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Watsonville Sloughs Pathogen and Sediment TMDL

Quality Assurance Project Plan And Field Sampling Plan

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Preface & Distribution List

This document is the Quality Assurance Project Plan (QAPP) and Field Sampling Plan for a project that provides technical assistance to the CCRWQCB for the development of the Watsonville Sloughs pathogen and sediment TMDLs. Following approval by the CCRWQCB staff, this document will be made available to any interested agencies and stakeholders on the CCoWS website. It contains an adaptive monitoring plan, which may be revised following the first synoptic monitoring runs.

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3. CCoWS Research Manager-Wendi Newman
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Relation to standard EPA document layout

The EPA publishes a guide to preparing QAPPs that is recommended for certain monitoring programs. This document is structured primarily according to conventional environmental research reporting practice, rather than the EPA guidelines. However, it contains all the elements of the EPA-recommended QAPP structure. The table below shows where each standard EPA QAPP element may be found in the present document:

EPA QAPP Element	This document
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1. Title and approval page	Cover page
2. Table of Contents	Table of Contents
3. Distribution	Preface and Distribution List
4. Project/Task Organization	6.1 Staff Structure and Training
5. Problem Identification/ Background	1.1 Background
6. Project/Task Description	1.2 Project Description
7. Data Quality Objectives for Measurement Data	5.3 Data Quality Objectives
8. Training Requirement/Certification	6.1 Staff Structure and Training
9. Documentation and Records	6.3 Reporting
Measurement/Data Acquisition	
10. Sampling Process Design	5 Field Sampling Plan
11. Sampling Methods	7 Sampling Protocols
12. Sample Handling and Custody Requirements	7.2 Protocol for sample management
13. Analytical Methods Requirements	8 Laboratory and Analytical Protocols
14. Quality Control Requirements	9 Quality Control
15.Instrument / Equipment Testing etc.	7.9 Protocols for equipment management
16. Instrument Calibration and Frequency	7.9 Protocols for equipment management
17.Inspection and Acceptance Requirements for Supplies	7.9 Protocols for equipment management
18. Data Acquisition Requirements	9.3 Data Acquisition Requirements
19. Data Management	6.2 Protocol for data management
Assessment and Oversight	
20. Assessment and Response Actions	9.4 Assessment and Response
21. Reports	6.3 Reporting
Data Validation and Usability	
22.Data Review, Validation and Verification Requirements	9.5 Data Review and Verification
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1 Introduction

1.1 Background

Watsonville Slough is listed on the California 303d list under the Federal Clean Water Act as being impaired due to pathogens and sediment. Accordingly, the Central Coast Regional Water Quality Control Board is required to develop and implement a Total Maximum Daily Load (TMDL) specification for pathogens and sediment.

The area is typical of Central California coastal watersheds. It contains relatively steep headwaters with some natural land uses, draining through undulating rural residential land with septic sewerage, then to a rapidly growing industrial-agricultural city, down to a broad alluvial flood plain with intense irrigated agriculture, and finally through a small residential dunes complex to the small Pajaro Lagoon, and thence to Monterey Bay and the Pacific Ocean. Watsonville Slough itself is the remnant of a once more-extensive wetland and estuarine complex that was ditched and drained a century ago to provide land for agriculture.

There is a lack of quantitative information on the extent, severity, and origins of pathogens and sediment in Watsonville Slough. A number of water resources management and environmental studies have been completed in the area. However, none have done targeted work on quantifying concentrations, loads, and sources of pathogens and/or sediment. The primary studies include:

- *Watsonville Sloughs Watershed Conservation and Enhancement Plan* (Swanson Hydrology & Geomorphology, 2002)
- *Pajaro River Watershed Water Quality Management Plan* (Applied Science and Engineering Inc., 1999)
- *Aquatic Toxicity in the Pajaro River Watershed: Tributary Sources and Chemicals of Concern* (Hunt et al., 1998)
- *Water Resources Management Plan for Watsonville Slough System Santa Cruz County* (Questa Engineering Corporation, 1995)
- *State Mussel Watch Program* (State Water Resources Control Board, 1977-2000)
- *Toxic Substances Monitoring Program* (State Water Resources Control Board, 1977-2000)

Additional water quality monitoring has also been conducted in the Watsonville Sloughs system by the following organizations:

- Santa Cruz County Environmental Health
- City of Watsonville
- Central Coast Regional Water Quality Control Board
- Pajaro Valley Water Management Agency
- University of Santa Cruz-Marc Los Huertos
- Watershed Institute (1995-1997)-John Oliver
- Santa Cruz County Resource Conservation District
- Coastal Watershed Council
- California Department of Fish and Game

Despite these monitoring efforts, there are very limited data on suspended sediment concentrations and sedimentation rates, and the specific manner in which the beneficial uses of Watsonville Sloughs may be adversely affected by sediment is not well documented. Some studies have made indications as to the nature of the sedimentation problem. A study conducted by Questa Engineering Corporation (1995) concluded that sedimentation in the sloughs was a major water quality problem because deposited sediments " obstruct and alter drainage patterns, reduce water clarity, blanket vegetation and aquatic organisms, and transport attached nutrients and pesticides into the receiving waters." The study also concluded that agricultural lands were the primary source, based on erosion estimates determined by the Universal Soil Loss Equation, although some erosion also occurred as a result of urban and rural development. However, there were no data collected to determine actual sedimentation rates or the direct effect on beneficial uses.

The Watershed Institute at California State University, Monterey Bay is contracted by the Central Coast Regional Water Quality Control Board (CCRWQCB) to provide technical assistance in the development of a TMDL for sediment and pathogens in the Watsonville Slough system, including monitoring, a problem statement and a preliminary source analysis. Table 1-1 is a timetable outlining the proposed completion dates for project tasks. The specific objectives of this project are as follows:

- Review in report form, previous studies and existing data on the hydrology, geometry, and water quality of Watsonville Slough

- Collect, analyze, and present in report form, field data on the hydrology, geometry, and water quality of Watsonville Slough
- Produce in report form, a problem statement for pathogens and sediment in Watsonville Slough, suitable for inclusion in a Technical TMDL document
- Produce in report form, a preliminary source analysis for pathogens and sediment in Watsonville Slough, suitable for inclusion in a Technical TMDL document

1.2 Project Description

The Watsonville Slough system TMDL project will include the following data collection, analysis, and reporting:

- A review and description of the study area, previous studies relating to water quality, and an inventory of existing data including: pathogen and sediment data, spatial data, channel cross-sections, and hydrologic rating curves and level data.
- A review of water quality standards relevant to pathogens and sediment in the study area.
- Develop in collaboration with and subject to the approval of CCRWQCB staff, a Field Sampling Plan and Quality Assurance Plan that, within the budget of the Contract, will result in field collection and laboratory analysis of sufficient additional hydrologic, geometric, and water quality data to allow the development of a problem statement and preliminary source analysis of pathogens and sediment in Watsonville Slough. The Plan shall be submitted to, and approved by CCRWQCB prior to commencement of field data collection.
- Ambient (non-winter) and storm-based (winter) field sampling of additional hydrologic, water quality, and channel geometry data in the Watsonville Sloughs ($\geq$ 5 sites).
- Statistical analysis of all data and comparison with water quality standards.
- A statement of the extent, importance, and severity of pathogen and sediment impairment of Watsonville Slough.
- Review of regional data on land-use-specific loadings of pathogens and sediment.

- Analysis of new and existing water quality data from multiple sites in the Watsonville Slough watershed in order constrain the estimated location of sources of pathogens and sediment.
- A statement of the most likely sources of pathogens and sediment that impair the beneficial uses of Watsonville Slough, and suggestion of the mechanisms of production, transport, storage, growth, and export of pathogens and sediment in the Slough.

The final products of this project will include:

1) Final report containing: review of the study area, review of previous studies, inventory of existing data, summary of all new data collected, analysis of hydrologic and water quality data, problem statement, and preliminary source analysis.
2) Electronic water quality database containing all previously existing water quality data reviewed as part of this project and all new data collected as part of this project.

1.3 Development of Project Plan

This Quality Assurance and Field Sampling Plan details the approach that will be taken to achieve the primary objectives of this project: development of a problem statement and completion of a preliminary source analysis for sediment and pathogens in the Watsonville Slough system.

The following documents were reviewed and used as a template for the following Quality Assurance Project Plan:

- *The Volunteer Monitor's Guide to Quality Assurance Project Plans*
  USEPA (September, 1996)
- *The Clean Water Team Model Quality Assurance Project Plan*
  SWRCB (January, 2002)
  http://www.swrcb.ca.gov/nps/docs/model_qapp32701.doc
- *Quality Assurance Project Plan for Volunteer Monitoring of Suspended Sediment Concentration and Turbidity Sonoma Creek Watershed, Sonoma County California*
  Sonoma Ecology Center (December, 2001)

The following QAP and field sampling plan outlines an adaptive strategy. The plan is dependent on numerous factors such as rainfall, budget constraints, and data results. Therefore, the following procedures and plan outlined in this document may be improved at any time.

Table 1-1. Project Timetable

Task	Description	Proposed Completion Date
1.	Project Management and Administration	
	1.1 Administration	on-going
	1.2 Progress Reports (every four months)	02/15/03, then every four months
	1.3 Project Survey Form (final)	02/01/04
2.	Data Collection and Analysis	
	2.1 Review and describe study area, previous studies	02/15/03
	2.2 Review water quality standards	12/01/02
	2.3 Field Sampling Plan and QAPP	12/01/02
	2.4 Ambient and storm-based field sampling	10/01/03
	2.5 Statistical analysis	12/01/03
	2.6 Problem statement	12/01/03
	2.7 Review loadings from regional data	12/01/03
	2.8 Analysis of data to estimate sources	12/01/03
	2.9 Preliminary source identification	12/01/03
3.	Electronic Water Quality Database	01/01/04
4.	Final Report	02/01/04

2 Study Area

The Watsonville Slough system is located in Santa Cruz County and is comprised of Harkins, Gallighan, Hanson, Struve, and Watsonville Sloughs (Fig. 2-1). Watsonville Slough is listed on the California 303d list as being impaired due to pathogens and sediment. Although the tributary sloughs are not currently listed, this study will investigate entire system including the four tributaries.

The system drains an area of approximately 50 km^2 (13,000 acres) (Fig. 2-1). The system originates in the foothills of the Santa Cruz Mountains and surrounding coastal hills and drains into the Pajaro Lagoon and finally to the Pacific Ocean. The upper reaches are more stream-like, whereas the lower areas are low gradient and sluggish. The lowest reach of the Watsonville Slough, near the confluence with the Pajaro Lagoon, is tidally influenced.

The system has been historically modified to meet the needs of adjacent land uses such as agriculture and urban development. For instance, some areas of the slough system have been channelized to drain surface water. Several pump stations were also installed to prevent tidal influences upstream and manage floodwaters. The two pump stations are located at Shell Road and at the confluence of Harkins Slough. Culverts were also installed at the major road crossings to prevent flooding. Additionally, there has been a history of land subsidence, which is most likely the result of shallow groundwater pumping and the decomposition of underlying peat (Swanson Hydrology and Geomorphology 2002). The primary land uses are row crop agriculture, grazing, residential, urban, and commercial and are illustrated in Fig. 2-1.

A more detailed review of the study area including a detailed description of monitoring locations will be included in future reports.

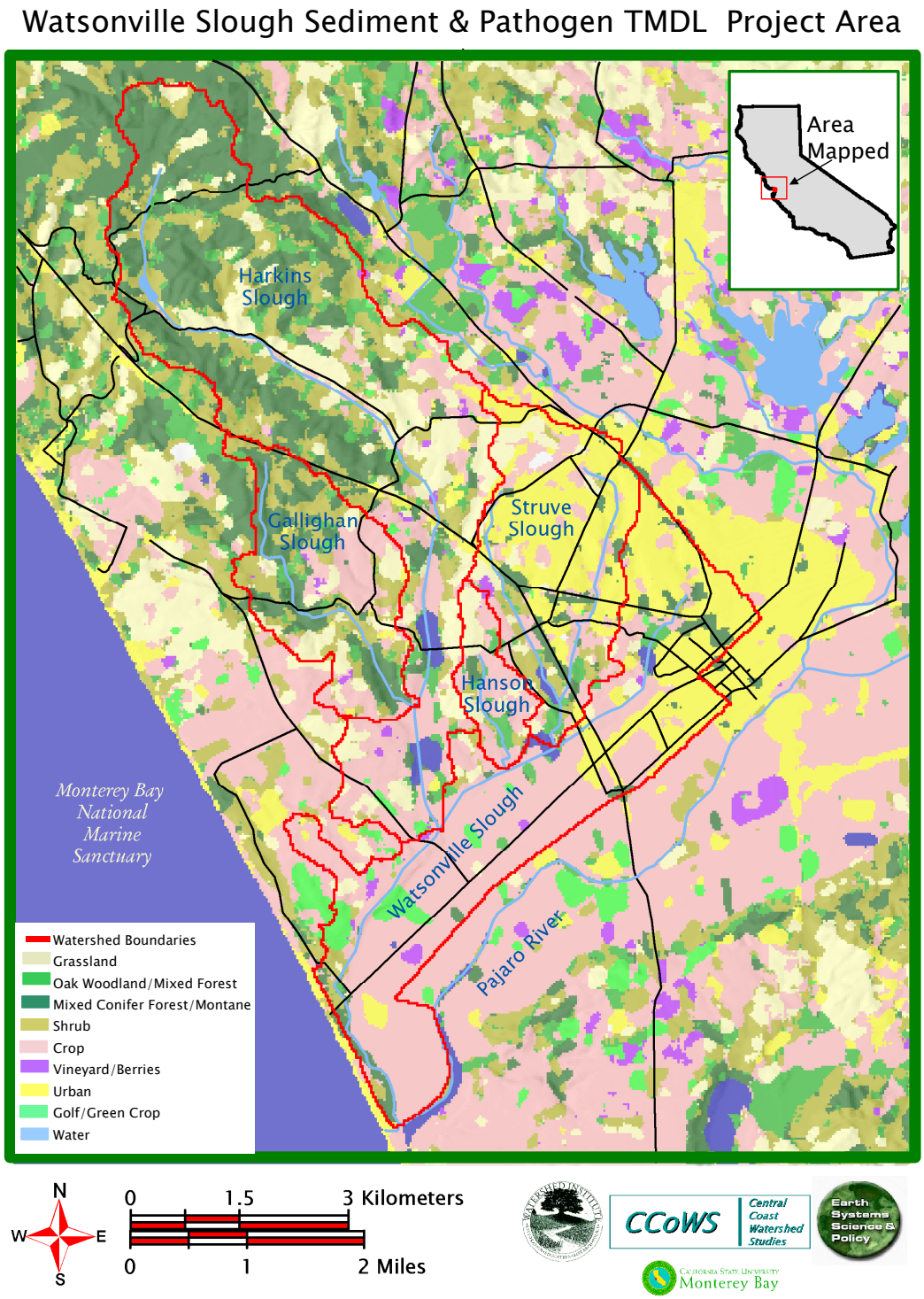

Figure 2-1. Map showing the Watsonville Sloughs project area and watershed boundaries.

3 Review of Water Quality Standards

The main water quality standards that apply to sediment and pathogen levels for Watsonville Sloughs are outlined in the Basin Plan for the California Regional Water Quality Control Board Central Coast Region (1994). This plan, as mandated by the California Porter-Cologne Water Quality Control Act (1969), outlines "water quality objectives" that apply to Watsonville Slough.

The Basin Plan (1994) states that:

> Suspended Material: Waters shall not contain suspended material in concentrations that cause nuisance or adversely affect beneficial uses.
>
> Settleable Material: Waters shall not contain settleable material in concentrations that result in deposition of material that causes nuisance or adversely affects beneficial uses.
>
> Sediment: The suspended sediment load and suspended sediment discharge rate of surface waters shall not be altered in such a manner as to cause nuisance or adversely affect beneficial uses.
>
> Turbidity: Water shall be free of changes in turbidity that cause nuisance or adversely affect beneficial uses.
>
> > Increase in turbidity attributable to controllable water quality factors shall not exceed the following limits:
> >
> > 1. Where natural turbidity is between 0 and 50 Jackson Turbidity Units (JTU), increases shall not exceed 20 percent.
> >
> > 2. Where natural turbidity is between 50 and 100 JTU, increases shall not exceed 10 JTU.
> >
> > 3. Where natural turbidity is greater than 100 JTU, increases shall not exceed 10 percent.

Allowable zones of dilution within which higher concentrations will be tolerated will be defined for each discharge in discharge permits.

Bacteria (REC-1)*: Fecal coliform concentration, based on a minimum of not less than five samples for any 30-day period, shall not exceed a log mean of 200/100 mL, nor shall more than ten percent of total samples during any 30-day period exceed 400/100 mL.

Bacteria (REC-2)*: Fecal coliform concentration, based on a minimum of not less than five samples for any 30-day period, shall not exceed a log mean of 2000/100 mL, nor shall more than ten percent of samples collected during any 30-day period exceed 4000/100 mL.

Bacteria (SHELL)*: At all areas where shellfish may be harvested for human consumption, the median total coliform concentration throughout the water column for any 30-day period shall not exceed 70/100 mL, nor shall more than ten percent of the samples collected during any 30-day period exceed 230/100 mL for a five-tube decimal dilution test or 330/100 mL when a three-tube decimal dilution test is used.

*Numeric standards were developed using the Multiple Tube Fermentation technique.

An additional review of water quality standards was conducted in search of numeric standards for this region. However, only one project, which provides numeric criteria, was found. Future reviews of water quality standards may include searching for comparable standards from different regions.

- USEPA: *Ambient Water Quality Criteria Recommendations for Rivers and Streams in Nutrient Ecoregion III* (December 2000)

  This plan divides the United States into various Ecoregions. The Watsonville Sloughs are in Ecoregion III (Xeric West). Each Ecoregion is then divided into various levels. For instance,

Ecoregions III has 12 levels. Watsonville Sloughs are level 6 (Southern and Central California Chaparral and Oak Woodlands).

- Aggregate Nutrient Ecoregion III Reference Conditions:
  (based on 25th %)
  - Turbidity – 1.84 NTU, 2.34 FTU

- Range of level III Subecoregions reference conditions:
  (based on 25th%)
  - Turbidity – 1.93 to 5.13 FTU

- Reference conditions for level III ecoregion 6 streams:
  (based on 25th percentiles)
  - Turbidity – 1.9 NTU, 2.65 FTU

For this project, turbidity will be measured a using a turbidimeter and the resulting unit will be NTU (Nephelometric Turbidity Unit). The previous standards list several different units for turbidity, which include NTU, JTU (Jackson Turbidity Unit), and FTU (Formazin Turbidity Unit). Historically, JTU was the common unit for turbidity measurements using the Jackson candle turbidimeter. This visual method has been removed from the Standard Methods manual (APHA, 1998) and has since been replaced by methods that utilize instruments such as nephelometers. Turbidity measurements made with nephelometers result in either NTU or FTU. FTU is a unit used when formazin is the primary reference standard. FTU and NTU will be approximately equal when measuring a formazin standard, but may vary when measuring an environmental sample (Hach, 2002).

4 Beneficial Uses

4.1 Specific beneficial uses of Watsonville Sloughs

The Watsonville Sloughs area is recognized as the largest wetland complex between Pescadero Marsh and Elkhorn Slough. The sloughs are home to diverse plant ecosystems, with unique plants that provide nesting sites and habitat for a variety of migratory and wetland birds, many of which are threatened, endangered, or California species of concern (Busch 2000; Swanson 2002). These birds depend on a healthy functioning aquatic ecosystem free from excessive pollutants. Wetland birds depend on abundant fish and macroinvertebrates for survival. Similarly higher organisms such as falcons and hawks depend on the wetland birds for survival. Humans also enjoy this wetland area for pastimes such as fishing, nature walks, and bird watching. Struve Slough and Harkins Slough, which has an extensive deepwater section, are especially popular areas for this.

The general beneficial uses that apply to Watsonville Slough and its tributary sloughs are outlined in Basin Plan for the Central Coast Region (1994) and are presented in Table 4-1. More detailed inventories of the flora and fauna of Watsonville Sloughs have recently been compiled by J. Busch (2000) and by Swanson Hydrology and Geomorphology (2002) containing supplementary work by the Biotic Resources Group, Dana Bland and Associates, and Hagar Environmental Sciences.

Table 4-1. Beneficial uses that apply to Watsonville Sloughs (Basin Plan 1994)

REC-1	Water contact recreation
REC-2	Non-contact water recreation
WILD	Wildlife habitat
WARM	Warm fresh water habitat
SPWN	Spawning, reproduction, and/or early development
BIOL	Preservation of biological habitat of special significance
RARE	Rare, threatened, or endangered species
EST	Estuarine habitat
COMM	Commercial and sport fishing
SHELL	Shellfish harvesting

4.2 Potential impacts to beneficial uses

4.2.1 Pathogens

The presence of pathogens in water bodies has been demonstrated to pose significant health risks to humans (EPA 2001). The beneficial uses most likely to be directly affected by pathogens and for which numeric water quality objectives have been established (Section 3) are SHELL, REC-1, and REC-2.

4.2.2 Sediment

Unlike pathogens, sediment impacts to beneficial uses are not as straightforward. No studies have investigated the direct impacts that concentrations of sediment and/or accumulation rates can have on the specific beneficial uses for Watsonville Sloughs. Furthermore, as previously discussed in Section 2, the primary CCRWQCB water quality standards that pertain to sediment are narrative stating that sediment "shall not cause nuisance or adversely affect beneficial uses" (Basin Plan 1994). The precise nature of sediment impairment to Watsonville Sloughs is not well understood. It is presumed that sediment can transport attached pesticides and nutrients, which may affect aquatic organisms. Studies, such as Hunt et al. (1998) conducted in the Watsonville Sloughs Watershed, have demonstrated that elevated pesticide levels can lead to toxicity in macroinvertebrates. Swanson (2002) observed that impairments of water quality factors such as dissolved oxygen may be the result of excessive nutrient loading and could have led to a fish kill that was observed in Watsonville Slough in January 2001. However, impacts such as these are not directly caused by sediment and therefore must be addressed in other TMDLs.

Several studies have shown that sediment can directly and adversely affect aquatic organisms in several ways. For instance, Newcombe and MacDdonald (1991) summarized that suspended sediment can impact salmonids by causing mortality, reducing growth rate, reducing resistance to disease, limiting egg and larvae development, disrupting movement and migration, reducing food availability, and disrupting feeding. The study also reported that suspended sediment can affect benthic invertebrates, which feed on periphyton, by disrupting algal growth and secondary production as light penetration is reduced; and that suspended sediment can also affect filter feeding benthic invertebrates by clogging feeding structures, reducing feeding efficiency, and

reducing growth rates, which could therefore lead to stress and possible mortality. Appendix Q and Appendix R contain the results of a literature review pertaining to the effects that sediment concentrations and turbidity levels can have on several fish and invertebrate species. It should be noted that these studies were conducted in different areas, and the numbers may not be directly applicable to Watsonville Sloughs. The listed concentrations and responses are not intended for use as a reference to exact concentrations that may affect fish in Wastsonville Sloughs, but more so to gain an understanding of the general range that can be expected to have adverse affect on various organisms. Many factors can influence the degree of sediment impact such as sediment composition and size, species adaptation to a given area, and simultaneous presence of different stressors.

The only known study to date that has surveyed fish species in Watsonville Sloughs was conducted by Hagar Environmental Sciences as part of a conservation plan for Watsonville Slough by Swanson (2002). Hagar found Sacramento blackfish (native), threespine stickleback (native), carp (non-native), mosquitofish (non-native), and black crappie (non-native) in a deepwater section of Harkins Slough. Visual observations of mosquitofish (non-native), threespine stickleback (native), and prickly sculpin (native) were made in the headwaters of Harkins Slough near Larkin Valley. Threespine stickleback (native) were also found in Struve Slough. Other native species that could be expected in Watsonville Sloughs but that have not been observed include: California roach, Sacramento sucker, pikeminnow, and possibly hitch (Swanson 2002).

Historically, steelhead and coho may have occupied the upper reaches Watsonville Sloughs, but no longer do today because of a barrier located at the Shell Road pump station. Federally endangered Tidewater goby and federally threatened steelhead have been observed in the lower estuarine reaches below Beach Road (Smith, 1993 as cited in Swanson, 2002).

As illustrated in Appendix Q, many of the studies that have examined the effects of sediment on fish have been conducted on salmonids. Since the primary water quality standards for sediment are narrative and steelhead are known to utilize the lower reach of Watsonville Slough, the numbers presented in Appendix Q could potentially be used as a guideline for suspended sediment concentrations until a specific study on beneficial uses is conducted for

Watsonville Sloughs. The various studies, with differing parameters such as duration time and sediment composition, showed a wide range of sediment concentrations and related impacts to steelhead. In summary, sediment concentrations ranging from 16.5 to 110 mg/L resulted in altered behavior such as reduced feeding, and concentrations from 500 to 2,000 mg/L revealed signs of stress. Turbidity ranges from 22 to 265 NTU resulted in displacement and avoidance behavior. A range of concentrations from 50 to 17,500 mg/L resulted in internal changes and physical damage such as gill tissue damage and increased rates of ventilation and coughing. Concentrations ranging from approximately 70 to 500 mg/L resulted in some mortality or a slight decrease in survival rates, whereas sediment concentrations ranging from 1,000 to 160,000 mg/L often resulted in significant population reductions. For all studies, duration times and sediment composition (i.e. grain size, kaolin clay, diatomaceous earth), temperature, and other factors may have had a significant impact on fish response.

A similar range of sediment concentration is given in a fisheries handbook developed by the U.S. Army Corps of Engineers, which states that streams with concentration ranging from 80 to 4,000 mg/L are not expected to support healthy fisheries (Bell, 1986).

Another important way that sediment can impact fish species is by altering spawning habitat. Many native fish of this region have specific substrate requirements for spawning. High rates of sediment accumulation, especially fines in spawning areas, may detrimentally impact fish reproduction. Table 4-2 summarizes specific spawning requirements for native fish that have been observed or are expected to occupy Watsonville Sloughs.

Furthermore, native fish, many wetland birds, and amphibians such as the federally threatened California red-legged frog rely heavily on aquatic insects for food. Appendix R summarizes the results of several studies that have examined sediment impacts to various aquatic invertebrates. The results show that ranges of sediment concentrations similar to those found for fish can also have lethal effects on invertebrates which may in turn have an indirect adverse affect on higher organisms. Table 4-3 lists several families of aquatic invertebrates likely to be found in central California that were found to be intolerant to disturbances such as sedimentation (Harrington and Born, 2000).

Table 4-2. Specific spawning requirements for selected native fish

Native Fish Species	Preferred Spawning Substrate	Preferred Spawning Location
Threespine stickleback*	sand and small pebbles with twigs and debris nearby	among beds of aquatic plants in estuaries and adjacent coastal streams, bays, and sloughs
Prickly sculpin*	large cobbles or flat rocks; artificial substrates such as concrete blocks and jetty crevices	flowing water with loose rocks
Speckled Dace**	gravel	shallow water; gravel edges of riffles
California roach**	30 to 50 mm	shallow flowing areas
Hitch**	clean fine to medium gravel	riffles of tributary streams; reservoirs and ponds
Sacramento sucker**	Sand, gravel, and cobble	tributary streams mostly in gravel riffles
Pikeminnow**	rocks and gravel	Gravel riffle streams and small foothill streams
Sacramento blackfish*	beds of aquatic vegetation and/or rocks	open, shallow water such as in sloughs, ponds, and reservoirs
Tidewater goby***	sand burrows or ditches with gravel, sand, or clay mud bottom	shallow weedy areas along coastal streams and lagoons; ditches
Steelhead***	Coarse gravel	Cool water streams in tail of pool or riffles

Sources: Moyle, 2002; Wang, 1983

* observed by Hagar, 2001.

**not observed, but could potentially exist

***observed in lower reaches of Watsonville Slough by Smith, 1993.

Table 4-3. Aquatic invertebrate species found to be intolerant to disturbances such as sedimentation

Order	Family
Diptera (aquatic flies)	Athericidae
	Blephariceridae
	Deuterophlebiidae
	Dixidae
Megaloptera (hellgrammites and alderflies)	Corydalidae
Trichoptera (caddisflies)	Calamoceratidae
	Goeridae
	Lepidostomatidae
	Odontoceridae
	Rhyacophilidae
	Uenoidae
Ephemeroptera (mayflies)	Ameletidae
	Ephemerellidae
	Isonychiidae
	Leptophlebiidae
Plecoptera (stoneflies)	Capniidae
	Chloroperlidae
	Leuctridae
	Peltoperlidae
	Perlidae
	Pteronarcyidae
Coleoptera (aquatic beetles)	Amphizoidae

Source: Harrington and Born, 2000.

5 Field Sampling Plan

The sampling plan for sediment works toward an answer to the following question:

1. Does sediment adversely impact the beneficial uses of the Watsonville Sloughs?

Many beneficial uses are listed for Watsonville Sloughs (REC1, REC2, WILD, WARM, SPWN, BIOL, RARE, EST, COMM, SHELL). However, based on previous studies, it is most efficient to base the sediment sampling plan around a more focused group of beneficial uses: SPWN, RARE, EST, and WARM. This results in specific sampling questions:

> 1a. Do sediment **concentrations** reach levels that may be high enough to adversely impact SPWN, RARE, EST, and/or WARM, given the current understanding of sediment impacts on these beneficial uses (see Section 4)?
>
> 1b. Do sediment loads cause benthic **accumulation** of sediment that may lead to an adverse impact on SPWN, RARE, EST, and/or WARM (given current understanding of sediment impacts on benthic habitat)?
>
> 1c. If yes to either 1a or 1b, what are possible sources?

The sampling plan for pathogens is driven by the following question:

2. Are pathogens in exceedance of Basin Plan standards (CCRWQCB 1994)?

> 2a. If so, what are the sources?

Given these questions, the proposed sampling plan is based around a series measurements of sediment and pathogen concentrations during storm-event and ambient conditions; and surveys of sediment accumulation at key sites of potential benthic habitat or sites where total volume of habitat may be threatened by sediment accumulation. Table 5-1 lists the potential sites throughout the Watsonville Sloughs that will be monitored for this project. Section 7.1 outlines the naming scheme, which allows for easy incorporation of

other sites from different agencies, such as the Regional Board and the United States Geological Survey, into the CCoWS database.

Table 5-1. Potential Monitoring Sites

CCoWS Site Code	Site Description
WAT-PAJ	Watsonville Slough mouth at Pajaro Dunes Colony
WAT-SHE	Watsonville Slough at Shell Road pump station
WAT-AND	Watsonville Slough at San Andreas Road bridge
WAT-LEE	Watsonville Slough at Lee Road bridge
WAT-HAR	Watsonville Slough at Harkins Slough Road crossing
HAR-CON	Harkins Slough at confluence with Watsonville Slough (pump station)
HAR-HAR	Harkins Slough at Harkins Slough Road crossing
HAR-RAU	Harkins Slough upstream of Ranport Road crossing
GAL-BUE	Gallighan Slough at Buena Vista Road (near landfill exit)
HAN-HAR	Hanson Slough at Harkins Slough Road crossing
STR-LEE	Struve Slough at Lee Road crossing
STR-HAR	Struve Slough at Harkins Slough Road crossing
STR-CHE	Struve Slough at Cherry Blossom Drive

Watsonville Slough Sediment & Pathogen TMDL

Project Area

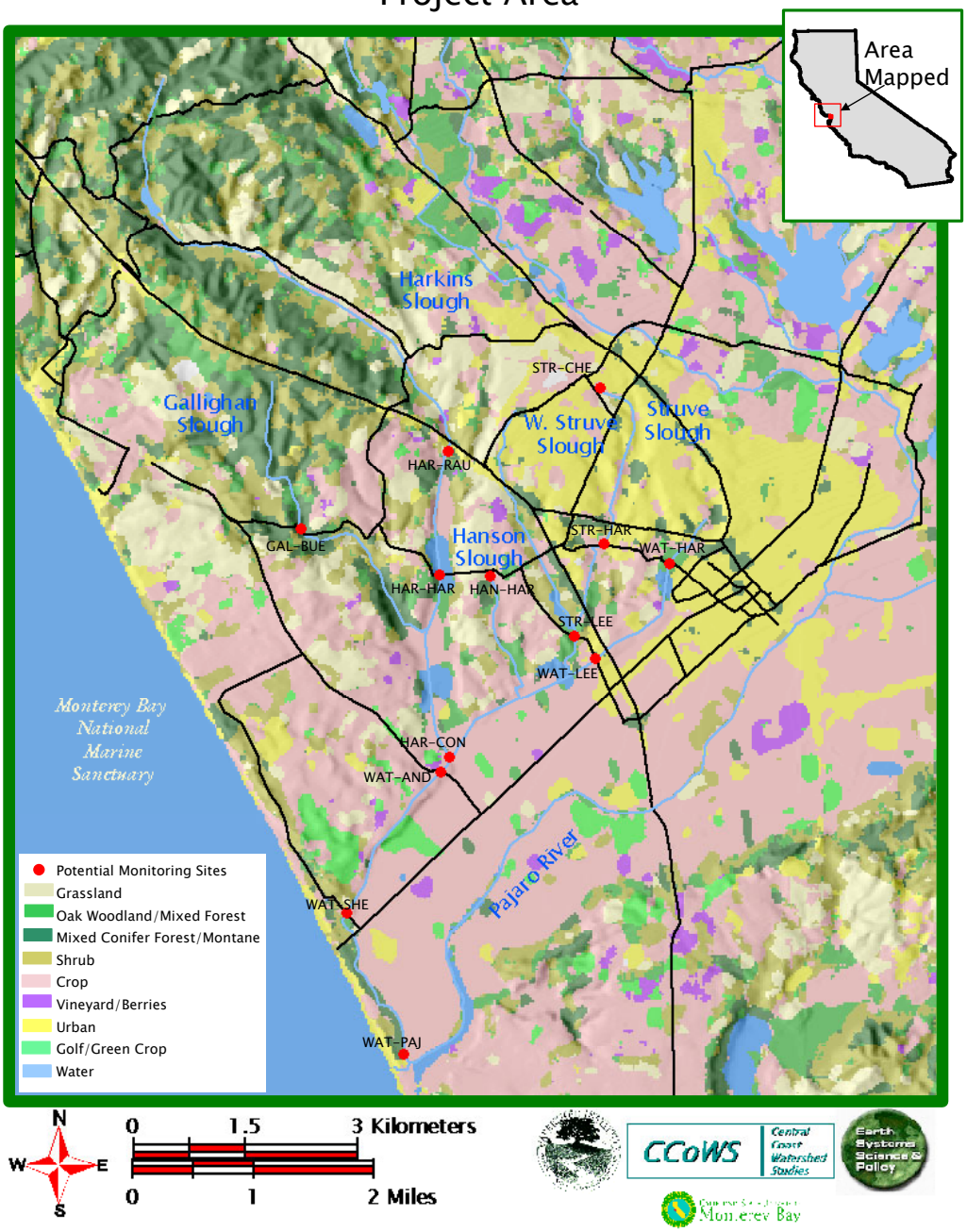

Figure 5-1. Map showing Watsonville Slough project area and potential monitoring sites.

5.1 Pathogens

The basis for the California 303d listing of Watsonville Slough for pathogens is not well documented and the extent of the impairment is unknown. The only consistent pathogen monitoring conducted in the Watsonville Slough system has been sampling for fecal coliform, an indicator of fecal contamination, by the Santa Cruz County Environmental Health Department (1977 to 2000). There have been several instances in which values detected by the Santa Cruz Environmental Health Department were in exceedance of the CCRWQCB's Basin Plan (1994) standard of 400 MPN/100mL for water contact recreation (Fig. 5-2). However, consecutive monitoring in a 30-day period is needed in order to confirm that Watsonville Sloughs are in exceedance of the Basin Plan standard for fecal coliform.

The approach for investigating pathogens in the Watsonville Sloughs watershed will be to sample for the indicator bacteria fecal coliform and *Escherichia coli.* The first stage of the monitoring plan will be to investigate current fecal bacteria levels and to determine if there is a potential pathogen problem in the Watsonville Slough system. This will involve 2 monitoring campaigns at 13 sites throughout the watershed for fecal coliform, *E. coli*, and possibly *Enterococcus* depending on budget constraints. The first monitoring campaign will take place during the rainy season (mid-February to mid-March) and will likely involve storm-event monitoring. The second monitoring campaign will occur during the dry season (July or August) and will involve monitoring during ambient conditions. Each monitoring campaign will consist of 5 synoptic sampling runs within a 30-day period.

The protocols for sample collection and analysis of pathogens are detailed in Sections 7 and 8. If the Watsonville Slough system is found to be in exceedance of the Basin Plan for fecal coliform and pathogenic contamination poses a risk to the beneficial uses, the next phase of the study will be to conduct a preliminary source analysis.

The second stage of the monitoring plan, a preliminary source analysis, will involve conducting genetic analysis of samples from 1 to 2 sites. A sampling run will be conducted in the late spring (May or early June) immediately following the rainy season and an additional sampling run will be conducted during the dry season (July). Ideally, the first sampling run would be collected

during the rainy season. However, the genetic analysis must follow the first stage of monitoring for pathogens, during which hot spots are identified and it is determined whether or not Watsonville Sloughs are in exceedance of the Basin Plan objectives for coliform. Rainy season genetic analysis is therefore not possible due to the constraints of the contract schedule, which requires that all monitoring be completed by October 2003.

In order to determine the genetic origin of *E. coli*, the chosen indicator organism for pathogen presence, approximately 12 to 20 samples will be analyzed by the laboratory group led by Dr. Betty Olson at the Department of Environmental Analysis and Design at the University of California, Irvine using the Toxin Gene Biomarker method. This method was selected following a detailed review of various methods for genetic source tracking. The following key documents were reviewed:

- *Identifying the Sources of Escherichia coli Contamination to the Shellfish Growing Areas of the Morro Bay Estuary*
  Kitts, C. et al., 2002
- *Little Soos Creek Microbial Source Tracking*
  Samadpour, M. et al., 1995
- *Detecting Bacteria in Coastal Waters*
  Hager, M.C., 2003
- *Comparison of DNA Fingerprinting Methods of E. coli, Genotyping Male Specific Phage Serotypes, and the Use of Toxin Genes as Biomarkers to Differentiate Human and Animal Waste*
  Olson, B. et al., 2001
- *A biomarker for the identification of cattle fecal pollution in water using the LTIIa toxin gene from enterotoxigenic Escherichia coli*
  Khatib, L.A. et al., 2002

The review of these documents revealed that while the two primary methods, genetic ribotyping and the Toxin Gene Biomarker method, are capable of detecting potential sources of *E. coli*, both methods have some limitations.

5.1.1 Genetic Ribotyping

The major limitation associated with the genetic ribotyping method is that only a small sample of the total population is tested when working on a limited budget. Success of source identification is also dependent on the size of the genetic library. If a certain ribotype for a given isolate, which varies geographically, is not present in the library, the source will be unidentifiable.

5.1.2 Toxin Gene Biomarker Method

The Toxin Gene Biomarker method screens a larger proportion of the population of the sample and is geographically stable. However, only a limited number of toxin genes have been identified for various animals, which include: human, cow, bird, rabbit, and dog. Sources other than these are not identified using this method. The Toxin Gene Biomarker method results in a presence/absence reading for each of the host specific biomarkers for each *E. coli* screened. The results are then used to determine the frequency of *E. coli* in the sample that carry the trait and the frequency of *E. coli* that do not. The Toxin Gene Biomarker method was selected for Watsonville Slough preliminary source analysis, as it was most aligned with the scope and budget of this project.

Santa Cruz County Environmental Health
Fecal Coliform Data (CFU/100mL)

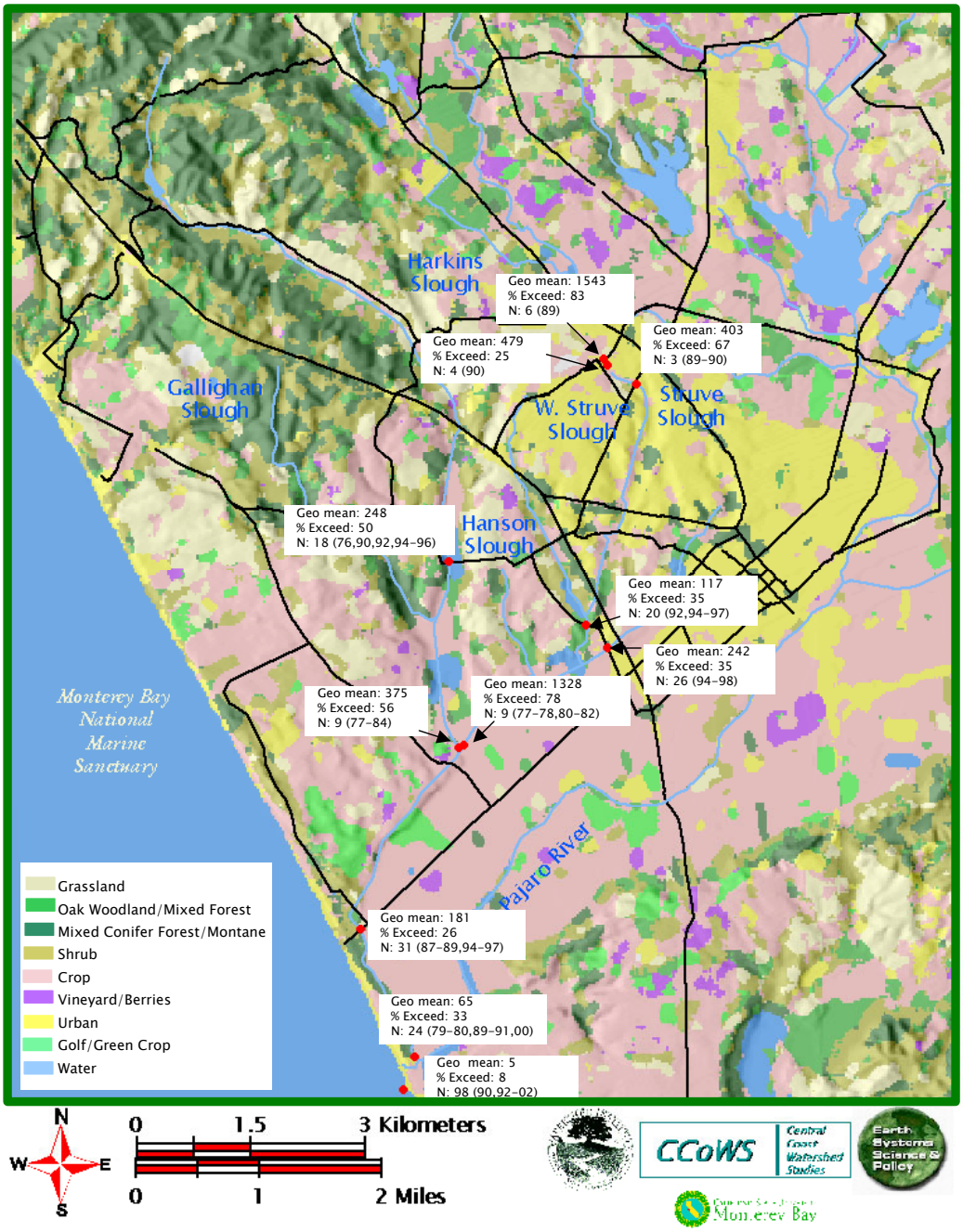

Figure 5-2. Santa Cruz County Environmental Health fecal coliform data map showing geometric mean and % exceedence of Region 3 Basin Plan recreation standard (400 MPN/100 mL).

5.2 Sediment

The California 303d listing of Watsonville Slough for sediment was based on a study by Applied Science and Engineering Inc. (1999), "The Pajaro River Watershed Water Quality Management Plan." No monitoring in Watsonville Sloughs was conducted for this study, but there was a review of limited previous sediment data collected by the Pajaro Valley Water Management Agency, City of Watsonville, County of Santa Cruz, and Questa Engineering Corporation (1995). Previous studies have described Watsonville Sloughs as a low gradient, sluggish system, with land subsidence occurring in many places, and indicate that sedimentation may be a problem.

The sediment monitoring plan will involve 2 approaches:

1) Suspended sediment concentration & turbidity level sampling
2) Investigation of sediment accumulation

The two sampling approaches are intended to answer the specific questions, 1a and 1b, previously outlined in Section 5. The first method for investigating sediment will involve synoptic monitoring to investigate current suspended sediment concentrations and turbidity levels. This will coincide with the exceedance monitoring for pathogens and will involve 2 monitoring campaigns at 13 sites throughout the watershed. The first monitoring campaign will take place during the rainy season (mid-February to mid-March) and will likely involve storm-event sampling. The second monitoring campaign will occur during the dry season (July or August) and will involve sampling during ambient conditions. Each monitoring campaign will consist of 5 synoptic sampling runs within a 30-day period.

Samples will be collected and analyzed by CCoWS for suspended sediment concentration (SSC) and turbidity. The protocols for sample collection and analysis are detailed in Sections 7 and 8. When possible discharge measurements will also be taken, but this is only likely to occur at sites in the upper watershed during storm events. The extent of possible impairment will be measured by the number of samples whose suspended sediment concentrations are higher than the concentration ranges presented in Section 4,

which were shown to have a negative impact on steelhead/rainbow trout, which occupy the lower reach of Watsonville Slough.

The second monitoring approach will involve conducting sediment accumulation surveys throughout the Watsonville Sloughs. The surveys will consist of field observations throughout the watershed, and photo documentation of areas with visible sediment accumulation or supply. The surveys will primarily be conducted during storm events, but will also coincide with the scheduled sampling runs for the pathogen exceedance monitoring.

The survey will involve wading at specific sites and visual channel inspections for features and conditions such as:

- recent (unvegetated) sediment accumulation
- gully, sheet, or rill erosion
- channel instability and/or visible bank erosion
- sediment plumes
- obvious sources of sediment supply such as ditches, pipes, and drains
- changes in bed level at staff plate locations
- depositional features such as islands or bars
- changes in substrate
- overbank sediment deposits
- sediment accumulation in culverts or on road crossings
- buried aquatic vegetation

It is possible that based on the initial monitoring, a delisting for sediment may be recommended.

5.3 Data Quality Objectives

The primary objective of this project is to collect valid quantitative information on the extent, severity, and origins of pathogens and sediment in Watsonville Sloughs. Water quality samples will be collected and analyzed to determine concentrations of various water quality constituents and sources for both pathogens and sediment. The data will then be used to develop a problem statement and preliminary source analysis suitable for inclusion in a technical TMDL document. In order to ensure that the data used to develop the problem

statement and source analysis are accurate and reliable, appropriate methods will be used and all data will satisfy the quality objectives outlined in Table 5-2.

Data quality objectives express the desired level of data quality for each measurement parameter (e.g. turbidity, temperature, …) in the following areas:

- Precision
- Accuracy
- Measurement Range
- Completeness
- Representativeness
- Comparability

5.3.1 Precision, Accuracy, and Range

Precision is defined by the EPA (1996) as "the degree of agreement among repeated measurements of the same characteristic, or parameter, and gives information about the consistency [of] methods". Accuracy is defined as a "measure of confidence that describes how close a measurement is to its 'true' value" (EPA, 1996).

Table 5-2 lists the precision, accuracy, and measurement range desired for each measured parameter. Where available, the table lists known or previous precision and accuracy for each instrument or method. Then, the data quality objective (DQO) that satisfies the aims of the present study is listed below the known or previous values. This DQO value is usually based on the known or previous value, plus some additional margin of flexibility in order to account for additional variance that may not be quantified by the known or previous value. In cases where some degree of bias is expected (such as bias do to instrument drift since time of last calibration), the DQO for accuracy is given as a wider range (large % value) than the DQO for precision. Notes on certain items in the table requiring further explanation are given after the table.

The details for assurance and assessment of accuracy and precision are summarized in Section 9.1 to Section 9.4 of this document.

Table 5-2. Data Quality Objectives for Precision, Accuracy, and Range

Water Quality Constituent	Analysis Instrument/ Method	Units	Range	Precision	Accuracy
fecal coliform	Multiple Tube Fermentation (15 tube) SM 9221E	MPN/100 mL	Low: 2 -1,600 MPN/100 mL High: 20-16,000 MPN/100 mL	TBD	See 95% Confidence Limits-Appendix F
	Membrane Filtration SM9222D	CFU/100 mL	To be determined once laboratory is selected	TBD	See 95% Confidence Limits- Appendix G
E. Coli	Multiple Tube Fermentation (15 tube) SM 9221E	MPN/100 mL	Low: 2 -1,600 MPN/100 mL High: 20 -16,000 MPN/100 mL	TBD	See 95% Confidence Limits-Appendix F
	Membrane Filtration- SM9222G	CFU/100 mL	To be determined once laboratory is selected	TBD	See 95% Confidence Limits-Appendix G
E. Coli source	Toxin biomarker using polymerase chain reaction	presence/ absence	Unknown	Uknown	Unknown
SSC	Vacuum filtration Comparable to ASTM D 3977	mg/L	Lower limit is 1mg/L	Analytical repeatability: ±1% for standards. Field repeatability: ~20%. **DQO**: **±25%**	±7.5% tested on 27 samples of know concentration; sample size dependent Can be biased due to poor depth integration. **DQO: ±40%**
turbidity	Hach2100P Turbidimeter USEPA 180.1, SM 2130B	NTU	0-1000 NTU 0-9.99 NTU 0-99.9 NTU	Analytical repeatability: ±1% of reading or 0.01 NTU for standards. Field repeatability: ~20%. **DQO**: **±25%**	±2% reading plus stray light; stray light < 0.02 NTU Can be biased due to dilutions, and poor depth integration. **DQO**: **±40%**
temperature	Raytek laser thermometer	° C	-18-275 ° C	Instrument precision: 0.1 deg. Variation due to observer choice of sampling location: ~1 deg. **DQO: ±5%**	Instrument accuracy: -1 to 260°: ±2% of reading, or ±2° C -18 to -1°: ±3° C. Bias due to sampling of surface and upper layers only: ~1 deg. **DQO**: **±10%**
conductivity	*Oakton TDSTestr* 20 SM 2510	uS/cm mS/cm	0-1990: uS/cm 2.0-19.9: mS/cm	Instrument resolution: 1 uS or 0.01 mS for standards Field variation due to poor mixing: ~20% **DQO**: **±25%**	Instrument accuracy: ±2 factory set Bias occurs due to poor depth integration **DQO**: **±50%**
pH	*Oakton pHTestr* 1 SM 4500B	pH units	(-1) to 15.0	Instrument is 0.1 pH for standards **DQO**: **±5%**	Instrument accuracy is ±0.2 pH **DQO**: **±10%**

5.3.2 Completeness

"Completeness is the comparison between the amount of data [planned to be collected] and the amount of useable data [that was actually collected]" (EPA, 1996). The objective for completeness in this project is 80%. Factors that could potentially prevent collection of the planned number of samples include:

- access due to flooding
- safety concerns
- staff laboratory or field errors
- rejection of invalid data

5.3.3 Representativeness

Representativeness is the extent to which measurements actually represent the true environmental condition of a waterbody (EPA, 1996). The degree of Representativeness of water quality samples is usually difficult to determine, and in some cases, can be significant. The sampling techniques used follow common practice, and thus will be equally as representative as data that are commonly reported by government monitoring agencies.

Specific parameters where representativeness may be lacking include coliforms and suspended sediment. It is thought that coliform abundance can be highly heterogeneous within an otherwise apparently homogeneous waterbody (B. Olson pers. comm.). The degree to which this problem will affect the present study will be evaluated through data from field duplicate samples. Suspended sediment varies both vertically and laterally within streams. The present study will follow common practice in using DH-48 samplers to correct for vertical heterogeneity, and immersing the DH-48 multiple times in different locations to account for lateral heterogeneity when applicable.

5.3.4 Comparability

"Comparability is the degree to which data can be compared directly to similar studies" (EPA, 1996). Field sapling methods used in this study are based on common practice in environmental science, such as is documented by the USGS reports on Techniques for Water Resources Investigations. Analytical methods used in this study are either an accepted Standard Method (APHA 1998), a

USEPA certified method (USEPA 1997), or a very similar method. An exception is methods for genetic coliform analysis, which are too new to have become standards yet, but reflect current practice. All methods used result in common data units that are comparable with data collected by other agencies and organizations.

6 Project Management

Research services will be provided by the Watershed Institute of California State University, Monterey Bay to the State Water Resources Control Board for development of the Watsonville Sloughs pathogen and sediment TMDL. The project leader at the Watershed Institute is Dr. Fred Watson, and the primary research technician for this project is Julie Hager. The project representative for the State Water Resources Control Board is Dominic Roques.

A full description of the management protocols used by CCoWS including staff structure and training, sample management, equipment management and database management is given in Watson et al. (2002). The following sections review key passages from that document and highlight the research management activities that pertain to this study.

6.1 Staff Structure and Training

The CCoWS staff structure for this study is as follows:

- Project leader: Fred Watson
- QA & field manager: Julie Hager
- Research manager: Wendi Newman
- Laboratory manager: Don Kozlowski
- Field Support: Joel Casagrande (senior technician)
  Thor Anderson (senior technician)
  Jon Detka (technician)
  Eve Elkins (technician)
  Joy Larson (technician/CSUMB student)
  Suzanne Gilmore (technician/CSUMB student)
  Jessica Wikoff (technician/CSUMB student)
  Regina Williams (technician/CSUMB student)
  Dennis Beaudoin (technician/CSUMB student)

6.1.1 Laboratory training

- The laboratory manager shall oversee laboratory analysis and staff training.
- Technicians shall be knowledgeable of all equipment and tests before analyzing samples independently. This shall include both training with the laboratory manager and/or an experienced technician as well as the study of instrument and procedure manuals. Staff training shall be documented on the *Technician Training Tracking Sheet* (Appendix B) and kept on file by CCoWS for 3 years.
- The laboratory manager shall be responsible for laboratory safety. It is their responsibility to assure that all technicians performing lab analysis have participated in a safety training session.
- Training on laboratory safety procedures is provided by the Earth Systems Science and Policy (ESSP) laboratory staff at CSU Monterey Bay and is a requirement prior to laboratory use. Documentation of lab safety training is kept on file by the ESSP laboratory staff.
- All accidents and incidents shall be reported to the lab manager and the ESSP lab director. Accidents and incidents shall be documented on the *Accident/Incident Report Form* (Appendix C).
- Students shall not undertake any potentially dangerous activity without staff supervision.
- Management and senior staff shall be responsible for the accuracy of analyses performed by students.

6.1.2 Field training

- The field management staff shall oversee field activities and staff training for field procedures.
- The field coordinator or a senior technician shall be responsible for safety in the field.
- Staff shall not undertake any field activity without prior training by the field manager or designee.
- Management and senior staff shall be responsible for the accuracy of field data collected by students.

6.2 Protocol for data management

- The primary data storage shall be on a central University server.
- The data shall be backed up on CD at least every 6 months. Backup CDs or tapes shall be stored at the Watershed Institute building in a fireproof safe for 3 years.
- A new master version of the *MS Access* database file shall be copied and renamed each time significant modifications are made.
- The data file names shall contain the last date on which they were significantly modified (in the format Name_YYMMDD_initials of user.*).
- Previous versions (with earlier dates) shall be maintained on the server as intermediate backups until they are backed up to CD (see above).
- All initial data from field books shall be entered into the appropriate database on the day following field sample collection.
- After laboratory analysis is complete, all results should be immediately entered into the database record for that particular field monitoring campaign.
- All laboratory data sheets are then kept on file for 3 years in the wet lab at the Watershed Institute.
- As a QC check, the Quality Assurance/Field manager will review the database by comparing entries to the original field books. This check is scheduled to follow each monitoring campaign.
- CCoWS shall keep all original data sheets and field books on file at the Watershed Institute for 3 years.
- Primary water quality data shall be maintained in the CCoWS *MS Access* database.
- The following exception applies:
    - Individual flow and depth measurements within stream flow cross-sections shall be maintained in *MS Excel* spreadsheets (as opposed to the total calculated discharge that results from these measurements which is maintained in the CCoWS *MS Access* database).
- The CCoWS *MS Access* database shall be a relational database, with tables for:
    - Site information (e.g. site code, Bridge/Road crossing, GPS coordinates)
    - Site visit information (e.g. Date/Time, container ID, sample type)

6.3 Reporting

Progress reports in hardcopy and electronic (PDF and CD) format will be submitted to the Regional Board Project Representative once every four months beginning on 15 Feb 03. Progress reports will describe all activities undertaken, accomplishments of milestones, any problems encountered in the performance of the work, and the results of quality control evaluations. Progress reports will also contain any required intermediate products.

A final report containing: review of the study area, review of previous studies, inventory of existing data, summary of all new data collected, analysis of hydrologic and water quality data, results of quality control evaluations, problem statement, and preliminary source analysis will also be submitted in hardcopy and electronic (PDF and CD) format to the Regional Board Project Representative. A draft report will be submitted no later than 10 Dec 03 and the final report will be submitted by 10 Feb 04. The report will also be published on the CCoWS web site.

Electronic water quality database (MS Access) containing all previously existing water quality data reviewed as part of this project and all new data collected as part of this project will be delivered to the Regional Board Project Representative by 1 Jan 04.

7 Sampling Protocols

The following sections outline general sampling protocols used by CCoWS including:

- site selection
- sample management
- field notes and data sheets
- suspended sediment and turbidity sampling
- pathogen sampling
- pH measurements
- conductivity measurements
- flow measurements
- equipment management

More detailed protocols for weather forecasting, storm event monitoring, agricultural monitoring, and necessary field equipment are detailed in Watson et al., 2002.

The sampling methods requirements are summarized in Table 7-1.

Table 7-1. Sampling Methods Requirements

Sample Type	Collection Instrument	Sample Container	Preservation Method	Maximum Holding Time
fecal coliform	grab	sterile polypropylene 125 mL bottle	ice cooler <10 °C	8 hr (MoCo) 24 hr (BioVir)
E. Coli	grab	sterile polypropylene 125 mL bottle	ice cooler <10 °C	8 hr (MoCo) 24 hr (BioVir)
SSC	DH-48	500 mL plastic DH-48 bottle	refrigeration	1 wk
turbidity	DH-48	500 mL plastic DH-48 bottle	refrigeration	1 wk
temperature	direct	direct measurement	field measurement	field measurement
conductivity	grab	*Nalgene* 1000 mL plastic beaker	field measurement	field measurement
pH	grab	*Nalgene* 1000 mL plastic beaker	field measurement	field measurement

7.1 Protocols for site selection

7.1.1 Site selection

- Sites should be established at multiple locations throughout the slough system.
- Sites should be established on major tributaries.
- Sites should be established primarily at bridges, to allow for monitoring during flood events.
- Bridges should be safe from traffic, with broad shoulders.
- Sites should be safe from nighttime social dangers.
- Sites should be accessible by public roads.
- A single vehicle should be able to visit all sites in a single day.
- Sites should allow convenient parking.
- If a site is privately owned, permission to access shall be obtained from the landowner.
- Each selected site shall be given a unique Site Code (e.g. SAL-DAV). The first three letters of the Site Code are the first three letters of the water body or stream name. The second three letters of the Site Code are the first three letters of the bridge or nearest road crossing. For instance, the Site Code SAL-DAV represents the monitoring location on the Salinas River at Davis Road bridge. A list of all CCoWS monitoring sites as well as other agency sites incorporated into the CCoWS database is included in Appendix O.

Potential monitoring sites have been selected throughout the Watsonville Sloughs Watershed and are listed in Table 5.1.

7.1.2 Site preparation

Staff plates measure river 'stage' and are the most robust, accurate record of river level available. Their permanency is vital.

- Except for existing USGS sites or sites installed by other agencies, sites shall be equipped with a set of one or more 1-meter metric metal staff plates.
- Where possible, these should be mounted on the concrete of bridge foundations. In cases where the concrete is too hard, or there is no

bridge, staff plates should be mounted on steel piles driven into the substrate.
- Note that "zero" stage does not need to be set to any particular level, such as the level of zero river discharge recorded at a particular time.

7.2 Protocol for sample management

- Sample containers shall be labeled with a unique ID before being taken into the field.
- Containers that may be used include:
  - DH-48 bottles (500mL)
  - *Nalgene* beakers (1000 mL)
  - Sterile bottles provided by external laboratories (125 mL)
- Containers shall be kept in groups, where all containers in a group have a similar ID.
- Containers shall be weighed before use. The weight shall be recorded in the CCoWS database for recurring use.
- Upon taking a sample, the container ID shall be recorded in a *Rite-in-the-Rain* field book (see Section 7.3).
- The combination of Site Code (see Section 7.1.1), Date/Time, and container ID shall serve as the unique identifier of a sample.
- Large samples may be distributed between more than one container, in which case all container IDs shall be recorded.
- Samples shall be transported directly to the CCoWS laboratory. If necessary, samples shall be kept on ice in a cooler during transport.
- Upon returning from the field, all samples shall be deposited in the CCoWS laboratory. When necessary, samples shall be refrigerated or frozen. Frozen or refrigerated samples shall be logged in and out on the *Sample Storage Management Log* (Appendix A).
- On the first office day following field sample collection, all available data for each sample shall be entered into the CCoWS MS Access database (see Section 6.2). Fields for results of laboratory analyses shall be left blank until laboratory results are obtained.
- Samples shall be analyzed prior to the holding times outlined in Table 7-1.
- If samples are to be analyzed at a laboratory other than CCoWS, samples shall be transported to the external laboratory and transferred according

to that laboratory's chain of custody procedures. An additional container ID for the external laboratory may be given to the original container.

- Samples analyzed in the CCoWS laboratory may also result in the use of additional containers and/or container IDs.
- Samples may be split in the CCoWS laboratory, such that the resulting fractions may be analyzed using either multiple techniques and/or multiple laboratories. Any fractional sample shall be transferred to new containers, with new container IDs.
- Upon completion of laboratory analysis, the analysis results shall be immediately recorded in the CCoWS *MS Access* database.
- CCoWS sample containers shall then be cleaned and prepared for future sampling.

7.3 Protocol for field notes and data sheets

- Field data collection and notes shall be organized as follows:
  Site Visit >> **Sample Run** (~5) >> **Monitoring Campaign** (2)
- A record of each visit shall be made in a numbered *Rite-in-the-Rain* field book.
- The record for each visit shall included the following information:
  - Name of field trip leader
  - Name/s of field party
  - Date of visit, with month written in letters (e.g. 2 Apr 02)
  - Time of visit, using 24 hr time **and** AM/PM notation (to reduce possibility of ambiguity)
  - Site code
  - Site observations and notes
  - Present weather conditions
- For stream visits, the following information shall also be recorded:
  - Presence/absence of water
  - Presence/absence of flow
  - Stage (where a staff plate is installed)
  - Type of sample collected
  - Collection or measurement time
  - Instrument type and ID, if applicable
  - Container ID (Section 5.2)
  - Method of collection (e.g. "grab" or "DH-48")

A sample field book entry is presented in Appendix N.

7.4 Protocol for sampling suspended sediment and turbidity

This section describes field-monitoring protocol for collecting suspended sediment and turbidity samples. Depending on a number of factors such as stream conditions, safety, and timing of rainfall, the method for collecting a suspended sediment sample in a stream may vary.

Depending on the magnitude of stream flow, the concentration of suspended sediment can range from well mixed to being vertically and horizontally stratified. To ensure that an accurate representation of the water column is collected, a DH-48 integrated suspended sediment sampler shall be used. When using a DH-48 sampler, a vertically integrated sample should be taken from several evenly spaced stations along a transect. At each collection station, the instrument shall be inserted, with the intake nozzle facing upstream, vertically downward through the water column and then back to the surface in a uniform motion. Special caution should be taken not to disturb sediment on the channel bottom. The same motion should be used at each station along the transect. However, due to the nature of the continuous sampling method used and the resulting restraints on time, a single sample may be taken in the thalweg, or the deepest portion of the stream channel. Each sample shall be taken immediately following the stream height, or stage reading.

When stream conditions are too dangerous for wading, the thalweg cannot be accessed, or the water is too shallow for the instrument, a surface water sample or 'grab' shall be collected. Grab samples shall be taken by simply reaching out from the bank and inserting the sample bottle into the water column in a quick downward motion with the mouth of the bottle facing upstream. A quick downward motion will facilitate the collection of a relatively integrated sample, rather than only water from the surface. Once again, special caution should be taken not to disturb bottom sediment. If a bridge is present, a sample bottle may be strapped to a rope and lowered into the thalweg to collect a 'grab' sample. Fast moving streams tend to be well mixed as opposed to slower moving streams, which are more stratified. A grab sample is not as accurate as a DH-48 sample. However, when collected in fast moving streams it can provide a fairly accurate representation of the stream concentration.

7.5 Protocol for sampling pathogens

CCoWS will sample Watsonville Sloughs for the following pathogen indicators:

- *E. coli*
- Fecal coliform

When sampling for coliform bacteria, the following steps shall be taken:

- Sterile, sealed sampling containers shall be obtained from the external laboratory. These shall remain sealed until the sample is taken.
- The container shall be labeled and the ID shall be recorded in the field notebook along with the time of collection.
- Technician shall wear latex gloves to prevent contamination of the sampling container and for health safety.
- Remove seal from bottle.
- Insert the sample bottle just below the water surface with the mouth of the bottle facing upstream & fill bottle (leaving enough space for sample to be shaken later). Take caution not to disturb bottom sediment.
- The sample shall be placed into a cooler with ice.
- The sample shall be immediately delivered to the external laboratory with an accompanying chain-of-custody form after the sampling run is complete.

7.6 Protocol for taking a pH measurement

The pH, negative logarithmic (base 10) hydrogen ion concentration, shall be measured in the field using an *Oakton pHTestr* probe. This probe is capable of measuring pH within the range of -1.0 to 15.0 using the SM 4500B technique (APHS Standard Methods, 1998). Instrument specifications and calibration instructions are kept on file by CCoWS. pH probes will be calibrated prior to each monitoring campaign.

A pH measurement should be made as follows:

- Use a clean Nalgene beaker (1000mL) for sample collection
- Rinse beaker in sample water 3 times
- To collect sample, insert beaker into water column just long enough to collect approximately 100 mL of water (enough to submerge the

electrode on the pH probe). Take caution not to disturb sediment on the bottom of the channel.

- Remove cap of probe, turn instrument ON, rinse with DI water, and insert into sample container.
- After reading has stabilized, approximately 1 minute, record reading in the field book.

7.7 Protocol for taking a conductivity measurement

Water conductivity will be measured in the field by an *Oakton TDSTestr* 10 conductivity probe with automatic temperature compensation. The *Oakton TDSTestr* 10 is capable of measuring conductivity in the ranges of 0 to 1990 uS and 2.0 to 19.90 mS using the SM2510 technique (APHS Standard Methods, 1998). The conductivity probes will be calibrated prior to each monitoring campaign.

Conductivity measurements shall be made with the Oakton TDSTestr 10 as follows:

- Using a clean Nalgene beaker (1000mL) for sample collection
- Rinse beaker in sample water 3 times
- To collect sample, insert beaker into water column just long enough to collect approximately 100 mL of water (enough to submerge the electrode on the conductivity probe). Take caution not to disturb sediment on the bottom of the channel.
- Remove cap of probe, turn instrument ON, rinse with DI water, and insert into sample container.
- After reading has stabilized, approximately 1 minute, record reading (uS or mS) in the field book.

7.8 Protocol for taking flow measurements

A number of techniques for flow (discharge) measurement may be used, depending on the nature of the flow. Protocols for each technique are listed below, in increasing order of flow magnitude. In all cases, the type of measurement used shall be recorded.

7.8.1 Presence/Absence

The simplest possible measurement pertaining to flow is whether or not any water is present. This should be a visual observation usually made by an observer standing at a site. It may also be made from a vehicle, although there are times when this is inaccurate. It may be made by interpolation between observations made above and/or below the site, although again, this can be inaccurate at times.

7.8.2 Flow / no-flow

The next simplest measurement of flow is whether or not the water in a channel can be seen to be moving in a net downstream direction. Again, this should be a visual observation made by an observer standing at a site. Unless obvious, the observation shall not be made from a vehicle. The observation shall never be made by interpolation. On one instance in a central coast stream, approximately 30 m^3/s of flow was observed at a site below which there was no flow or water present at a site approximately 5 km downstream along the same, single channel.

7.8.3 Visual estimation

In situations where logistics prevents all the methods listed below, flow rate should be visually estimated based on personal experience. Conversely, personal experience should be calibrated by memorizing the visual characteristics of flows for which discharges are known. Appropriate visual characteristics are the estimated width, depth, and surface velocity of a flow. Additional characteristics include turbulent features and standing waves, turbidity, sound, and the presence of waterborne litter and debris.

7.8.4 Calibrated bucket

A 5-gallon bucket may be used to measure discharge from flows falling over a vertical drop under which the bucket can be placed. The bucket should be marked on the inside surface at 1 liter intervals by pouring twenty 1-liter water samples into it. Care should be taken to record the exact duration and volume of each sample. The longer the duration, the more accurate the measurement will be.

Smaller flows with small vertical drops may be measured using a calibrated jug.

7.8.5 Rapid filling bucket

Where flows are so great as to overtop a bucket or jug in less than 2 seconds, a number of repeated measurements of the time taken to fill the bucket completely should be made using a stopwatch. Estimates made in this way are relatively inaccurate.

7.8.6 Rapid filling bin

Flows overtopping a bucket or jug in less than half a second may be measured using a 20-gallon bin. If the bin is overtopped in less than half a second, the bin may be placed successively under separate parts of the flow. Estimates made in this way are relatively inaccurate, but may be more accurate than current meter measurements where very slow flowing streams spill over broad crested weirs.

7.8.7 Estimation based on surface velocity and depth

In many natural channels, the mean velocity of a stream at a given point across its width is 85% of the surface velocity at that point (Gordon et al., 1992). Bright, floating objects such as a fluorescent wooden dowel may be used to estimate the surface velocity. In large rivers, orange peels may be thrown from bridges and the velocity estimated from a) the time taken for a peel to traverse under the bridge, and b) the measured width of the bridge. In smaller streams, a useful measuring device is a fluorescent orange 5 cm wooden dowel, measured against a 2 m stadia rod. Where possible, the velocity at three points across the width of the stream should be measured. In this case, the flow rate (m^3/s) shall be estimated as the sum of the products of the width represented by each surface velocity measurement, the depth of the water at each measurement, and 85% of the surface velocity. In cases where only one surface measurement is possible, the flow rate shall be estimated as half of the sum of the products of the width, depth, and estimated mean velocity in the center of the channel.

7.8.8 Cross section with a current meter

The most common method of measuring flow rate in small streams is to wade and record cross-sectional measurements using a current meter (flow probe). A number of different types of current meter may be used:

- Pygmy meter. This is the standard meter for small streams in the US. Three stainless steel cones are mounted on arms extending from a vertical axle with pointed ends mounted within a precision smooth conical bearing. The meter is sensitive to very slow flow but works well in fast flow. It is very expensive. It easily becomes un-calibrated when bumped during transport from a vehicle to the stream, or when in contact with bedload of significant size. It is expensive to re-calibrate. It requires partial dismantling before transport, and partial assembly before use; a small pin must be re-installed. It is thus generally unsuitable for conditions where:

    - Many measurements must be taken at many sites in a single day
    - At night
    - By new operators
    - Transport by small vehicles
    - Streams with large bedload particles
    - Extremely muddy conditions

    However, it is useful for calibration of more robust meters.

- Plastic impellor meters. CCoWS uses plastic impellor meters purchased from retailers such as *Global Water*, but also has developed the capability to construct this type of plastic impellor meter (flow probe) by using model boat propellers. These can be fitted inside PVC plumbing housings of various sizes and mounted on various tubes for handheld use. Bike computers may be used to count the rotations of the propellers, and calibrated internally to display flow rate in m/s. Methods for the calibration of CCoWS constructed flow probes are summarized in Watson et al., 2002. Additionally, a comparison study of the three types of current meters was conducted. The results are summarized are also summarized in Watson et al., 2002. The impellors can sometimes become blocked or jammed with sand or leaves, but are easily cleared.

Short mounting tubes may be constructed for wading use. Longer, triple-extendible tubes are useful for reaching in from stream banks, or down from low bridges. Impellors may also be mounted on heavy instrument packages suspended from large bridges. The instruments are inexpensive, easily repairable, and robust.

The following steps shall be taken when measuring stream flow rate by wading with a current meter:

- It shall be determined that the deepest part of the stream is safe to wade, and that no dangerous debris is likely to enter the site. One team member should serve as a spotter for any debris moving downstream.
- One end of a tape measure shall be firmly anchored at any low point on one bank of the stream. The other end shall be firmly anchored to the other bank. Intermediate supports shall be used in wide streams, such as metal stakes driven into the streambed, with clamps on the upper ends.
- A table shall be drawn up in a field book with columns for 'offset', 'depth', and 'velocity' (See Appendix N).
- The times of commencement and completion of measurements shall be recorded, as shall the river stages at those times.
- Two people shall be employed, one as recorder, the other as measurer.
- Where time permits, an even measurement interval shall be used, and at least 10 velocity measurements should be taken across the width of the stream. When time is scarce, an uneven measurement interval may be used, with most measurements taken at points of rapid change in velocity, and at points of high velocity and/or high depth.
- Starting from one bank, preferably the left bank, the offset at which the free water surface begins shall be recorded.
- Velocity measurements shall then be taken across the width of the stream until the opposite bank is reached and the offset at which point the free water surface end is recorded.
- Streams with multiple channels shall be measured as the sum of multiple streams.
- Each velocity measurement shall be taken as follows:
    - The measurer should stand well downstream of the instrument
    - The instrument should be placed in the water and rested against the bed such that the flow depth shall be recorded.

- The current meter shall be mounted on a top-setting rod such that it may be held steadily at 60% of the flow depth above the bed.
- The impellor shall be checked for blockages and free-running operation, and the computer shall be reset to zero average velocity.
- The impellor shall be allowed to run freely while the average velocity is observed over a period of 10 seconds to 1 minute in order to measure a steady mean value. This value shall be recorded as the (vertically-averaged) mean velocity of the stream at that offset across the stream.

The total flow rate for the stream shall be estimated in the laboratory using a Microsoft Excel spreadsheet as follows:

- The field book table shall be copied to the spreadsheet.
- Each velocity measurement is assigned a representative width, calculated as the difference in offset between the halfway points to adjacent measurement points either side of the point at which the velocity was measured.
- The flow rate for each measurement point shall be the product of the velocity and the representative width.
- The total stream flow rate shall be the sum of that for all measurement points across the stream.

7.9 Protocols for equipment management

All sampling equipment that will be used for this project has been previously used by CCoWS in similar sediment TMDL study for the Salinas Valley (Watson et al., 2003) and has proved to be both reliable and adequate for project needs. Any additional supplies needed for this project such as sample bottles, standards, and filters will be ordered by the research, field, or laboratory manager. All equipment is inspected by management upon arrival from the supplier and given a unique ID. Factory manuals, specifications, and instructions are kept on file by CCoWS at the Watershed Institute.

Prior to each sampling run, all equipment is visually inspected and assembled into field kits. Following each sampling run, field equipment is cleaned and stored until future use.

Various pieces of CCoWS sampling equipment require periodic calibration and maintenance to assure accuracy and reliability. This equipment includes:

- *Hach 2100P* Turbidimeter
- *Oakton* Total Dissolved Solids (TDS) probes
- *Oakton* pH probes
- *Global Water* and *CCoWS* water velocity meters (flow probes)
- *Mettler Toledo* Balance

The scheduling of the calibration and maintenance varies according to the amount of use and manufacturer's requirements. All equipment used by CCoWS is calibrated according to instructions provided by the manufacturer, with the exception of the water velocity meters constructed by CCoWS. CCoWS maintains an "Equipment Calibration & Maintenance Records" document that outlines specific calibration and maintenance schedules/procedures along with logs for the recording of calibrations and all maintenance performed. Sample calibration logs are presented in Appendix J to Appendix M. These records may be reviewed upon request. An equipment inspection and calibration schedule is outlined in Table 7-2.

Table 7-2. Equipment Inspection and Calibration Schedule

Equipment Type	Inspection Frequency	Inspection Method	Calibration Frequency	Calibration/Maintenance Method
Hach 2100P turbidimeter	prior to each use	accuracy check with *Gelex* factory standard	quarterly	factory Formazin standards, cell compartment cleaning
Oakton TDS probes	prior to each sampling run	battery check, accuracy check with factory standard	prior to each monitoring campaign; if problems are detected	factory prepared standard, electrode cleaning with alcohol
Oakton pH probes	prior to each sampling run	battery check, accuracy check with factory standard	prior to each monitoring campaign; if problems are detected	factory prepared standard, electrode soak in buffer solution
Global Water and *CCoWS* flow probes	prior to each sampling run	visual propeller check, computer calibration function check	annually	propellor cleaning; metered velocity vs. actual velocity test conducted at swimming pool
Mettler Toledo balance	prior to each use	level	balance is auto-calibrating	If problems are detected during quality assurance checks, maintenance will be provided by manufacturer

8 Laboratory and Analytical Protocols

This section contains protocols relating to laboratory analyses, including:

- Sediment analysis
- Pathogen analysis

Staff training on laboratory safety procedures is provided by the Earth Systems Science and Policy (ESSP) laboratory staff at CSU Monterey Bay and is a requirement prior to laboratory use. Documentation of lab safety training is kept on file by the ESSP laboratory staff. It is CCoWS responsibility to assure that all technicians performing the following tests have attended a safety training session.

Technicians shall be familiar with the equipment and tests before analyzing samples independently. This should include both training with the laboratory manager and/or an experienced senior technician and study of the instrument and procedure manuals. This training shall be documented, *Technician Training Tracking Sheet* (see Appendix B), and kept on file at the Watershed Institute.

8.1 Protocol for analyzing Suspended Sediment (SSC)

The following SSC procedure is employed to determine the concentration of suspended sediment in a water sample. A filtration process is used, based on Woodward and Foster (1997) and ASTM method D3977. The protocol for SSC analysis is as follows:

1) Sample bottles are pre-weighed (to the nearest 0.01g) and assigned a container ID prior to sampling events.
2) After the sample is obtained, the outside of the sample bottle is rinsed and dried, then weighed to the nearest 0.01g.
3) A small amount of sodium hexametaphosphate is added to the sample and shaken thoroughly. This helps to suspend particles and prevent flocculation.
4) Samples are first filtered through a 63-micron sieve to separate the sand component.
5) Pre-dried and pre-weighed (to the nearest .001g), disposable glass filters (filter size, 1.5 micron) are used to vacuum filter the water sample and

the fine sediment component. The sand component is transferred to a disposable glass filter.

6) The filters containing the sand component and fine sediment portion of the water sample are then dried for 2 hours at 100°C to evaporate any remaining water.
7) The filters are reweighed to determine the amount of sediment in the sample (to the nearest 0.001g).
8) The volume of the sample is determined from its weight and the density of water.
9) Concentrations of samples are recorded in mg/L.
10) All information shall be recorded on the *Lab Processing of SSC Water Samples* data sheet (Appendix D).

8.2 Protocol for analyzing turbidity

Turbidity samples are analyzed using a Hach 2100P portable turbidimeter, SM2130B. Samples are analyzed according to directions outlined in the factory manual. The protocols is as follows:

1) Fill sample cell with sample, cap the cell, taking care to handle cell by the top.
2) Wipe the cell with a soft, lint-free clot the remove water spots and fingerprints.
3) Apply thin film of silicone oil. Wipe with a soft cloth to obtain an even film over entire surface.
4) Place turbidimeter on a flat surface and turn on.
5) Insert sample cell in to instrument cell compartment so the diamond mark aligns with the raised orientation mark in front of the cell compartment. Close the lid.
6) Select automatic range. This measures turbidity from 0.01 to 1000 NTU.
7) Press: Read. The display will show the final turbidity in NTU after the lamp turns off.
8) All information is recorded on the *Lab Processing of TSS Water Samples* data sheet (Appendix D).

The scheduled calibration for the turbidimeter is once every three months according to manufacturer protocol. As a secondary accuracy check, *Gelex* factory standards are used before each series of measurements are taken. If the

reported measurement is within the *Gelex* standard range, samples are then measured according to protocol. If out of range, the turbidimeter shall be calibrated prior to analysis of samples.

8.3 Protocol for analyzing pathogen samples

At present, CCoWS does not analyze pathogen samples. Samples may be analyzed by the following state certified laboratories:

Monterey County Health Department Laboratory
1270 Natividad Rd.
Salinas, CA 93906
(831) 755-4516

Bio Vir Laboratories
685 Stone Road, Unit 6
Benicia, California 94510 -1126
1-800-GIARDIA (442-7342)
(707) 747 - 5906

The primary technique for coliform analysis will be Multiple Tube Fermentation (SM9221). Membrane Filtration may be a more appropriate method for coliform testing because it results in a direct colony count, but Multiple Tube Fermentation will primarily be used for this project in order to allow for comparisons to the Basin Plan objectives (1994). For the exceedance monitoring portion of the study, samples will be analyzed by the Monterey County Health Department according to procedures detailed in Appendix E. Additional information on laboratory protocols can be obtained from the above address. All samples will be placed on ice and delivered immediately to the external laboratory with an accompanying chain-of-custody form (Appendix I).

If the exceedance monitoring indicates that microbial source analysis is needed, genetic analysis will then be conducted in collaboration with Dr. Betty Olson, Department of Environmental Analysis and Design, University of California, Irvine (UCI). Samples will first be analyzed at the Monterey County Health Department Laboratory using the Membrane Filtration technique (SM9222), which is detailed in Appendix S. *E. coli* colonies will be isolated on mTEC media,

enumerated, and then immediately shipped on ice to the UCI laboratory for genetic analysis using the Toxin Gene Biomarker method.

A total of 12 to 20 samples will be tested for the occurrence of the toxin genes included in Table 8, as well as the *pap*G III allele that has been shown to be associated with dogs.

Table 8-1. Organisms identified using the Toxin Gene Biomarker method

Organism Identified	Source of Biomarker	Accession Number	Reference
Human	heat stable enterotoxin (STh)	M34916	Oshiro et al. 1997
Cow	heat labile enterotoxin (LTIIa)	M17894	Khatib et al. 2002
Bird	Hemolysin E (*Hly*E)/ *Tsh* strain	AF052225/L27423	Reingold et al. 1999 Provence et al. 1994
Rabbit	Enteroaggregative heat stable enterotoxin (EAST1) *ral*G	U84144.1	Adams et al. 1997

The method for the toxin gene biomarker analysis is summarized in Appendix T.

9 Quality Control

9.1 Suspended Sediment & Turbidity

All samples will be collected and analyzed according to the previously outlined CCoWS protocols. Suspended sediment sample will be analyzed by vacuum filtration (SM2540D) and turbidity samples will be analyzed using a Hach 2100P Portable Turbidimeter (SM2130B). All equipment will be inspected and calibrated according to the schedule outlined in Table 7-2. Additional methods for quality control will include:

- Sample replicates: Replicate SSC and turbidity samples (3) will be collected at one site per sampling run or for 10% of the total samples for the project.
- Inter-laboratory comparison: A duplicate turbidity sample (at least one per run or 10% of the total samples for the project) will be sent to an external laboratory and analyzed using the same method.
- Prepared Standard: 1 SSC sample per sample run or 10% of the total samples for the project will be prepared with a known amount of sediment and analyzed for recovery.
- Field blank: 1 field blank per sample run or for 10% of the total samples for the project will be analyzed to evaluate field methods.

Additional quality control for turbidity analysis will include analyzing a set of 3 factory-prepared standards prior to sample analysis. The turbidity reading must be within 2% of the known standard value before sample analysis can begin. Regular instrument calibration is also performed according to the Hach factory protocol.

Additional quality control for SSC analysis involved analyzing 27 samples of known sediment concentrations prior to commencement of this project. Three replicate samples for nine different sediment concentration ranges were analyzed to determine the precision of the vacuum filtration technique used by CCoWS. Samples were processed by trained technicians, who will also be analyzing the samples for this project. The relative standard deviation (coefficient of variation) was 0.6%. For the total 27 samples, the percent error

was 7.38%. The experiment is fully described in the CCoWS protocols document (Watson et al. 2002).

9.2 Pathogens

All samples collected for the pathogen exceedance monitoring, Section 5.1, will be analyzed using Multiple Tube Fermentation by the Monterey County Health Department Laboratory. Information on the quality assurance measures taken by the Monterey County Health Department Laboratory is summarized in Appendix E. Additional quality control measures taken by CCoWS will include:

- Sample replicates: Replicate samples (3) will be collected at one site per sampling run or for 10% of the total site samples for the project.
- Inter-laboratory comparison: A duplicate sample (at least one per run or for 10% of the total samples for the project) will be sent to BioVir Laboratory and analyzed using the same method.
- Field blank: 1 field blank per sample run or for 10% of the total samples for the project will be analyzed to evaluate field methods.

If stage one of the pathogen monitoring suggests that a source analysis is needed, then genetic analysis will be conducted by Dr. Betty Olson and her laboratory staff at the University of California, Irvine. All samples will first be delivered to the Monterey County Health Department Laboratory for initial analysis using Membrane Filtration (SM 9222). Information on the quality assurance measures taken by the Monterey County Health Department Laboratory is summarized in Appendix S. Samples will then be shipped overnight on ice to the UCI laboratory for analysis using the Toxin Gene Biomarker method. Quality assurance methods taken by UCI are summarized below. The following methods were written and provided by Dr. Betty Olson in a proposal from UCI to perform this analysis as part of a subcontract with CSUMB.

- General Laboratory Procedures: For quality control purposes, laboratory equipment like refrigerators (1 to 4°C) and freezers (-20 to -80°C) are monitored daily and temperature is recorded. The biohazard hood is monitored and tested once a month by exposing plate count agar plates to the airflow in hood to confirm no bacterial growth. The thermal cycler, pipettes, centrifuges, and spectrophotometer are calibrated prior to performing tests to ensure accuracy.

- Processing of DNA: There is no standardized protocol for quality control in regard to processing DNA. This laboratory has developed the following procedures to insure quality control. A positive and negative control is included with each set of extractions that are performed. The minimum detection limit is determined for each set of water samples.
- PCR Analysis: Interference with the PCR reactions is determined by a protocol reported in Oshiro et al. (1997) and Khatib et al. (2002). PCR assays are performed in a designated hood with UV light. The hood is sanitized with 10% bleach, followed by 75% ethanol, and then UV radiation for 5 minutes. Carry over in the PCR reaction is controlled for by a set of positive and negative controls, which are included with each PCR run.
- Confirmation of PCR Product: Protocols have been established to determine that the fragment produced does not have similar sequences to those in the model gene using restriction enzyme digests. The restriction enzyme, BstAPI (New England Biolab), is used to produce two fragments with the sizes of 121bp and 45 bp from PCR amplicon for the STh toxin gene. Two restriction enzymes, PstI and AluI are used to confirm PCR amplicons for the LTIIa toxin genes. PstI produces two fragments with sizes of 245bp and 113 bp, and AluI produces two fragments with sizes of 222 bp and 136 bp. Southern Blot hybridization is used to confirm PCR amplicons using previously designed probes specifically for each toxin.
- Additional Quality Control: If results are obtained that are unclear, the fragment is excised from the gel, sequenced, and the sequence is then compared to the Genbank target sequence to authenticate the result.

Additional quality control measures taken by CCoWS for the genetic portion of the pathogen sampling will include the collection of replicate samples (3) from each site.

9.3 Data Acquisition Requirements

Additional data, other than those collected and analyzed directly by CCoWS, may be used for this project.

External quality control and coliform analyses as summarized in Section 9.1 and Section 9.2 will be performed only by state certified laboratories using either

EPA approved or Standard methods (APHA 1998). Additional genetic analyses will only be performed by laboratories approved by the Regional Board Project Representative. Procedures and quality assurance documents for each laboratory will be submitted to the Regional Board once appropriate laboratories have been selected.

Additional data may be acquired primarily for planning purposes. Methods and quality assurance procedures will be reviewed for any data used for purposes other than planning, and this use is first subject to approval by the Regional Board. These data may include the following:

- Historical data collected from government agencies such as: County of Santa Cruz, Pajaro Valley Water Management Agency, State Water Resources Control Board, AMBAG, and the City of Watsonville
- Historical data from environmental consulting firms such as Questa Engineering, Hagar Environmental Science, Applied Science and Engineering, Inc., and Swanson Hydrology and Geomorphology
- USGS topographic maps
- USGS aerial photos
- GIS layers obtained from County of Santa Cruz Planning Department and the USGS dataset
- Landsat ETM imagery for land use classification

9.4 Assessment and Response

Project activities such as field techniques, laboratory procedures, and data management will be assessed as follows:

- The field manager will oversee all fieldwork, field training, and ensure that field equipment is inspected and calibrated as scheduled. Each sampling run will be assigned a team leader responsible for assuring that procedures are followed and that data is accurately recorded.
- The laboratory manager will oversee laboratory analysis, training and is also responsible for ensuring that calibrations of laboratory equipment are performed as scheduled.
- Quality control exercises will be conducted as previously described in Section 9.1 and Section 9.2. Following each exercise, a quality control evaluation will be completed. The evaluation form is presented in

Appendix P. The QA manager will review each evaluation sheet. If problems are detected, such as failure to meet accuracy and precision objectives, immediate action will be taken (see below).

- All quality control evaluations will be reviewed every four months. A summary of evaluations will be included in progress reports to the Regional Board Project Representative (Section 6.3).
- The project leader will hold Quarterly CCoWS team meetings to discuss the project.
- The project leader is required by CSUMB Foundation to perform annual employee evaluations. Any problems directly related to staff performance will be addressed in this evaluation.
- The Regional Board Project Representative may evaluate activities by accompanying the team on sampling runs or observing laboratory activities at any time.

Any problem encountered during assessment may lead to the following responses:

- Equipment calibration prior to scheduled date
- Equipment repair
- Supplemental training for team members
- Discussion at CCoWS team meeting
- Consultation with project leader
- Reevaluation of methods

9.5 Data Review and Verification

All data will be reviewed and verified in the following manner:

- Field books will first be reviewed by team leader following each sampling run to make sure all samples were collected and information was accurately recorded.
- The QA/field manager will compare all database entries to original field books following each monitoring campaign.
- Data will be reviewed by the QA/field manager during data analysis following each monitoring campaign. All data for each monitoring campaign will be queried by analyte to look for any gaps and outliers. Data will then be reviewed in graphic format

- Following data analysis, data will be reviewed once again by the project leader.
- Any detected data errors will be flagged in the database.
- The QA/field manager will review all calibration records, QC evaluations, and quarterly reports to assure that data complies with the QAP and DQOs.
- Percent completeness, accuracy, and precision will be calculated and compared to original objectives.
- Any data limitations will be addressed in the final report.

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Appendix A

Sample Storage Management Log

Campaign	# of Samples	Date Collected	Date/time Stored	Initial	Frozen? (y/n)	Date Frozen	Initial	Date Thawed	Initial

Appendix B

Technician Training Tracking Sheet

Technique	Trainee (print)	Trainee (signature)	Trainer (print)	Trainer (signature)	Date

Appendix C

Earth Systems Science & Policy
California State University, Monterey Bay

Accident/Incident Report Form

Date of Incident:_____ **Time** of Incident:_____
Location Where Incident Occurred: _____
_____

Identity of any involved persons:

Name _____ _____
Address _____ _____
_____ _____
_____ _____
Contact Info _____ _____

Identity of any witnesses:

Name _____ _____
Contact Info _____

Description of Incident: _____
_____
_____
_____
_____
_____
_____
_____

Actions Taken: _____
_____
_____
_____
_____
_____

Name of Person Completing Report _____Date_____

Staff/Faculty Signature _____Date_____

Supervisor Signature _____Date_____

Appendix D

Lab Processing of TSS Water Samples																	
Pre vacuum		Date:	Date:			Sand		Coarse Filter		Fine Filters		Post Vacuum and Oven					
Sample ID	**Bottle #**	**TDS (uS)**	**Transp (cm)**	**Turb (NTU)**	**Total Bottle (w/sample) wt (g)**	**AP40 Filter ID**	Filter wt (g)	**AP40 Filter ID**	AP40 Filter Dry wt w/tin (g)	**934-AH Filter ID**	934-AH Filter Dry wt w/ tin (g)	**Sand Filter ID**	**Sand Filter wt (g)**	**AP40 Filter ID**	AP40 Filter sample wt w/tin (g)	**934-AH Filter ID**	934-AH Filter sample wt w/ tin (g)

Appendix E

Monterey County Health Department Laboratory
Standard Total and Fecal Coliform and E. coli
Multiple-Tube Fermentation Technique
Fifteen Tube Test (SM 9221B&E)

The Standard Total Coliform Multiple-Tube Fermentation Technique is used to determine the most probable number of total and fecal coliforms and E. coli in shellfish growing water or wastewater samples. For recreational samples and raw water samples use Colilert chromogenic substrate method.

Samples are collected in presterilized 125 mL capacity sterile polypropylene bottles. Sample volume should be approximately 100 mL (larger volumes do not allow adequate mixing). Samples, which cannot be delivered to the laboratory within one hour, should be transported in an ice cooler at <10°C. A temperature blank should be included in the cooler and the temperature recorded on the requisition form. Samples should be refrigerated at 1-4°C upon arrival and processed as soon as possible. At no time should the elapsed time between collection and processing exceed 8 hours. All samples processed or received later than 8 hours are to be reported as unsatisfactory; notify the public health chemist or laboratory director.

Quality Control

Media: All media must be less than two weeks old, stored at room temperature (media racks and baskets should be labeled with date of preparation). Check media on each day of use with known positive control (E. coli; growth with production of gas), and two negative controls (E. aerogenes; growth with no gas and Ps. Aeruginosa; no growth). A temperature profile must be performed on the water incubator annually.

1) 10 mL sterile Lauryl Tryptose broth in 19 x 150 mm slip-cap test tubes. Fifteen tubes are required for each water sample tested. Each tube must contain one inverted vial.

2) 10 mL sterile Brilliant Green Lactose Bile broth in 19 x 150 mm slip-cap test tubes. One tube is required for each positive Lauryl Tryptose tube. Each tube must contain one inverted vial.

3) 10 mL sterile EC-MUG broth in 19 x 150 mm slip-cap test tubes. One tube is required for each positive Lauryl Tryptose tube. Each tube must contain one inverted vial.

4) 9.0 mL ± 0.2 mL sterile phosphate buffered dilution water in screw-capped test tubes. One dilution tube is required for each sample tested for a 'High' dilution. More dilution tubes may be necessary for samples that are very turbid. Check with the chemist for the number of dilutions necessary.

Completed Test: For all wastewater samples, the completed test is performed on 10% of positive samples or quarterly, whichever is more frequent. Transfer a loop of inoculum from Brilliant Green tube with gas to MacConkey plate and streak for isolation. After 24 hours incubation, pick a well-isolated lactose fermenting (pink) colony and transfer back to Lauryl Tryptose Broth. Incubate for twenty-four hours and examine for gas production. Record results on multiple tube fermentation worksheet.

Equipment:

1) Sterile 2.0 mL pipets in 0.1 mL subdivisions.

2) Sterile inoculating loops, 3 mm in diameter.

3) Incubator set at 35°C ± 0.5°C. Check and record incubator temperatures daily.

4) Water bath set at 44.5°C ± 0.2°C. Check and record water bath temperatures twice on each day of use, one time in the morning and again in the afternoon.

Procedure

Day One (Set-up and Inoculation of Samples):

1) Match samples with forms. Check to insure that sample collection/processing time does not exceed the 8-hour deadline.

2) Record on the lab form the day and time the samples were inoculated.

3) Check lab form to determine the tests requested: whether just total coliforms and/or fecal coliforms and E. coli.

4) Arrange samples in numerical order whenever possible.

5) Arrange fifteen test tubes per test of sterile Lauryl Tryptose broth in racks according to Figure 1. Do not use any tubes showing growth or gas inside the vial.

6) Insert two sterile dilution blanks in the spots marked "A" and "B."

7) Shake samples vigorously in a one-foot arc 24 times. If samples are not processed within ten minutes they must be reshaken.

8) Transfer the sample number on the bottle to the first test tube in the fifteen tube series.

9) To inoculate Sample A for high dilution, use a 2.0 mL pipet in 0.1 mL subdivisions to add 1.0 mL of the undiluted sample to each of the first five test tubes (#1-5) in the series.

10) Add 1.0 mL undiluted sample to the dilution blank at the end of the series. Rinse pipet several times with the dilution and replace cap. Shake tube vigorously several times.

11) Add 1.0 mL sample from the dilution tube to the next five test tubes (#6-10) in the series.

12) Add 0.1 mL sample from the dilution tube to the last five test tubes (#11-15) in the series.

13) Remove the dilution tube from the rack and discard. Use only one pipet per sample for all dilutions.

14) If the sample is extremely turbid, it may be necessary to continue to dilute the sample in order to get a working range (instead of a '> number'). Ask the public health chemist or water quality specialist for number of dilutions to be made.

15) To inoculate Sample B, rotate the rack 180° and start with tube #1. Follow the instructions the same as for Sample A.

15) Incubate Lauryl Tryptose tubes for 24 ± 2 hours.

FIGURE 1. ARRANGEMENT OF TEST TUBES

Sample B

10	9	8	7	6	5	4	3	2	1
				B	15	14	13	12	11
11	12	13	14	15	A				
1	2	3	4	5	6	7	8	9	10

Sample A

Day Two (Reading and Subculturing Tubes):

1) Remove racks containing the Lauryl Tryptose tubes from the incubator and gently shake to liberate trapped gas and resuspend organisms.

2) Working from left to right read all Lauryl Tryptose tubes after 24 ± 2 hours incubation, if gas is present in the vial record as positive on lab form and subculture to Brilliant Green Broth and EC-MUG Broth as outlined in steps 3 through 12 below. If no gas is present in the vial record as negative on the lab form and reincubate for an additional 24 ± 2 hours. Note: Record results of tubes as they actually occur (i.e. in the proper sequence). See Figure 2.

3) Remove the first positive 24 hour Lauryl Tryptose tube in the sample series.

4) Remove one tube each of Brilliant Green Broth and EC-MUG Broth from their storage baskets.

5) If tube #1 in the series is positive, transfer the specimen label from the Lauryl Tryptose tube to the Brilliant Green Broth tube. Using small adhesive labels make a copy of the sample number and attach it to the top of the EC-MUG tubes' caps.

6) Remove caps and with a sterile inoculating loop, transfer one loopful of the bacterial suspension from the Lauryl Tryptose tube to the EC-MUG tube.

7) Using the same inoculating loop, transfer a second loopful of the suspension to the Brilliant Green tube.

8) Discard loop, replace caps, and discard Lauryl Tryptose tube. Use diluted Osyl in a small container for disinfection of inoculating loops.

9) Place the inoculated Brilliant Green tube into the spot in the rack previously occupied by the positive Lauryl Tryptose tube. Place the inoculated EC-MUG tube into the first spot of a separate test tube rack.

10) Continue working from left to right and subculture all remaining positive Lauryl Tryptose tubes in the sample series.

11) Place all subsequent, inoculated EC-MUG tubes in the next available spot to the right of the first EC-MUG tube. Always leave one empty space between each sample series. Incubate all EC-MUG tubes in a 44.5°C waterbath within 30 minutes after inoculation. Maintain sufficient water depth in water bath incubator to immerse tubes to upper level of the medium.

12) Place racks containing negative Lauryl Tryptose and any inoculated Brilliant Green tubes into the incubator for 24 ± 2 hours. Place racks containing EC-MUG tubes in the waterbath for 24 ± 2 hours.

Day Three (Reading and Subculturing Tubes):

1) Remove racks containing the 48 hour Lauryl Tryptose and 24 hour Brilliant Green tubes from the incubator and gently shake to liberate gas and resuspend organisms.

2) Remove the racks of EC-MUG tubes from the waterbath and gently shake to liberate gas.

3) Read all EC-MUG tubes first. Record results on lab form and discard tubes. For fecal coliforms, record as positive only the tubes containing growth with gas inside the inverted vial. (Turbidity alone does not constitute a positive result). For E.coli, record as positive only the tubes that fluoresce under a 6-watt, 365 nm UV light in a dark environment.

4) Read all 24 hour Brilliant Green tubes from all racks next. If gas is detected, record as positive on lab from and discard tubes. If negative, record results, place in a separate rack labeled "48 hour Brilliant Green tubes," and incubate for an additional 24 $\pm$ 2 hours (total incubation 48 $\pm$ 3 hours). Label cup of first tube in sample series with lab number using small adhesive labels. Note: Record date tubes were inoculated on the rack label.

5) Read all remaining 48 $\pm$ 3 hours Lauryl Tryptose tubes last. If positive, record results and transfer to Brilliant Green and EC-MUG Broth and discard Lauryl Tryptose tubes. Label racks of Brilliant Green tubes as "24 hour Brilliant Green tubes." Label cap of first tube in sample series with lab number using small adhesive labels. If the Lauryl Tryptose tubes are negative after 48 hours of incubation, record results and discard. (See section on "Reporting and Interpretation" for reporting out results of completed tests.)

6) Place racks containing inoculated Brilliant Green tubes in the incubator for an additional 24 $\pm$ 2 hours (total incubation 48 $\pm$ 3 hours). Place racks containing EC-MUG tubes in the water bath for 24 $\pm$ 2 hours. Note: Record date tubes were inoculated on the rack label.

Day Four (Reading and Recording Results):

1) Remove racks containing the 24 and 48 hour Brilliant Green tubes from the incubator and gently shake to liberate gas and resuspend organisms.

2) Remove the racks containing the 24 hour EC-MUG tubes from the water bath and gently shake to liberate gas.

3) Read all EC-MUG tubes first. Record results on lab form and discard tubes.

4) Read all 48 hour Brilliant Green tubes next. Record results and discard tubes.

5) Read all 24 hour Brilliant Green tubes from all racks last. If positive, record results and discard tubes. If negative, record results, place in the rack labeled "48 hour Brilliant Green tubes," and incubate for an additional 24 ± 2 hours. If necessary, transfer sample number label to first tube of sample series.

Day Five (Reading and Reporting Final Results):

1) Remove racks containing the 48 hour Brilliant Green tubes from the incubator and gently shake to liberate gas and resuspend organisms.

2) Record results of all tubes and discard.

3) After interpreting and recording final results, recheck form for accuracy, initial, date, and place lab form in laboratory director's office to be checked.

Reporting and Interpretation

1) If Lauryl Tryptose or Brilliant Green tubes are negative after 48 ± 3 hours, report out as <20 Total Coliforms/100 mL for the dilution procedure mentioned above.

2) If EC-MUG tubes are negative after 24 ± 2 hours, report out as <20 Fecal Coliforms/100 mL and/or <20 E.coli/100 mL.

3) If any Brilliant Green are positive after 48 ± 3 hours and any EC-MUG tubes are positive after 24 ± 2 hours, proceed as follows to determine the MPN counts:

a) Record results of tubes as they actually occur, and in the proper sequence within each dilution. Wait until all tubes have been read before determining MPN counts. See Figure 2 for an example of the actual reading, interpretation, and recording of results.

b) Count separately the number of positive tubes within each of the three dilutions. This will give a three digit number. Sample A in Figure 2 would be 3-1-1 for Total Coliforms, and 0-0-0 for Fecal Coliforms and E. coli.

c) Find the sequences on the MPN chart in the current edition of Standard Methods. For shellfish growing water use table from "Recommended Procedures for the Examination of Shellfish." See Supplemental Information.

d) After determining the correct sequence, look to the right side of the column for a MPN number.

e) The correct interpretation of the MPN count for Sample A is 140 Total Coliforms per 100 mL, <20 Fecal Coliforms per 100 mL, and <20 E.coli per 100 mL.

Written by: Richard Smith
Date: May 1990
Revised: December 2002 by T. Lam

Approved by: _____
Laboratory Director's Signature

FIGURE 2A. READING

No Gas	No Gas	No Gas	No Gas	Gas					
Gas	Gas	No Gas	No Gas	Gas	No Gas	No Gas	No Gas	Gas	No Gas

Sample A

FIGURE 2B. REPORTING AND INTERPRETATION

SAMPLE ID	TEST	AMOUNT OF SAMPLE IN MILLILITERS															TOTAL COUNT MPH/100 ML
		1.0	1.0	1.0	1.0	1.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
A	TC	+	+	–	–	+	–	–	–	+	–	–	–	–	–	+	140
				–	–		–	–	–		–	–	–	–	–		
		+	+	–	–	–	–	–	–	+	–	–	–	–	–	–	
				–	–	+	–	–	–		–	–	–	–	–	+	
	FC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	<20
	EC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	<20

Appendix F

Multiple-Tube Fermentation MPN Index and 95% Confidence Limits for Various Combinations of Positive Results [Dilutions 10mL, 1.0mL, 0.1mL]

Table from Standard Methods (1998)

Combination of Positives	MPN Index/100 mL	95% Confidence Limits		Combination of Positives	MPN Index/100 mL	95% Confidence Limits	
		Lower	Upper			Lower	Upper
0-0-0	<2	-	-	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	10	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1	33	15	77
				4-4-0	34	16	80
1-0-0	2	1.0	11	5-0-0	23	9.0	86
1-0-1	4	1.0	15	5-0-1	30	10	110
1-1-0	4	1.0	15	5-0-2	40	20	140
1-1-1	6	2.0	18	5-1-0	30	10	120
1-2-0	6	2.0	18	5-1-1	50	20	150
				5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-1	300	100	1300
4-0-1	17	7.0	45	5-5-2	500	200	2000
4-1-0	17	7.0	46	5-5-3	900	300	2900
4-1-1	21	9.0	55	5-5-4	1600	600	5300
4-1-2	26	12	63	5-5-5	≥ 1600	-	-

Appendix G

Confidence Limits for Membrane Filter Coliform Results using 100mL sample

Table from Standard Methods (1998)

Number of Coliform Colonies Counted	95% Confidence Limits	
	Lower	Upper
0	0.0	3.7
1	0.1	5.6
2	0.2	7.2
3	0.6	8.8
4	1.0	10.2
5	1.6	11.7
6	2.2	13.1
7	2.8	14.4
8	3.4	15.8
9	4	17.1
10	4.7	18.4
11	5.4	19.7
12	6.2	21
13	6.9	22.3
14	7.7	23.5
15	8.4	24.8
16	9.2	26
17	9.9	27.2
18	10.7	28.4
19	11.5	29.6
20	12.2	30.8

Appendix H

BioVir LABORATORIES ®

SAMPLE DATA SHEET

SHIPPING ADDRESS: BIOVIR LABORATORIES, INC., 685 STONE ROAD, UNIT 6, BENICIA CALIFORNIA 94510
1-800-GIARDIA (442-7342) FAX: 707-747-1751

COMPANY OR UTILITY:	DATE OF SAMPLING:
TELEPHONE# EXT# FAX#	PURCHASE ORDER #:
NAME OF SAMPLER: (Please print and sign)	Matrix: (Drinking Water, Wastewater, Biosolid, etc.)

UTILITY ID#	TIME	VOLUME	TREATMENT	SAMPLING LOCATION	ANALYSIS REQUESTED

RELINQUISHED BY (SIGNED)	RECEIVED BY (SIGNED)	DATE	TIME

F:\WP\FORMS\Datasheets\DATASHT.GEN030201.wpd 10/2/00

Appendix I

ENVIRONMENTAL ANALYSIS REQUEST FORM
MONTEREY COUNTY CONSOLIDATED CHEMISTRY LABORATORY
1270 NATIVIDAD ROAD, SALINAS, CALIFORNIA – PHONE (831) 755-4516
SHADED AREAS ARE FOR LABORATORY USE ONLY

Collected by (Print & sign):	Received by:	Date & Time:
Relinquished by:	Received for Laboratory:	Date & Time:

Client Name:	Report Attention		ANALYSES REQUESTED
Address:	Copy to:		
City, State, Zip	Phone	FAX	

Laboratory Number	Sample ID or (Sweeps #)	Sample Site or Description	Collection Date & Time	Matrix(1) 1-Routine 2-Repeat 3-Replacement	No. of Containers	Coliform : □□□ MMO Quanti Low-D	Nitrate					

(1) D=Drinking Water (Specify as routine, repeat or replacement); W=Wastewater (Specify as grab or composite; O=Other (identify)

[ ] Payment received with delivery	Amount:	Sample comments (irregularities/preservation, billing information if different than reporting):
Check: _____	Initials:	
Receipt #: _____	Date:	

LAB60

Appendix J

OAKTON TDSTestr

Calibration & Maintenance Record

CALIBRATION					
A		**B**		**C**	
initials	date	initials	date	initials	date

BATTERIES CHANGED		
probe ID	**initials**	**date**

ELECTRODES CLEANED		
probe ID	**initials**	**date**

Appendix K

OAKTON pH Testr

Calibration & Maintenance Record

CALIBRATION									
A		B		C		D		E	
initials	date	initials	date	initials	date	initials	date	initials	date

BATTERIES CHANGED		
probe ID	initials	date

ELECTRODE REPLACEMENT		
probe ID	initials	date

Appendix L

FLOW PROBE

Calibration & Maintenance Record

CALIBRATION									
A		**B**		**C**		**D**		**E**	
initials	date	initials	date	initials	date	initials	date	initials	date

BATTERIES CHANGED		
probe ID	**initials**	**date**

PROPELLOR CLEANED		
probe ID	**initials**	**date**

Appendix M

HACH Portable Turbidimeter Model 2100P

Calibration & Maintenance Record

CALIBRATION

initials	date

BATTERIES CHANGED

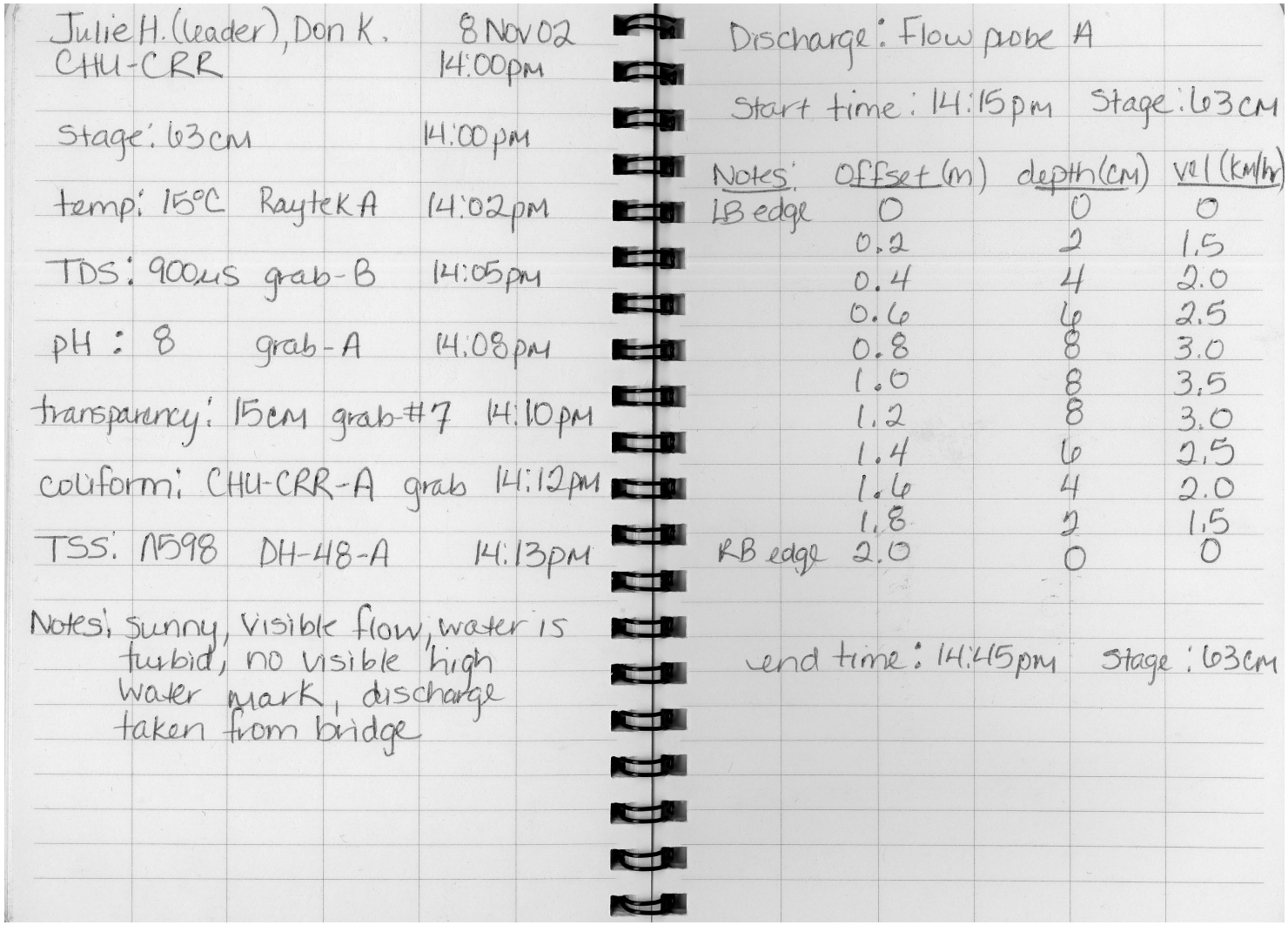
initials	date

CELL COMPARTMENT CLEANED

initials	date

Appendix N

CCoWS Sample Field Book Entry



Julie H. (leader), Don K. 8 Nov 02
CHU-CRR 14:00pm

stage: 63cm 14:00 pm

temp: 15°C RayteK A 14:02pm

TDS: 900uS grab-B 14:05pm

pH : 8 grab-A 14:08pm

transparency: 15cm grab-#7 14:10pm

coliform: CHU-CRR-A grab 14:12pm

TSS: N598 DH-48-A 14:13pm

Notes: sunny, visible flow, water is turbid, no visible high water mark, discharge taken from bridge

Discharge: Flow probe A

start time: 14:15pm Stage: 63cm

Notes:	Offset (m)	depth (cm)	vel (km/hr)
LB edge	0	0	0
	0.2	2	1.5
	0.4	4	2.0
	0.6	6	2.5
	0.8	8	3.0
	1.0	8	3.5
	1.2	8	3.0
	1.4	6	2.5
	1.6	4	2.0
	1.8	2	1.5
RB edge	2.0	0	0

end time: 14:45pm Stage: 63cm

Appendix O

CCoWS Site Code

SiteCode	WaterwayCode	Bridge/Road	USGSStationID	CCAMPID
AAA-AAA	?	Non-existent site for testing purposes		
ADC-PIE	ADC	No bridge?, Nr Piedras Blancas	11142500	
ADR-SEA	ADR	Unknown bridge, At Seaside	11143300	
ALI-AIR	ALI	Airport Rd		309ALU
ALI-OSR	ALI	Old Stage Rd	11152570	309UAL
ANT-101	ANT	Hwy 101		309SAN
ANT-CON	ANT	Just before confluence with Salinas River		
ANT-LOC	ANT	Interlake Rd	11149900	
ANT-PLE	ANT	Unknown old br. nr Pleyton (submerged)	11150000	
ANT-SAM	ANT	Sam Jones Rd	11149700	
ANT-SCO	ANT	Scott's Restoration Site, Naciemento Rd near Brown Ranch		
ARR-ARR	ARR	Arroyo Seco Rd	11152050	
ARR-CAM	ARR	Los Padres NF Campground	11151870	
ARR-CON	ARR	Just before confluence with Salinas River		
ARR-ELM	ARR	Elm Rd (USGS stn) (Green br.)	11152000	309SEC
ARR-THO	ARR	Thorne Rd		309SET
ATA-GOL	ATA		11120000	
ATA-H41	ATA	Hwy 41 in town of Atascadero		309ATS
BIT-PAR	BIT	Parkfield Rd		
BLA-COO	BLA	Blanco drain at Cooper Rd		
BLA-PUM	BLA	Pump station		
BOC-OSR	BOC	Old Stage Rd		
BSN-CON	BSN	Just before confluence with Salinas River		
BSU-BSU	BSU	Pfeiffer Big Sur SP, Weyland Camp br.	11143000	
BUL-WEO	BUL		11476600	
CAR-ESQ	CAR	Esquiline Rd	11143200	
CAR-HWY	CAR	Hwy 1		
CAR-LAG	CAR	Midwater		
CAR-VIA	CAR	Via Mallorca	11143250	
CAW-BOL	CAW	Bolhm Rd		306CAR
CH1-RWY	CH1	Railway Culvert		
CHA-CON	CHA	Just before confluence with Salinas River		
CHA-MET	CHA	Metz Rd		309TOP
CHO-BIT	CHO	Bitterwater Rd		317CHO
CHO-H46	CHO	Hwy 46	11147800	
CHT-RWY	CHT	Railway Bridge	11147700	
CHU-CCR	CHU	Chualar Canyon Rd		
CHU-CON	CHU	Just before confluence with Salinas River		
CHU-CRR	CHU	Chualar River Rd		
CHU-FOL	CHU	Foletta Rd		
CHU-OSR	CHU	Old Stage Rd		
CLR-CLR	CLR	Clear Creek Rd	11154700	
COR-COR	COR	Unknown bridge, Nr Corralitos	11159150	
COR-GVR	COR	Green Valley Rd	11159200	
COW-198	COW	Hwy 198 ?	11150800	
DRN-ALI	ALI	near Airport Rd		309AXX
DRN-BLA	DRN	Drains into Salinas R 30m S of Blanco Rd on R bank		

SiteCode	WaterwayCode	Bridge/Road	USGSStationID	CCAMPID
DRN-DAV	SAL	300m upstream from Davis Rd		309SDR
EEL-DOS	EEL		11472500	
ELK-KIR	ELK	Kirby Park		306ELK
ELT-USG	ELT	USGS stn	11152540	
EP1-ROG	EP1	Rodgers Rd		
EPL-EPL	EPL	Espinosa Lake		
ESC-RIV	ESC	River Rd / Escolle Rd		
EST-AIR	EST	Airport Rd	11148500	317EST
EST-CON	EST	Just before confluence with Salinas River		
EST-H46	EST	Hwy 46	11148000	
EST-RIV	EST	River Rd		317ESE
ESZ-HWY	ESZ	Between Hwy 101 & Railway, South of Esperanza Rd		
ESZ-OSR	ESZ	Old Stage Rd		
GAB-BOR	GAB	Boronda Rd		309GAB
GAB-CRA	GAB	Crazy Horse Rd		
GAB-HER	GAB	Herbert Rd	11152600	
GAB-NAT	GAB	Natividad Rd		
GAB-OSR	GAB	Old Stage Rd		
GAB-VET	GAB	Veterans Park Bridge		
GAL-BUE	GAL	Buena Vista Rd		
GVC-GVR	GVC	Green Valley Rd	11159400	
HAM-CON	HAM	Just before confluence with Salinas River		
HAN-HAR	HAN	Harkins Slough Rd.		
HAR-CON	HAR	Confluence with Watsonville Slough		305HGS
HAR-HAR	HAR	Harkins Slough Rd		305HAR
HAR-RAU	HAR	Upstream Ranport Rd		
HUE-CON	HUE	Just before confluence with Salinas River		
HUE-CRE	HUE	Creston Rd	11147600	
JAC-JAC	JAC	No Bridge?, Nr Templeton	11147000	
JOS-GOL	JOS		11120510	
LEW-LON	LEW	King City Rd-Lonoak		
LIT-LAK	LIT	Lake Driveway		
LIT-PA1	LIT	Parkfield Rd (1st above Parkfield)		
LIT-PA2	LIT	Parkfield Rd (2nd above Parkfield)		
LIT-PA3	LIT	Parkfield Rd (~3rd above Parkfield)		
LLA-BLO	LLA	Bloomfield Ave		305LLA
LLA-BVA	LLA	Buena Vista Ave		305VIS
LLA-CHE	LLA	Chesbro Reservoir		305CHE
LLA-HOL	LLA	Holsclaw Rd		305HOL
LLA-LUC	LLA	Lucchessa Rd		305LUC
LLA-MAS	LLA	Masten Ave		305MAS
LLA-MCR	LLA	Monterey County Rd		305MON
LLA-OGA	LLA	Oak Glen Ave		305OAK
LOP-ARR	LOP		11141280	
MAR-GUA	MAR		11141000	
MCS-HW1	MCS	HWY 1		306MOR
MCS-MOS	MCS	Moss Landing Rd		306MOS
MIS-BAR	MIS		11119745	
MOR-HWY	MOR	Hwy 1	11142080	
MOS-SAN	MOS	Moss Landing Harbor at Sandholt Rd		
NAC-101	NAC	Hwy 101		309NAC

SiteCode	WaterwayCode	Bridge/Road	USGSStationID	CCAMPID
NAC-BLD	NAC	? (below dam)	11149400	
NAC-BRY	NAC	Bryson Hespiera Rd	11148800	
NAC-CON	NAC	Just before confluence with Salinas River		
NAC-HIG	NAC	Highwater Bridge		
NAC-M07	NAC	Mile 7	11149500	
NAC-SAP	NAC	No bridge, Below Sapaque Ck	11148900	
NAT-BOR	NAT	Boronda Rd		
NAT-FRE	NAT	Freedom Blvd.		
NAT-LAS	NAT	Las Casitas		
NAT-LAU	NAT	Laurel Drive		
OLS-MON	OLS	Monterey Dunes Colony Rd		309OLD
OLS-POT	OLS	Potero Rd (Tide Gates)		309POT
PAC-156	PAC	HWY 156		305PAC
PAJ-BET	PAJ	Betabel Rd		305PAJ
PAJ-CHI	PAJ	Chttenden Rd		305CHI
PAJ-FRA	PAJ	Frazier Lake Rd		305FRA
PAJ-MAI	PAJ	Main St (Watsonvlle)	11159500	
PAJ-MCG	PAJ	McGowan Rd		305THU
PAJ-MUR	PAJ	Murphy's Ck Rd		305MUR
PAJ-PAJ	PAJ	Pajaro Rd ?	11159000	
PAJ-SAR	PAJ	Unknown bridge, Nr Sargent	11154500	
PAN-CON	PAN	Just before confluence with Salinas River		
PAN-SAR	PAN	Sargents Rd		
PAS-CON	PAS	Just before confluence with Salinas River		
PEC-129	PEC	Near Hwy 129	11158900	
PEC-CCA	PEC	CCAMP?		305PES
PEP-CIE	PEP	Cienaga Rd	11156700	
PER-PER	PER	No bridge ?, Perry Ck	11142240	
PIN-CON	PIN	Just before confluence with Salinas River		
PIP-KER	PIP	Drains into Rec. DItch near Kern Rd		
PIP-MOL	PIP	Ag. pipe on R bank of Tembladero Slough at Molera Rd		
PIP-NM1	PIP	Pipe into Rec. Ditch at North Main, Pipe flow coming from Rd		
PIP-NM2	PIP	Pipe into Rec. Ditch at North Main, Pipe flow coming from Industrial Wasteland		
PIP-NMA	PIP	Culvert under bridge and pipe at North Main Street		
PIP-SJB	PIP	Drains from Ag. land at Cnr San Jon Rd and Boronda Rd		
PIP-SJU	PIP	Drains from Ag. land Along San Juan Rd		
PIP-VIC	PIP	Drains into Rec. Ditch at Victor Rd		
QUA-OSR	QUA	Old Stage Rd		309UQA
QUA-POT	QUA	Potter Rd		309QUA
R001-01	CON	Confidential		
R001-02	CON	Confidential		
R001-03	CON	Confidential		
R001-04	CON	Confidential		
R002-01	CON	Confidential		
R002-02	CON	Confidential		
R003-01	CON	Confidential		
R003-02	CON	Confidential		
R003-03	CON	Confidential		
R005-01	CON	Confidential		
R006-01	CON	Confidential		
R006-02	CON	Confidential		

SiteCode	WaterwayCode	Bridge/Road	USGSStationID	CCAMPID
R006-03	CON	Confidential		
R006-04	CON	Confidential		
R006-05	CON	Confidential		
R006-06	CON	Confidential		
R006-07	CON	Confidential		
R007-01	CON	Confidential		
R007-02	CON	Confidential		
R007-03	CON	Confidential		
R007-04	CON	Confidential		
RAT-HWY	RAT	Hwy 1	11142800	
REC-183	REC	Hwy 183		
REC-BOR	REC	Boronda Rd		309ALD
REC-JON	REC	San Jon Rd	11152650	
REC-KER	REC	Kern Street		
REC-NMA	REC	North Main Street		
REC-RIC	REC	Rico Rd		
REC-VIC	REC	Victor Way		
RIT-TEM	RIT	Templeton Rd	11147070	
RTT-H46	RTT	Unnamed old bridge?, Nr Hwy 46	11147040	
SAL-ARD	SAL	San Ardo		
SAL-ARU	SAL	Just upstream of influence from Arroyo Seco River		
SAL-BLA	SAL	Blanco Rd		
SAL-BLD	SAL	No bridge, Below Dam	11144600	
SAL-BRA	SAL	Bradley Rd		309USA
SAL-CAT	SAL	along Cattleman Rd (?CCAMP Site)		309DSA
SAL-CHU	SAL	Chualar River Rd	11152300	309SAC
SAL-CRE	SAL	Creston Rd	11147500	309PSO
SAL-DAV	SAL	Davis Rd		309DAV
SAL-GAR	SAL	River Rd (Nr East Garrison)		309SUN
SAL-GON	SAL	River Rd Gonzales Bridge		
SAL-GRE	SAL	Greenfield		309GRN
SAL-H41	SAL	Hwy 41		309SAT
SAL-KIN	SAL	King City		309KNG
SAL-LAG	SAL	Lagoon		
SAL-LOC	SAL	Lockwood		
SAL-MIG	SAL	San Miguel		
SAL-MON	SAL	Del Monte Rd		
SAL-MOU	SAL	mouth of lagoon		
SAL-MOV	SAL	Near Blanco?		
SAL-MRG	SAL	Unknown bridge, Nr Santa Margarita	11145500	
SAL-PAU	SAL	Just before inflow from Paso Robles Creek		
SAL-PIL	SAL	Las Pilitas Rd	11145000	
SAL-POZ	SAL	Pozo Rd	11143500	
SAL-SOL	SAL	Hwy 101 at Soledad	11151700	
SAL-SPR	SAL	Hwy 68	11152500	
SAL-WUN	SAL	Wunpost Rd	11150500	
SAR-CON	SAR	Just before confluence with Salinas River		
SAW-RIV	SAW	Riverside Rd		305COR
SBR-156	SBR	Hwy 156	11158600	305SAN
SBR-H25	SBR	Hwy 25	11156500	
SBR-HER	SBR	Unnamed ? Jeep Trail, Nr Hernandez	11156000	

SiteCode	WaterwayCode	Bridge/Road	USGSStationID	CCAMPID
SBR-SOU	SBR	Near Southside Rd	11158500	
SCC-SCC	SCC	Old Bridge, San Carpoforo Ck	11142550	
SLC-BIT	SLC	No bridge, Bitterwater Rd (USGS stn)	11151300	309LOR
SLC-CON	SLC	Just before confluence with Salinas River		
SLC-FIR	SLC	First Street (G15, King City)	11151500	309LOK
SLC-LON	SLC	No bridge ?, Nr Lonoak	11151000	
SLP-SLP	SLP	Bridge unknown, Salsipuedes Ck	11144200	
SLR-BIG	SLR		11160500	
SRC-SRC	SRC	No bridge ?, Santa Rosa Ck	11142200	
SSC-SSR	SSC	San Simeon Rd	11142300	
STANDAR	LS	Laboratory Standard		
STR-HAR	STR	Harkins Slough Rd.		305SSE
STR-LEE	STR	Lee Rd		305SSV
SUL-SUL	SUL	Sulphur Spring Rd	11149650	
TEC-GOL	TEC		11120530	
TEM-HAR	TEM	Tembladero Slough at Haro Rd.		
TEM-MOL	TEM	Molera Rd		309TDW
TEM-PRE	TEM	Preston Rd		309TEM
TES-FAI	TES	Fairview Rd		305TES
TOM-TOM	TOM	Toro Creek Rd	11142100	
TOP-TOP	TOP	No bridge ?, Toro Ck Nr Pozo	11144000	
TOW-OSR	TOW	Small br nr Old Stage Rd		
TRE-SOU	TRE	Southside Rd		305TRE
TRE-TRE	TRE	No bridge, Near Hwy 25	11157500	
U01-INL	U01	Confluence with Salinas River		
U02-INR	U02	Confluence with Salinas River		
UVA-BLO	UVA	Bloomfield Ave		305UVA
UVA-MOR	UVA		11153900	
V01-001	CON	Confidential		
V02-001	CON	Confidential		
VIE-OSR	VIE	Old Stage Rd		
VIN-CON	VIN	Just before confluence with Salinas River		
WAT-AND	WAT	San Andreas Rd		305WSH
WAT-HAR	WAT	Harkins Slough Rd		305WSE
WAT-LEE	WAT	Lee Rd		305WSW
WAT-PAJ	WAT	Pajaro Dunes Colony near confluence with Pajaro R		
WAT-SHE	WAT	Shell Rd at pump station		305WAT
WCT-H25	WCT	Hwy 25	11156450	
WIL-V6R	WIL	V6 Ranch dirt Rd		
STR-CHE	STR	Cherry Blossom Drive		
ZAY-ZAY	ZAY	Zayante Rd ?	11160300	

Appendix P

Proposed Quality Control Evaluation Form

(may be improved throughout the course of the project)

Technicians:
Sample collection date:
Lab analysis date:

Suspended Sediment Concentration:

Sample Replicates (precision or environmental variability):			
Sample ID	SSC Conc. (mg/L)	Standard Deviation	
		Coefficient Of Variance (%)	

Prepared Standard (accuracy) :			
Sample ID	Sediment Added (mg)	Sediment Recovered (mg)	Percent Difference (%)

Field Blank (field method assessment) :			
Sample ID	Blank SSC Conc. (mg/L)	Original SSC Conc. (mg/L)	Absolute Difference
		0	

Turbidity:

Sample Replicates (precision or environmental variability):			
Sample ID	Turbidity (NTU)	Standard Deviation	
		Coefficient Of Variance (%)	

Gelex Standard (accuracy):		
Standard Range (NTU)	Turbidity Reading (NTU)	Within Range (yes/no)

Field Blank (field method assessment) :			
Sample ID	Blank Turbidity (mg/L)	Original Turbidity (mg/L)	Absolute Difference
		0	

Inter-Laboratory Comparison (laboratory method assessment)			
Sample ID	CCoWS Turbidity (NTU)	MCHD Turbidity (NTU)	Percent Difference (%)

Fecal Coliform:

Sample Replicates (precision or environmental variability):			
Sample ID	Fecal Coliform (MPN/100mL)	Standard Deviation	
		Coefficient Of Variance (%)	

Field Blank (field method assessment):			
Sample ID	Blank Fecal Coliform (MPN/100mL)	Original Fecal Coliform (MPN/100mL)	Absolute Difference
		0	

Inter-Laboratory Comparison (laboratory method assessment)			
Sample ID	MCHD Fecal Coliform (MPN/100mL)	BioVir Fecal Coliform (MPN/100mL)	Percent Difference (%)

E. Coli:

Sample Replicates (precision or environmental variability):			
Sample ID	*E. Coli* (MPN/100mL)	Standard Deviation	
		Coefficient Of Variance (%)	

Field Blank (field method assessment) :			
Sample ID	Blank *E. Coli* (MPN/100mL)	Original *E. Coli* (MPN/100mL)	Absolute Difference
		0	

<u>Inter-Laboratory Comparison</u> (laboratory method assessment)			
Sample ID	MCHD *E. Coli* (MPN/100mL)	BioVir *E. Coli* (MPN/100mL)	Percent Difference (%)

Quality Assurance Manger:

_____

Date:

_____

Appendix Q

Source	Species	Life Stage	Exposure Concentration (mg/L)	Exposure Duration (h)	Fish Response	Reference
Newcombe and Jensen 1996	Smelt (rainbow)	Adult	3.5	168	Increased vulnerability to predation	Swenson (1978)
	Steelhead	Adult	500	3	Signs of sublethal stress	Redding and Schreck (1982)
	Steelhead	Adult	500	9	Blood cell count and blood chemistry change	Redding and Schreck (1982)
	Trout	Adult	16.5	24	Feeding behavior apparently reduced	Townsend (1983); Ott (1984)
	Trout	Adult	75	168	Reduced quality of rearing habitat	Slaney et al. (1977b)
	Trout	Adult	270	312	Gill tissue damaged	Herbert and Merkens (1961)
	Trout	Adult	525	588	No mortality (other end points not investigated)	Griffin (1938)
	Trout	Adult	300	720	Decrease in population size	Peters (1967)
	Trout (rainbow)	Adult	66	1	Avoidance behavior manifested part of the time	Lawrence and Scherer (1974)
	Trout (rainbow)	Adult	665	1	Overhead cover abandoned	Lawrence and Scherer (1974)
	Trout (rainbow)	Adult	100	0.10	Fish avoided turbid water	Suchanek et al. (1984a,1984b)

Source	Species	Life Stage	Exposure Concentration (mg/L)	Exposure Duration (h)	Fish Response	Reference
Newcombe and Jensen 1996	Trout (rainbow)	Adult	100	0.25	Rate of coughing increased	Hughes (1975)
	Trout (rainbow)	Adult	250	0.25	Rate of coughing increased	Hughes (1975)
	Trout (rainbow)	Adult	810	504	Gills of fish that survived had thickened epithelium	Herbert and Merkens (1961)
	Trout (rainbow)	Adult	17,500	168	Fish survived; gill epithelium proliferated and thickened	Slanina (1962)
	Trout (rainbow)	Adult	50	960	Rate of weight gain reduced	Herbert and Richards (1963)
	Trout (rainbow)	Adult	810	504	Some fish died	Herbert and Merkens (1961)
	Trout (rainbow)	Adult	270	3240	Survival rate reduced	Herbert and Merkens (1961)
	Trout (rainbow)	Adult	200	24	Test fish began to die on first day	Herbert and Richards (1963)
	Trout (rainbow)	Adult	18	720	Abundance reduced	Peters (1967)
	Trout (rainbow)	Adult	4,250	588	Mortality rate 50%	Herbert and Wakeford (1962)
	Trout (rainbow)	Adult	49,838	96	Mortality rate 50%	Lawrence and Scherer (1974)
	Trout (rainbow)	Adult	80,000	24	No mortality	D. Herbert, personal comm. to Alabaster and Lloyd (1980)
	Trout (rainbow)	Adult	3,500	1,488	Catastrophic reduction in population size	Herbert and Merkens (1961)

Source	Species	Life Stage	Exposure Concentration (mg/L)	Exposure Duration (h)	Fish Response	Reference
Newcombe and Jensen 1996	Trout (rainbow)	Adult	160,000	24	Mortality rate 100%	D. Herbert, personal comm. to Alabaster and Lloyd (1980)
	Trout (rainbow)	Yearling	90	456	Mortality rates 0-20%	Herbert and Merkens (1961)
	Trout (rainbow)	Yearling	90	456	Mortality rates 0-15%	Herbert and Merkens (1961)
	Trout (rainbow)	Yearling	270	456	Mortality rates 10-35%	Herbert and Merkens (1961)
	Trout (rainbow)	Yearling	810	456	Mortality rates 35-85%	Herbert and Merkens (1961)
	Trout (rainbow)	Yearling	810	456	Mortality rates 5-80%	Herbert and Merkens (1961)
	Trout (rainbow)	Yearling	270	456	Mortality rates 25-80%	Herbert and Merkens (1961)
	Trout (rainbow)	Yearling	7,433	672	Mortality rate 40%	Herbert and Wakeford (1962)
	Trout (rainbow)	Yearling	4,250	672	Mortality rate 50%	Herbert and Wakeford (1962)
	Trout (rainbow)	Yearling	2,120	672	Mortality rate 100%	Herbert and Wakeford (1962)
	Trout (rainbow)	Juvenile	4,887	384	Hyperplasin of gill tissue	Gouldes (1983)
	Trout (rainbow)	Juvenile	4,887	384	Parasitic infection of gill tissue	Gouldes (1983)
	Trout (rainbow)	Juvenile	171	96	Particles pentrated cells of branchial epithelium	Gouldes (1983)

Source	Species	Life Stage	Exposure Concentration (mg/L)	Exposure Duration (h)	Fish Response	Reference
Newcombe and Jensen 1996	Trout (rainbow)	Juvenile	4,315	57	Mortality rate ~100%	Newcombe et al. (1995)
	Carp (common)	Adult	25,000	336	Some mortality	Wallen (1951)
	Sunfish (green)	Adult	9,600	1	Rate of ventilation increased	Horkel and Pearson (1976)
	Stickleback (threespine)	Adult	28,000	96	No mortality in test designed to identify lethal threshold	LeGore and DesVoigne (1973)
Lloyd 1987	Rainbow Trout (Great Britain)	Juvenile	270 (ppm)		Reduced survival (marked)	Herbert and Merkens (1961)
	Rainbow Trout (Great Britain)	Juvenile	200 (ppm)		Reduced survival (marked)	Herbert and Richards (1963)
	Rainbow Trout (Oregon)	Juvenile	1,000–2,500 (ppm)		Reduced survival (marked)	Campbell (1954)
	Rainbow Trout (Great Britain)	Juvenile	90 (ppm)		Reduced survival (slight)	Herbert and Merkens (1961)
	Rainbow Trout (Great Britain)	Juvenile	50 (ppm)		Reduced growth (slight)	Herbert and Richards (1963)
	Rainbow Trout (Arizona)	Juvenile	<70 (JTU)		Reduced food conversion	Olson et al. (1973)

Source	Species	Life Stage	Exposure Concentration (mg/L)	Exposure Duration (h)	Fish Response	Reference
Lloyd 1987	Rainbow Trout (Arizona)	Juvenile	70 (JTU)		Reduced feeding	Olson et al. (1973)
	Rainbow Trout (Great Britain)	Juvenile	110		Reduced condition factor	Scullion and Edwards (1980)
	Rainbow Trout (Great Britain)	Juvenile	110		Altered diet (terrestrial instead of aquatic)	Scullion and Edwards (1980)
	Steelhead (Oregon)	Juvenile	2,000		Stress (increased plasma cortisol, hematocrit, and susceptibility to pathogens)	Redding and Schreck (1980)
	Rainbow Trout (Great Britain)	Juvenile	270 (ppm)		Disease (fin rot)	Herbert and Merkens (1961)
	Rainbow Trout (Great Britain)	Juvenile	100 (ppm); 200 (ppm)		Disease (fin rot)	Herbert and Merkens (1961)
	Steelhead (Idaho)	Juvenile	22–265 (NTU)		Avoidance	Sigler (1980), Sigler et al. (1984)
	Steelhead (Idaho)	Juvenile	40–50 (NTU)		Displacement	Sigler (1980)
	Rainbow Trout (Great Britain)	Juvenile	110		Displacement	Scullion and Edwards (1980)
	Trout		25 JTU		Altered behavior (feeding)	Langer (1980)

Source	Species	Life Stage	Exposure Concentration (mg/L)	Exposure Duration (h)	Fish Response	Reference
Newcombe and MacDonald (1991)	Rainbow trout		68	720	25% reduction in population size	Peters (1967)
	Rainbow trout		1,000–6,000	1,440	85% reduction in population size	Herbert and Merkens (1961)
	Steelhead		84	336	Reduction in growth rate	Sigler et al. (1984)
	Rainbow trout		50	1,848	Reduction in growth rate	Sykora et al. (1972)
Bell (1986)	Mosquitofish			181,500 (average)	fatal	Bell (1986)
	Largemouth bass			101,000 (average)	fatal	Bell (1986)
	Black crappie			145,000 (average)	fatal	Bell (1986)

Appendix R

Source: Newcombe and MacDonald (1991)

Taxon	Exposure Concentration (mg/L)	Exposure Duration (h)	Fish Response	Reference
Zooplankton	24	0.15	Reduced capacity to assimilate food	McCabe and O'Brien (1983)
Benthic invertebrates	8	2.5	Lethal: increased rate of drift	Rosenberg and Wiens (1978)
Macro invertebrates	53-92	24	Lethal: reduction in population size	Gammon (1970)
Benthic invertebrates	1,700	2	Lethal: alteration to community structure and drift patterns	Fairchild et al. (1987)
Zoobenthos	10-15	720	Lethal: reduction in standing crop	Rosenberg and Snow (1977)
Benthic invertebrates	8	1,440	Lethal: up to 50% reduction in standing crop	Rosenberg and Wiens (1978)
Cladocera	82-392	72	Lethal: survival and reproduction harmed	Robertson (1957); from Alabaster and Lloyd (1982)
Benthic fauna	29	720	Lethal: populations of Trichoptera, Ephemeroptera, Crustacea, and Mollusca, disappear	M.P. Vivier, personal comm. in Alabaster and Lloyd (1982)
Benthic invertebrates	16	1,440	Lethal: reduction in standing crop	Slaney et al. (1977b)
Cladocera and Copepoda	300-500	72	Lethal: gills and gut clogged	Stephan (1953) cited in Alabaster and Lloyd (1982)
Benthic invertebrates	32	1,440	Lethal: reduction in standing crop	Slaney et al. (1977b)
Zoobenthos	>100	672	Lethal: reduction in standing crop	Rosenberg and Snow (1977)
Benthic invertebrates	62	2,400	Lethal: 77% reduction in population size	Wagener and LaPerriere (1985)

Taxon	Exposure Concentration (mg/L)	Exposure Duration (h)	Fish Response	Reference
Benthic invertebrates	77	2,400	Lethal: 53% reduction in population size	Wagener and LaPerriere (1985)
Bottom fauna	261–390	720	Lethal: reduction in population size	Tebo (1955)
Benthic invertebrates	390	720	Lethal: reduction in population size	Tebo (1955)
Benthic invertebrates	278	2,400	Lethal: 80% reduction in population size	Wagener and LaPerriere (1985)
Stream invertebrates	130	8,760	Lethal: 40% reduction in species diversity	Nuttall and Bielby (1973)
Benthic invertebrates	743	2,400	Lethal: 85% reduction in population size	Wagener and LaPerriere (1985)
Benthic invertebrates	5,108	2,400	Lethal: 94% reduction in population size	Wagener and LaPerriere (1985)
Stream invertebrates	25,000	8,760	Lethal: reduction or elimination of populations	Nuttall and Bielby (1973)

Appendix S

MEMBRANE FILTRATION FOR MEMBERS OF THE COLIFORM GROUP (SM 9222)

The membrane filter (MF) technique is highly reproducible, can be used to test relatively large sample volumes, and usually yields numerical results more rapidly than the multiple-tube fermentation procedure. However, the MF technique has limitations, particularly when testing waters with high turbidity or large numbers of noncoliform (background) bacteria.

PRINCIPLE:

A sample volume which will yield 20 to 80 but no more than 200 colonies is filtered through a 0.45 um pore diameter membrane which is then incubated on an Endo-type medium containing lactose for 24 h at 35°C. Bacteria that produce a red colony with a metallic (golden) sheen are considered members of the coliform group. The coliform group is defined as those facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that develop red colonies with a metallic (golden) sheen. Atypical coliform colonies produce dark red, mucoid, or nucleated colonies without a metallic sheen. Generally, pink (non-mucoid), blue, white, or colorless colonies lacking sheen are considered non-coliform by this technique.

MATERIAL AND CULTURE MEDIA:

The lab assistant in the section will be responsible for ordering and preparing the necessary media following manufacturer's directions for rehydration. We are currently using mEndo LES Agar from Criterion.

QUALITY CONTROL PROCEDURES:

1) Agar should have final pH of 7.2 ± 0.2 and be stored in the refrigerator in the dark, preferably in sealed plastic bags to reduce moisture loss.

2) Performance check (analyst) - Test each batch of laboratory-prepared MF medium for performance with positive and negative culture controls. Check for coliform contamination at the beginning and end of each filtration series by filtering 20 to 30 mL of dilution or rinse water through the filter. If controls indicate contamination, reject all data from affected samples and request resample.

3) Discard unused agar after 2 weeks from the date of preparation.

SAMPLE CRITERIA:

1. Size of sample will be governed by expected bacterial density. In drinking water analyses, sample size will be limited only by the degree of turbidity or by the noncoliform growth on the medium. For regulation purposes, 100 ml is the official sample size and should be collected in pre-sterilized bottles.

2. When sample dilution is required for quantitative testing (i.e. recreational water) use sterile water not buffered water.

3. Samples that cannot be delivered to the laboratory within 1 hour should be transported in an ice cooler at 4^0C. A temperature blank should be included in the cooler and the temperature recorded on the requisition form. Samples should be refrigerated upon arrival and processed as soon as possible. At no time should the elapsed time between collection and processing exceed 8 hours.

4. All samples processed or received later than 8 hours are to be brought to the attention of the senior chemist or laboratory director.

5. Laboratory will invalidate samples and record on the worksheet "Notification for Resampling and Sample Invalidation" log whenever:
   a. Hold times for total coliforms are exceeded:
      1. Potable water – 30 hours after collection
      2. Wastewater – 6 hours after collection
      3. Source water – 8 hours after collection
   b. Insufficient sample volume (< 97.5 ml for drinking water).
   c. Improper collection container.

PROCEDURAL NOTES:

1. From Standard Methods, Table 9222:I Suggested Sample Volumes for MF Total Coliform Test
   Drinking Water = 100 mL
   Wells, springs = 100 – 50 – 10 mL
   Water supple intake = 10 – 1 – 0.1 mL
   Bathing beaches = 10 – 1 – 0.1 mL
   River water = 1 – 0.1 – 0.01 – 0.001 mL
   Chlorinated sewage = 1 – 0.1 – 0.01 mL
   Raw sewage = 0.1 – 0.01 – 0.001 – 0.0001 mL

MEMBRANE FILTRATION PROCEDURE:

1) Specimens are set-up on the day they arrive. Check each sample bottle for proper sample quantity. For drinking water a minimum of 97.5 ml of sample is required. For other waters, three different volumes (diluted or undiluted) will be used depending on the expected bacterial density. When less than 10 mL of sample (diluted or undiluted) is to be filtered, add approximately 10 mL sterile dilution water to the funnel before filtration or pipet the sample volume into a sterile dilution bottle, and then filter the entire dilution.

2) Sterilize smaller size funnels and supports (250 ml) for a minimum of two minutes using the UV box. Make sure that the funnels are dry before placing in box. For larger volumes (1 L) the funnels and supports are individually packaged and autoclaved.

3) Place the supports in the filter base unit, making sure not to contaminate the tops of the base units.

4) Dip the forceps in the 70% alcohol, flame and let cool. Or one can sterilize the forceps by placing them in the UV box for a minimum of two minutes. Aseptically open the filter package (0.45 +- 0.02 um pore size) and remove the filter with the forceps. Place the filter on the filter base, grid side up.

5) Carefully place the funnel on the support, keeping the filter centered on the support. Place the clamp on the funnel/support to seal the unit.

6) Check for coliform contamination at the beginning and end of each filtration series by filtering 20 to 30 mL of dilution or rinse water through the filter. If controls indicate contamination, reject all data from affected samples and request resample.

7) Shake the sample bottle vigorously about 25 times and measure the desired volume of sample in to the funnel with the vacuum off. To measure the sample accurately and obtain good distribution of colonies on the filter surface, use the following procedure.

   a) Sample volumes of 20 ml or more: Measure the sample in a sterile graduated cylinder and pour it into the funnel. Rinse the graduated cylinder and pour it into the funnel. Rinse the graduate cylinder twice with sterile dilution water; add the rinse to the funnel.
   b) Sample volumes of 10-20 ml: Measure the sample with a sterile 10 ml or 20 ml pipet into the funnel.
   c) Sample volumes of 1-10 ml: Pour about 10 ml of sterile dilution water into the funnel without vacuum. Add the sample to the sterile water using appropriate sterile pipet and filter the sample.
   d) Sample volumes of less than 1.0 ml: Prepare appropriate dilutions in sterile dilution water and proceed as applicable.
   e) To reduce the chance for carryover, when analyzing a series of samples or dilutions, filter sample in the order of increasing volumes of original sample. The time elapsing between preparation of sample dilutions and filtrations should be minimal and never more than 30 minutes.

8) After adding the sample to filter funnel, turn on vacuum and filter the sample. Rinse the sides of the funnel walls at least twice with 20-30 ml of sterile dilution water. Turn off the vacuum and remove the funnel from the filter base.

9) Flame forceps, cool and aseptically remove the membrane filter from the filter base. Place filter, grid side up, on the mEndo agar using a rolling motion to prevent air bubbles. Reseat the filter if bubbles occur.

10) If there are any water droplets adhering to the surface of the funnel, wipe dry with tissue. Place funnel in UV box and sterilize for a minimum of 2 minutes. Equipment will be ready for next filtration sample.

11) Insert a sterile rinse water sample (100 mL) after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water. Incubate the rinse water control membrane culture under the same conditions as the sample.

12) Place the plates, lid side down in the 35 $\pm$ 0.5^{o}C incubator and maintain a humid environment (60% relative humidity) for 24 hours. Note: Organisms from undisinfected sources may produce sheen at 16 to 18 h, and the sheen subsequently may fade after 24 to 30 h. To maintain a 60% relative humidity, place plates in an enclosed plastic (Rubbermaid) container on damp paper towels.

13) After 24 hours, remove the agar plates. Examine the plate for pink to dark-red colonies with a metallic (golden) surface sheen, which are typical coliform colonies. Atypical coliform colonies produce dark red, mucoid, or nucleated colonies without a metallic sheen. Generally, pink (non-mucoid), blue, white, or colorless colonies lacking sheen are considered non-coliform by this technique.

14) Count the number of typical and atypical coliform colonies using a low-power (10-15 magnifications) binocular wide-field dissecting microscope or other optical device, with a cool white fluorescent light source directed to provide optimal viewing of sheen. Note: a high count of noncoliform colonies may interfere with the maximum development of coliforms, if present, refrigerate cultures (after 22 h incubation) with high densities of

noncoliform colonies for 0.5 to 1 h before counting may deter spread of confluence with aiding sheen discernment. Do not use a microscope illuminator with optical system for light concentration from an incandescent light source for discerning coliform colonies on Endo-type media.

CALCULATION OF COLIFORM DENSITY AND REPORTING:

1) Using membrane filters with 20 to 80 coliform colonies and not more than 200 colonies of all types, compute the count by the following equation:

(Total) coliforms/100 mL = (coliform colonies counted x 100)/ mL sample filtered.

2) If no coliform colonies are observed, report the coliform colonies counted as "<1 coliform /100 mL."

3) For verified coliform counts, adjust the initial count based upon the positive verification percentage and report as "verified coliform count/100 mL."

Percentage verified coliforms = (number of verified colonies)/(total number of coliform colonies subjected to verification) x 100

4) To confirm the presence of coliforms, either transfer a few colonies or place the entire membrane filter culture into a sterile tube of brilliant green lactose bile broth. If gas is produced within 48 h at 35 ± 0.5oC, coliforms are present.

5) If confluent growth occurs, covering either the entire filtration area of the membrane or a portion thereof, and colonies are not discrete, report results as "confluent growth with (or without) coliforms."

6) If the total number of bacterial colonies, coliforms plus noncoliforms, exceeds 200 per membrane, or if the colonies are not distinct enough for accurate counting, report results as "too numerous to count" (TNTC) or "confluent," repectively.

7) After results have been entered into the computer the typist clerk will return worksheet to chemist. The chemist will review results and return to binder.

Notes:

1. Although the precision of the MF technique is greater than that of the MPN procedure, membrane counts may underestimate the number of viable coliform bacteria. Table 9222.II illustrates some 95% confidence limits.

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Revised for MF Testing by: T. Lam
Date: February 2003

Approved by:_____
Laboratory Director

Appendix T

Toxin Gene Biomarker Method
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Bacteria strains and DNA preparations: Enterotoxigenic *E. coli* strains containing the pTC201 plasmid with the *LTIIa* toxin gene, pDAS100 plasmid with the *STh* toxin gene, and pXE39 plasmid with the *ralG* toxin gene were grown in LB media (Difco, Detroit, Michigan) amended with 20 ug/mL of ampicillin. The plasmid pNS1 with the *hly*E was grown in LB media supplemented with 50 ug/mL of kanamycin. All strains, in LB media, were incubated at 37°C with agitation at 150 rpm overnight. A 1 mL aliquot of each bacterial culture was centrifuged at 12,000 x*g* for 5 minutes using a bench top Eppendorf Centrifuge (Model 5415D Netheler-Hinz, Hamburg, Germany). The pellet was collected and DNA extracted immediately or stored at 4°C until analysis.

Extraction of DNA: The pellet was collected for direct total DNA extraction by a freeze-thaw and phenol-chloroform method as described by Tsai and Olson (1991). The cultures of *E. coli* strains were stored in 30% glycerol at -80°C.

Nested PCR Amplification: Outer primers for the cow biomarker (Khatib et al., submitted 2001a) and human biomarker were obtained from previous research. The specificity of primers was determined in earlier studies (Khatib et al., submitted 2001a,b; 20). A second set of primers for each toxin trait was developed for nested PCR. These primers were tested for cross-reactivity by screening all sequences contained in GenBank (http://www.ncbi.nlm.nih.gov/) using BLAST Amplification was performed using 1-10 uL of DNA sample extract for each 50 uL reaction, which consisted of 5mM Tris-HCl (pH 8.3), 25 mM KCl, 1.0 mM $MgCl_2$, 100 uM of each dNTP, 0.2 uM of each primer, and 2.5 U of *Amplitaq* DNA polymerase (Perkin-Elmer or Promega). These mixtures were heated to 95°C for 1 minute followed by 30 cycles of 95°C for 30 s, 61°C (LTIIa) for 30 s or 57°C (STII) for 30 s, and 72°C for 30 s with final extension at 72°C for 6 minutes. Annealing temperatures for nested PCR of the LtIIa biomarker was at 56°C and 47°C, respectively. Amplification of the human biomarker was done at 94°C for 1 minute followed by 35 cycles of 94°C for 30 s, 45°C (50°C for nested PCR) for 30 s and 72°C for 30 s with final extension at 72°C for 6 minutes. All reactions were amplified in a Perkin Elmer (model 9600, version

1.05) DNA thermal cycler. All PCR amplicons were visualized through gel electrophoresis.

Real Time PCR: Amplification was performed using 10 mL DNA sample extract for each 50 mL reaction, which consisted of 5 mM Tris-HCl (pH 8.3), 25 mM KCl, 3.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each primer, 1 U of AmpliTaq DNA polymerase (Perkin-Elmer or Promega) and 50 nM Taqman Probe. These mixtures were heated to 94°C for 3 minutes followed by 45 cycles of 94°C for 15 s and 53°C for 1 minute with final extension at 72°C for 2 minutes. All reactions were amplified in ABI prism (Model 7000) sequence detection system. Positive and negative controls were run with each reaction.

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