Identification of Sources of Fecal Pollution Impacting Pillar Point Harbor

Proposition 50 CBI Grant Program

Grant Agreement No. 07-574-550-0

Environmental Monitoring Project Plan

Version 1

Prepared by:

San Mateo County Resource Conservation District

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Section 1: Plan Approval and Distribution.

Table 1: Project Personnel, their Roles, and Contact Information

Name	Organizational Affiliation	Title	Contact Information (Telephone number, fax number, email address.)	Date	Signature
Kellyx Nelson	SMCRCD	Project Manager	ph. 650-712-7765 Fax 650-726-0494 Email kellyx@sanmateorcd.org		
Carolann Towe	SMCRCD	Resource Specialist/QA Officer	Ph. 650-712-7765 Fax. 650-726-0494 Email carolann@sanmateorcd.org		
Jennifer Toney	SWRCB Board Contract Manager	Contract Manager	Ph. 916-341-5646 Fax. Email jtoney@waterboards.ca.gov		
Stefan Wuertz	University of California, Davis	Professor of Environmental Engineering	Ph. 530-754-6407 Fax. 530-752-7872 Email swuertz@ucdavis.edu		
Bruce Fujikawa	SMC Public Health Laboratory	Laboratory Director	Ph. 650-573-2500 Fax. 650-573-2147 Email bfujikawa@co.sanmateo.ca.us		
Douglas Coffman	SMC Public Health Laboratory	Lead PH Lab Tech	Ph. 650-573-2456 Fax. 650-573-2147 Email dcoffman@co.sanmateo.ca.us		
Barry	Balance	Principal	Ph. 510-704-1000		

Hecht	Hydrologics	Hydrologist	Fax. 510-704-1001 Email bhecht@balancehydro.com		

Section 2: Introductions and Overview.

Introduction

Bacterial monitoring in Pillar Point Harbor has revealed potential impairment to beneficial uses linked to contamination of the water with fecal matter. The San Mateo County Resource Conservation District (SMCRCD) received a grant to characterize the water quality conditions and identify potential sources of fecal bacteria within Pillar Point Harbor and its associated watersheds using Microbial Source Tracking (MST) technology and Fecal Indicator Bacteria (FIB) enumeration, for the purpose of developing and prioritizing specific objectives for implementation of water quality remediation projects based on the research and analysis. The SMCRCD will collaborate with other agencies and organizations that have an interest in this watershed to pool monitoring resources for the benefit of all stakeholders including community members, landowners, visitors and regulatory agencies.

This Monitoring Plan describes the monitoring efforts that will be undertaken by the SMCRCD pursuant to Grant Agreement No. 07-574-550-0 between the State Water Resources Control Board (SWRCB) and the SMCRCD.

The Grant Agreement stipulates that all monitoring will be compatible with the Surface Water Ambient Monitoring Program (SWAMP) data collection effort, and this Plan has been developed with full consideration of current SWAMP requirements. The Plan is structured from the tools and guidance documents developed by the SWRCB's Clean Water Team (CWT) and the SWAMP.

The SMCRCD is a non-regulatory public benefit district established to help people protect, conserve and restore natural resources through information, education and technical assistance programs

As the grantee, SMCRCD will liaise with the Wuertz Laboratory UC Davis, San Mateo Public Health Laboratory (SMCPHL) and Balance Hydrologics to conduct the field monitoring and the sample collection techniques, to determine sampling site locations, sampling frequency and duration. SMCRCD is responsible for the collection of water samples for FIB enumeration and their delivery to SMCPHL. SMCRCD will create and populate a database of project results, and maintain copies of field sheets and chain-of-custody (COC) forms. SMCRCD will maintain contact with the Wuertz Laboratory UC Davis, Balance Hydrologics and SMCPHL to notify of intent to sample, and provide the SWRCB with updates on sampling progress. At the completion of the program, SMCRCD in collaboration with the Lead Researcher (Wuertz Laboratory UC Davis), SMCPHL and Principal Hydrologist will prepare a final report for the SWRCB.

SMCPHL will conduct all FIB enumeration, the Wuertz Laboratory UC Davis will conduct all MST studies and Balance Hydrologics will be responsible for the terrestrial and aquatic hydrology. Field analyses will be conducted by both SMCRCD and the Wuertz Laboratory UC. All analyzes will be conducted in accordance with all methods and quality assurance requirements found in the Quality Assurance Project Plan (QAPP).

Section 3: Problem Statement and Monitoring Objectives.

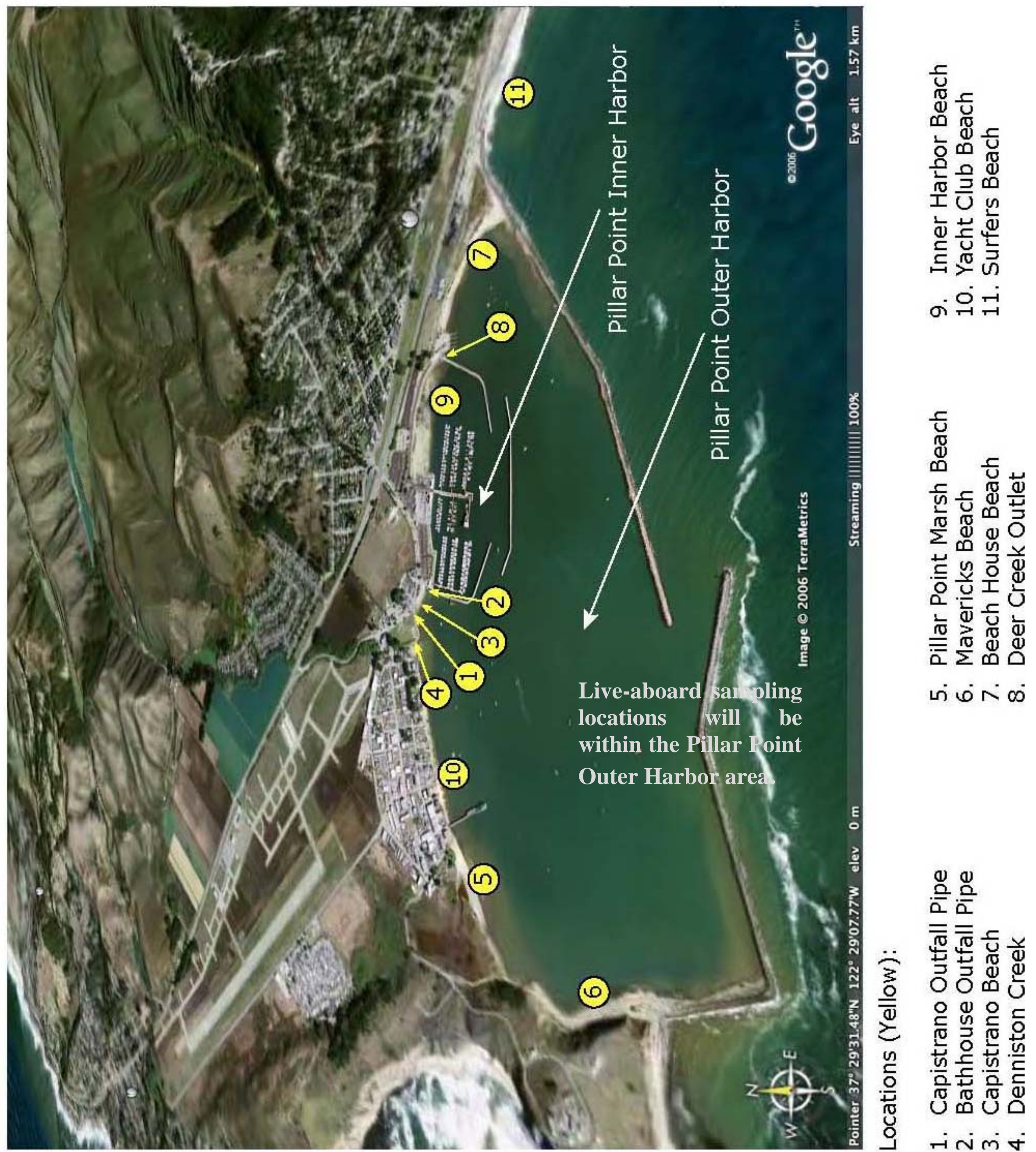
3.1 Geographical Setting.

Pillar Point Harbor is an enclosed watershed with complex inputs and water flows. It contains an inner boat harbor, pier, and saltwater/brackish tidal marsh (Pillar Point Marsh). It receives drainage from Denniston and Deer Creeks, storm drains, outflow pipes, and large, mixed use areas including an airport, agricultural, commercial and residential sections. Pillar Point Harbor contains five beaches: Capistrano Beach, Yacht Club Beach, Marsh Beach, Mavericks Beach, Inner Harbor Beach, and Beach House Beach.

Pillar Point Harbor comprises an inner harbor and outer harbor. The inner harbor berths 180 commercial fishing vessels and approximately 200 recreational boats, and is encompassed within the much larger outer harbor area. The harbor area houses commercial ventures including several restaurants, hotels, shops, a fertilizer plant, three commercial fish buyers, sport fishing concessions, a yacht club, two kayak rental companies, a recreational vehicle park, and a Naval Station situated on the bluff overlooking the Outer Harbor. Outside of the Outer Harbor area but within the project study area are conference facilities, residential areas, and additional commercial ventures as well as a pump station for the Sewer Authority Mid-coastside

The project area is also within the boundaries of the James V. Fitzgerald Marine Reserve Critical Coastal Area (CCA) Pilot Project. The Marine Reserve is a designated Area of Special Biological Significance and the CCA pilot project, jointly administered by the SWRCB and the California Coastal Commission, is to foster collaboration among local stakeholders and government agencies to better coordinate resources and focus efforts on coastal watersheds in critical need of protection from polluted runoff. The CCA will develop an action plan to reduce non-point source pollution by 2010 and intends to coordinate closely with this project to identify and remediate sources of fecal contamination in Pillar Point Harbor.

Figure 1: Aerial perspective of the Study Area including watersheds and key locations



3.2 Problem Statement.

Pillar Point Harbor water quality chronically is so poor that the SWRCB recently listed the location as impaired by coliform bacteria on the 303(d) list submitted to the United States Environmental Protection Agency. Capistrano Beach has elevated levels of FIB, such as *E. coli* and *Enterococcus* on over 95% of sampling events in wet and dry conditions. This beach has been ranked for several years by Heal the Bay's Report Card as a "Beach Bummer," meaning that it is in the top ten most polluted beaches in California in dry weather conditions. In 2005/2006, Capistrano Beach ranked sixth on the "Beach Bummer" list. It was the worst ranked beach in Northern California and is a Clean Beaches Task Force Priority Beach with regard to fecal pollution. Capistrano Beach is permanently posted by the San Mateo County Environmental Health Department as a potential health hazard.

The public health impact of the impaired waters may impact commercial ventures, harbor activities, tourism, recreation, ecological habitat, and sources of drinking water for municipal utilities in the watershed. The harbor area has approximately 100,000 visitors annually and is heavily used recreationally by boating enthusiasts, kayakers, windsurfers, campers, hikers, dog walkers, bird watchers, swimmers, waders, families, clam diggers, surfers, and thousands of spectators for the world famous Mavericks big wave surf break.

In 2005 and 2006, San Mateo County Department of Environmental Health repeatedly posted beaches in the Harbor as potential health hazards. Capistrano Beach was posted most frequently, approximately 50 weeks in each year. Marsh Beach was posted over 20 weeks in each year i.e. 42% to 51% of sampling events. Mavericks Beach was posted approximately 15 weeks each year, approximately 30% of sampling events. The county terminated sampling for Capistrano Beach and permanently posted the beach as a potential health hazard in March of 2006.

Within the local community there are numerous opinions as to the primary sources of fecal pollution impacting the harbor, including but not limited to human contamination from leaking sewer lines, avian contamination from resident and migratory bird populations including large flocks of gulls and other birds, and lack of flushing in the harbor due to the presence of two breakwalls. Although much effort has been expended on studying the locations of fecal pollution impacting the harbor, including water sampling and fecal indicator enumeration studies, data on identification of primary sources and their relative contributions to the overall magnitude of the pollution problem are lacking. There is an urgent need for a comprehensive study of all of the possible sources of pollution in this watershed and how these flows interact in the confines of the enclosed Pillar Point Harbor bay.

A primary objective of the study is to determine which inlets constitute a significant source of pathogen indicators to Pillar Point Harbor, including the near shore area and adjacent beaches. A multi-tiered approach will be undertaken to identify sources and to quantify fecal loads. It is intended to go beyond the traditional methods of evaluating the receiving water based solely on fecal pathogen indicators. Fecal pathogen indicators are commonly used to indicate the presence of fecal pathogens due to the cost and numerous obstacles associated with the direct measurement of pathogens. Numerous investigators are expressing concern with regard to the ability of the traditional fecal pathogen indicators to accurately reflect the risks to human health. Studies have shown that the correlation between the pathogen indicators and human health risks may be poor primarily due to persistence of the indicators in the natural environment and the presence of indicators in feces from non-human sources. It should be noted, however, that fecal pathogen indicator data can provide valuable information in the analysis of the problem, and that non-human pathogen sources may also pose a human health risk.

3.3 Monitoring Objective.

A primary objective of the study is to determine which inlets constitute a significant source of pathogen indicators to Pillar Point Harbor, including the near shore area and adjacent beaches. A multi-tiered approach will be undertaken to identify sources and to quantify fecal loads. It is intended to go beyond the traditional methods of evaluating the receiving water based solely on fecal pathogen indicators. Fecal pathogen indicators are commonly used to indicate the presence of fecal pathogens due to the cost and numerous obstacles associated with the direct measurement of pathogens. Numerous investigators are expressing concern with regard to the ability of the traditional fecal pathogen indicators to accurately reflect the risks to human health. Studies have shown that the correlation between the pathogen indicators and human health risks may be poor primarily due to persistence of the indicators in the natural environment and the presence of indicators in feces from non-human sources. It should be noted, however, that fecal pathogen indicator data can provide valuable information in the analysis of the problem, and that non-human pathogen sources may also pose a human health risk.

The objective of the Source Identification Survey is divided into four tasks:

1) Assess and analyze existing data relevant to potential sources of fecal contamination impacting Pillar Point Harbor.
2) Investigate sources of fecal contamination impacting Pillar Point Harbor.
3) Conduct public outreach and community participation.
4) Develop implementation goals and strategies.

The project will provide quarterly progress reports, including collected data, during the life of the project. At the end of the project, SMCRCD will provide a full listing and summary of the data collected. From these data SMCRCD will develop and prioritize specific objectives for implementation of water quality remediation projects based on the research and analysis including but not limited to.

3.3.1 Identify specific constraints, impediments, opportunities, and priorities to remediate water quality on the beaches in Pillar Point Harbor.

3.3.2 Offer specific cost-effective, feasible water quality improvement projects.

3.3.3 Develop milestones and timeline for implementing the proposed methods to achieve specific water quality objectives and the parties responsible for meeting these objectives.

3.3.4 Draft monitoring plan and specific performance measures to track implementation of specific projects.

Some opportunities for remediation that can be implemented during the project may be identified and undertaken, potentially earlier than March 2010.
The grant agreement stipulates all monitoring will be compatible with the Surface Water Ambient Monitoring Program (SWAMP) data collection effort. This plan is developed with full consideration of current SWAMP requirements using the tools and guidance documents developed by the SWRCB's Clean Water Team (CWT) and SWAMP.

Section 4: Project Personnel, Roles, and Responsibilities.

San Mateo County Resource Conservation District (SMCRCD)

The SMCRCD is a non-regulatory public benefit district established to help people protect, conserve and restore natural resources through information, education and technical assistance programs. The SMCRCD received a grant from the SWRCB to identify of Sources of Fecal Pollution Impacting Pillar Point Harbor

The SMCRCD is the lead agency in this effort, as it is the grant recipient. SMCRCD will create and populate a database of project results for FIB enumeration, and maintain copies of field sheets and COC forms. SMCRCD will maintain contact with the SWRCB, the Wuertz Laboratory, SMCPHL and Balance Hydrologics to notify of intent to sample, and provide the SWQCB with updates on sampling progress. At the completion of the program, SMCRCD will prepare a final report for the SWRCB (see table 3 for timeline).
All samples will be analyzed in accordance with methods and quality assurance requirements found in the Quality Assurance Project Plan (QAPP).

Kellyx Nelson, SMCRCD Executive Director will be responsible for all project management tasks including invoicing and reporting, management of contracts with the laboratories and hydrologist and oversight of project progress. Invoices and reports will be sent to Jennifer Toney, Grant Manager with SWRCB. She will work closely with the Quality Assurance (QA) Officers, receive their reports, and ensure that any problems are solved promptly. Table 1 lists the representatives of the various organizations who will assume project personnel roles to assure data quality and timely delivery of reliable and usable monitoring data. They will be responsible for all project tasks and deliverables.

California State Water Resources Control Board

Jennifer Toney is the Grant Manager. Ms Toney's responsibilities are to review the deliverables specified in Table of Items for Review within the Grant agreement, including monitoring results and receive the quarterly reports and invoices for the project.

Department of Civil and Environmental Engineering, UC Davis

Professor Stefan Wuertz is the Program Professor of Environmental Engineering at the Wuertz Laboratory UC Davis. He is the lead researcher for this project. His responsibilities include guiding the research based on a set of testable hypotheses using MST, FIB enumeration and hydrology both terrestrial and aquatic. He is a non-voting member of the Technical Advisory Committee.

San Mateo County Public Health Laboratory

The SMCPH is a California ELAP certified laboratory. Bruce Fujikawa is the Laboratory Director and is responsible for oversight of all laboratory activities. Douglas Coffman as the Lead PH Lab Tech is responsible for the receipt, processing and QA/QC of all FIB enumeration samples. Both Bruce and Doug are voting members of the Technical Advisory Committee

Balance Hydrologics

Balance Hydrologics focuses on analysis of watershed, channel, ground-water and wetland dynamics, providing site specific hydrology. Barry Hecht is the Principal Hydrologist and will be responsible for oversight of the project as well as guiding the dye circulation study. Bonnie Mallory De Berry is the Project Manager and is also responsible for the oversight of the terrestrial hydrology. Barry is a non-voting member of the Technical Advisory Committee.

Technical Advisory Committee

A Technical Advisory Committee (TAC), including Lead Researcher Professor Stefan Wuertz, guides and contributes expertise to all aspects of the project. The TAC meets three times per year as a group, while subgroups and individuals may contribute expertise to the project as needs and opportunities arise. The Lead Researcher will provide oversight for the design of the research components of the project. The TAC establishes appropriate methodology to identify the sources of fecal pollution and recommends strategies for remediation. As such, expertise on the TAC includes harbor function and infrastructure, wastewater treatment function and infrastructure, abatement of FIB, hydrology and geology, microbiology, public health, MST, and water quality (see Table 2).

Table 2: **Technical Advisory Committee**

	Member	Expertise
1	Barry Hecht Balance Hydrologics	Hydrology and geology of region
2	Bruce Fujikawa, PhD Public Health Laboratory San Mateo County	Public health
3	Carmen Fewless Regional Water Quality Control Board	Water quality
4	Chuck Duffy Granada Sanitary District	Sanitary District function
5	Dean Peterson Environmental Health Services San Mateo County	Environmental health
6	Doug Coffman Public Health Laboratory San Mateo County	Public Health laboratory function Regional knowledge
7	Sarah Corbin Surfrider San Mateo County Chapter	Regional volunteer water quality monitoring activities Surfrider laboratory function
8	Jill Murray, PhD Creeks Division City of Santa Barbara	MST in Santa Barbara region
9	Jim Howard Natural Resources Conservation Service	Upland uses, conservation practices, and best management practices NRCS programs and conservation standards
10	John F. Foley, III Sewer Authority Mid-Coastside	Sewer authority function
11	John Oram, PhD San Francisco Estuary Institute	Near-shore and harbor circulation, pollutant transport
12	Keith Mangold	Regional watershed knowledge
13	Lisa Sniderman California Coastal Commission	Knowledge of marina water quality issues Critical Coastal Areas Program
14	Peter Grenell/Dan Temko Harbor District San Mateo County	Harbor function
15	Stefan Wuertz *(lead researcher for project)* Civil and Environmental Engineering UC Davis	Biofilms, aerobic wastewater treatment, bacterial and viral pathogens, and microbial source tracking
16	Steve Peters Environmental Health Services County of Santa Cruz	MST at Santa Cruz County beaches

Section 5: Project Tasks and Schedule.

The Source Identification Survey is divided into four tasks:

1) Assess and analyze existing data relevant to potential sources of fecal contamination impacting Pillar Point Harbor.
2) Investigate sources of fecal contamination impacting Pillar Point Harbor.
3) Conduct public outreach and community participation.
4) Develop implementation goals and strategies.

Numerous types of flows and drainages are to be investigated, including outflows from Denniston and Deer Creeks, outfall pipes, storm drains, runoff from blacktop and impervious surfaces, agricultural concerns and commercial operations; flows originating from wave action and/or wave generated surges broaching harbor walls, water circulation within the harbor, tidal surges, currents, other issues associated with enclosed beaches, and potential drainage from sewer lines. Effects of wet and dry weather upon these flows will be analyzed. Water flows draining into the harbor with well documented elevated levels of fecal indicators include Capistrano and Bathhouse outfall pipes and Denniston Creek, and these flows will be a primary focus of the project.

5.1 Assess and analyze existing data relevant to potential sources of fecal contamination impacting the Harbor.

The project team will perform a full search for all existing data, review and analyze with respect to the following:

a) Hydrology of contributing watersheds, marsh, and harbor
b) Geologic setting
c) Natural and human-made conditions
d) Sedimentation and beach sand shifting
e) Sewer and other sewerage inflow and infiltration studies
f) Existing MST data
g) Enumeration data for fecal indicators with regard to tidal and flow conditions, temporal issues, weather, rain events, seasons, first flush events, etc.
h) Watershed sanitary surveys and similar analyses and related watershed data
i) Residency time for harbor water circulation

5.2 Investigate sources of fecal contamination impacting Pillar Point Harbor.

Several strategies can be undertaken to implement a multi-tiered approach. One method that shows great promise for the accurate evaluation of sources and human health risks is MST source tracking. To develop a cost-effective plan that will provide the greatest benefit toward understanding and eliminating sources of contamination, the approach must take advantage of all the available data, must account for all sources in the watershed, and also must focus on the areas of greatest concern.

MST is based on the assumption that characteristics of fecal bacteria vary according to their source. By identifying and accounting for the differences, the source of fecal pathogens can be ascertained. Generally, sources can be traced to warm blooded organisms such as human, canine, avian, bovine, or porcine. These linkages can enable the analyst to trace nonpoint sources of pathogens to a distinct land use or region and allows for a site-specific approach to deal with each source.

5.1.1 Investigate sources of fecal contamination known to impact Capistrano Beach.

5.1.1.1 Perform MST to identify and estimate relative contributions of fecal pollution arising from human, avian, dog, horse, cattle and other host sources.

5.1.1.2 Track pollutants to their sources by scoping storm drains and sewer lines and conducting hydrologic studies and sanitary surveys with FIB enumeration.
5.1.1.3 Investigate role of sediment as a potential source of elevated levels of fecal indicators, including sampling Denniston Creek and the Pillar Point Marsh during storms.
5.1.1.4 Investigate role of wrack as a potential source of elevated levels of fecal indicators, including sampling Denniston Creek and the Pillar Point Marsh during storms.

5.2.2 *Investigate potential sources of fecal contamination to remaining beaches in Pillar Point Harbor: Mavericks, Yacht Club, Beach House, Inner Harbor, and Pillar Point Marsh beaches.*

5.2.2.1 Sample beaches, lagoons, creeks, monitoring wells, bluff seeps, and storm drains, and assess by fecal indicator enumeration of *E. coli* and *Enterococcus* and by watershed sanitary surveys.
5.2.2.2 For sites which are identified as major sources of fecal pollution, perform MST to identify and estimate relative contributions of fecal pollution arising from human, avian, dog, horse, cattle and other host sources.

5.2.3 *Investigate other factors which may impact fecal contamination in Pillar Point Harbor with respect to the following and perform MST as required.*

5.2.3.1 Hydrologic parameters, including currents, flow, and salinity where relevant.
5.2.3.2 A fluorometric dye tracing study will be conducted to observe flow direction, velocity and dispersion in the harbor to allow estimation of expected residence times of constituents in the harbor under late-summer or early-fall conditions.
5.2.3.3 Impact of birds, domestic animals, recreational vehicles, leach fields, and boats.
5.2.3.4 Sewer line integrity.
5.2.3.5 Inflow sources, including the marsh, surface runoff, discharge from Denniston Creek and Deer Creek, storm drains and storm sewers.

5.3 Conduct public outreach and community participation.
SMCRCD will facilitate public outreach and education about the project and coordinate stakeholders by notifying and encouraging the participation of affected and interested public and private landowners, utilities, resource agencies, and others as necessary and appropriate, providing opportunities for public input into the project effort. Outreach activities will include a public workshop, creation and maintenance of a web page for the project, and enabling/encouraging any member of the public to ask questions and provide input on the project at regularly scheduled public meetings of the SMCRCD Board of Directors.

5.4 Develop implementation goals and strategies.
The project will provide quarterly progress reports, including collected data, during the life of the project. At the end of the project, SMCRCD will provide a full listing and summary of the data collected. From these data SMCRCD will develop and prioritize specific objectives for implementation of water quality remediation projects based on the research and analysis.

5.4.1 Identify specific constraints, impediments, opportunities, and priorities to remediate water quality on the beaches in Pillar Point Harbor.

5.4.2 Offer specific cost-effective, feasible water quality improvement projects.

5.4.3 Develop milestones and timeline for implementing the proposed methods to achieve specific water quality objectives and the parties responsible for meeting these objectives.

5.4.4 Draft monitoring plan and specific performance measures to track implementation of specific projects.

Some opportunities for remediation that can be implemented during the project may be identified and undertaken, potentially earlier than March 2010.

Table 3: Project Major Tasks and Completion Dates

Activity	Anticipated Date of Initiation	Anticipated date of completion	Deliverable	Deliverable Due Date
Assess and Analyze Existing Data	Oct 2007	May 2008	Summary List and review of existing data	May 2008
Investigate Sources of Fecal Contamination	January 2008	November 2009	Sample enumeration and MST data	January 2010
Conduct Public Outreach and Community Participation	January 2008	January 2010	a public workshop, a web page for the project, enabling/encouraging the public to ask questions and provide input on the project at regularly scheduled public meetings of the Resource Conservation District Board of Directors	January 2010
Develop Implementation goals and strategies	October 2009 or sooner	February 2010	Complete data set	February 2010
Draft final report	December 2008	February 2010	Draft final report for review	January 2009
Final report	February 2010	March 2010	Final report	March 2010

Section 6: Monitoring Strategy and Design.

Data collection will be based on a set of guiding hypotheses established by the lead researcher and Technical Advisory Committee. (See Appendix) They will be based on the following assumptions: Numerous types of flows and drainages exist in the harbor and its watersheds, including outflows from Denniston and Deer Creeks, outfall pipes, storm drains, runoff from blacktop and impervious surfaces, agricultural concerns and commercial operations; flows originating from wave action and/or wave generated surges broaching harbor walls, water circulation within the harbor, tidal surges, currents, other issues associated with enclosed beaches, and potential drainage from sewer lines. Effects of wet and dry weather upon these flows will be analyzed. Water flows draining into the harbor with well documented elevated levels of fecal indicators include Capistrano and Bathhouse Outfall Pipes and Denniston Creek, and these flows will be a primary focus of the project.

Table 4: Water Quality Characteristics, Number of Samples, and Logistics Planned for this Project

Site	Approach	Frequency		
		events per yr	samples per event	Years
Mavericks Beach (#6 on fig.1)	2 events per wet season	2	3	3
	2 events per dry season	2	3	3
	2 events per first flush	2	3	3
	possible upper watershed survey if spike found	3	8	1
	possible dog survey	2	2	1
	possible sediment/wrack survey	2	9	1
	possible avian survey	2	5	1
Marsh Beach (#5 on fig.1)	2 events per wet season	2	3	3
	2 events per dry season	2	3	3
	2 events per first flush	2	3	3
	possible upper watershed survey if spike found	3	8	1
	possible dog survey	2	5	1
	possible sediment/wrack survey	2	9	1
	possible avian survey	2	5	1
Yacht Club Beach (#10 on fig.1)	2 events per wet season	2	3	3
	2 events per dry season	2	3	3
	2 events per first flush	2	3	3
	possible upper watershed survey if spike found	3	8	1
	possible dog survey	2	5	1
	possible sediment/wrack survey	2	9	1
	possible avian survey	2	5	1

Denniston Creek (#4 on fig.1)	sanitary survey with 3 consecutive sampling events at 25 sites	3	25	1
	20 events at approximately 10 sites selected for follow-up	20	10	1
	biweekly sampling	26	10	2
	possible sediment/wrack survey	2	9	1
	possible dog survey	2	5	1
	possible avian survey	2	5	1
	enumeration for MST, 4 events (wet, dry, first-flush, and other) at 5 sites with 2 samples per site	4	10	1
Capistrano Pipe (#1 on fig.1)	sanitary survey with 3 consecutive sampling events at 25 sites	3	25	1
	20 events at approximately 10 sites selected for follow-up	20	10	1
	biweekly sampling	26	10	2
	possible sediment/wrack survey	2	9	1
	possible dog survey	2	5	1
	possible avian survey	2	5	1
	enumeration for MST, 4 events (wet, dry, first-flush, and other) at 5 sites with 2 samples per site	4	10	1
Capistrano Beach (#3 on fig.1)	25 events at 1 site	25	1	1
	biweekly sampling	26	7	3
	possible sediment/wrack survey	2	9	1
	possible dog survey	2	5	1
	possible avian survey	2	5	1
Bathroom Outfall (#2 on fig.1)	sanitary survey with 3 consecutive sampling events at 25 sites	3	25	1
	20 events at approximately 10 sites selected for follow-up	20	10	1
	biweekly sampling	26	10	2
	possible sediment/wrack survey	2	9	1
	possible dog survey	2	5	1
	possible avian survey	2	5	1
	enumeration for MST, 4 events (wet, dry, first-flush, and other) at 5 sites with 2 samples per site	4	10	1
Beachhouse Beach (#7 on fig.1)	2 events per wet season	2	3	3
	2 events per dry season	2	3	3
	2 events per first flush	2	3	3
	possible upper watershed survey if spike found	3	8	1
	possible dog survey	2	2	1
	possible sediment/wrack survey	2	9	1
	possible avian survey	2	5	1

Live-aboard Boats (located in PPH Outer Harbor area on fig.1)	3 events at 10 sites	3	10	1
	possible sediment/wrack survey	2	9	1
	possible dog survey	2	5	1
	possible avian survey	2	5	1
Contingent MST	MST for any of the above sites as follow-up to elevated bacteria levels as determined from enumeration studies. Preliminary estimates are for 5 triggers at 6 locations with 2 samples per site.	5	12	1

Sampling Design Principles Used to Select Locations and Timing.

The sampling design principle used to select initial stations is based on the project team (Lead Researcher and SMCRD Resource Specialist) analysis of existing data with known concentrations of FIB in exceedance of state standards. Data from these sites will be used to categorize initial sites and perform more detailed sampling upstream with the use of sanitary surveys.

Each site will consist of three different sets of data. FIB, MST data and field measurements that will document the water quality conditions at the station at the same time the samples were collected. These include pH, temperature, flow where possible, conductivity and turbidity as well as Total Suspended Solids (TSS). This latter dataset is for the specific intent of supporting the lab data. This data can be critical in interpreting lab data and will also include notes and visual observations (expressed in verbal categories) captured during each station visit. In addition a global set of hydrology data will be gathered at a separate discreet set of locations as well as a circulation study of the flow dynamics of the harbor. This data will be complementary to the three first sets described in defining the final dataset.

Total coliform, *E. coli* and *Enterococcus* will be determined by the Colilert-18® and Enterolert ® QuantiTray-2000 system.
Bacteroidales will be detected using a library-independent MST method based on quantitative PCR (qPCR) as detailed below.

Streamflow will be measured in Denniston Creek, Deer Creek, Pillar Point Marsh, and at accessible outfalls according to U.S. Geological Survey standard methods (Buchanan and Somers, 1976) which are available through the USGS website (http://pubs.usgs.gov/twri/) and upon request from Balance Hydrologics. At two or more stations, a continuous record of water stage (depth) will be obtained by installing instruments that sense and record water pressure. Streamflow measurements, made at various stages, will be used to develop stage-discharge rating curves at these stations in order to develop a continuous streamflow record.
Circulation data will be collected during two runs in late summer or early fall using a fluorometric dye study. First will be a small run to test the vertical distribution of dye in the water column in this harbor which has an average depth of 15'. The second run will be a full scale assessment of dye released from three points of interest in defining the circulation within the harbor. Sampling sites are allocated to a series of transects perpendicular to shore. The information gathered will be used during the analysis of FIB data as well as MST data to determine the effects of harbor circulation dynamics on FIB sources. The test and full scale events are planned for the summer/fall of 2008, a period when surface runoff is negligible, uncoupled from specific meteorological events. By sampling close to the onset of the winter rainy season, we hope to characterize the water quality characteristics of the harbor

Sampling Station Location.

Pillar Point Harbor is an enclosed watershed with complex inputs and water flows. It contains an inner boat harbor, pier, and saltwater/brackish tidal marsh (Pillar Point Marsh). It receives drainage from Denniston and Deer Creeks, storm drains, outflow pipes, and large, mixed use areas including an airport, agricultural, commercial and residential sections. Pillar Point Harbor contains five beaches: Capistrano Beach, Yacht Club Beach, Marsh Beach, Mavericks Beach, Inner Harbor Beach, and Beach House Beach.

Pillar Point Harbor comprises an inner harbor and outer harbor. The inner harbor berths 180 commercial fishing vessels and approximately 200 recreational boats, and is encompassed within the much larger outer harbor area. The harbor area houses commercial ventures including several restaurants, hotels, shops, a fertilizer plant, three commercial fish buyers, sport fishing concessions, a yacht club, two kayak rental companies, a recreational vehicle park, and a Naval Station situated on the bluff overlooking the Outer Harbor. Outside of the Outer Harbor area but within the project study area are conference facilities, residential areas, and additional commercial ventures as well as a pump station for the Sewer Authority Mid-coastside

The project area is also within the boundaries of the James V. Fitzgerald Marine Reserve Critical Coastal Area (CCA) Pilot Project. The Marine Reserve is a designated Area of Special Biological Significance and the CCA pilot project, jointly administered by the SWRCB and the California Coastal Commission, is to foster collaboration among local stakeholders and government agencies to better coordinate resources and focus efforts on coastal watersheds in critical need of protection from polluted runoff. The CCA will develop an action plan to reduce non-point source pollution by 2010 and intends to coordinate closely with this project to identify and remediate sources of fecal contamination in Pillar Point Harbor.

Multiple field crews will visit various locations in the Harbor and watersheds. Stations for this project will be at key points, as well as upstream and downstream of most prominent communities, to provide representation of potential sources of fecal bacteria. Planned sampling stations will be selected by reviewing a list of points that have already been established on Figure 1 coupled with historical data sets.

Actual sample sites will be determined "on the run" during the first sampling trip and the same spots will be visited on consecutive trips if accessible and relevant. Outfalls will be sampled if they are found to be discharging when visited. In these cases additional samples will be collected upstream of the outfall. Resulting data will guide the choice of site location on consecutive trips. This will take the form of sanitary surveys upstream from locations defined as hot spots. All new stations will be added to the database.

Section 7: Measurement Quality Objectives (MQOs).

Data acquisition activities will include both field and laboratory analyses, and the quality objectives depend on the amount of error that can be tolerated. Data quality objectives for this project have been refined to reflect the opportunity for comparability with other data sets in accordance with SWAMP requirements.

Table 5: Data quality objectives for laboratory measurements.

Group	Parameter	Accuracy	Precision	Recovery	Method Detection Limit	Completeness
Bacterial Analyses Colilert-18 (Idexx)	Total coliforms and *E. coli*	Positive results for target organisms. Negative results for non-target organisms	The San Mateo Public Health Lab is ELAP certified Cert. #1591. Lab follows all of section 9020, Standard Methods 20th Edition	See appendix IDEXX 6I&6J, 12E&Budnick	10-24,191 MPN/100 mL	90%
Bacterial Analysis Enterolert (Idexx)	Enterococci	Positive results for target organisms. Negative results for non-target organisms	The San Mateo Public Health Lab is ELAP certified Cert. #1591. Lab follows all of section 9020, Standard Methods 20th Edition	See Appendix Idexx 12E & Budnick	10-24,191 MPN/100 mL	90%
UCD	PCR assays for *Bacteroidales* source markers (universal, human, cow, dog)	positive/negative controls	Lab duplicates same results (presence/ absence); one duplicate every day of testing	> 50% true recovery based on filtration and extraction	1-4 gene copies per PCR reaction	90%
UCD	New PCR assays for seagull source	positive/negative controls	Lab duplicates same results (presence/ absence); one duplicate	> 50% true recovery based on	To be determined	90%

	markers		every day of testing	filtration and extraction		

Table 6: Data quality objectives for field measurements.

Group	Parameter	Representativeness	Accuracy	Precision	Method Detection Limit	Completeness
UCD/Balance Hydrologics	TSS	With each MST monitoring event, based on 1Liter sample	± 0.5 mg or 10%	± 0.1 mg	0.1 mg	90%
UCD/ Balance Hydrologics/SMCRCD	Conductivity	With each monitoring event and then as part of the circulation study	± 0.5% FS	± 5%	200 µS conductivity	90%
UCD/ Balance Hydrologics/SMCRCD	pH	With each monitoring event and then as part of the circulation study	+ 0.1 pH unit within + 10°C of calibration temperature or + 0.2 pH unit within + 20°C of calibration temperature	± 0.1 pH unit	0	90%
UCD/ Balance Hydrologics/SMCRCD	Water Temperature	With each monitoring event and then as part of the circulation study	± 0.1 °C or ± 1 LSD	± 0.1°C	0.1°C	90%
UCD/ Balance Hydrologics	Harbor currents	With the preliminary and major dye-tracing events chosen to represent spatial, temporal and meteorological variation at a diagnostic time of year	Qualitative Comparison to Waverider Buoy; Calibrated Annually	1% Measured Value	0.5 cm/s to 10 m/s	each of two monitoring events
Balance Hydrologics	Water Pressure	Every 15 minutes throughout the assigned flow monitoring period	0.05% FS	.002-0.0006% FS	0.001ft	90%
Balance	Flow	With each flow	+/- 8% depending on	+/- 8%	0cfs	90%

Hydrologics		monitoring event	field conditions	depending on field conditions		
Balance Hydrologics	Stream Depth	With each flow monitoring event, and in addition if necessary	+/- 0.01feet	+/- 0.01feet	0feet	90%
Balance Hydrologics	Rhodamine WT	Positive/negative controls	Quality control samples agreed to within 5% of measured undiluted samples	Not applicable	.1-100 micrograms per liter	80%

Section 8: Instruments and Methods for Sample Handling and Laboratory Analysis.

Enumeration samples:
After collection the samples will be immediately placed on ice in a cooler for transport to SMCPHL, coolers to contain freezer packs. All samples should be delivered to the SMCPHL in a suitable time for which the holding time (6 hours) will not be exceeded before analysis can begin. All specimen and test data will be captured in the Laboratory Information System. The COC form is used as a shipping record.

Bacteroidales:
Water samples will be placed on ice and transported to the Wuertz Laboratory. Analysis will occur within 24hours. COC forms will be filled out for all samples submitted to the Wuertz laboratory. Sample data, sample location, sample collection crew names, and analysis requested shall be noted on each COC.

Sample Handling
Identification information for each sample will be recorded on the label when the sample is collected. Samples will be labeled with the water body name, sample location, sample number, date and time of collection, sampler's name.

Disposal
All of the SMCPHL waste is handled as medical waste and discarded in accordance with a Medical Waste Management Plan and permit which meet all CLIA and California ELAP requirements. All of the UCD waste resulting from qPCR analyses will handled as hospital waste and discarded in accordance with Hospital Waste Regulations and meets the California ELAP requirements.

Transport to Lab
Samples will be stored in coolers with ice and delivered to:
SMCPHL, San Mateo - Enumeration-
The Wuertz Laboratory, UC Davis, Davis – qPCR

Table 7: Laboratory analytical methods – Field Samples

Analyte	Laboratory/ Organization	Project Action Limit (units, wet or dry weight)	Project Quantitation Limit (units, wet or dry weight)	Analytical Method		Achievable Laboratory Limits	
				Analytical Method/ SOP	Modified for Method yes/no	MDLs (1)	Method (1)
Surface water temperature	UCD/ SMCRCD/Balance	Not applicable	Not applicable	YSI handheld model manual	No	0°C	0°C
Conductivity	UCD/ SMCRCD/Balance	Not applicable	Not applicable	YSI handheld model manual	No	10 µS/cm	10 µS/cm
Dissolved Oxygen	UCD/ SMCRCD	Not applicable	Not applicable	YSI handheld model manual	No	0 mg/L	0 mg/L
pH	UCD/ SMCRCD	Not applicable	Not applicable	YSI handheld model manual	No	pH=0	pH=0
Fluorescence	Balance Hydrologics	Not applicable	Not applicable	10-AU Field Fluorometer manual	No	10ppt Rhodamine WT in potable water	10ppt Rhodamine WT in potable water

Table 8: Laboratory analytical methods – Enumeration

Analyte	Laboratory/ Organization	Project Action Limit (units, wet or dry weight)	Project Quantitation Limit (units, wet or dry weight)	Analytical Method		Achievable Laboratory Limits	
				Analytical Method/ SOP	Modified for Method yes/no	MDLs (1)	Method (1)
Total Coliform	SMCPHL	<1000 MPN/100mL	10-24,191 MPN/100mL	Standard Methods 9223B Enzyme substrate: IDEXX and SMCPHL SOP	None	10 MPN/100mL	10 MPN/100mL
E. coli	SMCPHL	<200 MPN/100ml	10-24,191 MPN/100mL	Standard Methods 9223B Enzyme substrate: IDEXX and SMCPHL SOP	None	10 MPN/100mL	10 MPN/100mL
Enterococcus	SMCPHL	<35 MPN/100ml	10-24,191 MPN/100mL	Standard Methods 9223B Enzyme substrate: IDEXX and SMCPHL SOP	None	10 MPN/100mL	10 MPN/100mL

Table 9: Laboratory analytical methods – *Bacteroidales*

Analyte	Laboratory/ Organization	Project Quantitation Limit (units, wet or dry weight)	Analytical Method		Achievable Laboratory Limits	
			Project Quantitation Limit (units, wet or dry weight)	Analytical Method/ SOP	Modified for Method yes/no	MDLs (1)
Filtration and concentration of target	UCD	10 gene copies per mL	Rajal et al. 2007a	No	1 gene copy per reaction	Rajal et al. 2007a
Bacteroidales Genetic markers	UCD	10 gene copies per mL	Kildare et al. 2007	No	1 gene copy per reaction	Kildare et al. 2007

Table 10: Sample handling and custody – *Bacteroidales*

Parameter	Container	Volume	Initial Preservation	Holding Time
Bacteroidales	Autoclavable plastic container (polycarbonate)	4-100 L	Keep on ice	24 h

Table 11: Sample handling and custody – Enumeration

Parameter	Container	Volume	Initial Preservation	Holding Time
E. coli	125 ml sterile plastic (high density polyethylene or polypropylene) container	100 ml volume sufficient for both *E. coli* and Enterococci analyses	Cool to 4°C; dark.	STAT: 6 hours at 4°C, dark; lab must be notified well in advance
Enterococci	125 ml sterile plastic (high density polyethylene or polypropylene) container	100 ml volume sufficient for both *E. coli* and *Enterococcus* analyses	Cool to 4°C; dark.	STAT: 6 hours at 4°C, dark; lab must be notified well in advance
TOTAL COLIFORM	125 ml sterile plastic (high density polyethylene or polypropylene) container	100 ml volume sufficient for both fecal and total coliform analyses	Cool to 4°C; dark.	STAT: 6 hours at 4°C, dark; lab must be notified well in advance

Section 9: Quality Assurance Plan.

This section describes how the quality of the measurement data collected during this effort will be assured, and is dependent on competent operators, good capture of all supporting documentation and effective protocols. This is not the official Quality Assurance Plan (QAPP) for this project, but includes tables and information from the official 24-Elements QAPP.

9.1 Competent Operators.

Specialized training or certifications.

No specialized training or certifications are required for SMCRCD field staff, for this project. All staff involved will be familiar with the field guidelines, fully trained in the aseptic technique of bacterial sample collection and procedures. Additional training will be provided by Professor Wuertz for the collection of the samples for MST. The Resource Specialist has access to the SWAMP Field Methods Course on CD_ROM and will review course materials prior to field work. The SWAMP Quality Assurance Management Plan (QAMP), Appendix D, Field collection of water samples (Appendix 1) will also be consulted prior to field work where applicable. The Resource Specialist has had volunteer training for two other SWAMP compliant field water quality sampling projects.

The Department of Health Services certifies San Mateo County Public Health Laboratory (SMCPHL). The Laboratory provides training to its staff as part of its Standard Operating Procedures. California ELAP Certificate No. 1591 CLIA certificate No. 05D0857622.

The Department of Civil and Environmental Engineering, UC Davis provides training to all personnel engaged in scientific research on an ongoing basis. An illness and injury prevention protocol is updated regularly by the department safety committee. Professor Wuertz is chair of the committee. Specific laboratory training is given to all personnel, including staff and students by the Laboratory Manager and Principal Investigator associated with the research in question.

Balance Hydrologics provides training to all personnel engaged in field investigations and hydrologic analysis on an ongoing basis. All work is conducted under supervision of a registered professional. Barry Hecht is registered in California as a Geologist (#3664). He is also certified in California as an Engineering Geologist (#1245) and a Hydro geologist (#50).

Training and certification documentation.

SMCPHL maintains records of its training. Those records can be obtained if needed from SMCPHL through the Quality Assurance Officer. The SMCPHL is a certified Clinical and Environmental Microbiology Laboratory.

The Department of Civil and Environmental Engineering, UC Davis maintains training logs for all personnel engaged in scientific research as is required by law. Those records can be obtained if needed from the Safety Coordinator, Professor Wuertz.

Training personnel.

The SMCRCD Resource Specialist provides training in field sampling for enumeration samples.

SMCPHL's Lead and Senior personnel, along with the Laboratory Safety Officer, provide all necessary training to laboratory personnel.

The Department of Civil and Environmental Engineering UC Davis will provide training by the Laboratory Manager and Primary Investigator.

The Principal Hydrologist will direct training activities at Balance Hydrologics.

9.2 Documentation.

All field parameters will be recorded at the time of collection, using the field data sheets. Data sheets will be reviewed for errors and omissions before leaving the sample site. Field data sheets as well as copies of all records held by SMCPHL and the Department of Civil and Environmental Engineering UC Davis, will be stored in hard copy form at the SMCRCD office. Field data sheets are archived for three years from the time they are collected. All data will be entered into an MS Excel or MS Access database. If data entry is ever performed at another location, duplicate data sheets will be used, with the originals remaining at the SMCRCD's office site. Hard copies of all data as well as computer back-up disks are maintained by the Resource Specialist.

Samples being sent to SMCPHL and the Wuertz Laboratory, UC Davis will include a COC. SMCPHL and Wuertz Laboratory will generate records for sample receipt and storage, analyses and reporting.

The Resource Specialist will maintain sample collection, sample transport, and COC forms as well as maintain the database. Douglas Coffman and Lab Information Services at SMCPHL and graduate student, Dan Wang at The Wuertz Laboratory, UC Davis will maintain all records associated with the receipt and analysis of samples.

Copies of the records, for sample collection, and laboratory analysis will be maintained at the SMCRCD for 3 years after the final grant payment. Copies of records at the SMCPHL and the Wuertz Laboratory, UC Davis will be **maintained for a minimum of 5 years.**

All records will be delivered to the SWRQCB'S Grant Manager, Jennifer Toney, at project completion.

Copies of the QAPP will be distributed to all parties involved with the project directly or by mail. Copies will be sent to the SMCPHL and the Wuertz Laboratory, UC Davis directors/managers for distribution within the laboratory. Any future amended QAPPs will be held and distributed in the same fashion. All originals of the first and subsequent amended QAPPs will be held at the SMCRCD office. The Resource Specialist will be responsible for the distribution of the QAPP.

A final data report will be prepared containing data collected for this project and summarizing activities conducted to generate that data. The data report package will include:

- Field data sheets and field notes
- COC of custody forms
- Geographical locations of sampling sites determined by a Global Positioning System (GPS) device
- SMCPHL and the Wuertz Laboratory UC Davis, records of sample receipt and storage, analyses and reporting.

The data will be presented in a tabular format in a technical report to the Grant Manager. The report will include results of the analyses of the QC samples and an assessment of the overall quality of the data in comparison to the objectives described in the QAPP.

9.3 Protocols

Field Protocols

Field operators using instruments with multiple probes will follow SWAMP protocols and manufacturer instructions. Field crews (2 persons per crew, minimum) will only be mobilized for sampling when weather conditions and flow conditions are considered to be safe. For safety reasons, sampling will occur during daylight hours. A sampling event should proceed in the following manner:

1. Before leaving the sampling crew base of operations, notify laboratory, confirm number and type of sample bottles as well as the complete equipment list.
2. Proceed to the first sampling station.
3. Fill-out the general information on the field log sheet.
4. Take field measurements and observations, and record on the field log sheet.
5. Take the samples indicated on the field log sheet in the manner described in this study plan. Take additional volume and blank samples for field-initiated QA/QC samples, if required. Place bottles in the coolers with ice. Double check against the log sheet that all appropriate bottles were filled.
6. Repeat the procedures in steps 3, 4, and 5 for each of the remaining sampling stations.
7. Complete the COC forms using the field notes.

After collection is completed, deliver the samples to Wuertz laboratory within 6 hours of the first sample collection.

Bacteroidales

Microbial Source Tracking Methodology:

The Microbial Source Tracking Method selected for this project is the direct measurement of specific genetic markers of Bacteroidales in water samples using available quantitative PCR assays. This approach is geographically independent, the assays have been widely used in California by the Wuertz laboratory, and there is no need to build a library for each study or cultivate the bacteria first using a traditional enumeration method. Analysis is relatively fast and methods are precise and can be coupled with direct pathogen assays. In addition data will be interpreted using a conditional probability approach developed for this purpose.

TaqMan® Analysis Procedures

Quantitative PCR will be used for to determine filtration recovery of virus and bacteria based on a surrogate Acinetobacter strain and the benign bacteriophage PP7 and the detection of *Bacteroidales*. The following section will outline the general treatment used for TaqMan® analysis, as well as specific assays used in detection.

General Guidelines for all Samples Analyzed using TaqMan®

The following is basic protocol that is to be used for any TaqMan® assay, regardless of target sequence or organism type.

Protocol

1. Thaw samples to be analyzed, and mix well with a vortex. Extract nucleic acid using the Qiagen QiaAmp Viral RNA Mini Kit (small-scale extraction) according to manufacturer's instructions or the large-scale extraction protocol outlined previously.
2. Prepare the appropriate dilutions of sample RNA using RNase, DNase free molecular grade water. The dilutions for the extractions should not be made more than a half an hour ahead of time, and should remain covered at $4^{o}C$ until used.
3. Determine the total number of reactions needed (all dilutions in duplicate, plus a negative control, and a positive control when appropriate) and prepare a master mix appropriate for the microbe of interest. The negative control should be composed of the same water used to make the master mix. The master mix should always be made in a DNA/RNA free zone.
4. Load the master mix into a 96 well plate, and cover with foil adhesive cover before leaving the DNA/RNA free zone.
5. Load extract dilutions into the 96 well plate, which contains the previously made mastermix. Cover the plate with an optical adhesive cover, making sure that the adhesive cover is sealed across each well and at all sides of the cover. Pulse centrifuge to collect all liquid to the bottom of each well, and to remove any bubbles that may have been produced during the nucleic acid transfer.
6. Run the appropriate thermocycling profile for the microbe of interest.
7. Use Ct values to calculate total concentration of the microbe of interest per reaction by applying a standard curve (see, "Quantification with TaqMan® Results").
8. Calculate the corresponding concentration of the microbe of interest in the sample volume added to the TaqMan® reaction. Apply the appropriate equation to determine the original concentration of the microbe in the environmental water sample (see "Calculation of Target Quantity in Sample Retentate").
9. If no target is detected in a TaqMan® reaction, determine the detection limit for the microbe of interest (see, "Calculation of Sample Detection Limits").

Filtration and Processing of Large Water Samples

The samples collected for MST are to be concentrated by a two-step ultrafiltration process. The first step involves concentrating the water sample from 10 to 100 liters to approximately 1.5 liters using the "large" filtration system. The second step involves further concentrating the 1.5 liters of large system retentate to approximately 100 mL with the "small" filtration system. Both systems utilize the same principles and are discussed in more detail in the following sections of this report.

Large Filtration System

The following section will outline the procedures required during the concentration of each sample with the large filtration system. Strict application of this filtration protocol will ensure the quality control and quality assurance of the results of each sample relative to each other.

Protocol

1. Rinse filter with 20 liters of nanopure water (they were stored with 0.025% NaOH). Make sure that both the drain and the flow valves are closed before filling tank with the rinse water. Connect the feed line of the tank to the pump. Do not connect the return line to the feed tank, instead route it to waste. Open the permeate port, and allow a line to run to waste.
2. Turn pump on at 240 rpm. Run rinse water to waste with recirculate valve open so that the recirculate line alone is rinsing for 10 liters of water. Then close the recirculate valve slowly, without turning off the pump, to achieve an inlet pressure of 15-17 psi. Allow the rinse water to go to waste through the recirculate line and the permeate line simultaneously.
3. Pump until dry. Drain all liquid from permeate and retentate. All of the lines, and feed tank should be completely empty.
4. Connect the feed line of tank to the pump. Also, connect return line to feed tank.
5. Using sieves and funnel fill the tank to the 100-liter mark inside the tank, again making sure that the tank's flow valve and drain valves are closed. Take 2-liter water sample for suspended solids (Raw, see "Description of Filtration Subsamples"). If filtering in lab refrigerate "Raw" sample until analysis is performed. If filtering in field, place sample on dry ice and store at –20°C until analysis in the lab. Refill the feed tank to the 100-liter mark.
6. Spike with 100 µL of bacteriophage PP7, and 400 µL of Acinetobacter sp. Each spike is to be prepared in advance, and stored in individual tubes in liquid nitrogen.
7. Mix well at full speed for 10 minutes. Take a subsample (Feed, see "Description of Filtration Subsamples"). Store as appropriate (see step 5).
8. Take a second feed sample. For this sample, place 10 ml of feed on lysis buffer for extraction. See "Large-Scale DNA/RNA Extraction" steps 3-6 in "TaqMan® Analysis Procedures" section. Store as appropriate (see step 5).
9. Open permeate port. Check that permeate line goes to clean and disinfected permeate tank, and that recirculate valve is open. Open the flow valve on the feed tank. Turn on pump at 240 rpm. Close recirculate valve slowly to achieve an inlet pressure of 15-17 psi.
10. When the permeate tank fills up, take a sample from the permeate tank (Permeate, see "Description of Filtration Subsamples"). Store as appropriate (see step 5). Run the permeate line to waste, and discard the remaining permeate.
11. Filter until the retentate in the feed tank is approximately near the black line in the bottom of the tank. This is important so that the volume of collected retentate will be about 1 liter. Turn pump off.
12. Drain the permeate port first, and discard the collected liquid.
13. Collect all of the retentate. Recovery will strongly depend on making sure all it collected. Open all drains on the feed tank and filter cart, collecting the retentate from within. Make sure that all lines are empty. Retentate volume should be around 1 liter. Measure the exact volume, and record in filtration check list. Place retentate in a clean beaker with a clean stir bar, and mix on a stir plate for 2 minutes. Take a sample while still on the stir plate. (Retentate, "Description of Filtration Subsamples"). Store as appropriate (see step 5).
14. Add "glycine 1" bottle to retentate (see "Filtration Bottle Definitions"). Bring the volume up to 1.5 liters with nanopure water. Make sure the feed tank drain valve is again closed, as well as the flow valve. Carefully pour retentate mixture back into feed tank. Avoid splashing the liquid, as it will cause bubbling to occur.
15. Close the permeate port and open the recirculate valve. Now open the tank flow valve, and turn the pump on at 130 rpm. Recirculate for 10 minutes to elute the membrane. Turn pump off. Disconnect feed and completely drain all lines, making sure to carefully collect all liquid within. Measure and record volume in the filtration check list.

Place recirculate in a clean beaker with a clean stir bar, and mix on a stir plate for 2 minutes. Take a sample while still on the stir plate. (Recirculated, see "Description of Filtration Subsamples"). Store as appropriate (see step 5).

16. Place the remaining liquid, covered with clean foil, aside. Drain permeate liquid if necessary. Due to pressure some liquid can cross the membrane during the recirculation step.
17. Disconnect filter for a second elution step and later cleaning (see "Cleaning and Disinfection of Microza Filters"). Replace filter with dummy steel tube to clean the lines and feed tank.
18. For the second elution of the filter, add "glycine 2" bottle (see "Filtration Bottle Definitions") to one end of the filter, making sure that the other end is capped. Bubbles will be slowly displaced while the liquid fills the fibers. Cap the open end, and put the filter in the shaker. Shake at maximum speed for 20 minutes at room temperature. Drain the liquid completely from the filter, and register the volume in the filtration checklist. Place the liquid in a clean beaker with a clean stir bar, and mix on a stir plate for 2 minutes. Take a sample while still on stir plate. (Membrane, see "Description of Filtration Subsamples"). Store as appropriate (see step 5).
19. Add the remaining membrane sample to the recirculated sample that was set aside. This will be further concentrated with the small filtration system. If processing in the lab, cover and store at 4°C until small system processing. If processing in field, carefully transfer liquid to a disinfected storage container with a tightly fitting lid. Label the container with sample name, sample date, and volume of retentate collected. Place sample on dry ice and store at –20°C until analysis in the lab.
20. Add 200 mL of "storage solution" (see "Filtration Bottle Definitions") to the filter for storage until cleaning.
21. To disinfect the lines and the feed tank, add approximately 3 liters of "disinfectant" (see "Filtration Bottle Definitions") to the feed tank. Make sure that the dummy bar is properly in place where the filter usually goes. Connect return line to feed tank, and open permeate port with a return line to feed tank also. Check that the recirculate valve is open. Turn pump on at maximum speed and recirculate for 10 minutes.
22. Spray the feed tank with 10% bleach solution, and scrub well with a brush. Rinse the feed tank with Deionized water thoroughly. The system is now ready for processing the next sample.

Small Filtration System

This section will outline the procedures required during the concentration of each sample with the small filtration system. Again, strict application of this filtration protocol is required to ensure the quality control and quality assurance of the results of each sample relative to each other.

Protocol

1. Divide the recirculate and membrane mixture between four large centrifuge tubes. Centrifuge at 2000 rpm for 5 minutes. If large system filtration was performed in field, the retentate will have to thaw at room temperature previous to this step.
2. Pour supernatant from each of the four centrifuge tubes into a clean beaker, with a clean stir bar. Cover and set aside.
3. Remove the solids from the centrifuge tubes. Measure and record volume in the filtration check list. Place solids in a labeled container. The label should contain the following information: sample location, Solids-SS, date collected, and volume collected.
    a. Example: ALP (Arroyo Las Posas), Solids-SS, 2/10/04, 14 mL.
4. Store solids at –20°C.
5. Place beaker with supernatant on a stir plate, and mix for two minutes. Collect subsample while still mixing (Feed, see "Description of Filtration Subsamples").
6. Pour feed into the feed tank of the small system. Make sure that the tank drain line is closed, and that the recirculate valve is open. Also, check that the permeate line is connected to the permeate port on the small filter, and that it is draining to a permeate tank. The recirculation line should feed into top of feed tank.
7. Turn on pump, and increase pump speed until it registers a reading of 10.
8. Make sure that the feed is recirculating properly, and then close recirculation valve slowly until an inlet pressure of about 18 psi is reached.
9. Allow system to filter liquid until retentate is at proper volume.
10. Collect a permeate subsample. (Permeate, see "Description of Filtration Subsamples").

11. Completely drain all of the retentate from all lines and from feed drain valve. Place in a clean beaker and set aside.
12. Remove filter, and place rubber fittings to each end. Fill a 60 CC syringe with 50 ml of a 1:10 dilution of glycine 1 solution (see "Filtration Bottle Descriptions"). Dilution should be made with nanopure water.
13. Insert the filled syringe into the end of one of the rubber fittings on the filter. Insert an empty syringe in the rubber fitting on the opposite end of the filter. Force liquid out of the syringe, through the filter, and allow it to fill the empty syringe. Continue this step, turning the filter upside down each time, for about 10 cycles.
14. Collect all liquid from the syringes and the filter. Use one of the syringes to force any of the remaining liquid from the filter.
15. Combine the eluted liquid, and the retentate liquid. Measure and record the volume in the filtration checklist. Place the mixture in a clean beaker, with a clean stir bar. Mix on stir plate for 2 minutes, and collect a subsample (Retentate, see "Description of Filtration Subsamples").
16. Also, place 10 ml of retentate on lysis buffer for extraction. See "Large-Scale DNA/RNA Extraction" steps 3-6 in "TaqMan® Analysis Procedures" section.
17. Place remaining liquid into a labeled container. The label should contain the following information: sample location, Retentate-SS, date collected, and volume collected.
    a. Example: ALP (Arroyo Las Posas), Retentate-SS, 2/10/04, 126 mL.
18. Store solids at -20°C.
19. Clean filter, and the small filtration system, by pouring about 2 liters of "cleaning solution" (see "Filtration Bottle Descriptions") to feed tank. Recirculate with the recirculation valve open at a speed of 30. Make sure that lid to feed tank is secured properly for this step! Run for at least 20 minutes.
20. Drain all liquid and rinse the system well with Deionized water. It is now clean for the next processing.
21. Rinse the filter well with Deionized water. The filter can be directly attached to the Deionized water faucet with a hose and allowed to run clean for at least 10 minutes.
22. Fill the filter with 50 mL of "storage solution" (see "Filtration Bottle Descriptions") until next use.

Filtration Bottle Definitions
Glycine 1: 150 mL of 0.5 M glycine/NaOH, pH 7.0 + 15 mL of 10% Tween 80.
It is added to the retentate to obtain (in 1.5 L volume) a final concentration of 0.05 M glycine/NaOH and 0.1% Tween 80.

Glycine 2: 200 mL of 0.05 M glycine/NaOH, pH 7.0

Storage Solution: 0.025% NaOH solution.

Disinfectant: 0.025 % NaOH + 200 ppm chlorine bleach.

Cleaning Solution: 4g/L NaOH with 7.5 ml/L of bleach.

Description of Filtration Subsamples
Raw: From original water sample, after sieving, and before any processing with the pump.

Feed-Large System**:** Collected from feed tank, after addition of 100 L of sample, all appropriate spikes, and the completion of the mixing step for 10 minutes.

Permeate-Large System**:** Collected from the permeate tank after at least 45 liters has accumulated.

Retentate-Large System**:** The retentate after complete filtration of the whole volume of water sample. Subsample should be taken after mixing for 2 minutes on a stir plate.

Recirculate-Large System**:** Retentate with the addition of glycine and Tween 80, after recirculation step. Subsample should be taken after mixing for 2 minutes on a stir plate.

Membrane-Large System**:** The resultant liquid from eluting membrane with glycine, after shaking. Subsample should be taken after mixing for 2 minutes on a stir plate.

Feed-Small System**:** The supernatant that remains from the centrifugation (at 2000 rpm for 5 minutes) of the mixture of the recirculate and the membrane from the large system. Subsample should be taken after mixing of supernatant for 2 minutes on a stir plate.

Permeate-Small System**:** Collected from the permeate tank after the entire volume of the sample has been filtered.

Retentate-Small System: The mixture of the retentate remaining in the small system after filtration, and the volume that result from the elution of the small filter. Subsample should be taken after mixing for 2 minutes on a stir plate.

Subsampling Procedure
The subsample will consist of 10 mL.

To remove the subsample, use a sterile pipette each time. Place subsample in a sterile plastic 15 mL centrifuge tube, tighten the cap and label the tube with a waterproof sharpie. The label should contain the following information: sample location, type of subsample, small or large system, and date.

Example: ALP (Arroyo Las Posas), Feed - LS or SS, 2/10/04.

Subsamples shall be collected only from well-mixed sample volumes, and shall be stored at $4^{o}C$ if being analyzed within 8 hours, or at $20^{o}C$ for long-term storage.

Cleaning and Disinfection of Microza Filters

The Microza filters were designed for reuse. The disinfection of the filters is a very important step, due to the possibility of cross-contamination if the filter is not cleaned properly. The following is the protocol for disinfecting the Microza filters for the large system (small system filter disinfection is located in steps 17 and 18 of the "Small System Filtration" section of this report). This protocol is to be followed for the cleaning and disinfection of all Microza filters for each time they are used.

Protocol

Secure filter to stand and attach recirculation line. Route hose back to feed tank. Do not open permeate port.

1. Pour 2-3 liters of "cleaning solution" (see "Filtration Bottle Definitions") into feed tank. Turn on pump to maximum speed.
2. Pulse liquid through the lines and the filter. Stop pump, unhook feed line, and drain filter and lines.
3. Reconnect all lines and filter, and repeat steps 2-4 several times until liquid draining from filter and lines becomes clear. Repeat 5 more times.
4. Run cleaning solution through lines and filter continuously for about 20 minutes.
5. Stop pump, unhook feed line, and drain filter and lines.
6. Rinse feed tank well, and fill with about 50 liters of Deionized water. Repeat steps 2-4 and 6, this time with the Deionized water instead of the cleaning solution.
7. Open permeate port, and close recirculate valve slowly to achieve an inlet pressure of 15 psi. Allow 10 liters of water to pass through permeate before checking the flux of the filter. Flux must be at least 368 L / h. (9.7 sec per liter). Step 13 has detailed instructions for measuring the flux of the Microza filters.
8. Drain water from all lines. If flux is satisfactory, remove filter and fill with 200 mL of "storage solution" (see "Filtration Bottle Definitions"). If not, continue with steps 10-15. It is usually the case that the flux requirement has not been met by this point.
9. Fill feed container with 2-3 liters of citric acid solution (20 g/L citric acid). Turn on pump to maximum speed, with permeate port closed and recirculation valve open. Recirculate for 20 minutes at maximum speed. Then, open the permeate port, with the hose routed back to the feed tank, and slowly close the recirculation valve to reach an input pressure of 15 psi.
10. Run for as long as it takes to secure the approximate proper flux (368 L/hr).
11. Upon reaching the approximate proper flux, rinse the feed tank very well, and fill it with 60-70 liters of Deionized water.
12. Run a hose from the recirculation valve to waste. With the permeate port closed, allow 20-30 liters of Deionized water to pass through the open recirculation valve to waste with the pump set at 190 rpm. Turn off pump, connect a hose from the permeate port (now open) to waste. Turn on pump (again at 190 rpm), and slowly close the recirculate valve to achieve an input pressure of 15 psi.
    - After lines fill and equilibrate, place permeate line in a large graduated cylinder and measure time needed to pump 1 liter. Repeat.
    - Calculate flux in L/ hour. Flux must be at least 368 L / h. (9.7 sec per liter).
13. If flux is acceptable, test the pH of the permeate. The pH should be in the range of 6.0 – 7.0. Remove filter, drain all water, and fill with 200 mL "storage solution".
14. Cap and store at 4°C.

Nucleic Acid Extraction

Two different procedures are used for all DNA and RNA extraction needed for this project. The first procedure is for the extraction of nucleic acid from our samples requiring only "small scale DNA/RNA extraction". These consist mainly of subsamples taken and used for recovery calculations at differing times throughout the filtration process. The second procedure is for those samples that require a larger volume of nucleic acid extract, and is thus referred to as "large scale DNA/RNA extraction".

Small-Scale DNA/RNA Extraction Samples requiring only small-scale extraction were extracted using the QIAamp Viral RNA kit (Qiagen, Valencia, CA) according to the manufacturer's directions. Final eluted volumes were approximately 80L.

Large Scale DNA/RNA Extraction

In order to analyze a larger fraction of the original sample, which is required for all final retentate samples, (and ultimately those samples contributing to global recovery calculations) a modified protocol for the Qiagen QIAampDNA Blood Kit (Qiagen, Valencia, CA) was developed. This new protocol involves the production of large quantities of lysis buffer (see preparation of lysis buffer in "Required Media and Solutions" section of this report). The following outlines the protocol to be used for all large-scale DNA/RNA extractions.

Protocol

1. Label a to a 200 ml conical plastic centrifuge bottle with the sample name and date of collection.
2. Turn on Bambino incubator to allow it to warm to 70°C. The incubator will be used later.
   Note: Steps 3-6 are performed in advance of extraction (See "Lab Filtration and Processing of Samples with Large System" and "Lab Filtration and Processing of Sample with Small System").
3. Add 40 mL of lysis buffer (1:4 ratio of sample: buffer) to the centrifuge tube. Make sure that the solids in the lysis buffer (which form due to refrigeration) are completely dissolved.
4. Completely mix the sample that will be extracted with a vortex (F-LS or R-SS).
5. While sample is still well mixed, and using a sterile pipette, transfer 10 ml of the sample into the centrifuge tube.
6. Pulse vortex 15 times, and let sit for 10 minutes at room temperature.
   Note: If steps 3-6 were previously performed, allow frozen sample to thaw at room temperature. Continue with steps 7-27.
7. Add 40 mL of ethanol (ethyl alcohol) to the centrifuge tube that contains the sample and the lysis buffer. Make sure to use either a sterile pipette or a sterile plastic tube to measure the ethanol. (1:1 ratio of ethanol: buffer).
8. Pulse vortex 15 times.
9. Centrifuge for 5 minutes at 4000 rpm.
10. Label all three pieces of a large extraction tube from the Qiagen QIAampDNA Blood Kit. Label with sample name and subsample collection date.
11. Place the spin column into an opening in a vacuum manifold. Replace the lids on the correctly labeled catch tube, and set aside. The vacuum apparatus should be very clean and sterile before use. Place tops on all un-used openings of the vacuum manifold.
12. Apply vacuum while slowly pouring centrifuged mixture into it. DO NOT pour the pellet into the spin column.
13. Add 5 ml of AW1 wash buffer from the Qiagen kit, and vacuum through. Apply evenly over filter. Use a sterile pipette when applying the wash buffer to each extraction column.
14. Add 5 ml of AW2 wash buffer from the Qiagen kit, and vacuum through. Use the same precautions as in step # 13.
15. Place the spin column in its appropriate catch tube, and centrifuge for 15 minutes at 4000 rpm.
16. Remove the lid, and place spin column on top of upside-down lid. Place both in 70°C Bambino incubator for 5 minutes to burn off excess ethanol. Discard catch tube.
17. Perform "smell test" for ethanol on each spin column. Place columns back into incubator until all ethanol smell is gone.
18. Upon validation of the absence of ethanol in the column, place the spin column into new catch tube with a new lid. Make sure to label both correctly.
19. Dilute filter evenly with 600 µl of Genemate DEPC treated water (nuclease free). Use a clean pipette tip for each spin column. Let spin column sit for 5 minutes.
20. Centrifuge 5 minutes at 4000 rpm.
21. Add another 600 µl of Genemate DEPC treated water, and let it sit again for 5 more minutes.
22. Centrifuge again for 5 minutes at 4000 rpm.
23. Measure the volume of the extract, and place into a labeled 1.5 ml centrifuge tube. Record volume in appropriate places.
24. Store samples at –20°C.

Hydrology measurement methods and computational procedures will conducted according to the U.S. Geological Survey (see Buchanan and Somers, 1976) and U.S. Army Corps of Engineers standards, enabling comparison with results of similar studies previously conducted by these agencies. The standards are available at the USGS website (http://pubs.usgs.gov/twri/) and upon request from Balance Hydrologics.

9.4 Procedures to Affect and Check Quality.
Quality control samples will be taken to ensure valid data are collected.
Depending on the parameter, quality control samples will consist of blanks, replicate samples, and split samples. In addition, quality control sessions (a.k.a. intercalibration exercises) will be held twice a year to verify the proper working order of equipment, refresh volunteers in monitoring techniques and determine whether the data quality objectives are being met.

Enumeration
The laboratory will analyze the field blanks submitted. The expected results are the absence of total coliforms, *E. coli* and Enterococci.

The laboratory will analyze the 5 replicates samples and record the individual results and an average. At a frequency of 10% of the samples received, the laboratory will split the five samples and run them in duplicate – providing two sets of five replicates. The averages of each 5 replicate set represents the data that will be used in assessment of the Precision criteria. This is done in accordance with Standard Methods 9020B.

The laboratory will analyze reference samples consisting of one sample that contains total coliform, but not *E. coli; E. coli*, a non coliform organism, and a sterile sample. The results expected are as follows. Total coliform/not *E. coli* – positive total coliform/negative *E. coli; E. coli* – positive for both total coliform and *E. coli*; non coliform organism and sterile sample – negative for both total coliform and *E. coli*.

Table 12: Analytical QC - Enumeration.

Matrix: water
Sampling SOP: Standard SMCPHL
Analytical Parameter(s): E. coli and *Enterococcus*
Analytical Method/SOP Reference: IDEXX Colilert-18, Enterolert/ SMCPHL SOP's
 # Sample locations: variable

Laboratory QC	Frequency/Number	Acceptance Limits
Method Blank	Each lot	Pos/Neg
Reagent Blank	Each lot	Pos/Neg
Storage Blank	Daily and each batch	2-4 degrees C
Instrument control	Twice daily	35 +/- .5C (TC&EC) 41 +/- .5 C (Enterococci)
Lab. Duplicate	Not applicable	Not applicable
Lab. Matrix Spike	Each lot	Pos/Neg
Matrix Spike Duplicate	Not applicable	Not applicable
Lab. Control sample	Pos/Neg/Ster. Controls	Pos/Neg for each
Surrogates	Not applicable	Not applicable
Internal Standards	To comply with certification	100% or full review
Others:	Routine PT's performed	100% or full review

Parameter	Blank	Duplicate Sample	Split Sample to lab	QC session
Water quality				
Biological Parameters				
Total Coliform and *E. coli* Bacteria	daily	5% or a minimum of once per day	twice a year	twice a year
Enterococci	daily	5% or a minimum of once per day	twice a year	twice a year

Blanks, Replicates, Split Samples, and Standardization

Field/Laboratory Blanks:
A laboratory blank will be performed for each sampling/analysis event for bacterial analysis.

Instructions for Field and Lab Blanks:
For bacterial analysis, the reagents are added to sterile distilled water (in the same manner as for a field sample) and that blank is then sealed in a quantitray and incubated along with the field samples. The blank should be below detection limits (i.e., no positive wells) at the end of the incubation period.

Replicate Samples:
For bacterial analysis lab duplicates will be run at least once per sampling day, and when there are more than 20 samples run per day then there will be a minimum 5% of the samples analyzed in duplicate.

Standardization of Instruments and Procedures:
At the Quality Assurance Sessions the temperature measurements will be standardized by comparing our digital thermometers to a SMCPHL certified calibrated thermometer in ice water and ambient temperature water.

Bacteroidales
Quality control measures will be taken for each sample filtration and subsequent qPCR analysis. These steps consist of running field blanks for each event and testing the resulting concentrated water for target organisms. It is expected that these blanks show non-detects for the specific qPCR assays. Laboratory blanks (negative controls) will also be run for qPCR assays. Positive controls are included as part of the calibration curve.

The Wuertz laboratory has developed methodology and analytical procedures for the determination of true sample limits of detection (SLODs) {Rajal, 2007a #94; Rajal, 2007b #209}{Kildare, 2007 #116} for qPCR using environmental samples. These procedures include an equation that accounts for filtration recovery, DNA extraction efficiency, and enzyme inhibition {Rajal, 2007b #209}. All quantitative *Bacteroidales* assays to be used have been designed in the Wuertz laboratory and validated using blind samples {Kildare, 2007 #116}.

Field Blank
Field blanks should be collected for the stations and events to be specified. The field crew will use blank water provided by the laboratory to generate field blanks by pouring blank water directly into the sample bottles. Field blanks should be submitted "blind" to the laboratory as Station PPH 1226-B designation. If detected values are reported for field blanks, the frequency of collection will be increased.

Field Duplicates
Field duplicates shall be collected for the stations and events specified. Field duplicates shall be collected immediately following the collection of normal samples. In cases where multiple intermediate bottles are used for a single analysis, field duplicates and normal sample containers should be filled in an alternating sequence (i.e., normal-duplicate-normal-duplicate). Field duplicates should be submitted "blind" to the laboratory as Station PPH1226-XXX (X's are for the duplicate field station).

Laboratory Duplicates
Laboratory duplicate analyses should be requested for all constituents for the stations and events specified. No special sampling considerations are required. However, additional sample volume must be collected, per laboratory requirements, for each analysis.

Table 13: Analytical QC - qPCR.

Matrix: Water or sediment/biofilm
Sampling SOP: Attached
Analytical Parameter(s): Host-specific gene markers
Analytical Method/SOP Reference: Rajal et al. 2007 a,b; Kildare et al 2007
Sample locations: Variable

Laboratory QC	Frequency/Number	Acceptance Limits
Method Blank	Each event	Pos/Neg
Reagent Blank	Each run	Pos/Neg
Storage Blank	Daily	2-4 degrees C
Instrument Blank	Each run	Pos/Neg
Lab. Duplicate	Each run	Pos/Neg
Lab. Matrix Spike	Each lot	Pos/Neg
Matrix Spike Duplicate	Each event	Pos/Neg
Lab. Control sample	Pos/Neg/PCR controls	Pos/Neg for each
Surrogates	Each run	Pos/Neg
Internal Standards	Each run for DNA extraction efficiency	50% or full review
Others:	Check slope of sequential dilutions	90% or full review

9.4.1 Accuracy and Precision.

Representativeness indicates how well the data represent environmental conditions. This is addressed through the overall sampling design. Sites were selected to identify potential contributors to fecal contamination and the sample schedule was designed to maximize representativeness by optimizing the sampling frequency and location.
Precision describes how well repeated measurements agree.
Accuracy describes how close a measurement is to its true value.
Completeness is the percentage of how much of the data are available for use versus the total amount of data collected. It is calculated as the number of analyses generating useable data for each analysis divided by the total number of samples collected for that analysis. Data may be unavailable for use due to unavoidable circumstances such as laboratory error, samples lost or contaminated, etc.
Sensitivity is the ability of the instrument to detect one concentration from the next.

9.4.2 Sample Integrity

Enumeration samples:
After collection the samples will be immediately placed on ice in a cooler for transport to SMCPHL, coolers to contain freezer packs. All samples should be delivered to the SMCPHL in a suitable time for which the holding time (6 hours) will not be exceeded before analysis can begin. All specimen and test data will be captured in the Laboratory Information System. The COC form is used as a shipping record.

Bacteroidales:
Water samples will be placed on ice and transported to the Wuertz Laboratory. Analysis will occur within 24hours. COC forms will be filled out for all samples submitted to the Wuertz laboratory. Sample data, sample location, sample collection crew names, and analysis requested shall be noted on each COC.

Sample Handling
Identification information for each sample will be recorded on the label on the plastic sample bottles when the sample is collected. Samples will be labeled with the water body name, sample location, sample number, date and time of collection, sampler's name.

Custody Procedures
The conventional water quality monitoring tests do not require specific custody procedures since they will be conducted immediately by SMCPHL. In special circumstances (such as severe weather conditions), sample collection will be rescheduled in a timely and orderly manner.

Disposal
All of the SMCPHL waste is handled as medical waste and discarded in accordance with a Medical Waste Management Plan and permit which meet all CLIA and California ELAP requirements.

All of the UCD waste resulting from qPCR analyses will handled as hospital waste and discarded in accordance with Hospital Waste Regulations and meets the California ELAP requirements.

9.5 Data Verification and Validation

UCD will be performing all the lab data acquisition except microbial indicator enumeration, which will be performed by SMCRCD. Flow data will be obtained by Balance Hydrologics and made available as raw data, calibrated parameters, and tabular and/or graphical format, tied to the three gage locations, suitable for use by others in GIS and Google Earth images or maps.

Field data sheets will be checked for completeness and samples and sample information will be examined, at the SMCPHL and Wuertz Laboratory, upon receipt to be sure samples are within holding times, sample identification information is correct, and samples have been held and handled appropriately. Any samples that are not acceptable will be marked and not entered into the database. The lab director also will review lab data sheets completed by laboratory technicians to be sure there are no omissions or errors and that all entries are clear. The graduate student Dan Wang will review all field sheets and laboratory results and enter data into a database. MS Excel will be used.

Review of all field and data activities is the responsibility of each team leader along with the technical advisors. All field personnel will be trained by the Lead Researcher at UCD and the SMCRCD Resource Specialist prior to sampling. The work of the field crews will be reviewed by the Lead Researcher and the Resource Specialist at the end of each sampling day. Anyone in need of more instruction will receive it on site prior to the next sampling day.

Data files will be reviewed on a quarterly basis to identify outliers, spurious results, or omissions. Any problems will be reported to the project director. The Lead Researcher and the Resource Specialist will review data for compliance with data quality objectives. Any problems with data quality will be included in the final report.

Each of the data components (enumeration, MST and hydrology) will have field data sheets reviewed for completeness and any unusual results the day of the monitoring event. Data validation is covered with signed entries by the field and laboratory technicians on field data sheets and laboratory data sheets, respectively; review for completeness and accuracy by the field and general laboratory director; review by the team leaders for outliers and omissions; and the use of quality control criteria to accept or reject specific data. Verification of data will be performed by a designee. Any errors found will be noted and discussed by the technical advisory team before being corrected in the database. Then the validation process will begin. The verified data in the database will be compared to the DQO as written in the QAPP. When that process is complete, a report will describe the findings

Review of all field and data activities is the responsibility of graduate student Dan Wang at UCD and the Resource Specialist. All field staff will be trained by Dan Wang and the Resource Specialist prior to the first sampling event. Dan Wang and the Resource Specialist will review the work of field staff at the end of each sampling day and additional instruction will be given if deemed necessary.

The Principal Hydrologist will be responsible for assessing all hydrologic data collected by Balance Hydrologics.

9.6 Administrative Reports

Table 14: QA management reports.

Type of Report	Frequency (daily, weekly, monthly, quarterly, annually, etc.)	Projected Delivery Dates(s)	Person(s) Responsible for Report Preparation	Report Recipients
Analysis of existing data		May 2008	Resource Specialist/	TAC
Public Notices and meeting attendance information	Quarterly		Resource Specialist/	
Grant Summary Form	90 days		Project Manager	SWRCB
Progress Reports by the end of the month following the end of the calendar quarter (March, June, September, and December)	Quarterly	Dec. 2007, March 2008, June 2008, Sep 2008, Dec. 2008, March 2009, June 2009, Sep 2009, Dec. 2009, March 2010, June 2010, Sep 2010, Dec. 2010	Resource Specialist/ Project Manager	TAC/SWRCB/Public Meetings
Natural Resource Projects Inventory (NRPI) Project Survey Form		Before final invoice	Project Manager	SWRCB
Draft Project Report		February 2010	Consultants and Resource Specialist/ Project Manager	SWRCB/TAC
Final Project Report		March 2010	Consultants and Resource Specialist/ Project Manager	SWRCB/TAC/Public Meetings

Section 10: Health and Safety.

Field crews will always consist of at least two persons, and will only be mobilized for sampling when weather conditions and flow conditions are considered to be safe. For safety reasons, sampling will occur during daylight hours. Field work will consist of collecting grab samples. No chemicals, such as acid preservatives, will be handled during sampling activities. The Project Manager will contact the landowner/operator prior to sampling and heed any warnings regarding recent applications of pesticides.

Field staff will wear disposable polyethylene, nitrile, or non-talc latex gloves and appropriate footwear during all sampling activities. Field personnel will be aware of unstable ground and potentially hazardous snakes and insects. Field personnel will possess a map showing the nearest hospital and quickest route to it from all project sites.

Section 11: Data Management, Interpretation, and Reporting

Each component set of data will be reviewed quarterly to determine if DQOs have been met. Any errors will be noted, the cause determined and corrective action suggested. If the problems cannot be corrected, then the DQOs will be reviewed for feasibility. If specific DQOs are not achievable, the lead researcher will recommend appropriate modifications. Any revisions would need approval by the project manager and TAC. Any revisions to DQOs will be appended to this QAPP with the revision date and the reason for modification

The Resource Specialist will be responsible for data management and data analysis. The data will include physical and chemical analysis results received from Soil Control Lab and all relevant field data and information collected by SMCRCD. Data generated from the project will be stored in Microsoft Excel spreadsheets and/or entered/imported directly into the SWAMP database when required. The monitoring data will be used by the SMCRCD Project Manager, RWQCB Grant Manager, technical specialists with the NRCS, SWAMP and the producers. The project manager will use the data to assess the overall effectiveness of the BMP. This assessment will be communicated to the producers in writing.

The data will be reported in a technical report to the Grant Manager within the quarterly progress report process. The technical report will include the following sections:

1.0 Introduction: background, problem, objective, question
2.0 Location and methods
3.0 Results and discussion
4.0 Conclusion and recommendations
5.0 References
Appendices, if necessary

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APPENDICES:

Appendix #1:

PRELIMINARY RESEARCH HYPOTHESES FOR PILLAR POINT HARBOR (PPH) PROJECT
STEFAN WUERTZ, DECEMBER 2007

Hypothesis 1:

Poor circulation in PPH results in long residence times of land-derived and sea-derived (seagulls) microbial pollution

Hypothesis 2:

Seagulls and other birds (e.g. ducks) account for a significant amount (≥ 50%) of fecal indicator bacteria (FIB) in PPH

Hypothesis 3:

Human fecal pollution is the predominant source of FIB (≥ 50%) in PPH

Hypothesis 4:

FIB survive at interfaces, such as sand and sediment particles, resulting in unpredictable release of viable FIB into the surrounding water column

Hypothesis 5:

FIB can not only survive but "re-grow" in suitable environmental niches in PPH that provide shelter and a food source (Examples: decaying seaweed, fecal droppings from birds and pets on shore, storm drains containing plant material)

Notes

If FIB survive in the harbor environment (i.e., there is a slow decay rate of cells) hypothesis 1 would suggest a gradual increase in FIB if there is a constant input of fecal matter from whatever source.

Research questions

- Do fecal indicator bacteria (FIB) like *E. coli* and *Enterococcus* survive in the harbor environment (water/sand/sediment/wrack/other)?
- Do FIB grow in the harbor environment?
- Are there biofilms in storm drains or other ecological niches (e.g. wrack line) that could harbor FIB and provide conditions conducive to growth?
- Are there significant numbers of FIB in sand and sediment that could lead to transient elevated microbial counts in the surrounding water exceeding water quality objectives?
- Can we model the fate of FIB or microbial source tracking signals based on hydrology and bathymetry of the harbor environment? In other words, what happens to a slug of FIB introduced to the harbor?

- What are the main sources of fecal pollution in the harbor? (Have quantitative assays for human, dog, cow, universal)
- Are there seasonal trends with respect to sources of fecal pollution?
- Which remediation measures are likely to reduce the input of fecal pollution
- Is there regular input of fecal contamination into the harbor? Which outfalls/storm drains/ creek outlets are the main contributors?