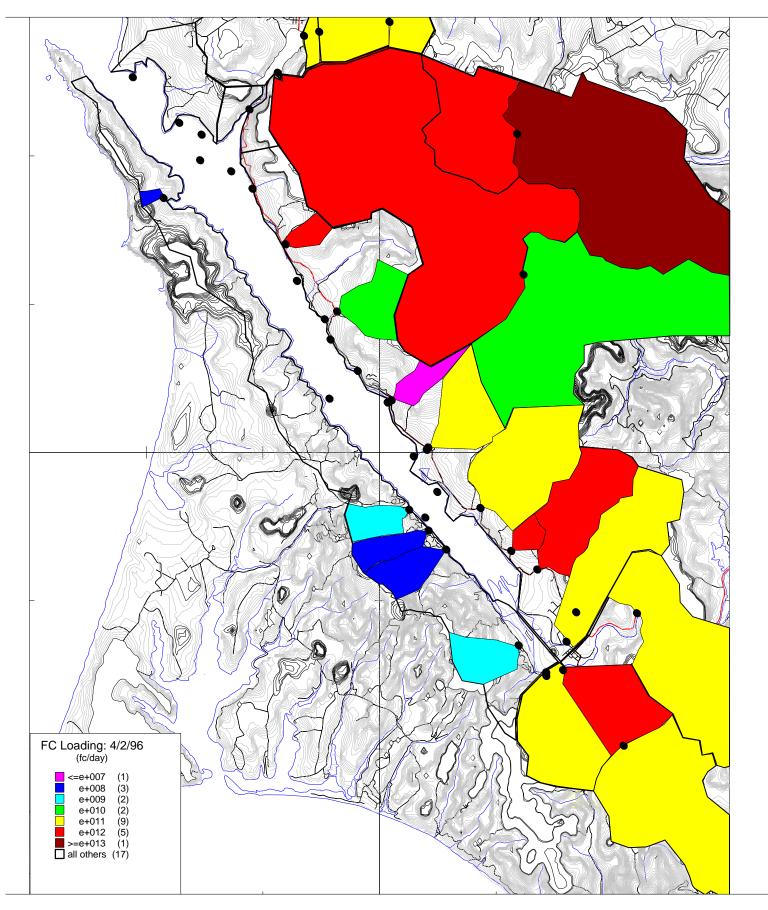


Figure 56. Fecal coliform loadings in the Tomales Bay watershed on 4/2/96 (event 4, day 2).

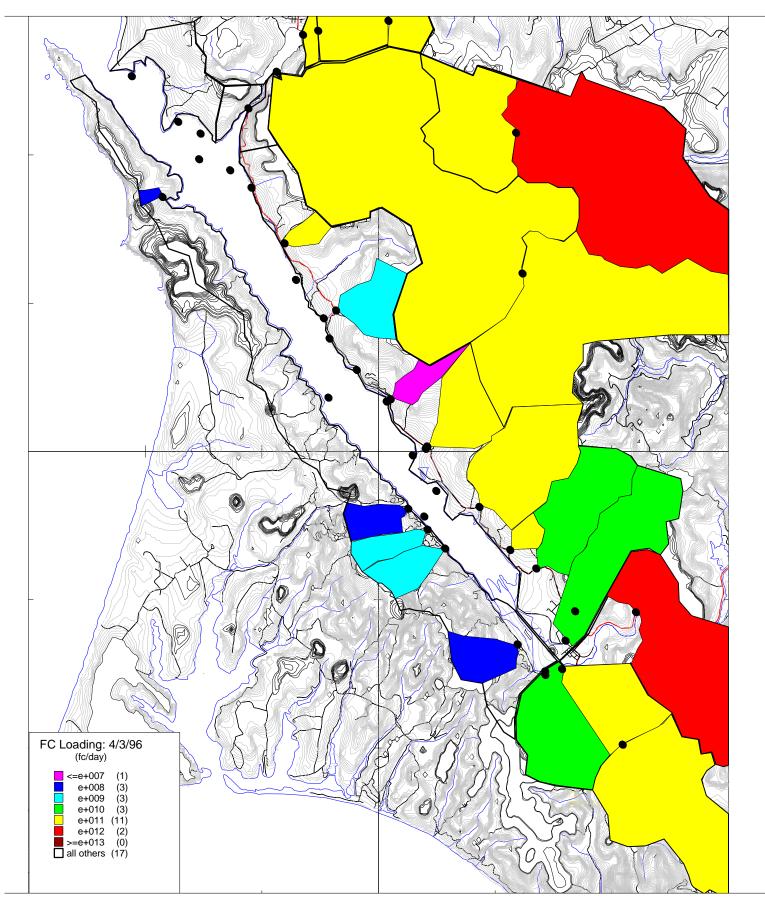


Figure 57. Fecal coliform loadings in the Tomales Bay watershed on 4/3/96 (event 4, day 3).

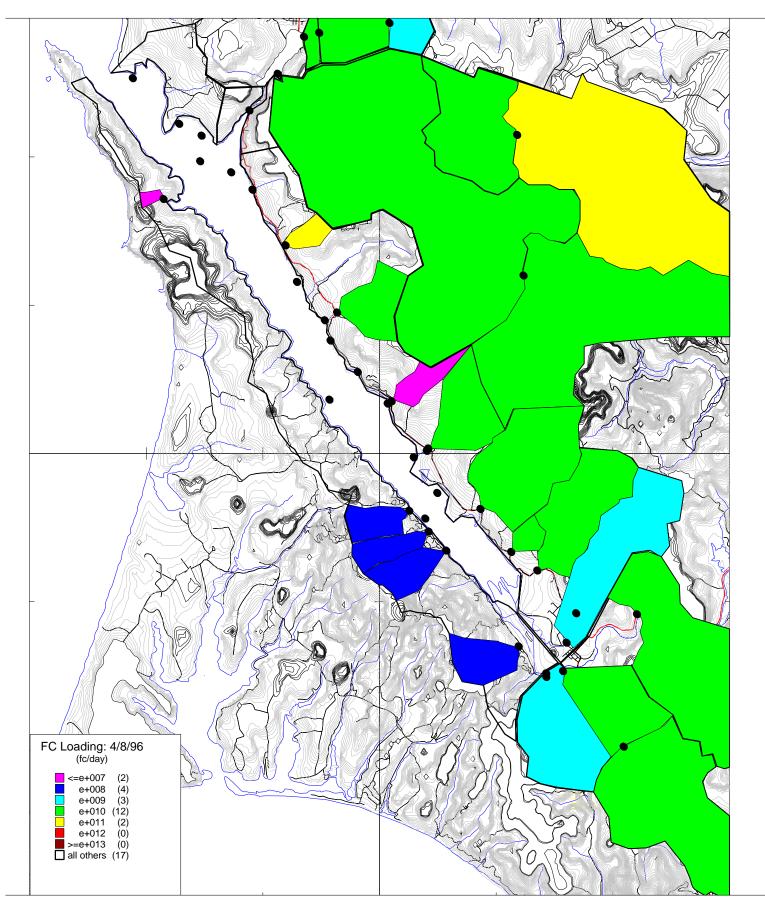


Figure 58. Fecal coliform loadings in the Tomales Bay watershed on 4/8/96 (event 4, day x).

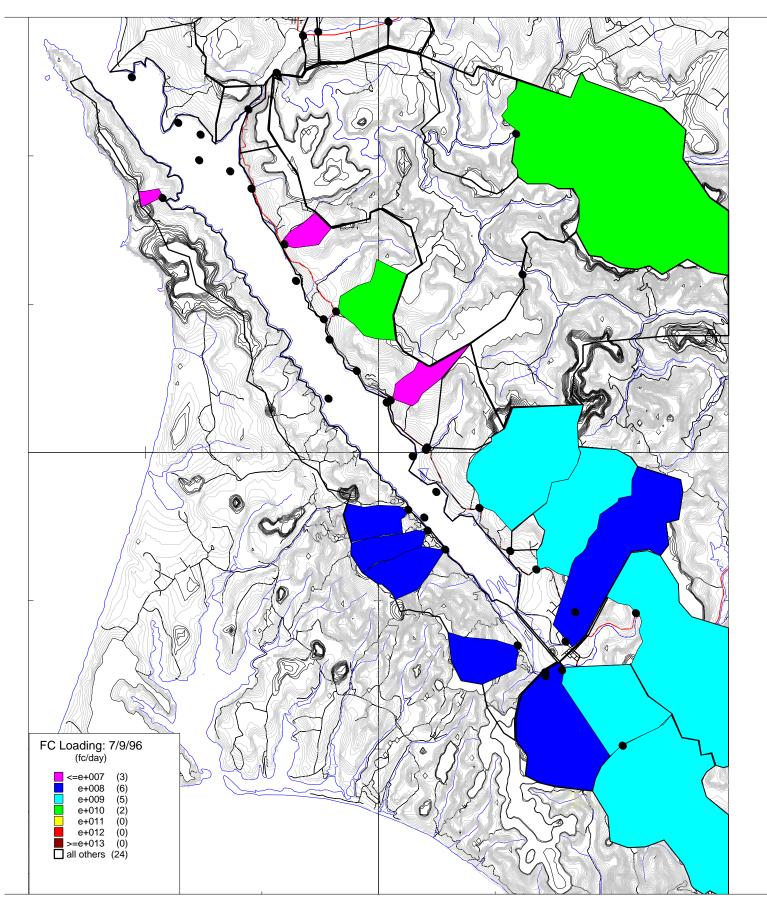


Figure 59. Fecal coliform loadings in the Tomales Bay watershed on 7/9/96 (dry season event 2).

# **APPENDIX A.**

Tomales Bay Shellfish Technical Advisory Committee study design approved on July 26, 1995 and finalized on September 5, 1995.

## TOMALES BAY SHELLFISH TECHNICAL ADVISORY COMMITTEE

Study Design Work Group

Revised Study Plan Outline

September 5, 1995

## I. DESCRIPTION.

The following outline is a final revision of the original study presented to the Tomales Bay Shellfish Technical Advisory Committee on April 5. This revised plan includes several changes made as a result of preliminary investigations conducted throughout the Tomales Bay watershed during the winter of 1994-95. This preliminary data, and the recommended changes to the study plan, were presented to the Shellfish Technical Advisory Committee on July 26, 1995. Final changes were discussed by the Study Design Work Group on August 7, 1995.

Briefly, the purpose of the study is to determine the various point and non-point sources of fecal contamination that are contributing to the degradation of water quality, hence threatening the shellfish growing areas, of Tomales Bay. It is apparent from past monitoring data from the California Department of Health Services that a large component of this contamination is associated with rainfall-related runoff. The preliminary monitoring conducted in conjunction with the current study during the winter of 1994-95 confirmed this conclusion. The need for such a study was approved by the Tomales Bay Shellfish Advisory Committee on February 15, 1994.

In summary, the study consists of 6 sampling periods (2 dry events, 4 rainfall events) and 40 primary sampling stations; additional secondary sampling stations will be added as needed. All samples collected from the primary stations will be analyzed for four standard indicators of microbiological water quality: total coliform, fecal coliform, enterococcus, and E. coli. Four of these primary stations, plus a seawater control site, were selected for use in the special indicator study work. Samples will be collected from these five special study sites twice per rainfall event and once per dry season event. All special study samples will be analyzed for two additional potential biological indicators of fecal contamination, Bacteroides vulgatus and bacteriophage, as well as the four standard indicators mentioned previously. Five sentinel oyster stations will be established: three stations will be used to monitor levels of contamination in the outer, middle, and inner sections of the Bay, and two stations will be used to measure the impact of birds. Shellfish samples will be analyzed for the four standard indicators and *B. vulgatus*. The previous study design proposed the use of a chemical determinant of human source contamination at the indicator study stations. Following preliminary investigations with this method, the TAC agreed to employ this method on an "as-needed" basis, targeting any area suspected of being contaminated by human source material (e.g., failing septic systems). The location of sampling stations and the sampling frequency are discussed in Section III. Details on these methods are provided in Section II.

Indicators were chosen on the basis of (i) potential for success, based on published data, National Indicator Study preliminary results, and/or personal communications with other researchers, and (ii) specificity for a given source material. The sampling stations were selected on the basis of (i) proximity to potential source of fecal contamination, (ii) past history of contamination problems, and (iii) accessibility. The number of stations and the frequency of sampling were dictated by the budget: a balance was reached such that all portions of the watershed are adequately represented while maintaining enough sampling days to gather meaningful data on the extent, duration, and source of fecal contamination in Tomales Bay. Details are provided below.

The Study Design Subcommittee agreed that the current study plan, while providing a good starting point, may be modified during the course of the study. Data from the first sampling events may provide insight that would allow the collection of more meaningful data by adding/deleting sampling stations, changing sampling days, etc. Any such changes will be agreed upon by the study design work group before implementation.

II. TYPES OF ANALYSES (biological indicators, chemical).

According to the National Shellfish Sanitation Program (NSSP), indicator microorganisms are to be employed for the determination of the presence of human fecal pollution of commercial shellfish growing waters. Coliform organisms are the only officially accepted microbiological indicator group. The California Department of Health Services (DHS), as a participant in the NSSP, uses fecal coliform (FC) densities in water samples as the primary regulatory tool for all commercial shellfish growing areas.

- A. Total Coliform (TC) and Fecal Coliform (FC)(Reference No. 1).
  - 1. Fecal coliform encompasses several species of aerobic and facultative anaerobic enteric bacteria. These determinations will act as the standard against which all other microbiological measurements will be compared.
  - 2. All water samples will be analyzed for TC and FC. Total analyses = 720: (see section IV.A.-B. for detailed outline of sampling schedule)
    - a. Rainfall Event: 4 events x 4 days/event x 40 stations = 640 analyses
    - b. Dry Season: 2 events x 1 day/event x 40 stations = 80 analyses
  - Shellfish samples from the two dry season runs and from day X of each rainfall event will be analyzed for TC and FC. Total analyses = 30: (see section IV.A.-B. for detailed outline of sampling schedule)
    - a. Rainfall Event: 4 events x 1 days/event x 5 stations = 20 analyses
    - b. Dry Season:  $2 \text{ events } x \ 1 \text{ day/event } x \ 5 \text{ stations} = 10 \text{ analyses}$
- B. Enterococcus (Reference No. 2).
  - 1. The Enterococci are associated with the fecal wastes of humans and other warm-blooded animals. Determinations of their concentrations will be compared with the total coliform and fecal coliform concentrations.

- 2. All water samples will be analyzed for enterococcus. Total analyses = 720: (see section IV.A.-B. for detailed outline of sampling schedule)
  - a. Rainfall Event: 4 events x 4 days/event x 40 stations = 640 analyses
  - b. Dry Season: 2 events x 1 day/event x 40 stations = 80 analyses
- 3. Shellfish samples from day X will be analyzed for enterococcus. Total analyses = 30: (see section IV.A.-B. for detailed outline of sampling schedule)
  - a. Rainfall Event: 4 events x 1 days/event x 5 stations = 20 analyses
  - b. Dry Season: 2 events x 1 day/event x 5 stations = 10 analyses
- C. E. coli (Reference No. 3, 4).
  - 1. *E. coli* is the most common species of enteric coliform bacteria. Although *E. coli* can be found in the feces of other mammals, it does not readily occur in the environment as do other coliforms. Therefore its presence implies recent fecal pollution.
  - 2. All water samples will be analyzed for *E. coli*, (i.e., a total of 720 determinations).
    - a. Rainfall Event: 4 events x 4 days/event x 40 stations = 640 analyses
    - b. Dry Season: 2 events x 1 day/event x 40 stations = 80 analyses
  - 3. Shellfish samples from day X will be analyzed for *E. coli*. Total analyses = 30: (see section IV.A.-B. for detailed outline of sampling schedule)
    - a. Rainfall Event: 4 events x 1 days/event x 5 stations = 20 analyses
    - b. Dry Season: 2 events x 1 day/event x 5 stations = 10 analyses
  - 4. Approximately 10% of all samples found positive for *E. coli* will be further analyzed by plating techniques for confirmation and determination of strain(s). Some strains of *E. coli* are known to be pathogenic to humans (e.g., *E. coli* 0157:H7).
  - 5. 5% of all samples found negative for *E. coli* will be further analyzed by plating techniques for confirmation. At least one pathogenic strain of *E. coli* (0157:H7) does not test positive with the standard procedure.
- D. Bacteroides (Reference No. 5).
  - 1. Anaerobic bacteria in the human intestine outnumber facultative bacteria such as coliforms by a factor of 1000:1. *Bacteroides vulgatus* is the predominant species of *Bacteroides* found in the human intestine, accounting for 70-80% of the total number present. *Bacteroides spp.* have been identified in animal feces, but *B. vulgatus* has been shown to be the least common species present. Although anaerobic, *B. vulgatus* is one of the most aero-tolerant species in this genera. A National Indicator Study research project conducted by D. Wadford (DHS) and Dr. B. Dixon (California State University, Hayward) has shown that *B. vulgatus* is one of the more promising indicators.
  - 2. All water samples from each of the 5 special study stations will be analyzed for *B. vulgatus*. Total analyses = 50.
    - a. Rainfall Event: 4 events x 2 days/event x 5 stations = 40 analyses
    - b. Dry Season: 2 events x 1 day/event x 5 stations = 10 analyses

- 3. Shellfish samples from day X will be analyzed for *B. vulgatus*. Total analyses = 30:
  - a. Rainfall Event: 4 events x 1 days/event x 5 stations = 20 analyses
  - b. Dry Season:  $2 \text{ events } x \ 1 \text{ day/event } x \ 5 \text{ stations} = 10 \text{ analyses}$
- E. Methylene-Blue-Active Substances (MBAS) (Reference No. 6).
  - 1. Linear alkyl sulfonate (LAS) is the most widely used surfactant in detergents. Methylene blue reacts with LAS, providing a means of detecting the presence of detergents in raw waters. The range of detection is approximately 0.025 to 100 mg/l. Various organic and inorganic compounds can interfere with the determination of LAS, thus the suitability of the MBAS method for estuarine waters needs to be determined.
  - 2. Samples will be collected and analyzed for MBAS as needed.
- F. Bacteriophage: Coliphage (Reference No. 7).
  - 1. Bacteriophage are viruses of bacteria; coliphage are viruses specific to the coliform group. Coliphage have been reported to occur in relatively large numbers in sewage and indicate the presence of coliform organisms. Also, coliphage are thought to emulate the environmental behavior of many human enteric viruses.
  - 2. All water samples from each of the 5 special study stations will be analyzed for coliphage. Total analyses = 50.
    - a. Rainfall Event: 4 events x 2 days/event x 5 stations = 40 analyses
    - b. Dry Season: 2 events x 1 day/event x 5 stations = 10 analyses
- G. Quality Assurance (QA).
  - 1. Field Duplicates: 10% of all water samples will be collected in duplicate (i.e., a total of 72 QA determinations for TC, FC, and *E. coli*). The purpose of the field duplicates is to provide a measure of variability in sample collection. All QA samples will be randomly selected for each sampling event.
  - 2. Lab Duplicates: To ensure integrity in sample analyses, 10% of all samples will be collected in larger volumes for replicate analyses (i.e., a total of 72 QA determinations for TC, FC, and *E. coli*). The purpose of the lab duplicates is to provide a measure of variability in sample analysis. QA stations will be randomly chosen for each sampling event.

#### III. SAMPLING STATIONS.

There are 40 sampling stations located within Tomales Bay and throughout its watershed, and one seawater control site located at the Bodega Marine Lab (Figure 1, Table 1). Four of these stations (plus the seawater control station) will be targeted for more intensive study with the suite of indicators, as described in section IV. Five sentinel oyster stations will be sampled to determine: (i) the level of contamination in oysters subsequent to rescinding a rainfall closure or (ii) the potential microbial contamination due to birds. In addition, there are currently seven secondary water sampling stations, with more to be added as needed. Any of the secondary sampling stations may be activated if data from nearby primary stations warrant closer investigation. The following is a summary of the general location and purpose of these stations.

- A. Tomales Bay (15 stations).
  - 1. 9 stations (#32, 34, 1, 39, 4, 12, 6, 7, 9): primarily located in shellfish growing areas along the east shore. Several of these stations are located near potential non-point sources, primarily creeks and ephemeral drainages.
  - 2. 1 station (#42): potential contamination from outer Bay (northeast of Tom's Pt. near Lawson's Landing).
  - 3. 4 stations (#43, 44, 45, 30): located in near-shore areas where human impact is possible.
  - 4. 1 station (#46): west shoreline approximately mid-Bay in main channel near Indian Beach, for comparison of contamination with east-shore stations.
- B. West Shore Tributaries (5 stations).
  - 1. 4 stations: creeks along west shoreline, from northwest of Inverness to Inverness Park. These represent direct input from the watershed area containing relatively dense human populations.
  - 2. 1 station (creek at White Gulch): control for freshwater inputs, where any fecal contamination should be low and restricted to wildlife sources.
- C. Lagunitas Creek/Olema Creek Watershed(5 stations).
  - 1. 5 stations: located to detect some of the various potential human and animal sources located in this portion of the watershed.
- D. East Shore Tributaries (8 stations).
  - 1. 8 stations: creeks along the east shoreline representing an immediate, direct input into the Bay. Potential sources of contamination represented are livestock and human.
- E. Walker/Keyes/Chileno Creek Watershed (7 stations).
  - 1. 4 stations: Walker Creek and Chileno Creek; primary source of potential contamination is livestock.
  - 2. 3 stations: Keyes Creek, two sites above and one site below the Tomales sewage treatment plant. These represent potential human and animal sources of contamination.
- F. Special Study Stations.
  - 1. Five stations were selected for a detailed analysis with the suite of indicators described in section II.
    - a. Walker Creek: potential livestock source material.
    - b. Keyes Creek below the town of Tomales sewage treatment plant: potential human source from treatment plant.
    - c. Nearshore Marshall (#44): potential human source from faulty septic systems.
    - d. Freshwater Control: creek at White Gulch.
    - e. Seawater Control: seawater from U.C. Bodega Marine Lab.

- G. Shellfish Stations (5 stations). the NSSP does not utilize tissue analysis as a monitoring or regulatory tool for evaluating shellfish growing areas. However, the time required for contaminated shellfish to cleanse themselves of fecal coliform contamination, once water quality has returned to acceptable levels, must be considered when determining the length of rainfall closures in commercial growing areas. Additionally, floating bags of oysters used by some commercial shellfish growers can attract large numbers of birds (e.g., sea gulls, cormorants). The study design work group deemed it important to evaluate the contribution of this component of fecal contamination both to Bay waters and to shellfish.
  - 1. 3 stations: sentinel oyster bags established in outer (#32), middle (#6), and inner (#9) Tomales Bay. Samples collected at day X (see section IV.B.3.b.) will be analyzed to determine the levels of contamination in shellfish from different areas on the first day of harvest reopening.
  - 2. 2 stations: floating and submerged sentinel oyster bags (#1). Samples collected at day X (see section IV.B.3.b.) will be analyzed to determine if a significant difference in microbial contamination exists between the two locations.

#### IV. SAMPLING EVENTS AND SAMPLING FREQUENCY.

- A. Dry Season.
  - 1. There will be 2 dry season sampling events consisting of one day each:
    - a. Prior to the rainy season, tentatively September 12 1995.
    - b. Subsequent to the rainy season, approximately May-June 1996.
  - 2. For each event:
    - a. Water samples from all 40 primary stations will be analyzed for TC, FC, *E. coli* and enterococcus. See details in section II.C. for explanation of additional *E. coli* confirmatory analyses.
    - b. Water samples from the 5 special study stations will be analyzed for two additional indicators.
    - c. Samples from the 5 shellfish stations will be analyzed for TC, FC, *E. coli*, enterococcus, and *B. vulgatus*.
- B. Wet Season (rainfall event).
  - 1. There will be 4 rainfall events that are sampled during the winter of 1995-96.
  - 2. The trigger for defining a rainfall event will be:
    - a. Precipitation exceeding 0.5" within a 24 hour period. This is the current threshold for rainfall closures imposed on the commercial growing areas.
    - b. The amount of rainfall versus freshwater flow at gauged sites will be examined whenever possible prior to calling a sampling event.
    - c. The 4 rainfall events will be distributed as evenly as possible throughout the rainy season.
  - 3. Each event will consist of 4 sampling days:
    - a. Days 1, 2, and 3 following the trigger of 0.5" per 24 hours (i.e., a rainfall closure).

- b. Day X, representing the first day of reopening (i.e., termination of rainfall closure). The opening day for the outer Bay will be used to determine day X.
- 4. For each rainfall event:
  - a. Days 1, 2, 3, and X: water samples from all 40 stations will be analyzed for TC, FC, enterococcus, and *E. coli*. See details in section II.C. for explanation of additional *E. coli* confirmatory analyses.
  - b. On days 2 and X: water samples from the 5 special indicator study stations will be analyzed for the suite of indicators.
  - c. On day X, oyster samples from the 5 sentinel bags at stations 32, 1, 6, and 9 will be analyzed for TC, FC, enterococcus, *E. coli*, and *B. vulgatus*. (Note: station 1 contains 2 different sentinel bags; see section III.G. for description).
- C. Ancillary Data.
  - 1. Stream gauge data will be collected at Lagunitas Creek gauging station and, possibly, on Walker Creek.
  - 2. Salinity measurements will be taken concurrently with all water samples.

## V. SAMPLING EFFORT.

The budget for this study does not permit the hiring of field personnel. Therefore all sampling will be conducted by volunteer staff from the Department of Health Services, the Regional Water Quality Control Board, the State Water Resources Control Board, and the Department of Fish and Game. Additional agency volunteers may be needed to aid the trained staff in the collection and transport of samples.

- A. Watershed.
  - 1. It is anticipated that a two-person team will be needed to sample all watershed stations.
- B. Tomales Bay.
  - 1. Routine Sampling: 1 boat and 2 samplers will be needed on each sampling day. Back-up boats and crews will be needed.
- C. Sample Delivery.
  - 1. Samples must be delivered to the DHS laboratory in Berkeley promptly on each sampling day. In addition, additional sampling materials (bottles, ice chests) will need to be returned to the field for the next day of sampling. Volunteers will be needed to provide this courier service.

# REFERENCES

- 1. APHA. <u>Recommended procedures for the Examination of Seawater and Shellfish, 4th Edition</u>. American Public Health Association, New York. 1970.
- 2. APHA. <u>Standard Methods for the Examination of Water and Wastewater</u>, 18th Edition. Fecal Streptococcus and Enterococcus Groups. American Public Health Association, Method 9230. New York. 1992.
- 3. USEPA. <u>National Primary Drinking Water Regulations: Analytical Techniques; Coliform Bacteria</u>. Final Rule. Fed. Reg. 57:24744-24747. 1992.
- 4. USEPA. <u>Manual for the Certification of Laboratories Analyzing Drinking Water. 3d Edition</u>. EPA/570/990/008. 1991.
- 5. Wadford, D. <u>Bacteroides vulgatus</u>, a Potential Indicator of Fecal Contamination in Shellfish. Master's Thesis, California State University, Hayward. 1993.
- 6. APHA. <u>Standard Methods for the Examination of Water and Wastewater</u>, 18th Edition. Anionic Surfactants as MBAS. American Public Health Association, Method 9223. New York. 1992.
- 7. Kott, Y. 1966. Estimation of low numbers of Escherichia coli bacteriophage by use of the most probable number method. Appl. Microbiol. 14:141-44.

 Table 1.
 Names and descriptions of designated sampling sites for the Tomales Bay Shellfish Technical Advisory Committee Study Plan.

STATION NAME	LOCATION/DESCRIPTION			
BAY STATIONS:				
42	Tomales Bay, Outer. Main channel near boat moorings at Lawson's Landing.			
32	Tomales Bay, Outer. Inside Tom's Pt. Northeastern extent of Lease M-430-15. Site of Outer Bay sentinel oyster bags.			
34	Tomales Bay, Outer. Eastern extent of Lease M-430-16, near northern channel of Walker Creek.			
1	Tomales Bay, Outer. West of Walker Creek delta at established aquaculture lease (M-430-02). Site of top and bottom cultured sentinel oyster bags.			
39	Tomales Bay, Outer. Southwest corner of Lease M-430-17, near southern channel of Walker Creek.			
43	Tomales Bay, Mid. East shoreline, near Blake's Landing.			
4	Tomales Bay, Mid. East shoreline, north of Cypress Pt. Lease M-430-04.			
12	Tomales Bay, Mid. East shoreline, near north end of Marshall at wet storage intake.			
44	Tomales Bay, Mid. East shoreline, Marshall. Indicator Study station.			
45	Tomales Bay, Mid. East shoreline, near Reynold's Cove.			
46	Tomales Bay, Mid. West shoreline, near Indian Beach.			
6	Tomales Bay, Mid. East shoreline, Marconi Cove. Lease M-430-06. Site of Outer Bay sentinel oyster bags.			
7	Tomales Bay, Mid. East shoreline, south of Marconi Cove. Lease M-430-12.			
9	Tomales Bay, Inner. East shoreline, between Tomasini Pt. and Millerton Pt. Lease M- 430-05. Site of Inner Bay sentinel oyster bags.			
30	Tomales Bay, Inner. West shoreline, near Inverness.			
	WESTERN WATERSHED STATIONS:			
White Gulch Ck.	Freshwater control site for Indicator Study.			
Teachers Beach Ck.	Tributary running through Teachers Beach, north of Inverness.			
Milepost 28.86	Tributary running under highway to Bay near Vision Road.			
Milepost 28.29	Tributary running under highway to Bay near Inverness gas station.			
Milepost 25.86	Tributary running under highway to Bay near Inverness Park			

LAGUNITAS/OLEMA WATERSHED STATIONS:					
Whitehouse Pool	Lagunitas Creek at Whitehouse Pool.				
Bear Valley Ck.	Lower portion of tributary near Lagunitas Creek at Whitehouse Pool.				
Olema Ck.	Lower portion of tributary near Lagunitas Creek.				
Olema Ck., upper	Tributary runs under bridge at Bear Valley Road near Olema.				
Lagunitas Ck.	Lagunitas Creek gaging station.				
EASTERN WATERSHED STATIONS:					
Tomasini Ck., lower	Tributary running under Mesa Road southeast of Bay.				
Grand Canyon Ck.	Tributary running under highway southeast of Bay.				
Milepost 32.12	Little Creek, running under highway southeast of Bay.				
Millerton Ck.	Tributary to Bay running under highway.				
Milepost 34.95	Tributary to Bay near Lease M-430-12; runs under highway south of Bay Ranch.				
Milepost 36.17	Tributary to Bay near Lease M-430-06; runs under highway at Marconi Cove.				
Milepost 38.54	Tributary runs under highway, leading to Bay station #12.				
Milepost 40.35	Tributary to Bay, running under highway near Zimmerman and Strauss ranches.				
WALKER/KEYES/CHILENO WATERSHED STATIONS:					
Walker Ck., lower	Below confluence of Walker and Keyes Creek, near railroad crossing.				
Keyes Ck., lower	Below town of Tomales wastewater treatment plant (WWTP).				
Keyes Ck., Irvin Rd.	Tributary crosses under Irvin Road above WWTP.				
Keyes Ck., upper	Near upper reach of Keyes Ck.				
Walker Ck.	Before confluence with Keyes Ck. above highway crossing.				
Walker Ck. Ranch	Walker Ck. below package treatment plant in Walker Creek Ranch.				
Chileno Ck., mid.	Adjacent to ranch road off of Chileno Valley Road.				
	INDICATOR STUDY STATIONS:				
Bodega Marine Lab	Seawater control site				
44	Tomales Bay, Mid. East shoreline, Marshall.				
White Gulch Ck.	Western watershed. Freshwater control site				
Keyes Ck., lower	Below town of Tomales wastewater treatment plant (WWTP).				
Walker Ck.	Before confluence with Keyes Ck. above highway crossing.				

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SHELLFISH SENTINEL STATIONS:				
32	Tomales Bay, Outer. Inside Tom's Pt. Northeastern extent of Lease M-430-15.			
1	Tomales Bay, Outer. West of Walker Creek delta at established aquaculture lease (M-430-02). Site of top and bottom cultured sentinel oyster bags.			
6	Tomales Bay, Mid. East shoreline, Marconi Cove. Lease M-430-06.			
9	Tomales Bay, Inner. East shoreline, between Tomasini Pt. and Millerton Pt. Lease M-430-05.			

ADDENDUM: Sample Volumes Required for Each Analysis.

- A. Water Samples
  - 1. TC, FC, *E. coli*: 100 mL
  - 2. Bacteroides: 100 mL
  - 3. Enterococcus, bacteriophage: 500 mL a. includes filtration and Idex methods for enterococcus
- B. Shellfish Samples
  - 1. 15 20 individuals per sample.

STATION TYPE	NUMBER	CONTAINER VOLUME	TOTAL NUMBER
Regular Stations: 40 sites	2	100 mL	80
	1	500 mL	40
Indicator Study Sites (5):			
4 sites (Bay, creeks)	1	100 mL	5
1 site (BML)	3	100 mL	3
	1	500 mL	1
Field Duplicates: 4 sites	1	100 mL	4
Lab Duplicates: 4 sites	1	500 mL	4
E. coli:0157: 10 sites	1	100 mL	10

Table 2. Summary of sample volumes required for daily sampling run.

# **APPENDIX B.**

Summary of the May, 1998 illness outbreak in Tomales Bay<sup>26</sup>.

# GASTROENTERITIS ASSOCIATED WITH TOMALES BAY OYSTERS

# Investigation, Prevention, and Control

The California Department of Health Services (DHS) was first informed about a foodborne illness outbreak associated with the consumption of raw oysters from Tomales Bay, Marin County, on May 13, 1998. When additional illnesses were reported the next day, DHS's Environmental Management Branch (EMB) immediately closed the six commercial shellfish growers in Tomales Bay to harvesting. The total number of cases eventually reached 171 in 44 clusters from 7 counties. DHS launched an investigation that involved participation by the Division of Communicable Disease Control, the Division of Drinking Water and Environmental Management, the Division of Food, Drug, and Radiation Safety, and several local county health departments. The activities pursued by these groups included clinical investigation, trace-back of product linked to illness from the marketplace back to the growing area leases, a sanitary survey of the growing areas and surrounding watershed, and the analysis of clinical specimens, oysters recovered from the market, and environmental samples to help determine the causative agent. Potential agents initially included viral pathogens, bacterial pathogens, and marine biotoxins. The following summarizes those findings and the steps that were taken to permit reopening of Tomales Bay to shellfish harvesting.

Tomales Bay is a long narrow estuary, approximately 11 miles long that varies from one to two miles wide, located 50 miles north of San Francisco in Marin County. There are currently six certified shellfish growers in the bay, and all but one operate on state leases located on the east shoreline. Immediately following the harvest closure, DHS requested all harvest logs from those growers. An audit of these logs, together with illness reports and the product trace-back data, helped DHS determine that all of the reported illnesses were linked to oysters harvested from the mid to outer Bay. Conversely, there was no evidence of a link between illness and shellfish harvested from the inner Bay leases. When water and shellfish samples collected after the harvest closure were found to consistently meet the National Shellfish Sanitation Program standards, DHS reopened the inner Bay leases for harvesting on June 9. DHS's field investigation then focused on the outer and mid bay regions, which remained closed until August 4.

Onsets occurred between May 1 and May 25, with the majority occurring by May 14. During that time there were approximately 84,500 oysters harvested from Tomales Bay; 52,000 of them originated from the implicated leases in the mid to outer Bay. There were 10 stool samples from patients submitted to DHS, but only three were suitable to analyze for viruses (such as caliciviruses) by electron microscopy. All three were negative. Likewise, these stool samples were found to be negative for a variety of bacterial pathogens, including *Salmonella*, *Shigella*, *Campylobacter*, and all species of naturally-occurring *Vibrio* when analyzed at various local health departments and at DHS. The *Vibrio* results were not surprising, as water temperatures within Tomales Bay during the outbreak period were between 15 and 18 °C, which is much cooler than temperatures normally associated with the proliferation of *V. parahaemolyticus*. In addition, the commercial growers had been conducting their own periodic analyses for *Vibrio* species prior to the outbreak, all of which were negative.

Two stool samples were sent to the Center for Disease Control and Prevention, in Atlanta, which used a reverse transcription polymerase chain reaction (RT-PCR) method for identifying small round structured viruses (SRSVs). One of the two samples was found to be positive for the Group 2 calicivirus (whereas Norwalk agent is in Group 1). Furthermore, convalescent sera sent for study to researchers at the University

<sup>&</sup>lt;sup>26</sup> Reprinted from the January 1999 issue of California Morbidity and Mortality, published by the California Department of Health Services' Division of Communicable Disease Control.

of Massachusetts revealed 2 of 17 samples from outbreak-related cases with very high IgM titers to Group 2 caliciviruses.

A variety of oyster samples recovered from the growers' voluntary recall efforts were submitted for study at the U.S. Food and Drug Administration (USFDA) laboratories in Alabama and California for SRSVs. Of three samples submitted, two (both harvested on May 5) were found to be positive for Group 2 calicivirus.

The potential link between the outbreak and a viral pathogen that has humans as its only known reservoir allowed DHS to focus on sanitary aspects of Tomales Bay, with an aim of finding human sources of contamination. Based on existing knowledge of the bay and additional shoreline survey work, DHS determined that the two most likely causes for the outbreak were the substandard and potentially failing septic systems along the shoreline or overboard discharge(s) of toilet wastes from a recreational or commercial boater. To address the concern of contamination by boaters, DHS interviewed all commercial kayaking companies that are known to operate on Tomales Bay. These companies obtain a use permit from the National Park Service (NPS), and are provided informational leaflets on the proper handling of waste. Each company claims to use sealed marine sanitation devices for overnight camping trips. All such devices are self-contained and are to be safely transported from the bay to an onshore disposal facility several miles from the bay. Private kayakers are not currently regulated by the NPS, and could represent the greatest potential risk, as could non-permitted commercial kayaking companies that may be operating in the bay. During the Department's investigation we received two reports from people who, over the past three years, had each found a plastic bag on the bay that possibly contained human waste. These incidents indicate a potential for contamination of the shellfish growing areas by improper handling and disposal of waste by users of Tomales Bay. Due to the lack of adequate sanitary facilities at the majority of undeveloped campsites on the west shore, there is probably a greater risk of improper waste disposal or transport there than elsewhere along the shoreline of the bay.

In addition to potential impact from kayakers and campers, the Department investigated the likelihood of contamination from recreational boats. Conversations with the California Department of Fish and Game revealed that most recreational fishing trips inside Tomales Bay are four to five hours in length and are based on tide changes. The preferred fishing grounds are primarily within two miles of the boat launch, where the Marin County Parks and Recreation Department (MCPRD) maintains several portable toilets. Additionally, many recreational boaters use the bay as a launch site, then exit the bay for open ocean fishing. For these reasons, the Department determined that this group of users was less likely to be a source of fecal contamination of the bay.

DHS convened a special meeting on August 5 with representatives from federal, state, and county agencies, and from the commercial shellfish industry to discuss short-term and long-term improvements related to boating and camping activity. The NPS had already implemented some improvements, e.g., the permit system for commercial kayaking companies, and NPS is in the process of developing others. For example, the NPS has also proposed a permit system for camping, as well as a reduction in the number of existing campsites. NPS stated that their patrol efforts will increase dramatically next spring in support of the kayaking and camping permit system. The NPS has also agreed to modify their kayaking leaflet to include information on the location of portable toilets around the bay. The NPS and the MCPRD will provide additional signs and information at major launch sites around the bay. The Regional Water Quality Control Board (RWQCB) and MCPRD are investigating the application process to obtain funds from the federal Clean Water Act to install marine pump-out stations at several locations. When this is accomplished, the RWQCB will initiate the process to have Tomales Bay officially declared a "no discharge" zone by the federal Environmental Protection Agency.

Calculations by DHS indicate that one person shedding SRSVs could contaminate a shellfish growing area one mile away with infectious quantities of virus, and waste from two or more people could contaminate an area at least 2.5 miles away. DHS's shoreline survey identified 35 houses within two miles of the affected leases, and 45 additional properties within two miles of other shellfish leases. DHS developed a "Plan of

Action for Determining the Impact from Onsite Sewage Disposal to Shellfish Growing Areas in Tomales Bay" and provided this to the county environmental health department and the RWQCB. Using this document as a guide, DHS reviewed 80 parcels on the east shore of Tomales Bay from Ocean Roar to Marconi Cove, ranking them on a scale from "0" to "3". A ranking of "0" indicated little potential of a negative impact while a ranking of "3" indicated a high likelihood of a negative impact from the onsite sewage disposal system to the water quality of the shellfish growing areas and the need for a field visit to confirm this potential. A total of 71 (89%) of the 80 parcels reviewed were classified as "3s".

DHS met with the commercial shellfish growers and established the criteria for reopening the closed leases in the mid and outer Bay. The first criterion was evidence that SRSVs were no longer present in the closed leases, and the second criterion was a strong commitment by the RWQCB and Marin County to work aggressively to correct identified problems with septic systems around the bay.

DHS, in cooperation with the shellfish growers, collected oysters from all affected leases on June 12 for SRSV analysis by RT-PCR at the USFDA lab in Alameda. Split samples were also sent to the University of North Carolina at Chapel Hill for RT-PCR analysis and detection of culturable enteric viruses by cell culture techniques. Both laboratories reported no detectable SRSVs in any of the shellfish samples. A second series of oyster samples were collected on July 13 and these were also submitted to USFDA for SRSV analysis. These samples were also negative for SRSVs.

DHS had earlier obtained a commitment from the Executive Officer of the San Francisco office of the RWQCB, which is responsible for protecting the waters of Tomales Bay for beneficial uses, to work aggressively to eliminate potential sources of human sewage contamination of Tomales Bay shellfish growing areas. On August 3, DHS received a similar commitment from Marin County, which is responsible for permitting and regulating onsite sewage disposal systems with oversight by the RWQCB. On August 4 DHS notified all commercial shellfish growers that the closed leases in the mid and outer Bay could again reopen for commercial shellfish harvesting.

DHS is hopeful that the combined efforts of all involved agencies, the commercial shellfish growers, and local community members will result in a significant reduction in the risk of contamination to the bay from human sewage. DHS has made it clear that serious measures may be necessary in cases where homes are found to have improper on-site wastewater treatment. Measures may be necessary to prevent these sewage systems from being used until such time as improvements or repairs have been made to eliminate or minimize the threat posed to the shellfish growing waters of Tomales Bay. Long-term solutions should also be explored, such as initiation of community wastewater treatment systems or other acceptable alternatives.

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