Controlling Pathogens in Tomales Bay, California

Total Maximum Daily Load:
Sources & Loadings

Progress Report

June 30, 2001

California Regional Water Quality Control Board
San Francisco Bay Region

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PREFACE

This document is a progress report from the California Regional Water Quality Control Board, San Francisco Bay Region (RWQCB), to U.S. EPA on a Total Maximum Daily Load (TMDL) for pathogens in Tomales Bay, California. This report includes the background information, summary of the past pathogen monitoring studies, and characterization and assessment of the potentially problematic sources and their associated loadings to Tomales Bay. Additional information will probably be added to these sections as the TMDL project progresses. The next steps will be to evaluate linkages between loadings and in-stream response and to calculate waste allocations. On a parallel track, we will focus on implementation actions and linking those actions to the proposed targets.

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INTRODUCTION

Description of Water Body

Tomales Bay is an estuary located in western Marin County, California, approximately 50 km (40 miles) northwest of San Francisco (Figures 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 the Bay 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. 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 lower-bay from the mouth to approximately Hog Island near the Walker Creek Delta, sluggish mixing in the mid-bay (Pelican Point to Double Point) and even less water exchange in the portion of the upper-bay (south of Double Point). 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).

The watershed drainage area for Tomales Bay is approximately 561 km^2 (216 square miles) with four major sources of input: (1) the immediate drainage from small tributaries along the west and east shores (73 km^2; 28 mi^2); (2) Lagunitas Creek (241 km^2; 93 mi^2) to the southeast; (3) Olema Creek (50 km^2; 19 mi^2), which flows into Lagunitas Creek close to the head of the Bay; and (4) Walker Creek (196 km^2; 76 mi^2) to the northeast (Table 1; Figure 2) (Fischer et al. 1996).

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 2). 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 1 & 3). 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 Bay shore 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/year. Approximately one third of the sediment is carried into the Bay from the Walker/Keyes Creek drainage.

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-1800s, although the number of dairies has been declining since there has been an increase in competition from large Central Valley dairies. The number of livestock and associated manure production in the watershed was estimated in 1990 (Table 4). However, since some dairies have switched to raising beef cattle and others have increased the size of their dairy herds, it is unclear, at this time, how the number of animals in the watershed has changed.

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 Reyes 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 small sewage treatment systems within the watershed, and one facility that accepts septage waste (Table 5). The Regional Water Quality Control Board (RWQCB) prohibits direct discharge from treatment systems into Tomales Bay or the creeks within the watershed. A number of the sewage treatment systems have holding ponds and are permitted to discharge to irrigation fields during the dry season.

Regulatory Authority and Water Quality Standards for Tomales Bay

As is the case with all surface bodies of water in the State of California, the RWQCB has been designated authority by U.S. EPA to administer the Clean Water Act (CWA) in Tomales Bay. Under this authority, the RWQCB designates beneficial uses for Tomales Bay and adopts standards to protect those beneficial uses. The RWQCB has adopted a Water Quality Control Plan (Basin Plan) that contains a list of beneficial uses for each water body in the Region and the standards and implementation measures necessary to protect those beneficial uses. The beneficial uses of Tomales Bay listed in the Basin Plan related to pathogens are shellfish harvesting, water contact recreation and non-contact water recreation. Numerical water quality objectives for fecal and total coliforms have been developed for each of these beneficial uses and are listed in the Basin Plan (Table 7). In accordance with section 303(d) of the CWA the RWQCBs are required to develop lists of impaired water bodies in their region, along with the causes of impairment. The RWQCB has listed Tomales Bay as an impaired water body for pathogens, sediments, and nutrients. The listing of Tomales Bay as impaired due to pathogens is based on the exceedence of water quality standards for shellfish harvesting, the listing of Tomales Bay as "threatened" under the State's Shellfish Protection Act, the prohibition on commercial harvesting during rainfall periods, regulated by the California Department of Health Services, and an illness outbreak from the consumption of shellfish that illustrated the inability to protect human health, under current conditions, even when coliform objectives are being met.

The California Department of Health Services (DHS) has separate authority and standards to regulate commercial shellfish growing areas. These standards supercede those contained in Regional Basin Plans. In the San Francisco Bay Region, Basin Plan standards for fecal coliform (FC) in shellfish-growing waters require that the concentration of FC in the ambient water cannot exceed a median of 14 MPN/100mL, or the 90th percentile cannot exceed 43 MPN/100mL. Although DHS used a median value in the past, they now use a geometric mean of 14 MPN/100mL. DHS standards follow criteria developed by the National Shellfish Sanitation Program (NSSP), which is administered by the U.S. Food and Drug Administration (FDA) (U.S.FDA, 1997). These standards allow for a median or a geometric mean to be used. The NSSP standards are based on acceptable levels of FC in shellfish and shellfish growing waters. The NSSP FC standard for shellfish is a market standard of 230 MPN/100 grams (U.S.FDA, 1995). DHS has developed rainfall closure rules, when shellfish cannot be harvested, for different areas of Tomales Bay based on the analysis of water column and shellfish data. These closure rules have become very site specific as the amount of data has increased and the data analysis has become more refined. Rainfall closure rules have also become more stringent. The latest and most stringent rules were issued in 1997.

On October 10, 1993, legislation was passed by the California legislature that enacted the Shellfish Protection Act of 1993. This legislation is incorporated in the Porter Cologne Water Quality Control Act (California Water Code, Division 7, Chapter 24, Section 14950-14958). Under this law the RWQCB is required 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 Tomales Bay Shellfish Technical Advisory Committee (TBSTAC). The RWQCB staff organized 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.

Aquaculture

The vast majority of shellfish harvesting in Tomales Bay is from commercial shellfish growing areas. There are currently eight certified commercial shellfish harvesters in Tomales Bay, with a combined aquaculture lease area of 483 acres (Table 6; Figure 2). 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. Shellfish cultivation in Tomales Bay is primarily devoted to Pacific oysters (*Crassostrea 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*). There is a fairly large amount of recreational harvesting for horseneck clams north of the Walker Creek Delta during the spring and fall. There is also a small bed of cockles and clams used for recreational harvesting near Hamlet, just south of the Walker Creek Delta.

Problem Statement

To summarize, the following arguments form the basis for listing Tomales Bay as impaired due to pathogens under the Clean Water Act section 303(d):

1. Tomales Bay exceeds water quality objectives set by (a) the RWQCB in the San Francisco Bay (Region 2) Water Quality Control Plan (Basin Plan); (b) the California DHS; and (c) the FDA through National Shellfish Sanitation Program standards. Since DHS rainfall closure rules are based on FC concentrations in water and shellfish, the number of days Tomales Bay is closed for harvesting can be used as a surrogate for the number of days FC concentrations exceed standards. It has been estimated that Tomales Bay is closed to harvesting approximately 90 days per year, and therefore it is assumed that FC standards are exceeded for approximately 90 days per year.

2. Under the State's Porter Cologne Water Quality Control Act (California Water Code, Division 7, Chapter 24, Section 14950-14958), the Shellfish Protection Act, Tomales Bay is considered "threatened" due to the conditions listed under Paragraph No. 1.

3. DHS prohibits shellfish harvesting during periods of rainfall based on the results of bacteriological studies. As stated in Paragraph No. 1, the Bay is closed to harvesting approximately 90 days per year. In 1997, closure rules for shellfish harvesting were made more stringent. Therefore, the Beneficial Use of Shellfish Harvesting is not protected during this season.

4. During a period without rainfall and when bacteriological objectives were met, there was a major human illness outbreak of a virus of human origin from consumption of

oysters; therefore, beneficial uses were not protected even when water quality objectives were met.

PAST STUDIES

Overview

Monthly water quality monitoring for FCs in Tomales Bay is conducted by shellfish growers under the authority of DHS. In addition, several intensive studies have been conducted on bacteriological water quality in relation to shellfish harvesting over the past 26 years. These studies were: 1) a shellfish and water quality study conducted in 1974 by the DHS (Sharpe, 1974), 2) a shoreline and watershed water quality survey carried out in 1976-77 and 1977-78 by the RWQCB (Jarvis et al., 1978), 3) a sanitary survey conducted by the Department of Health and Human Services of FDA (Musselman, 1980), 4) a pilot study conducted by DHS in the winter of 1994-95 to test sampling methods and locations for the 1995-96 study and 5) a State Water Resources Control Board (SWRCB) funded study conducted in 1995-96 by DHS and the RWQCB, under the auspices of the TBSTAC. The results of these studies are discussed briefly below.

1974 Study – California Department of Health Services

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 coliforms. Shellfish from six locations were also sampled for coliforms 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 the 90th percentile of samples may not exceed 43 MPN/100mL. Shoreline samples showed elevated total and FC 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 FC 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.

1976-78 Study – Regional Water Quality Control Board

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 FCs, 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.

1980 Study – U.S. Food and Drug Administration

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 FC and fecal streptococci. Shellfish samples were taken from two sites in the Bay and analyzed for total and FCs.

Results showed that the shellfish market standard for FCs 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. Tributary samples ranged from low FC 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 FC densities in the streams during dry weather were equal to sewage from about 150 to 200 people. During wet weather, FC 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 nonpoint pollution were considered to be the most likely cause of high FC densities in the Bay.

1994-95 Pilot Study – Department of Health Services

The pilot study conducted by the DHS in the winter of 1994-95 was a prelude to the study during 1995-96 (DHS, 1996). Both of these studies were a result of Tomales Bay being considered "threatened" under the Shellfish Protection Act and the formation of the TBSTAC. This study 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 FC, *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 FC 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 FC concentrations were observed: 1) sites that showed declining FC densities throughout the winter, suggesting a nonrenewable source and, 2) sites that exhibited high FC densities throughout the season, suggesting a renewable 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.

1995-96 Study – TBSTAC, SWRCB, DHS, RWQCB

In the winter of 1995-96 the RWQCB and DHS, under the auspices of the TBSTAC and funded by the SWRCB, conducted an intensive study of bacteriological and pathogen levels in the water of Tomales Bay and its watershed. Concentrations of FCs in oyster tissue were also measured. Samples were collected before and after the wet season and throughout rainfall events, including the day the Bay would normally be opened for shellfish harvesting (day X). 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, FC, 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 watershed. Slightly lower concentrations of FC were detected throughout the Lagunitas/Olema subwatershed. In contrast, bacterial levels at the western shoreline watershed stations were generally 10 to 100 times lower than those from all other subwatersheds.

FC loadings were calculated to estimate the amount of FC contributed by each subwatershed on a daily basis. The highest loadings occurred within the Walker/Keyes/Chileno Creek and the Lagunitas/Olema subwatersheds. The former region is primarily dairy and livestock 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 FC loadings occurred in the Chileno Creek subwatershed. Within the eastern shoreline watershed, the highest FC loadings generally occurred in the subwatersheds 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 subwatershed. FC loadings from the western subwatershed were less than that contributed by the other subwatersheds.

Bay Water Quality

Outer-bay sampling stations were adversely affected within the first two days following significant rainfall. FC concentrations often remained elevated three days after the rainfall event and did not always return to acceptable levels by the day shellfish growing waters were reopened for harvest (day X). This indicated either a long residence time in the outer-bay or a prolonged source of contamination. The highest FC 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 FC levels that were generally lower than either the outer or inner-bay regions, although all Bay stations experienced elevated concentrations of FC 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 FC 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 FC 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 the 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 FC 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 FC 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 subwatersheds consists of dairies and cattle grazing land. Primary land uses in the southern subwatersheds 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. As a result of this study and previous supporting data the rainfall closure requirements that DHS applies to harvesting shellfish in Tomales Bay were made more stringent. More detailed conclusions are included in the final report (TBSTAC et al., 2001).

Comparisons of FC Results Among Studies

In order to try to assess trends in FC numbers over time, data from all studies were compared for selected Bay and watershed stations as part of the report on the 1995-96 study (TBSTAC et al., 2000). 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 FC 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).

Lack of data on other environmental variables related to sampling (e.g., streamflow and precipitation) and variability in rainfall, streamflow, and soil saturation make it difficult to come to any clear conclusion about FC 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 FC 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 FC 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. Although initially there seemed to be an improvement in water quality between the 1974 and 1976-78 study, long-term there were no clear overall trends of increasing or decreasing FC 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.

2000-2001 STUDY

Overview

In 2001, the TBSTAC, in conjunction with the RWQCB designed and conducted a study to partially satisfy the TMDL requirements, and to also carry out some of the TBSTAC recommendations from the 1995-96 study. The specific goals of the study were to: i) verify the findings of previous studies regarding potential sources of fecal contamination to Tomales Bay, ii) collect fecal coliform data from some additional stations (points of interest) within the watershed, iii) characterize and assess the loadings of FC to Tomales Bay at the subwatershed scale, and iv) to compare FC and *E. coli* data obtained from two different methods, in order to evaluate the correlation between the two indicators.

Materials and Methods

Sampling Frequency

The study consisted of five sampling events, designed around the wet season. Two dry-weather sampling events were conducted, the first occurring prior to the wet season, and the second, following the wet season. Each dry-weather sampling event was carried out in one single day period. There were three wet season sampling events. Samples for the wet season events were collected over a two-day period that coincided with the first two days of a rainfall harvest closure (defined as 0.5 inch of rain within a 24-hour period). The sampling event conducted during Rainfall Event No. 1 was an exception; due to the short duration of the rainfall event, samples were collected over a one-day period only.

Wet Season Sampling Trigger

The beginning of the wet season was defined as the first rain event, after ground saturation had been attained, producing 0.5-inch rainfall within a 24-hour period. Ground saturation was determined by the onset of continuous flow in the smaller (seasonally ephemeral) tributaries of the watershed. Point Reyes National Park Service Staff monitored the flow in the ephemeral tributaries and called for the initiation of the rainfall period sampling, once a continuous flow was observed. At the time that the wet season sampling was initiated, approximately 30 cumulative inches of rain had fallen in the region.

Sampling Stations

A total of 20 sampling stations were selected throughout the watershed and the Bay—three inner-Bay stations, three outer-Bay stations, and fourteen watershed stations (Table 8, Figure 3). Station locations were selected on the basis of their i) proximity to potential sources of fecal contamination, ii) past history of contamination iii) areas of regulatory compliance (i.e., shellfish beds), and iv) site accessibility.

"Standard" FC Sampling

During each sampling event, FC samples were collected and analyzed for each of the 20 stations. Bay stations were sampled three times daily (see below), whereas the watershed stations were sampled only once in any given sampling day. To increase the statistical power of the FC results, triplicate FC sampling was conducted at selected watershed sampling stations (Table 8). The actual number of stations and the total number of daily samples, including the number of replicates, were dictated for each sampling event by the limited capacity of the contract laboratory analyzing the samples.

E. coli Time Series Sampling

In order to obtain samples from the complete hydrograph and to capture the peak flow and peak loadings at the Olema, Lagunitas, and Walker Creek Stations, *E. coli* time series samples were collected at those stations, in addition to the standard once-a-day FC sampling. Time series sampling, which consisted of collecting a sample approximately every few hours for the duration of the rainfall event, was conducted throughout each of the 3 rainfall sampling events. Within a sampling event, the time series sampling period varied from the "standard" FC sampling event (*E. coli* time series sampling typically began the evening prior to the "standard" sampling event, and in some occasions continued past the last FC collection time).

Bay Temporal Response Sampling
To assess the assimilative response of the Bay waters, in a temporal context, to FC loadings from major creeks and tributaries, all Bay sampling stations were sampled for FC three times on each sampling day, at 8:00 AM, 11:00 AM, and 2:00 PM, respectively.

Collection Method
Samples were collected in 100-mL sterile, screw-cap polypropylene bottles, which were then placed in watertight Zip-Loc bags. Once in the bags, the bottles were immediately placed in an insulated ice chest containing sufficient ice and water to maintain a temperature between 4^o and 10^oC. Samples were transported to the EPA Region IX and BioVir Laboratories where *E. coli* (using Colilert) and fecal coliform (using standard MPN) analyses were performed, respectively, within the required holding times.

Analytical Methods
All non-time series water samples were analyzed for FC using the Most Probable Number (MPN) estimate of bacterial density in a multiple tube fermentation test (Standard Methods, 18th ed., Part 9221, 1992). The laboratory conducting the MPN analyses had a processing capacity of 50 samples per day so the time series water samples were analyzed for *E. coli* using the Colilert method at EPA Region IX laboratory (Standard Methods, 18th ed., Part 9223, 1992). The reporting unit for both methods is Most Probable Number per 100 Milliliters of water (MPN/100 mL). 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).

To compare and relate the results of the two indicators, a subset of the time series samples, that were analyzed for *E. coli*, was also analyzed for FCs, using the standard MPN method. In the 1995-96 study, which used the standard MPN method for the detection of both FC and E. coli, *E. coli* and FC results were observed to be closely correlated. The procedure for the Colilert method (used in this study), however, involves a larger number of dilutions than the traditional MPN method, and therefore, is more precise than the MPN method. For this reason, use of the Colilert method may affect the previously observed (TBSTAC et al., 2000) relationship of *E. coli* and FC measurements.

Flow/Discharge Measurements
Utilizing calibrated rating curves provided by the United States Geological Survey (USGS 2001) and the Point Reyes National Park Service (PRNPS 2001), discharge data in 15-minute increments were obtained for Lagunitas, Walker, and Olema Creeks from preexisting gauging stations. For the remaining streams for which no automated gauging station and/or accurate rating curves were available, manual discharge measurements were conducted. In the manual method, surface water velocity was determined by placing a floatable object (i.e., orange peel) in the creek and timing its transport on the water surface along a known distance. The channel flow velocity was then calculated by using a gravel co-efficient of 0.8 as a multiplier to convert surface water velocity to channel flow velocity.

Staff gauges were used to measure the water height at each sampling station. Where possible, by measuring the cross sectional area of the creek (width times water height) and multiplying it by the channel flow velocity, the instantaneous discharge of the stream was calculated.

Rainfall Measurements

All rainfall measurements were obtained 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. Point Reyes National Park Service staff closely monitored rainfall throughout the study and contacted RWQCB for concurrence prior to initiating a sampling event.

Results and Discussion

Rainfall Record

The results of earlier studies have revealed that degradation of Bay water quality clearly coincided with the pulses of fecal contamination from the watershed after rainfall. In order to further elucidate the relationship, this study was designed to capture the peak loadings of FC and *E. coli* to the Tomales Bay during 3 rainfall events that produced greater than 0.5 inch of rain within 24 hours (shellfish harvesting closure threshold).

Figure 4 depicts the cumulative rainfall record from January 1, 2001, to February 12, 2001 (the duration of wet weather sampling). After ground saturation and occurrence of continuous stream flow in smaller tributaries was reached (the first condition for commencement of wet-season sampling events), the first significant rainfall that exceeded the closure threshold of 0.5 inch within 24 hours occurred on January 11, and produced 1.02 inches of rain. This rainfall initiated the first wet-weather sampling event which lasted only one day due to the fact that it stopped raining on the second day.

The second wet-weather sampling event began on January 25, 2001, during a rainfall event that produced a total of 0.52 inches of rain. This sampling event lasted for two days since an additional 0.24 inches of rain was deposited on the next day, January 26.

The third wet-weather sampling event began on February 9, 2001, and lasted for two days though February 10. This sampling event was initiated following a significant rainfall event that produced 0.4 inches of rain on February 9 and 0.23 inches of rain on February 10. Smaller rainfall amounts on February 11 and 12 prompted the continuation of E. coli time series sampling through February 12.

Fecal Coliform Results

Tables 9 through 14 contain the FC monitoring results for the watershed and Bay stations. Comparisons of FC densities and water quality objectives (WQO) for Shellfish Harvesting Waters are summarized in Figures 5 through 9.

Dry Weather Samples (Pre-Season; 1/3/01):
Seven of the 20 watershed and Bay stations were not sampled, due to insufficient water flow in some of the creek stations (because of a delayed wet season), and the unavailability of a sampling boat for Bay station sampling.

The pre-season dry weather samples that were collected were not analyzed at the correct dilutions at the laboratory, causing a loss of resolution in the resulting data. In the MPN procedure, in order to attain sufficient resolution to detect the low levels of FC typically found in dry season water samples, a dilution factor should be utilized that is orders of magnitude less than what is used for high concentration, wet season samples. The laboratory mistakenly processed the dry weather samples at the higher dilution appropriate for wet season samples, and therefore, the method did not achieve the resolution necessary to accurately define the concentrations of most samples, which were all reported as <200 MPN ("less than certain value"). Of the few samples with levels greater than 200 MPN (Chileno Creek, Walker Creek Ranch, Olema Creek, San Geronimo at White Horse Bridge, and San Geronimo at Roy's Pool), concentrations ranged from 200 to 400 MPN. The highest FC concentration detected, 400 MPN, was sampled at Roy's Pool station on San Geronimo Creek.

Rainfall Event No. 1 (1/11/01):
Due to the unavailability of a sampling boat for Outer-Bay station sampling, no samples were collected at the Outer-Bay Stations Nos. 4-6.

For all of the Bay and Watershed sites sampled, Rainfall Event No. 1 FC levels were greatly elevated and ranged from 200 MPN at Inner-Bay Station No. 2 (located at Tomales Bay Oyster Company lease area) to 1.27×10^5 MPN at the Chileno Creek station. Walker Creek Ranch and Keyes Creek at Tomales Village stations had the second and third highest FC levels.

Rainfall Event No. 2 (1/25-1/26/01):
Due to the unavailability of a sampling boat for Outer-Bay station sampling, no samples were collected at the Outer-Bay stations Nos. 4-6, on either day one or day two of the sampling.

The FC levels for Rainfall Event No. 2-Day 1 ranged from 200 MPN at the Walker Creek Ranch station, Samuel P. Taylor Park station, and Inner-Bay station No. 2, to 1.17×10^4 MPN at the Chileno Creek station. The Lagunitas Creek station at Gallagher had the second highest FC level, at 6.7×10^3 MPN. Although the Rainfall Event No. 2-Day 1 FC levels at most stations were somewhat lower than those measured during Rainfall Event No.1, they remained greatly elevated and exceeded the WQO for shellfish harvesting and, in many cases, exceeded the WQOs for water contact recreation and non-contact water recreation, throughout the Watershed and Bay stations.

Rainfall Event No. 2-Day 2 FC concentrations were significantly increased from Day 1 concentrations at all sampling stations, with the exception of the 3rd Street storm drain site at the Town of Point Reyes Station. Day 2 FC levels ranged from 333 MPN at 3rd Street storm drain station at Point Reyes Station, to 9.4×10^4 MPN at Walker Creek station at the Highway 1 bridge crossing. The Chileno Creek station had the second highest FC level at 3.2×10^4 MPN.

Rainfall Event No. 3 (2/9/-2/10/01):
Due to personnel shortage and insufficient water flow, no Day 1 samples were collected at Walker Creek station at the Highway 1 bridge crossing and 3rd Street storm drain station at Point Reyes Station. During Day 2 sampling, due to the unavailability of a sampling boat and insufficient water flow, no samples were collected at the Outer-Bay stations Nos. 4-6 or 3rd Street storm drain station at Point Reyes Station.

The Day 1 results for Bay stations Nos. 1-6 and Watershed Station No. 17 (Nicasio Creek at the Platform Bridge) fell under the detection limit of 200 MPN and were reported as <200 MPN. The FC levels for all other Day 1 samples ranged from 200 MPN at the Lagunitas Creek station at Samuel P. Taylor State Park to 1.1×10^4 MPN at the San Geronimo Creek station by Roy's Pool. Walker Creek Ranch and Keyes Creek at Tomales Village stations had the second and third highest FC levels at 7×10^3 MPN, and 6.7×10^3 MPN, respectively. Rainfall Event No. 3-Day 1 FC levels for all but one station (Walker Creek Ranch) were lower than those measured during Rainfall Event No. 2-Day 2. The FC levels for all stations; however, significantly exceeded the WQOs designated for shellfish harvesting and in most cases those for contact and non-contact water recreations.

The Day 2 results for Bay stations Nos. 1-6 and the Nicasio Creek station at the Platform Bridge fell under the detection limit and were reported as <200 MPN. Day 2 FC levels remained greatly elevated throughout the watershed stations, with a range of 300 MPN at the Walker Creek station at the Highway 1 Bridge crossing, to 6.3×10^4 MPN at the Chileno Creek station. Giacomini Levee and Keyes Creek station at the Village of Tomales had the second and third highest Day 2 FC levels at 5×10^3 MPN and 4.7×10^3 MPN, respectively.

Throughout all three wet-weather sampling events, the FC levels for all watershed and Bay station samples significantly exceeded the designated WQO for shellfish harvesting waters and in most cases for contact and non-contact water recreations. In general, FC levels increased during the second day of each wet-weather sampling event (with the exception of the first wet-weather sampling event which lasted only one day). The FC levels decreased from the rainfall event 1 (1/11/01) to the first day of the rainfall event 2 (1/25/01) but, increased to the similarly high levels in the second day of rainfall event 2 (1/26/01).

Dry Weather Samples (Post-Season; 6/1/01*):*
(No results have been received as of the date this document was prepared).

Bay Stations Temporal Sampling:
Over the 6-hour span during which the temporal sampling was conducted, FC levels at the Bay Stations (Figures 9-14) did not reveal any obvious trend in the assimilative response of the Bay to loadings from the creeks and tributaries. Due to unavailability of sampling boat, only one set of samples from Outer-Bay Stations Nos. 4-6 was collected during this study.

However, the FC concentrations in these samples fell below the detection limit of 200 MPN and were reported as <200 MPN. Results for the Inner-Bay Stations Nos. 1-3 for the Pre-Season Dry Weather Sampling Event and the Rainfall Event No. 3 samples also fell below the detection limit and could not be used in the analysis.

For the remaining Inner-Bay sampling locations, FC levels did not change between Rainfall Event No. 1 and Rainfall Event No. 2-Day 1, but increased significantly on Rainfall Event No. 2-Day 2. Of the Inner-Bay station samples, over all of the sampling events, the highest FC levels were consistently detected at the Inner-Bay Station No. 1 (located south of the Tomales Bay Oyster Company (TBOC) lease area) which is closer to the inlet of Lagunitas and Olema Creeks than the other two Inner-Bay stations. The FC levels at Inner-Bay Station No. 2 (located at the center of the TBOC lease area) were the lowest among samples collected from all three Inner-Bay stations.

E. coli Time Series Sampling Results

To obtain complete hydrographs and to capture the peak bacterial loadings for Olema, Lagunitas, and Walker Creeks, *E. coli* time series samples were collected at those 3 stations for each of the 3 rainfall sampling events. The results of the E. coli time series sampling are reported in Tables 15-18 and summarized in graphs in Figures 10-19.

Dry Weather Samples (Pre-Season):

Overall, the Olema Creek samples, with a range of 20 to 140 MPN, had the highest dry-season *E. coli* levels. The E. coli levels at Lagunitas Creek and Walker Creek were similar to each other, with ranges of 12 to 29 and 13-29 MPN, respectively. The hydrographs for the Olema and Lagunitas Creeks were similar to each other, showing high *E. coli* concentrations in the morning, decreasing to the lowest levels by noon, and increasing back to high levels by early afternoon. Rather than attributing this observation to a diurnal cycle in transport rates; it is possible that changes in water column temperature from morning to afternoon cause a fluctuation in the E. coli die-off rate. In the Walker Creek hydrograph, *E. coli* levels were highest in the morning and gradually decreased to their lowest levels in the afternoon.

Rainfall Event No. 1 (1/10-1/11/01):

Due to personnel shortage, no time-series samples were collected for the Walker creek station during this sampling event. For both Olema and Lagunitas Creeks, the *E. coli* levels were significantly higher than their dry-season background levels. With a range of 730-12,000 MPN, the Olema Creek samples had the highest *E. coli* levels. The range for the *E. coli* samples collected at the Lagunitas Creek station was 70-6600 MPN.

At the Olema Creek station, the highest *E. coli* concentration was sampled at 8:00 PM on 1/10/01, 3 hours after peak discharge occurred. Both the discharge and the *E. coli* concentrations decreased gradually after this point. The peak *E. coli* concentration at the Lagunitas Creek station was detected at 6:00 AM on 1/11/01, approximately 10 hours after the occurrence of peak discharge. As with the Olema Creek time series, the discharge and the *E. coli* levels for the Lagunitas Creek also gradually decreased after this point.

Rainfall Event No. 2 (1/25-1/26/01):
Over all three stations, *E. coli* concentrations observed in the second rainfall event were similar to those of the Rainfall Event No. 1. As had been observed during the first rainfall event, the Olema Creek samples showed significantly higher E. coli levels than the Lagunitas Creek samples. Walker Creek samples, however, had the highest *E. coli* concentration detected during Rainfall Event No. 2, with a range of 200 to 21,333 MPN. The *E. coli* ranges for Olema and Lagunitas Creeks were 130-12,000 MPN, and 190-5800 MPN, respectively.

The sample with the highest *E. coli* concentration at the Olema Creek station was collected at 4:00 PM on 1/25/01, coinciding with the peak discharge. The peak *E. coli* concentration at the Lagunitas Creek station was detected at 9:00 PM on 1/25/01, one hour after the peak discharge occurrence. For the Walker Creek station, the highest *E. coli* level was detected in the sample collected at 2:00 PM on 1/26/01—the only sample collected at the Walker Creek station on that day. Due to the lack of additional sampling, it is not possible to determine whether the 2:00 PM 1/26/01 sample accurately represents the actual peak *E. coli* concentration for that day.

Rainfall Event No. 3 (2/8/-2/12/01):
Due to personnel shortage, no *E. coli* time series samples were collected at Walker Station during Rainfall Event No. 3.

Overall, *E. coli* concentrations in the third rainfall event were significantly lower than those of the second rainfall event. *E. coli* levels were higher in the Olema Creek samples than the Lagunitas Creek samples. Olema Creek samples' *E. coli* concentrations ranged from 62-5500 MPN. *E. coli* concentrations in the Lagunitas Creek ranged from 10-1700 MPN.

The highest *E. coli* concentration detected in Rainfall Event No. 3 was collected at the Olema Creek station at 8:00 AM on 2/11/01, coinciding with peak discharge. The peak *E. coli* concentration at the Lagunitas Creek station was detected at 1:00 PM on 2/11/01, two hours after the peak discharge had occurred.

Overall, the Olema Creek samples had the highest *E. coli* levels throughout the study. Across the three locations, *E. coli* concentrations in the third rainfall event were significantly lower than those of the second rainfall event. A possible explanation of this trend is that the sources of *E. coli* to the three stations were not renewable. Under this explanation, *E. coli* that had accumulated in the watershed during the dry season would be washed off by each successive rainfall event, therefore gradually decreasing concentrations in the water samples as the study progressed.

For the first rainfall event, a lag time of a few to several hours was observed between peak discharge time and the time of peak *E. coli* concentrations. There was little or no lag between peak discharge and peak E. coli concentration times for the second or third rainfall events. These observations suggest that as the wet season progressed and degree of ground saturation increased in the watersheds, smaller ephemeral tributaries began to run continuously and the transport time for E. coli to travel from the watershed to the creeks decreased. Under these conditions, *E. coli* mobilization would occur very quickly in a rainfall event.

Comparison of FC *and E. coli data*

All non-time series water samples were analyzed for FC using the Most Probable Numbers (MPN) estimate of bacterial density in a multiple tube fermentation test (Standard MPN Method). The contract laboratory had a MPN sample capacity of up to 50 samples, and therefore, it was necessary to utilize a different method (Colilert Method) for the *E. coli* analyses of the time series sample sets. To compare and relate the results of the two indicators (FC and *E. coli*), a subset of time series samples were also analyzed for FCs using the standard MPN method. In the 1995-96 study, in which both FC and *E. coli* levels were measured using the Standard MPN Method, a very close relationship (correlation factor of 0.99) between FC and *E. coli* results was observed. The Colilert method used in this study to enumerate *E. coli*, however, differs from the standard MPN method by both employing a higher number of dilution factors in the processing of samples and using a different MPN table to determine the concentrations. In the samples collected for this study, therefore, a correlation between the concentrations of *E. coli* (processed by Colilert method) and FC (processed by MPN method) may be difficult to demonstrate.

Table 19 contains the results from the simultaneous FC and *E. coli* samples taken at three time series sampling stations throughout the rainfall events. Figures 20-24 illustrate the comparison of FC and *E. coli* levels in the simultaneous samples for each day of wet-weather sampling.

Overall, the correlation between the *E. coli* and FC results were much poorer than those observed in the 1995/96 study. The correlation factors calculated for *E. coli* and FC results ranged from 0.32 on 2/10/01, to 0.82 on 1/25/01. The overall correlation factor for all simultaneous *E. coli* and FC samples collected over the span of this study was 0.78. While *E. coli* levels, for the most part, were lower than FC levels, no clear pattern between the concentrations of the two indicators was observed. The significant decrease in a correlation between the two indicator populations, as observed in the present study, compared to the 1995/96 study, is most likely due to the current study's analysis of the two populations by two different analytical methods. It cannot be concluded, therefore, that the results of this study accurately represent the relationship between the concentrations of the two sampled indicator groupings.

FC Loadings

The incremental discharge data (m^3/s) recorded at the stream gauges on Walker, Lagunitas, and Olema Creeks, together with the FC density data (MPN/ 100 cm^3) reported above, were used to calculate daily FC loadings (FC/Day) at the following sampling stations: Walker Creek Ranch; Lagunitas at Samuel P. Taylor Park; Lagunitas at Gallagher; and Olema at Bear Valley Road. For the remaining stations, the instantaneous discharge was estimated based on manual measurements of relevant parameters (i.e., surface water velocity, water height, and cross-sectional area of the stream). For the Walker Creek station at Highway 1, a rating curve previously constructed by the Regional Board staff was utilized to estimate the discharge.

Table 20 presents the FC loadings data for each rainfall event. These loading values reflect the amount of FC contributed by each sub-watershed on a daily basis. Due to insufficient flow at certain sampling sites, FC loadings for some subwatersheds were not calculated for some of the sampling dates (see below).

Rainfall Event No. 1 (1/11/01):
Overall, FC loadings during Rainfall Event No. 1 were the second highest of all rainfall events. The FC loadings during this event ranged from 3.5×10^{11} FC/Day for the Olema Creek subwatershed to 9.2×10^{13} FC/Day for the Lower Walker Creek subwatershed. The Chileno Creek, Upper San Geronimo, and Lower Lagunitas subwatersheds recorded the next-highest FC loadings.

Rainfall Event No. 2 (1/25/-1/26/01):
Several subwatersheds contributed significant FC loadings on Rainfall Event No. 2-Day 1; the lower Lagunitas subwatershed showed the highest loadings at 9.6×10^{12} FC/Day. The Lower Walker Creek, upper San Geronimo, Olema Creek, and Chileno Creek subwatersheds had the next highest FC loadings. The upper Walker Creek subwatershed contributed the lowest Day 1 FC loadings, at 8.1×10^{10} FC/Day.

Overall, the FC loadings during the Rainfall Event No. 2-Day 2 (1/26/01) increased by 1-2 orders of magnitude from day 1 loadings and were the highest of all rainfall events. At 1.7×10^{15} FC/Day, the lower Walker Creek subwatershed recorded the highest FC loadings in the study. Lower and upper San Geronimo subwatersheds contributed the next highest FC loadings. The upper Walker Creek subwatershed again showed the lowest FC loadings, at 3×10^{12} FC/Day.

Rainfall Event No. 3 (2/9-2/10/01):
At several stations, the FC loadings decreased by 1-2 orders of magnitude during Rainfall Event No. 3. The Rainfall Event No. 3-Day 1 loadings ranged from 1.2×10^{11} FC/Day at the Chileno Creek subwatershed to 1.5×10^{13} FC/Day at Upper San Geronimo subwatershed. Lower San Geronimo and upper Walker Creek subwatersheds had the second and third highest FC loadings.

During Rainfall Event No. 3-Day 2, the Olema Creek subwatershed had the lowest loading at 1.4×10^{11}FC/Day, and the lower Walker Creek subwatershed had the highest loading, at 6.8×10^{12} FC/Day. Chileno Creek and lower San Geronimo Creek subwatersheds contributed the second and third highest FC loadings.

Overall Contributions:
Table 21 contains the overall ranking of all subwatersheds according to the total number of FC they each contributed over the span of the 3 rainfall sampling events. The lower Walker Creek subwatershed contributed the highest one-time and highest overall FC loadings. Lower and upper San Geronimo Creek subwatersheds rank as second and third

largest contributors of FCs. The Keyes Creek and Olema Creek subwatersheds, recorded the lowest FC loadings.

Peak E. coli Loadings
The peak *E. coli* concentrations from the time series sampling were used in conjunction with the incremental discharge data, to calculate the peak *E. coli* loadings for the Olema Creek, lower Walker Creek, and lower Lagunitas Creek subwatersheds. Table 22 summarizes the daily *E. coli* loadings for the above-mentioned subwatersheds during the three rainfall sampling events. The peak *E. coli* loadings are calculated based on the highest *E. coli* concentrations observed during each day of time series sampling, and the associated discharge rate at the time that the highest concentration is recorded.

Rainfall Event No. 2 produced the highest *E. coli* loadings among all rainfall events. On Rainfall Event No. 2-Day 2, the lower Walker Creek subwatershed recorded the highest overall *E. coli* loading, at 3.8×10^{14} *E. coli*/Day. The Olema Creek subwatershed contributed the lowest one-day E. coli loading during Rainfall Event No. 3-Day 1.

The overall ranking of watersheds based on their total *E. coli* contributions during the three rainfall events matched the ranking of the watersheds for their FC loadings (Table 22). The lower Walker Creek subwatershed contributed the highest overall FC loadings over the span of 3 rainfall sampling events. The Olema Creek subwatershed, recorded the lowest overall FC loadings to the Bay.

In general, the peak *E. coli* loadings were higher than the FC loadings calculated for the same watersheds. This is most likely due to the differences in the methods used for calculating the two loadings: the *E. coli* density value used for calculation of loadings was the highest detected daily *E. coli* concentration per sampling day, whereas the FC concentrations used to calculate loadings were grab-samples taken at random points during the rain event, and did not necessarily represent the highest FC concentration of the day.

Conclusions

- The data from this study verifies previous findings, showing that rainfall-related runoff has a deleterious effect on the water quality of the Bay. In this study, the effect is evident in the rain event-increases of FC and *E. coli* levels sampled from the tributaries discharging into the Bay, and exceedences of water quality objectives in shellfish growing waters.

- Throughout all three rainfall sampling events, the FC levels for all Watershed and Bay station samples significantly exceeded the designated water quality objective of 14 MPN for Shellfish Harvesting Waters, and in most cases, even the much higher value set by the water quality objective for Non-Contact Water Recreation (mean < 2000 MPN).

- In general, FC levels increased during the second day of each wet-weather sampling event (with the exception of Rainfall Event No. 1, which lasted only one day). The

FC levels decreased from Rainfall Event No. 1 (1/11/01) to Rainfall Event No. 2-Day 1 (1/25/01) but, increased to the similarly high levels in Rainfall Event No. 2-Day 2 (1/26/01).

- The FC levels and loadings remained high during all rainfall events sampled in all watersheds. This would suggest the presence of a renewable source, or the introduction of new sources, of fecal coliform throughout portions of the watershed. Failing onsite sewage disposal systems, discharge from overflowing or leaky waste ponds, or runoff from manure pastures could be some of the potential new or renewable sources of fecal coliform.

- The FC results from the temporal sampling of the Bay stations did not reveal any clear trends in the assimilative response of the Bay to FC loadings from the tributaries over the span of 6 hours that the sampling was conducted. However, it was not possible in the scope of this study to collect enough Bay samples, over a large enough period of time, to detect any potentially existing trend in a temporal response of the Bay to microbial loadings from the watersheds. Further studies would be required to address this issue adequately and conclusively.

- The results from the *E. coli* time series sampling revealed that overall, *E. coli* concentrations in Rainfall Event No. 3 were significantly lower than those of Rainfall Event No. 2. This could indicate, throughout the watershed, the absence of a renewable *E. coli* source; existing populations of *E. coli* in the watershed would be washed off by each successive rainfall event, therefore leading to diminishing *E. coli* concentrations in the tributaries as the wet season progressed.

- For the first rainfall event, a lag time of a few to several hours was observed between peak discharge time and the time of peak *E. coli* concentrations. There was no or little lag between peak discharge and peak *E. coli* concentration times for the second or third rainfall events. These observations suggest that as the wet season progressed and degree of ground saturation increased in the watersheds, smaller ephemeral tributaries began to run continuously and the transport time for *E. coli* to travel from the watershed to the creeks decreased. Under these conditions, *E. coli* mobilization would occur very quickly in a rainfall event.

- Overall, the correlation between the *E. coli* and FC results were much lower than those observed in the 1995/96 study. The correlation factors calculated for *E. coli* and FC results ranged from 0.32 on 2/10/01, to 0.82 on 1/25/01. The overall correlation factor for all simultaneous *E. coli* and FC samples collected over the span of this study was 0.78. While *E. coli* levels, for the most part, were lower than FC levels, no clear pattern was observed between the concentrations of the two bacterial indicators. The significant decrease in a correlation between the two indicator populations, as observed in the present study, compared to the 1995/96 study, is most likely due to the current study's analysis of the two populations by two different methods. It cannot be concluded, therefore, that the results of this study accurately represent the relationship between the two sampled bacterial groupings.

- The lower Walker Creek subwatershed contributed the highest one-time and highest overall FC loadings. Lower and upper San Geronimo Creek subwatersheds rank as second and third largest contributors of FCs. The Keyes Creek and Olema Creek subwatersheds, recorded the lowest FC loadings.

- The overall ranking of subwatersheds based on their total *E. coli* contributions during the three rainfall events matched the ranking of the subwatersheds for their FC loadings (Table 22). The lower Walker Creek subwatershed contributed the highest overall *E. coli* loadings over the span of the three rainfall sampling events. The Olema Creek subwatershed, recorded the lowest overall *E. coli* loadings to the Bay.

- Although the Chileno Creek subwatershed was only the fourth largest overall contributor of FC loadings, samples collected there showed very high levels of FC concentrations (the highest or the second highest overall FC levels, on four of the five sampling days). However, because the flow in Chileno Creek was much lower than in Walker or San Geronimo Creeks, FC loadings from this subwatershed were lower that those of the Walker and San Geronimo subwatersheds.

- Several past studies have indicated runoff from dairies and livestock grazing land to be the primary source of FC to Tomales Bay. The present study is consistent with past findings: the highest FC concentrations and/or loadings in the study were observed in the Chileno and Walker Creek watersheds—watersheds whose land use consists primarily of grazing lands and dairies. High FC levels detected in the storm drains of the town of Point Reyes Station, indicate that another likely source of fecal contamination to the Bay is urban runoff, commonly known to convey waste from domestic animals and residential sources. While livestock and domestic animals provide significant loadings of FC and *E. coli* to the Bay, failing residential septic systems cannot be discounted as a loading source. Given that the predominant land use in the San Geronimo Creek watershed is residential housing, it can be concluded that the high FC levels/loadings observed there are due mainly to failing/substandard residential septic systems and urban runoff containing waste from pets.

- In May of 1998, a food borne illness outbreak associated with the consumption of oysters from Tomales Bay occurred, affecting 171 people. In the subsequent investigation, the cause of the outbreak was traced to a virus of human fecal origin. An investigation determined that the oysters causing the illness were harvested from the mid and outer-bay. The outbreak occurred after a rainfall closure and there was no additional rainfall after this time. Data at the time showed that both water and shellfish met FC standards. 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. This outbreak reinforces the need to evaluate those sources of fecal contamination that were not adequately addressed in previous studies, including onsite sewage disposal systems and recreational and commercial boating and camping activities. It also reinforces the need to manage those sources and not to rely solely on the attainment of FC standards to protect human health and shellfish harvesting.

- The body of studies conducted on pathogen indicators in Tomales Bay, points towards a group of the most-likely main loading sources of pathogens to the Bay. The probable main loading sources are: 1) run-off from grazing lands and animal facilities; 2) leaking waste from failing residential septic systems, 3) domestic animal waste in urban runoff; 4) waste from wildlife; 5) human waste from campers and boaters; and 6) possibly, leakage and/or overflow from sewage treatment plants and sewage holding ponds. As did the study of 1995/96, this study evaluated general trends in water quality and sources of fecal contamination on a watershed and subwatershed scale. Individual or localized anthropogenic sources of FC, such as domestic sewage disposal systems or individual incidents of direct disposal of sewage from sources not associated with rainfall (i.e., recreational boating and camping) were not specifically evaluated in this study.

- Future work should focus on the status of septic systems in the Tomales Bay watershed, to determine explicitly whether systems are failing and leaking waste into the creeks and Bay. Possible studies could include the use of tracer dyes to track wastewater from leaking septic systems to receiving waters, and more specific (higher resolution) sub-watershed studies.

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Tables 1-22

Table 1. Tomales Bay watershed area estimates, including reservoirs (adapted from Fischer, 1996).

WATERSHED	AREA (km^2)	AREA (%)
Walker	196.35	35
Lagunitas	241.72	43
Olema	50.0	9
Remainder	72.93	13
TOTALS	**561**	**100%**

Table 2. 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 3. Estimates of watershed contributions to runoff into Tomales Bay (Fischer, 1996).

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

Table 4. Estimated numbers of livestock[1] and manure production in Tomales Bay watershed (totals/watershed/day)[2].

DRAINAGE	DAIRY (Cows and Heifers)	MANURE Lbs/Day	BEEF	MANURE Lbs/Day	SHEEP	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	---	---	2563	616,212
Totals	10,970	980,084	1320	86,490	1000	7200	13,031	1,448,018

[1] Approximate numbers based on rough estimates by the University of California Cooperative Extension
[2] Table adapted from R. Bennett and S. Larson, *Preventing Animal Wastes from Degrading Water Quality: The Case for Tomales Bay, California, 1990.*

Table 5. Permitted sewage treatment systems in the Tomales Bay watershed, which are regulated under Waste Discharge Requirements from the San Francisco Bay Regional Water Quality Control Board.

NAME	LOCATION	WASTE (GPD[3])	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

[3] GPD = Gallons per Day

Table 6. Commercial shellfish growers and wet storage operators in Tomales Bay.

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

Table 7. Water quality objectives for coliform bacteria[4]. (From Regional Water Quality Control Plan [Basin Plan], 1995).

Beneficial Use	Fecal Coliform	Total Coliform
Water Contact Recreation[5]	log mean < 200	median < 240
	90th percentile < 400	no sample > 10,000
Shellfish Harvesting[6]	Geometric Mean < 14	Geometric Mean < 70
	90th Percentile < 43	90th Percentile < 230[7]
Non-Contact Water[8,9]	Mean < 2000	
	90th Percentile < 4000	
Municipal Supply:		
surface water[10]	Log Mean < 20	Log Mean < 100
ground water		< 1.1[11]

[4] Based on a minimum of five consecutive samples equally spaced over a 30-day period.
[5] Freshwater and ocean water. Freshwater values are based on DHS recommended values.
[6] Source: National Shellfish Sanitation Program.
[7] Based on a five-tube decimal dilution test. Use 300 MPN/100 mL when a three-tube decimal dilution test is used.
[8] Source: Report of the Committee on Water Quality Criteria, National Technical Advisory Committee, 1968.
[9] Freshwater
[10] Source: DHS recommendation.
[11] 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. List of Sampling Sites for the 2001 Tomales Bay Bacterial Monitoring Study.

Table 9. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Pre Season Dry-Run, 1/3/01.

Table 10. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 1, 1/11/01.

Table 11. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 2, 1/25/01.

Table 12. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 2, 1/26/01.

Table 13. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 3, 2/9/01.

Table 14. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 3, 2/10/01.

Table 15. *Escherichia coli* concentrations in time series water samples collected from Olema, Lagunitas, and Walker Creeks; Pre Season Dry-Run, 1/3/01.

Table 16. ***Escherichia coli*** **concentrations in time series water samples collected from Olema and Lagunitas Creeks; Rainfall Event 1, 1/10-1/11/01.**

Table 17. *Escherichia coli* concentrations in time series water samples collected from Olema, Lagunitas, and Walker Creeks; Rainfall Event 2, 1/25-1/26/01.

Table 18. ***Escherichia coli*** **concentrations in time series water samples collected from Olema and Lagunitas Creeks; Rainfall Event 3, 2/8-2/12/01.**

Table 19. Comparison of fecal coliform and *Escherichia coli* data for Walker, Olema, and Lagunitas Creeks.

Table 20. Fecal coliform loadings for the subwatersheds of Tomales Bay.

Table 21. Ranking of subwatersheds based on their overall fecal coliform contributions over the span of this study.

Table 22. Peak *Escherichia coli* loadings for Olema, Lower Walker, and Lower Lagunitas Subwatersheds.

Figures 1-24

Figure 1. Tomales Bay, Marin County, California.

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Figure 2. General location of commercial shellfish growing area leases in Tomales Bay, California.

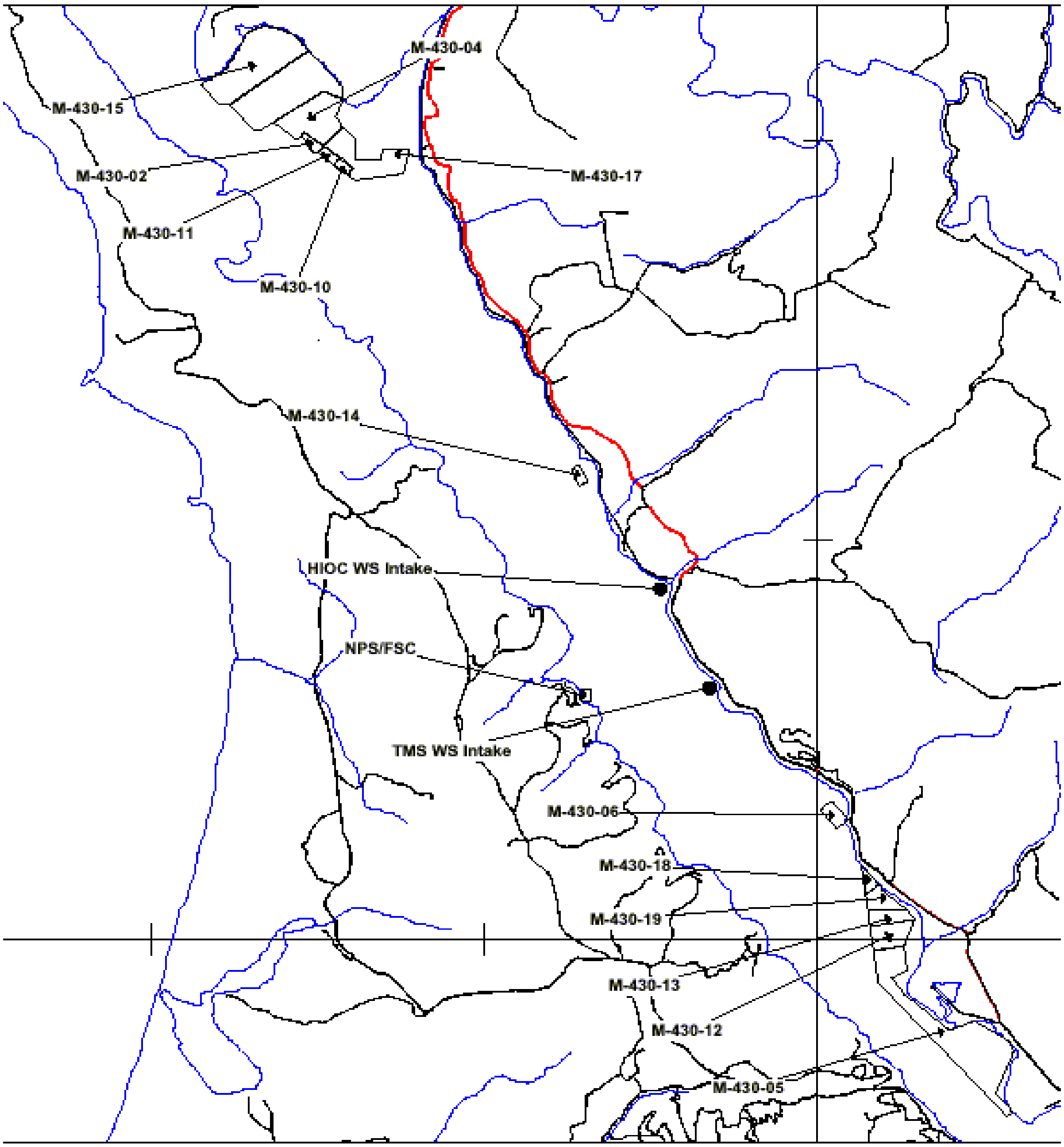

Figure 3. General location of commercial shellfish growing area leases in Tomales Bay, California.

Figure 3. Location of sampling stations for Tomales Bay and its watershed.

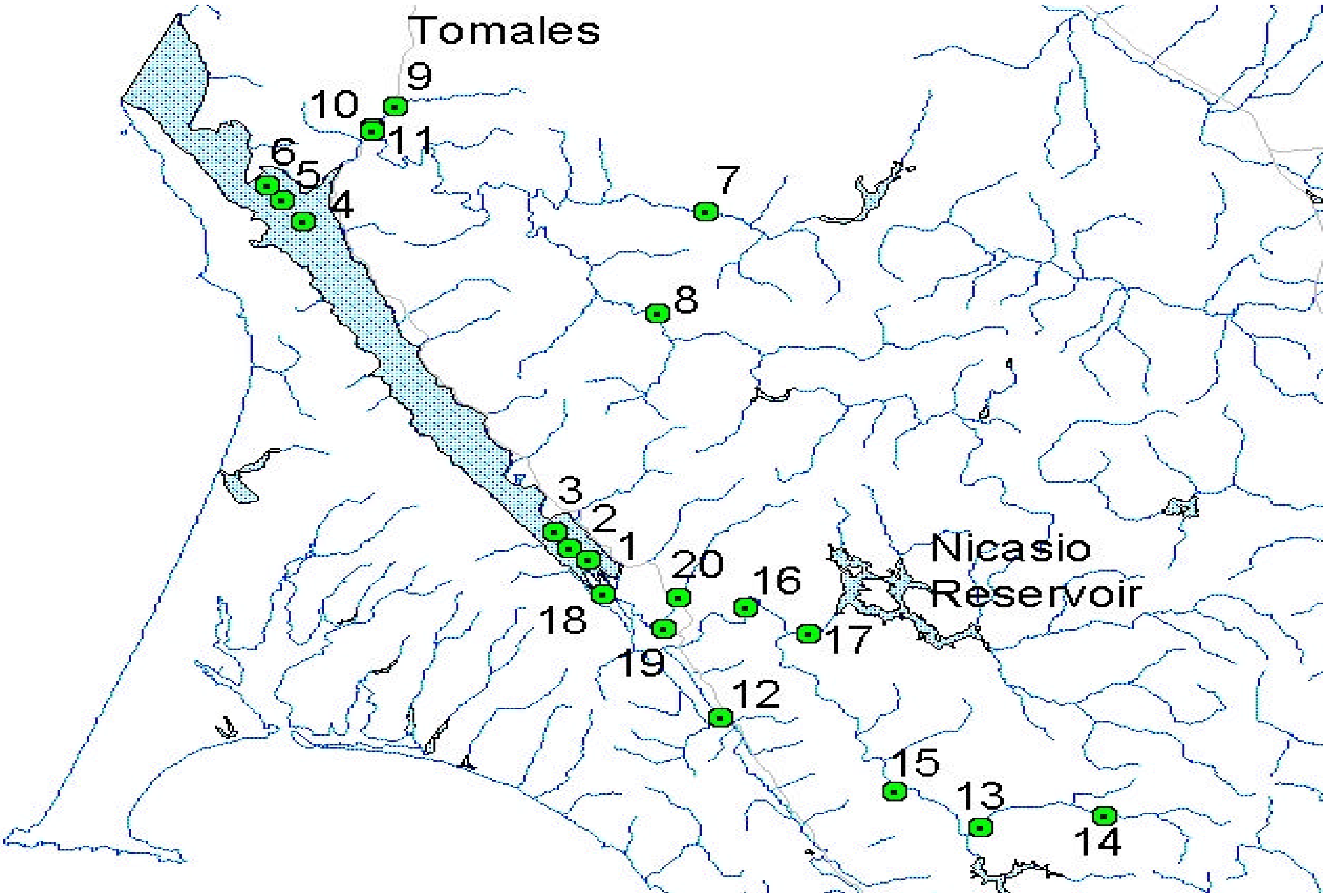

Figure 4. Cumulative rainfall record for the duration of study, obtained from the California Department of Health Services remote weather station at Tomasini Point, Tomales Bay.

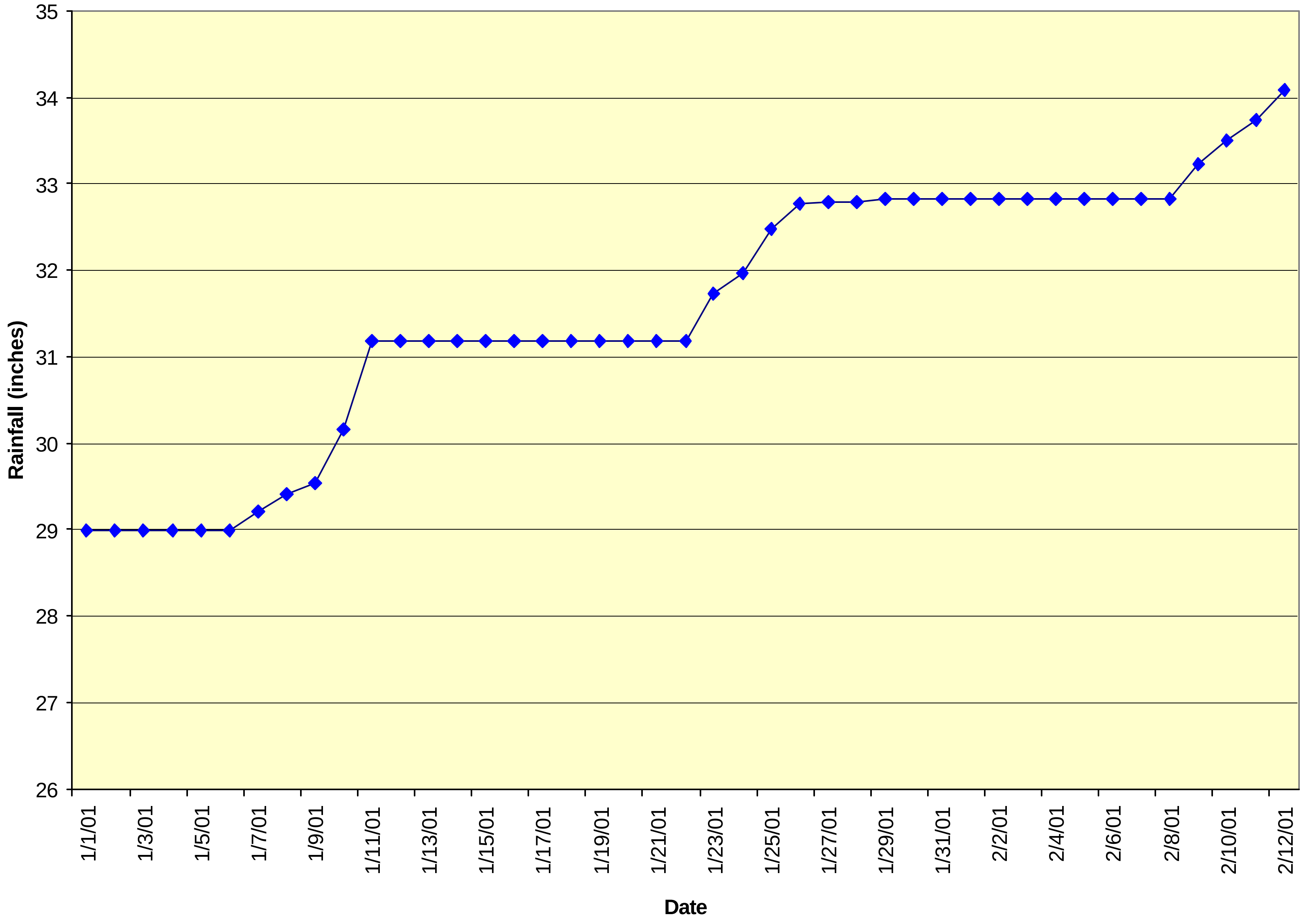

Figure 5. Fecal coliform concentrations in Bay and watershed water samples; Rainfall Event 1, 1/11/01.

Figure 6. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 2, 1/25/01.

Figure 7. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 2, 1/26/01.

Figure 8. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 3, 2/9/01.

Figure 9. Fecal coliform concentrations in water samples collected from watershed and Bay stations; Rainfall Event 3, 2/10/01.

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Figure 11. ***Escherichia coli*** **concentrations in time series water samples from Lagunitas Creek; Pre Season Dry-Run, 1/3/01.**

Figure 12. ***Escherichia coli*** **concentrations in time series water samples from Walker Creek; Pre Season Dry-Run, 1/3/01.**

Figure 13. ***Escherichia coli*** **concentrations in time series water samples from Olema Creek; Rainfall Event 1, 1/10-1/11/01.**

Figure 14. ***Escherichia coli*** **concentrations in time series water samples from Lagunitas Creek; Rainfall Event 1, 1/10-1/11/01.**

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Figure 23. Comparison of fecal coliform and *Escherichia coli* data for Olema and Lagunitas Creeks; Rainfall Event 3, 2/9/01.

Figure 24. Comparison of fecal coliform and *Escherichia coli* data for Olema and Lagunitas Creeks; Rainfall Event 3, 2/10/01.

The End