Characterizing Freshwater Inflows and Sediment Reservoirs of Fecal Coliforms and *E. coli* at Five Estuaries in Northern California

prepared for **North Coast Regional Water Quality Control Board**

prepared by **University of California School of Veterinary Medicine and Cooperative Extension in Sonoma and Marin Counties**

in completion of **Standard Agreement 03-097-551-0**

April 2007

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Acknowledgements: Study is funded by the Costa-Machado Water Act of 2000 (Proposition 13) under contract with the California State Water Resources Control Board and North Coast Regional Water Quality Control Board.

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EXCUTIVE SUMMARY

Microbial contamination of California's bays, estuaries, and near-shore marine environments continues to impact the beneficial uses of these waters. These beneficial uses are vulnerable to microbial contamination from contaminated freshwater inflows and if occurring, resuspension of contaminated riverine, estuarine, and bay sediments during the rainfall season. Segregating and quantifying these potentially different reservoirs of bacteria will further improve the validity of monitoring methods, such as microbial source tracking, when conducted during storm flow conditions. It is also important that water quality monitoring protocols be able to clearly identify sources and reservoirs of bacterial contamination so that programs of remediation, such as the implementation plans of Total Maximum Daily Loads (TMDLs) and bond-measure supported projects, can effectively reduce pathogen pollution of coastal waters. To address this information need, we conducted a water quality and estuarine sediment survey of five northern California estuaries.

From north to south, we conducted this study in the estuaries of the Russian River, Salmon Creek, Estero Americano, Walker Creek, and Lagunitas Creek. These five estuaries typify the diversity in estuary type, drainage area, land use, and water body beneficial uses in the study region.

At each estuary, we established a two-dimensional sampling grid of 15 points (5 transects × 3 positions, L×W) to span the saltwater-freshwater transition zone. The five transects were numbered 1 through 5, with 1 being located at the mouth of the estuary (primarily saltwater) and 5 being the furthest upstream (primarily freshwater), typically at the first riffle. The three sampling positions at each transect were located at 25%, 50%, and 75% of the channel width facing downstream and moving left to right. At each sampling point, we collected a 1 liter water sample at the top, middle, and bottom of the water column. In addition, we collected a sediment sample from the upper layer of the estuary or river bottom. Each water sample was split into suspended solids and a residual water fraction, with fecal coliforms and *E. coli* then enumerated for each fraction of the original water sample. We conducted similar indicator bacteria enumeration for the estuarine or riverine sediments that underlay the column of water from which the water samples were taken. We also measured water temperature, dissolved oxygen, and discharge in the field and analyzed water samples in the laboratory for salinity, tubidity, and total suspended solids. Sediment samples were processed for particle size distribution. We also used a genomic fingerprinting method, referred to as BOX-PCR, to determine which isolates of *E. coli* were approximately identical to each other across this suspended sediment-water-bottom sediment continuum.

Findings from this investigation include:

- In general, mean fecal coliforms concentrations were above water quality criteria for shellfish harvesting in all three flow seasons (wet season base flow, wet season storm flow, dry season base flow), although it is important to point out that the majority of our sampling locations were upstream and not in shellfish harvesting areas. Mean bacterial concentrations in all five estuaries were close to shellfish harvesting criteria in the dry season base flow conditions. Mean concentrations for fecal coliforms grouped by season were above the criteria for non-contact recreation in all estuaries during wet season storm flow conditions, with the exception of Lagunitas Creek as

discussed below. Bacterial concentrations for Estero Americano exceeded the criteria for contact recreation during the wet season storm flow. Comparing mean bacteria concentrations grouped by transect to beneficial use water quality criteria indicate that Estero Americano waters at all five transects were above shellfish harvesting and noncontact recreation criteria. This is also true for values at Lagunitas Creek transects one and two. Values for the remaining four estuaries at all five transects were between the criteria for contact recreation and shellfish harvesting with the exception of Lagunitas Creek as discussed below.

- Relative to the other estuarine systems in the project, Lagunitas Creek exhibited the highest counts of bacteria during dry season base flow conditions. This suggests that the source of bacteria for Lagunitas Creek during summer is not the result of a source that relies on precipitation and saturated soils to create overland flow conditions, but is instead a source of bacteria that is directly discharged into the creek. Alternatively, the tidal regime and high residence time of water in the lower portion of Tomales Bay and therein Lagunitas Creek may be concentrating bacteria. Extending the sampling network to sites further upstream may identify the point or non-point source of these bacteria given the likely proximity of this bacteria source to Lagunitas Creek.
- A year-round reservoir of fecal coliforms and *E. coli* was identified in estuarine sediments. Estuary and stream sediment mean concentrations of fecal coliforms and *E. coli* ranged from 7.3 to 11.6 and 5.0 to 13.0 cfu/gm, respectively. This translates to mean loads ranging from 1,758,730 to 2,766,634 and 1,196,634 to 3,121,576 cfu/0.15 m^3 for fecal coliforms and *E. coli*, respectively. There was a 10-fold increase in the concentration of bacteria in sediment going from dry season base flow to wet season (winter-spring) storm flow conditions. One would expect that the conditions for bacterial replication in estuarine sediment would be more prevalent in summer compared to winter, suggesting that the source of bacteria driving these higher concentrations during winter-spring are from contaminated freshwater inflows and not bacterial replication of local, indigenous bacteria surviving in the sediment. Contradicting this assertion is the observation that none of the 787 pairs of bacteria (*E. coli* from sediment compared to *E. coli* from H2O or TSS) that were isolated during the same day of collection had matching DNA fingerprints (Tables 15A-15E). Either the *E. coli* located within estuarine sediments are independent from the *E. coli* transiting the estuary in the water column and whose source was somewhere upstream, or, if most of the *E. coli* do in fact originate from sources upstream of the estuary, then high levels of genetic diversity for this population of *E. coli* result in very low probabilities of a DNA match. This technical issue will need more investigation.
- Concentration of fecal coliforms and *E. coli* was highest in suspended sediments compared to residual water or bottom sediments when bacterial concentrations were standardized across these three matrices on a per gram basis (cfu/g). Nevertheless, suspended solids typically constituted less than 1% of the mass of 100 ml of water sample, which limited the amount of fecal coliforms and *E. coli* associated with suspended solids to a maximum of 12 percent of total bacteria enumerated in the original 100 ml composite water sample. In other words, for the fresh-to-saltwater transition zone, the majority of bacteria in the water column were in the water

fraction as single or aggregates of bacteria and not associated with suspended sediments.

- In general, incoming tidal conditions, low summer streamflow conditions, sampling near the bottom of the water column, or locating a sampling site in a predominately saline location (e.g., transect 1 or 2 down in the estuary) resulted in lower mean counts for fecal coliforms and commensal *E. coli* compared to the alternative condition (outgoing or ebb tide, winter or spring stormflows, sampling in the mid to upper reaches of the water column, and sampling at the upper freshwater transect sites). Sampling lateral to the midline of the channel nearer to the shoreline did not influence the mean count of fecal coliforms or commensal *E. coli* compared to the alternative condition of sampling in the middle of channel.
- *E. coli* isolated from bottom sediments rarely matches the *E. coli* isolated from the water fraction or the TSS fraction, suggesting that biases may occur with DNA fingerprinting if bottom sediments are incorporated into a water sample for microbial source tracking efforts. Furthermore, *E. coli* transiting the fresh-to-saltwater zone rarely match each other regardless of where they were isolated from (water, TSS, sediment), indicating a high degree of genetic diversity for this waterborne population of *E. coli*.

If and when we secure more long-term funding, we would like to build upon these findings by conducting an investigation into the key processes (tidal, climate, etc.) that govern the rate of dispersion of freshwater bacterial plumes discharging into an estuary and identify key environmental predictors that signal when these bacterial contaminants are present or absent in cultured or wild shellfish.

PROBLEM STATEMENT & RELEVENT ISSUES

Microbial contamination of California's bays, estuaries, and near-shore marine environments continues to impact the beneficial uses of these waters. Beneficial uses of the aquatic resources along the California coast range from shellfish harvesting and body contact recreation to recovery of threatened and endangered aquatic species. All of these beneficial uses are vulnerable to microbial contamination from contaminated freshwater inflows and if occurring, resuspension of contaminated riverine, estuarine, and bay sediments during the rainfall season.

A central challenge for resource agencies such as the Regional Water Quality Control Boards is to accurately identify and prioritize sources and reservoirs of bacterial contamination for these dynamic freshwater-coastal interfaces. More specifically, when estuaries experience elevated counts of fecal coliforms and *E. coli* during base- or storm flow conditions, it is important to determine if elevated bacterial counts are due to local resuspension of bacterially-contaminated estuarine sediments or due to freshwater inflows contaminated by upstream terrestrial sources. Segregating and quantifying these potentially different reservoirs of bacteria (local sediment versus upstream terrestrial sources) will further improve the validity of such methods as microbial source tracking when conducted during storm flow conditions.

It is also important that water quality monitoring protocols be able to clearly identify sources and reservoirs of bacterial contamination so that remediation efforts such as the implementation plans of Total Maximum Daily Loads (TMDLs) and Bond measure supported projects can effectively reduce pathogen pollution of coastal waters. The design of a remediation plan to restore water quality will be quite different if the source of elevated bacteria is attributable to the local estuarine environment as opposed to the result of upstream terrestrial sources such animal agriculture and pasture runoff. Failure to resolve this distinction could substantially reduce the effectiveness of TMDL plans or water quality projects designed to reduce pathogen loading, and delay the restoration of beneficial uses of California's bays, estuaries, and near-shore marine environments.

With this context in mind, we conducted a water quality and estuarine sediment survey of five northern California estuaries. This report documents the methods and outreach tasks we have completed to carry out this study, and provides a summary of the results of our investigation.

PROJECT GOALS & OBJECTIVES

The goal of our study is to facilitate the development of an improved water quality monitoring protocol for quantifying sources and transport processes of bacterial contamination for estuaries and to extend this information to regulatory staff, conservation organizations, and allied watershed groups. We achieved this through five project objectives.

OBJECTIVE 1: Quantify the reservoir of fecal coliforms and *E. coli* associated with estuarine sediments that are under direct hydrologic influence of freshwater inflows.

OBJECTIVE 2: Measure and contrast the reservoir of sediment-associated bacteria to the amount of bacteria transported via freshwater inflows that collectively function to elevate bacterial concentrations at the freshwater-coastal interface.

OBJECTIVE 3: For conditions of base- and storm flow, determine whether waterborne fluxes of *E. coli* measured along the freshwater-saltwater transition zone originate from local, indigenous bacteria present in bottom sediments of the estuary (local sources) or result from contaminated freshwater inflows likely from upstream sources.

OBJECTIVE 4: Using information from Objectives 1 through 3, develop an improved water quality monitoring protocol for characterizing nonpoint source pathogen contamination at the freshwater-coastal interface.

OBJECTIVE 5: Conduct workshops for regulatory personnel, watershed and conservation organizations involved in restoring water quality at the freshwater-coastal interface.

PROJECT DESCRIPTION

Project Type

This is a monitoring project.

Project Costs

Project funding came from the State Water Resources Control and North Coast Regional Water Quality Control Board through the Costa-Machado Act and Proposition 13. Matching funds were provided by the University of California Agricultural Experiment Station. Project costs are presented below in Table 1.

Table 1: Project costs including funds from the Costa-Machado Act and the University of California.

Costa-Machado Act	U.C.	Total
$306,736	$85,685	$392,421

Methodology

Overview

Studied estuaries included the Russian River, Salmon Creek, Estero Americano, Walker Creek, and Lagunitas Creek (Figure 1). These five were selected because, as a group, they represent the diversity in estuary type, drainage area, landuse, and water body beneficial uses that are typical in the study region. A brief description of each estuary and location of sampling transactions is provided in the Study Location portion of this report.

At each estuary, we established a two-dimensional sampling grid of 15 points (5 transects × 3 positions, L×W) that attempted to span the entire saltwater-freshwater transition zone (Figure 2). The five transects were numbered 1 through 5, with 1 being located at the mouth of the estuary (primarily saltwater) and 5 being the furthest upstream (primarily freshwater), typically at the first riffle. While facing downstream and during base flow conditions, the three sampling positions at each transect were located at 25%, 50%, and 75% of the channel width moving left to right. At each sampling point, we collected a 1L water sample at the top, middle, and bottom of the water column. In addition, we collected a sediment sample from the upper layer of the estuary or river bottom. Each water sample was split into suspended solids and a residual water fraction, with fecal coliforms and *E. coli* then enumerated for each fraction and the composite water sample. We conducted similar indicator bacteria enumeration for the estuarine or riverine sediments that underlay the column of water from which the water sample was taken. To determine which isolates of *E. coli* were related to each other across this suspended sediment-water-sediment continuum we used a genomic fingerprinting method referred to as BOX-PCR.

In addition to indicator bacteria and BOX-PCR measurements we made a number of field and laboratory analytical parameter measurements to enumerate potential drivers of

bacterial transport. These are discussed in detail in the following Sampling and Analyses section.

Figure 1: Project area and location of five studied northern California estuaries.

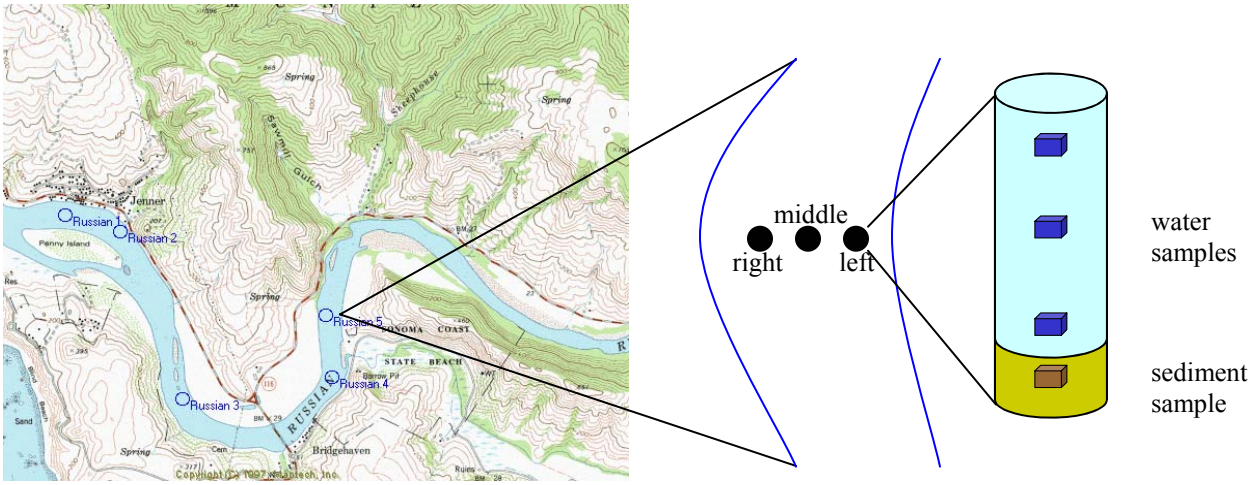


Figure 2: Map of the Russian River Estuary with the five sampling transect locations identified. Each transect consists of three sampling positions from which three water samples and one sediment sample will be collected.

Study Location

The five selected estuaries represent a variety of environmental conditions across the coast of Marin and Sonoma Counties. In a general sense, they have similar climate, precipitation, and hydrology. Specifically, this similarity is the result of the Mediterranean

climate in California, with cool wet winters and dry hot summers. As a result, all five estuaries experience an inflow of freshwater during the winter. The five estuaries differ in size, area of contributing watershed, and land use including agriculture, urbanization, and recreation. Additionally, the Russian River, Salmon Creek and Estero Americano are bar built estuaries while Walker and Lagunitas Creeks are part of the low inflow system of Tomales Bay. These differences and similarities provide a good context for understanding how these factors interact with bacteria movement in estuaries.

A brief description of each estuary is provided below. Complimenting these descriptions and the five study site maps (Figures 3 – 7), we have provided the coordinates of the five sampling transects within each of the studied estuaries in Appendix B. Photo point documentation of the sampling transects has been submitted under separate cover.

Russian River

The Russian River Estuary has a drainage area of 3,864 km^2. The estuary experiences varying periods of being closed and open, and of the five estuaries studied has the most inconsistent status in terms of estuary type but it predominately a bar-built system. Landuse includes dairy farming, livestock grazing, wine grape vineyards, urban and rural development, and some timber harvesting. The Russian River is a source of drinking water and is also popular for both contact and non-contact recreation.

Our five study transects for the Russian River start near the town of Jenner and end just upstream of the confluence with Willow Creek (Figure 3).

Salmon Creek

The Salmon Creek watershed and estuary is a small terminal system approximately one mile north of Bodega Bay (Figure 1). The estuary is generally closed with the exception of storm surges and peak high tide events. The village of Salmon Creek, California is located on the southern shore of the estuary. The total drainage area is 90 km^2. This estuary is primarily rural and dominated by livestock grazing and rural residential.

Our five study transects for the Salmon Creek Estuary start near Salmon Creek village with two on the west side of Highway 1 bridge and three on the east side (Figure 4). The study reach within the Salmon Creek Estuary was the shortest of the five we studied (Table 2).

Estero Americano

The Estero Americano is south and west of the town of Valley Ford, California (Figure 1). This estuary, like Salmon Creek, is typically closed expect for storm and tide events that open or over top the sand bar present at is terminus. It is the smallest of the five studied estuaries, with a drainage area of 80 km^2. Landuse in the watershed has traditionally been livestock grazing and dairy farming which continue today.

Our five study transects for the Estero Americano are all west of the Highway 1 bridge including the furthest downstream near the mouth (Figure 5). The study reach in Salmon Creek was the longest of the five we studied (Table 2).

Walker Creek

Walker Creek is one of the two major systems that drains into Tomales Bay (Figure 1). It is an open estuary that drains approximately 196 km^2. Similar to the Estero Americano, landuse in the watershed is typified by livestock grazing and dairy farming. Tomales Bay, including the mouth of Walker Creek are popular for contact and noncontact recreation and are used for commercial and recreational shellfish harvesting. The small town of Tomales is located north and east of the estuary on Keyes Creek, a tributary within the watershed.

Our five study transects for the Walker Creek are all west and north of the Highway 1 bridge (Figure 6).

Lagunitas Creek

Lagunitas Creek is the other major system contributing to Tomales Bay (Figure 1). It is an open estuary that drains approximately 241 km^2. Landuse in the watershed is a mix of recreational lands for hiking, livestock grazing and dairy farming, and several small towns including Point Reyes Station, Woodacre, and San Geronimo. The estuary has been popular for contact and noncontact recreation including swimming, canoeing, and kayaking. Shellfish harvesting is also conducted in the portion of Tomales Bay north of where Lagunitas Creek spills into it.

Our five study transects for the Lagunitas Creek are all west and north of the Highway 1 or "Green" bridge (Figure 7).

Table 2: Study reach and subreach length in the five northern California estuaries studied for bacterial transport dynamics.

	Length (kilometers)				
Reach	**Estero**	**Lagunitas**	**Russian**	**Salmon**	**Walker**
1 to 2	1.8	1.6	0.5	0.4	0.9
2 to 3	2.8	1.1	1.4	0.3	0.4
3 to 4	1.2	1.3	1.5	0.3	0.9
4 to 5	1.7	1.4	0.5	0.3	1.1
Total	7.4	5.3	4.0	1.3	3.3

Figure 3: Russian River sample transect locations.

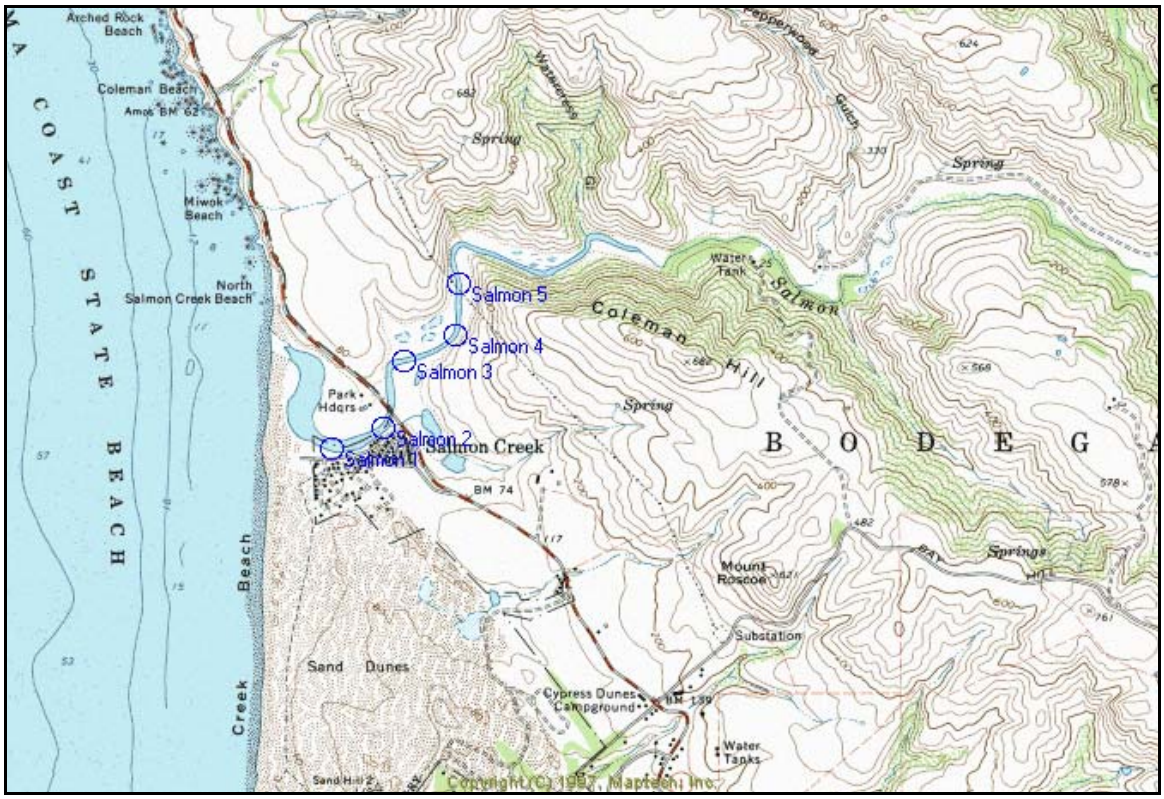


Figure 4: Salmon Creek sample transect locations.

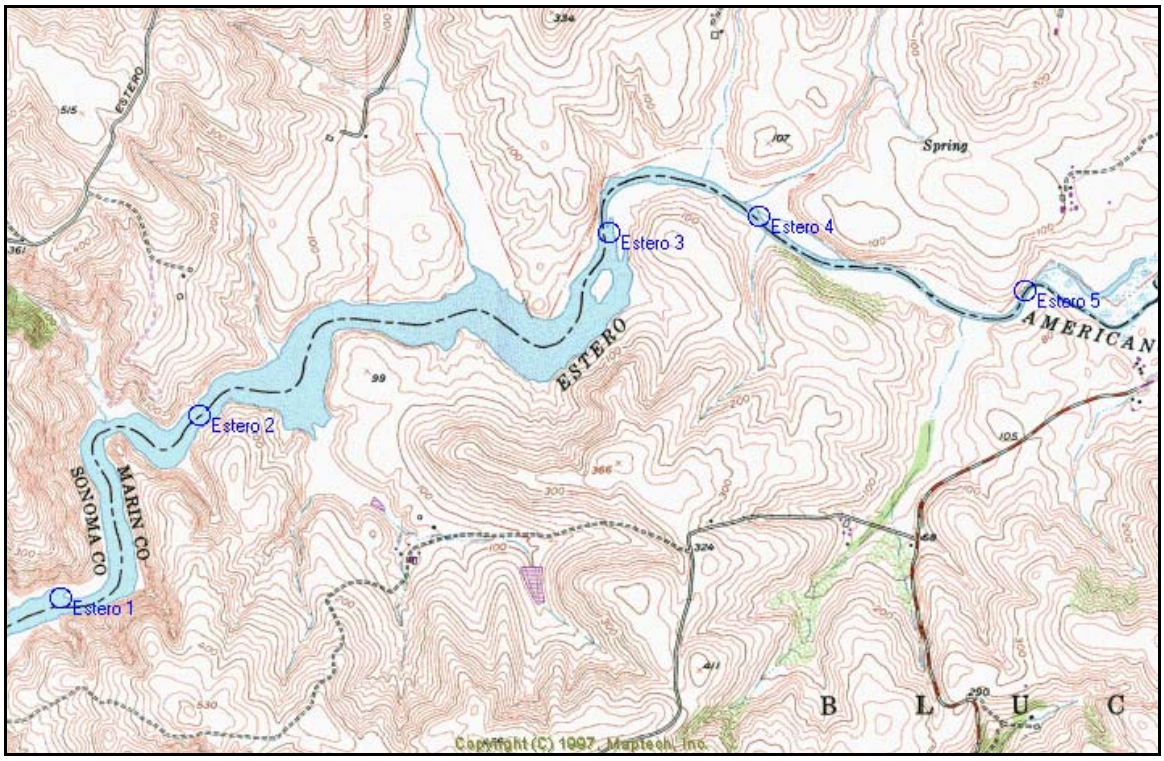


Figure 5: Estero Americano sample transect locations.

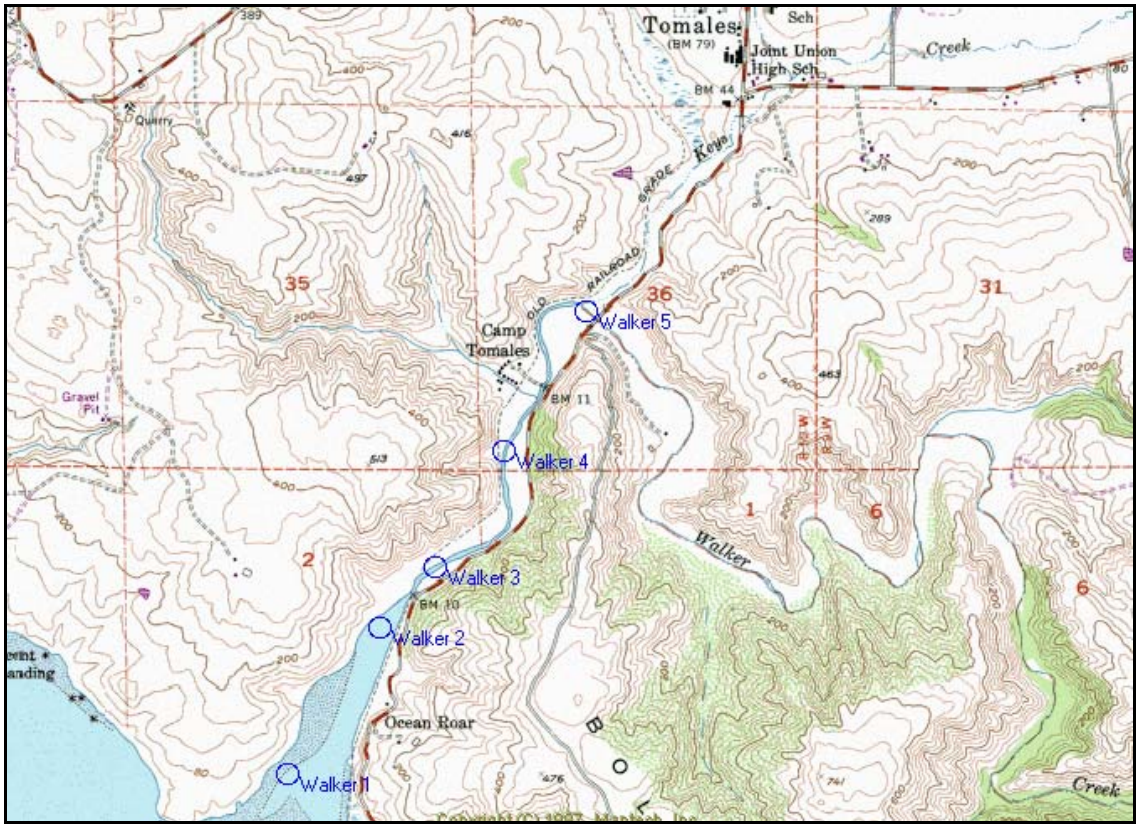


Figure 6: Walker Creek sample transect locations.

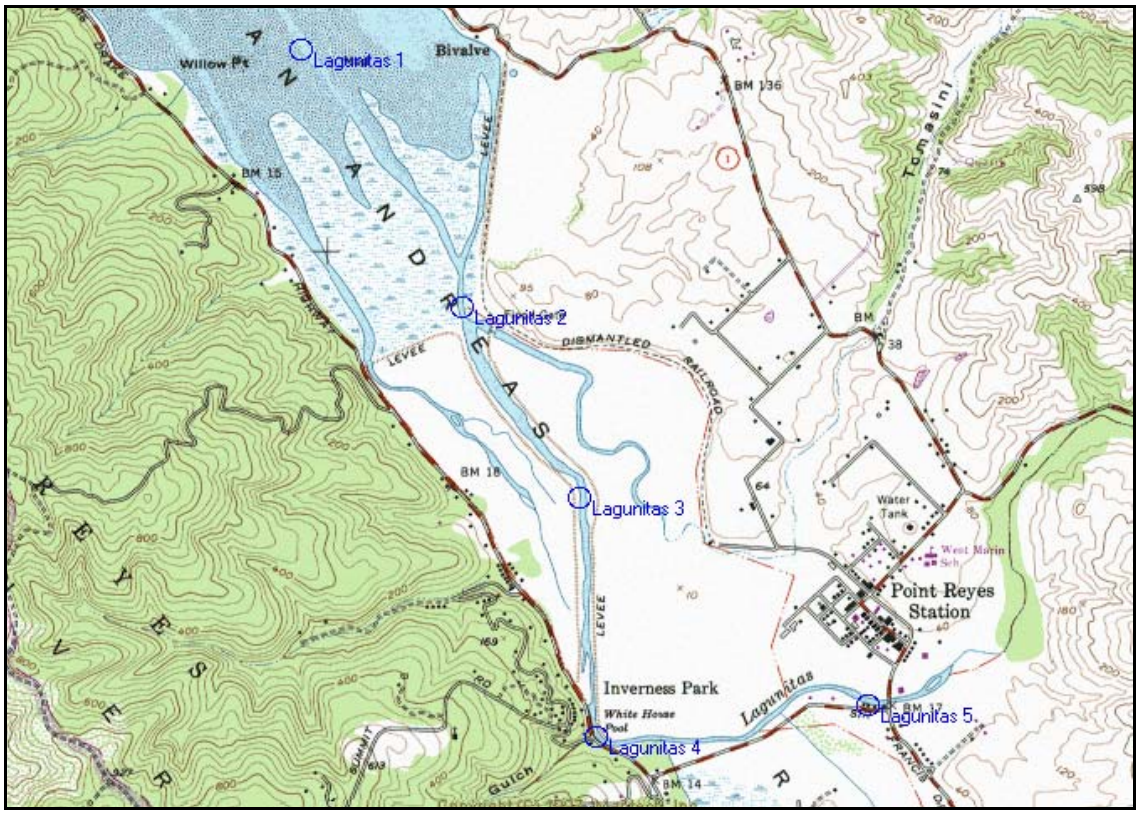


Figure 7: Lagunitas Creek sample transect locations.

Sampling and Analyses

For each of the five studied estuaries, we sampled each 15-point grid once per month for 10 months, beginning in August 2004 and ending June 2005 (Table 3). This allowed us to enumerate concentrations and loads of waterborne and sedimentborne bacteria for dry season base flow, wet season base flow, and wet season storm flow conditions.

Water samples were collected at each position from each of the three depths using an adapted depth sampler. With the sampler, we were able to place the sterile sample bottle at the desired depth and open it for collection of the sample. A 1L water sample was collected at ~30 cm (1 foot) below the surface, at the middle of the water column, and at ~30 cm (1 foot) above the bottom of the channel.

Estuarine and stream sediments were collected using an Eckman Dredge™ bottom sampler. At each sampling position, we dropped the sampler to the estuary or stream bottom and released is jaws to collect 250 to 500 gm of sediment from the top 15 cm of the sediment profile.

Parameters measured included six analytical laboratory and seven field parameters (Table 4), providing a useful combination of response and predictor variables that we used in our analysis to achieve the study objectives.

Table 3: Dates and season designations for the ten sampling events conducted in five northern California estuaries from August 2004 to June 2005.

Sampling Event	Estero Americano Sampling Date	Season Code	Lagunitas Creek Sampling Date	Season Code	Russian River Sampling Date	Season Code	Salmon Creek Sampling Date	Season Code	Walker Creek Sampling Date	Season Code
1	8/23/2004	3	9/13/2004	3	8/25/2004	3	8/16/2004	3	8/30/2004	3
2	9/27/2004	3	10/11/2004	3	9/29/2004	3	10/4/2004	3	9/20/2004	3
3	11/14/2004	3	11/1/2004	3	10/25/2004	3	10/19/2004	3	11/8/2004	3
4	12/12/2004	1	12/14/2004	2	12/6/2004	2	11/16/2004	2	11/29/2004	2
5	1/18/2005	2	2/7/2005	2	1/4/2005	1	1/24/2005	2	1/9/2005	1
6	2/16/2005	1	2/22/2005	1	1/31/2005	2	2/14/2005	1	3/7/2005	2
7	3/29/2005	1	4/3/2005	2	3/14/2005	2	3/28/2005	1	3/21/2005	1
8	5/10/2005	1	4/19/2005	2	4/5/2005	1	4/11/2005	2	4/18/2005	2
9	5/24/2005	2	5/3/2005	2	5/1/2005	2	4/26/2005	2	5/17/2005	2
10	6/7/2005	2	6/20/2005	2	6/13/2005	2	6/5/2005	2	6/27/2005	3

Wet-stormflow 1
Wet-baseflow 2
Dry-Baseflow 3

Table 4: Measured analytical laboratory and field parameters quantified for respective water, Total Suspended Solids (TSS), and estuarine or riverine sediment samples and estuary systems.

Parameter	Water	TSS	Sediment	Estuary
Analytical Laboratory Parameters				
Fecal coliforms	•	•	•	
E. coli	•	•	•	
Particle Size			•	
Turbidity	•			
Total Suspended Solids	•			
Salinity	•			
Field Parameters				
Dissolved Oxygen	•			
Temperature	•			
Discharge				•
Transect cross-sectional area				•
Annual Cumulative Precipitation				•
Tidal Stage				•
Windspeed				•

Analytical Laboratory Parameters

Water and total suspended solids. Water samples were analyzed within 6 to 96 hours after collection. Mean hour of analysis was 41 hours, with the extended times due to replating samples with too numerous colonies to county. Each 1 L sample was mixed by hand, partitioned into four 250 ml sterile bottles, and remixed on an automated wrist shaker (Burrell Scientific, Pittsburgh, PA) for 5 minutes at setting 7. Suspension was centrifuged (Thermo Electron Corp. Waltham, MA) at 1,000 *g* for 5 minutes. The supernatant (residual water) was removed using a sterile pipette and analyzed for fecal coliforms and *E. coli* as described below. The residual pellet was resuspended in 0.5 ml of sterile dionized water, the four aliquots pooled into a pre-weighed 2.0 ml microcentrifuge tube, and each 2.0 ml tube centrifuged at 14,000 *g* for 10 minutes. Supernatant was decanted and the weight of the pellet determined. The pellet was resuspended in 1,200 µl of phosphate buffered saline (Sigma-Aldrich, St. Louis, MO) (PBS), with 800 µl removed for *E. coli* enumeration and 400 µl removed for fecal coliform enumeration.

Sediment Analysis. Twenty grams of each sediment sample were placed into 250 ml conical bottle with 90 ml of PBS. Each suspension was mixed with an automated wrist shaker (Burrell Scientific, Pittsburgh, PA) for 5 minutes at setting 7 and then centrifuged at 500 *g* for 10 minutes. The supernatant was split into two separate 45 ml aliquots, with one analyzed for *E. coli* and the other for fecal coliforms.

Bacterial Enumeration. The residual water, suspended solids from the water sample, and the sediment suspension were analyzed for *E. coli* and fecal coliforms using direct membrane

filtration (American Public Health Association, 2005). For residual water, aliquots of up to 300 ml were filtered through a sterile membrane (47 mm diameter, 0.45 µm pore, Fisherbrand, Waltham, MA) using a sterile stainless steel manifold (Hydrolab, Waltham, MA). For *E. coli*, the membrane was placed onto CHROMagar EC (CHROMagar Microbiology, Paris, France) and incubated at 35°C for a 2hr resuscitation period, transferred to 44.5°C for another 23 h (± 2h), then colonies enumerated. For fecal coliforms, the membrane was placed onto mFC agar (Difco Laboratories, Detroit MI) and incubated at 44.5° C for 24hrs, then colonies enumerated and adjusted to cfu/100 ml. For suspended solids and sediment, 45 ml of sample suspension were processed as for residual water, described above.

DNA Extraction. Three suspect colonies of *E. coli* per sample were individually streaked onto MacConkey II Agar (Becton, Dickinson and Company, Sparks, MD) and incubated at 44.5EC for 24 hours. Biochemical confirmation was performed on a typical *E. coli* colony using Triple Sugar Iron, Urea, Citrate, Indol, Methyl-Red, and Voges-Proskauer. Gram staining was performed for confirmation of gram negative bacteria. Confirmed colonies of *E. coli* were grown at 37EC in 2 mL of Luria-Bertani broth Miller (EMD Chemicals Inc., Darmstadt, Germany). DNA was extracted using Qiagen DNeasy Tissue Kits (Qiagen Sciences, Valencia, MD) following the manufacturer's instructions for extraction of gram negative bacteria.

Box-PCR. PCR was performed on the extracted DNA samples as described by Rademaker and Bruijn (1997). Briefly, the master mix was comprised of 5 µL of 5H Gitschier buffer, 0.2 µl of 20 mg/ml Bovine Serum Albumin, 2.5 µl of 100% DMSO (Dimethyl sulfoxide), 1.25 µl of each dNTP (10 mM stock solution per dNTP), 1 µl of BOX primer (0.4 µM final concentration), and 13.65 µl of RNase and DNase-free water for each sample reaction. Twenty four µl of master mix was added to 1 µl of sample DNA (50 ng/µl) and the reaction tubes inserted into an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, CA). PCR started with a 7 minute incubation at 95ºC, followed by 35 cycles of 1 minute at 94 ºC, 1 minute at 52ºC, and 8 minutes at 65 ºC. Lastly, a 16 minute extension at 65 ºC was used to terminate the reaction. Samples were stored at 4 ºC.

Gel Electrophoresis. Gel electrophoresis was performed on the samples as described by Rademaker and Bruijn (1997). To prepare the gel, 3.75 g agarose (UltraPure™ Agarose, Invitrogen, Carlsbad, CA) was combined with 250 mL of 0.5H TAE (Tris-Acetate EDTA), and dissolved by microwaving. A 20H25 cm gel tray (Whatman Inc., Florham Park, NJ) and 30 tooth comb (1 mm) were used for the gel. Into each well, a mixture 6 µl of PCR product and 1.2 µl 6H loading dye were added. For the ladder, 2 µl of 1 kb DNA ladder (Invitrogen, Carlsbad, CA) 4 µl ddH_2O, and 1.2 µl 6H loading dye were combined and loaded. The gel was run at 35V for 18 hours. Gels were stained using 0.5 µg/ml ethidium bromide and destained in 0.5H TAE, each for 30 minutes. Band lengths were estimated under an ultraviolet light using a Gel Logic 200 Imaging System (Eastman Kodak Co., Rochester, NY).

DNA similarity analysis. Each single gel was saved as TIF file and imported into a software program, GelCompar II (Applied Maths, Inc., Austin, TX), for fingerprinting analysis. The following variables were entered for each *E. coli* isolate for statistical comparisons: system (Estero Americano, Lagunitas Creek, Salmon Creek, and Walker

Creeks, and Russian River), season (dry base, wet base, storm base), type of sample (water, suspended solids, and sediment), lateral position (left, middle and right), water depth (shallow, intermediate, deep for water and the suspended solids fraction and bottom for sediment), collection date, and transect (1, 3 or 5). The Box-PCR fingerprint was developed by first normalizing gels using standard markers, and selecting bands for comparison. For each isolate, an internal reference position was assigned and bands below 500 bases and above 4000 bases were excluded. Then, for each estuary, each set of isolates from a single collection date were analyzed for their molecular similarity by first setting values for the tolerance and optimization so that the two or more standards present on the gels were classified as similar. Using these values for the tolerance and optimization, comparisons of all isolates obtained from the collection date were compared for 100% similarity using the band similarity and the dendrogram constructed by the UPGMA.

Total Suspended Solids. A 100ml subsample of each composite water sample was analyzed for total suspended solids or the concentration (unit weight/unit volume; mg/L) of mineral and organic sediment. This was determined by the weight of dry solid material remaining after vacuum filtration of a known sample volume (50 to 100 ml). Samples for this study were filtered through a 0.45 micron filter in accordance with American Public Health Association protocols (Clesceri et al., 1998).

Turbidity. A 10ml subsample of each composite water sample was analyzed for turbidity or water clarity. We measured this with a HF Scientific, Inc. DRT-15CE turbidimeter and nephelometry methodology, recording values in nephelometric turbity units (ntu). Samples for this study were analyzed according to the American Public Health Association protocols (Clesceri et al., 1998).

Salinity. A 3ml subsample of each composite water sample was analyzed for salinity. This was done using a salinity refractometer with automatic temperature compensation (Fisher Scientific Catalog Number 12-946-27). Values were recorded for each sample in parts per thousand (ppt).

Sediment Particle Size Distribution. Using a series of Fisher Scientific, Inc. sieves and a Mienzer, Inc. sieve shaker, we processed each sediment sample, according to protocols outlined in Gee and Bauder (1986), to differentiate the respective contributions of coarse gravel, fine gravel, coarse sand, fine sand, silt, and clay to the entire sample. Results were recorded in units of mass and percentage of entire sample.

Field Parameters

Dissolved Oxygen. In the field we used a Yellow Springs Instruments Model 550A ™ to measure dissolved oxygen at each sample position. Values were recorded in mg/L.

Temperature. In the field we used a Yellow Springs Instruments Model 550A ™ to measure water temperature at each sample position. Values were recorded in degrees Celsius.

Discharge. At each site where a water sample was taken, instantaneous flow was measured using a Global Waters flow meter (Global Waters Inc., Gold River, California, USA) as well

as the sample transect cross-sectional area. These measurements were used in area-velocity method (velocity x channel width x channel depth) to calculate flow volume in cubic feet a second and cubic meters per second (Mosley and McKercher, 1993).

Annual Cumulative Precipitation. Annual cumulative precipitation for each sample event was recorded based upon existing precipitation monitoring systems. These include data from stations operated by Marin County in Point Reyes Station, by University of California Bodega Marine Laboratory in Bodega, and California Department of Foresty and Fire Prevention in Santa Rosa. Values were recorded in mm.

Tidal Stage. Tidal stage data was recorded based upon tide tables for Tomales Bay, Bodega Bay and Jenner using the appropriate and approved time adjustments for sample locations. In addition to maximum or minimum tidal stage

Windspeed. Average daily windspeed for each sample event was recorded based upon existing climate monitoring systems. This includes data from stations operated by Marin County in Point Reyes Station, by University of California Bodega Marine Laboratory in Bodega, California Department of Foresty and Fire Prevention in Santa Rosa and windspeed data reported at www.IWINDSURF.com.

Data Analyses and Statistical Approaches

Adjusting bacterial indicator concentration to a 24-hour standard

Differences in the number of hours needed to sample each estuary resulted in different intervals of time between sample collection and processing at the analytical laboratory. In order to adjust bacterial indicator (fecal coliforms, *E. coli*) enumerations to a single time-duration standard of 24 hours, we conducted a time-dependent decay analysis for fecal coliforms and *E. coli*. For each estuary site (n=5) and for each season (n=3, dry baseflow, wet baseflow, wet stormflow), two water and two sediment samples were collected on different dates (n=2). For each of these 30 sampling dates, fecal coliforms and *E. coli* were enumerated in residual water, suspended solids, and sediment supernatant using membrane filtration as described above at approximately 4, 8, 24, 30, 48, and 54 hours post-collection in order to generate the necessary raw data for statistical modeling of the decay coefficients in our source water.

A linear mixed effects regression model (Pinheiro and Bates, 2000) was used to estimate the magnitude and significance of the time-dependent decay coefficients for fecal coliforms and *E. coli* in our source water. The $\log_{10}$ concentration of each bacterial indicator was used as the outcome variable, site, season and time (duration in hours between water collection in the field and bacterial enumeration in the laboratory) were set as fixed effects, and the group of enumeration for the same sample date were set as a repeated measure (group random effect) to control for potential lack of independence of bacterial concentration. In addition, given marked heteroscedasticity of the error term across time, an exponential variance term was included in the model (Pinheiro and Bates, 2000). Level of significance for the various terms was set at P-value<0.05, based on either a likelihood ratio test for interaction terms or a conditional t-test for main terms. Results of this analysis identified significant decay coefficients for *E. coli* in water and sediment related to season and estuary (Table 5).

Table 5: Estimated time-dependent decay coefficients for adjusting fecal coliforms and *E. coli* to a 24 hour duration standard, stratified by matrix (residual water, suspended solids, sediment) and indicator bacteria (fecal coliforms, *E. coli*)

Factors	Coefficient[c]	*P*-value[b]
Water - Fecal coliforms	N.S.	>0.10
Water - *E. coli*		
Duration (hr)	-0.00021	0.92
Duration H Season interaction		
wet-storm[a]	0.0	--
wet-base	-0.0045	0.02
dry-base	-0.0078	0.0001
Duration H Site interaction		
Russian River[a]	0.0	--
Walker Creek	0.0023	0.37
Salmon Creek	-0.00034	.90
Estero Americano	0.0072	0.005
Lagunitas Creek	0.00063	0.82
Suspended solids - Fecal coliforms	N.S.	>0.10
Suspended solids - *E. coli*	N.S.	>0.10
Sediment - Fecal coliforms	N.S.	>0.10
Sediment - *E. coli*		
Duration (hr)	0.0043	0.04
Duration H Season interaction		
wet-storm[a]	0.0	--
wet-base	-0.0026	0.32
dry-base	-0.0054	0.03

[a] Referent condition for the categorical variable.
[b] Adjusted for potential lack of independence due to repeated sampling of high use areas across storms.

In order to adjust the bacterial indicator (BI) concentration in each sample matrix (residual water, suspended solids, sediment) tested *x* hours (t=x) after initial time of collection (t=0) to a single 24-hour standard (t=24), we first assumed the following basic model,

$$\log_{10}(BI_{t=x}) = \log_{10}(BI_{t=0}) + \beta(t = x) \qquad (1)$$

whereby $\log_{10}(BI_{t=x})$ is the observed $\log_{10}$ concentration of the bacterial indicator determined x hours (t=x) after initial time of collection, $\log_{10}(BI_{t=0})$ is the modeled $\log_{10}$ concentration of the bacterial indicator at the initial time of collection (t=0), and $\exists(t=x)$ is the fitted decay coefficient generated by the linear mixed effects model described above. The decay process is for samples held at approximately 4°C. Once $\exists(t=x)$ is obtained, equation (2) is used to adjust each sample to a single 24-hour standard (t=24), which is derived as follows,

$$\log_{10}(BI_{t=24}) = \log_{10}(BI_{t=0}) + \beta(24)$$
$$\log_{10}(BI_{t=24}) = \log_{10}(BI_{t=x}) - \beta(x) + \beta(24)$$
$$\log_{10}(BI_{t=24}) = \log_{10}(BI_{t=x}) + \beta(24 - x)$$
$$BI_{t=24} = (BI_{t=x})\, 10^{\beta(24-x)} \qquad (2)$$

whereby $BI_{t=24}$ is the fitted or expected concentration of the bacterial indicator at a 24-hour standard, $BI_{t=x}$ is the observed concentration of the bacterial indicator determined x hours (t=x) after initial time of collection, and $10^{\exists(24-x)}$ is the expected decay coefficient adjustment factor raised to the power of 10 which allows us to model concentrations of the bacterial indicator directly instead of $\log_{10}$ concentration values.

Summary Statistics

Summary statistics included mean and standard error values for indicator bacteria concentrations and loads in the water, suspended solids, and sediment fractions. This included summary values by estuary by season. We developed these using S-plus Professional Version 6.1 (Insightful Corporation, 2002, Lucent Technologies Inc.©).

Modeling

Linear mixed effects regression (Pinheiro and Bates, 2000) was used to identify factors such as transect location, depth of water sample, and season and flow regime classification (dry season base flow, wet season base flow, wet season storm flow) cumulative annual rainfall, and tidal factors associated with indicator bacteria values. Fecal coliforms and *E. coli* concentrations and instantaneous loads functioned as the outcome variables, with each sample location (estuary, transect, position, depth) as a group effect to adjust the *P*-values for repeated sampling at the same sites. We employed a forward stepping approach in final modeling to develop the multivariate regression model, with $P<0.05$ set as the criterion for inclusion of predictor variables in the final model.

Results and Discussion

Sedimentborne Bacteria

Concentration

A maximum of 150 sediment samples were collected from each estuary during the ten respective sampling events. Fecal coliforms was greatest in sediment from the Salmon Creek estuary followed by Estero Americano, Walker Creek, Lagunitas, and then Russian River (Table 6). Similarly, *E. coli* concentrations were also greatest in Salmon Creek Estuary sediments followed by Estero Americano, Walker Creek, Russian River, and then Lagunitas (Table 6). Regression analysis confirms a significant direct relationship between the percentage of clay and silt particles and fecal coliforms and *E. coli* concentrations in analyzed estuarine and riverine sediment samples.

Table 6: Mean fecal coliforms and *E. coli* concentrations (cfu/gm) in sediment from five northern California estuaries.

	Fecal coliforms (cfu/gm)		***E. coli*(cfu/gm)**	
Estuary	mean	standard error	mean	standard error
Russian	7.3	1.5	5.7	1.2
Salmon	11.6	3.0	13.0	3.2
Americano	10.4	1.9	8.2	1.5
Walker	9.5	2.0	7.8	1.9
Lagunitas	8.7	2.8	5.0	1.0

Load

We calculated the bacterial load within the upper layer of estuarine sediment by multiplying the measured bacterial concentrations in each sediment sample by one meter squared by 15 cm (6 inches) of depth. This depth value represents the maximum depth from which the sediment sampler retrieved a sample. These calculations resulted in 10 measurements of fecal coliforms and *E. coli* load at each position and 150 measurements per estuary.

To integrate these individual estimations, we also calculated the total bacterial load for fecal coliforms and *E. coli* for each of the ten sample events within each studied estuary. We did this by multiplying the mean of the individual load estimations by the study reach dimensions (Table 7) for each of the ten sample events conducted in each estuary.

Keeping with the patterns of the concentration results, the load of fecal coliforms and *E. coli* in the upper layer of estuarine sediment was greatest in the Salmon Creek Estuary followed by Estero Americano, Walker Creek, Lagunitas Creek, and Russian River, respectively (Table 7).

Table 7: Fecal coliforms and *E. coli* load per square meter in the upper layer of sediment within five northern California estuaries.

	Fecal coliforms (cfu/0.15 m^3)				***E. coli* (cfu/0.15 m^3)**			
Estuary	mean	standard error	minimum*	maximum	mean	standard error	minimum*	maximum
Russian	1,758,730	365,805	0	24,240,000	1,367,750	293,857	0	22,320,000
Salmon	2,766,634	706,665	0	76,800,000	3,121,576	767,024	0	81,600,000
Americano	2,489,560	455,796	0	34,320,000	1,968,592	348,089	0	24,480,000
Walker	2,269,703	486,954	0	39,120,000	1,882,993	448,636	0	43,680,000
Lagunitas	2,090,000	674,049	0	72,900,000	1,196,634	228,025	0	18,960,000

Notes:
* Value for indicator bacteria in sediment sample was below detection limit resulting in a concentration value of zero and therein a load value of zero.

The total load for fecal coliforms and *E. coli* within the studied reaches varied as a function of the reach length (Table 2) and the indicator bacteria concentrations (Table 6). As a result, the greatest total load estimated was in the Estero Americano, the longest reach

studied, and the lowest value estimated was in Salmon Creek, the shortest reach studied (Table 8).

Table 8: Total Fecal coliforms and *E. coli* load in the upper layer of sediment across the length of the studied reach within five northern California estuaries.

	Fecal coliforms				***E. coli***			
Estuary	mean	standard error	minimum*	maximum	mean	standard error	minimum*	maximum
Russian	$6.5X10^{11}$	$3.3X10^{11}$	$8.1X10^{09}$	$3.1X10^{12}$	$5.1X10^{11}$	$2.7X10^{11}$	$5.1X10^{10}$	$2.7X10^{12}$
Salmon	$7.4X10^{10}$	$3.8X10^{10}$	$2.9X10^{09}$	$3.2X10^{11}$	$8.4X10^{10}$	$4.3X10^{10}$	0	$3.7X10^{11}$
Americano	$5.6X10^{11}$	$3.1X10^{11}$	0	$3.5X10^{12}$	$4.4X10^{11}$	$2.6X10^{11}$	0	$2.7X10^{12}$
Walker	$3.9X10^{11}$	$1.8X10^{11}$	$4.6X10^{09}$	$2.2X10^{12}$	$3.2X10^{11}$	$1.8X10^{11}$	$2.4X10^{09}$	$1.6X10^{12}$
Lagunitas	$4.5X10^{11}$	$2.6X10^{11}$	$6.2X10^{10}$	$2.7X10^{12}$	$2.5X10^{11}$	$8.6X10^{10}$	$4.6X10^{10}$	$8.1X10^{11}$

Notes:
* Value for indicator bacteria in sediment sample was below detection limit resulting in a concentration value of zero and therein a load value of zero.

Waterborne Bacteria

Concentration

A maximum of 750 water samples were collected from each estuary during the ten respective sampling events. This resulted in three respective values for fecal coliforms and *E. coli* for each sample, one for suspended solids, one for residual water, and one for the composite water sample.

Mean fecal coliforms concentrations was greatest in suspended sediment from the Estero Americano estuary followed by Salmon Creek, Walker Creek, Lagunitas, and then Russian River (Table 9). Similarly, E. coli concentrations were also greatest in Estero Americano suspended sediments followed by Salmon Creek, Walker Creek, Russian River, and then Lagunitas (Table 9). Results were similar for residual (Table 10) and composite water (Table 11).

Table 9: Fecal coliforms and *E. coli* concentration in suspended sediment within five northern California estuaries.

	Fecal coliforms (cfu/gm)				***E. coli*(cfu/gm)**			
Estuary	mean	standard error	minimum*	maximum	mean	standard error	minimum*	maximum
Russian	11	1	0	240	7	0.8	0	179
Salmon	24	4	0	592	19	3	0	416
Americano	48	9	0	1,600	25	4	0	565
Walker	5	0.4	0	83	4	0.3	0	41
Lagunitas	5	0.3	0	100	5	0.5	0	198

Notes:
* Value for indicator bacteria in sediment sample was below detection limit resulting in a concentration value of zero and therein a load value of zero.

Table 10: Fecal coliforms and *E. coli* concentration in residual water within five northern California estuaries.

	Fecal coliforms (cfu/ml)				*E. coli*(cfu/ml)			
Estuary	mean	standard error	minimum*	maximum	mean	standard error	minimum*	maximum
Russian	61	4	0	667	47	4	0	585
Salmon	185	21	0	2,689	171	18	0	2,116
Americano	953	99	0	21,110	576	60	0	14,413
Walker	115	10	0	2,355	85	7	0	2,277
Lagunitas	111	5	0	933	136	10	0	1,894

Notes:
* Value for indicator bacteria in sediment sample was below detection limit resulting in a concentration value of zero and therein a load value of zero.

Table 11: Fecal coliforms and *E. coli* concentration in composite water sample within five northern California estuaries.

	Fecal coliforms (cfu/ml)				*E. coli*(cfu/ml)			
Estuary	mean	standard error	minimum*	maximum	mean	standard error	minimum*	maximum
Russian	71	5	0	709	54	4	0	620
Salmon	206	23	0	3037	189	20	0	2,279
Americano	1,033	106	0	21,846	601	62	0	14,730
Walker	120	10	0	2,439	88	7	0	2,313
Lagunitas	115	5	0	943	142	10	0	1,927

Notes:
* Value for indicator bacteria in sediment sample was below detection limit resulting in a concentration value of zero and therein a load value of zero.

Load

To explore the load of indicator bacteria within each estuary we calculated the instantaneous flux of fecal coliforms and *E. coli* at each transect during each sampling event for the suspended sediment, residual water, and composite water fractions. We did this by using the area velocity method (Mosley and McKercher, 1993). This involved multiplying the average of the nine values for fecal coliforms or *E. coli* concentration for each fraction by the cross-section area and velocity measured at each transect at the time of sampling. The result of these calculations is ten measurements of flux for fecal coliforms and *E. coli* at each transect or 50 measurements per estuary.

When analyzing the flux values across the five estuaries it is important to keep in mind the role that the cross-sectional area size and flow velocity have in driving flux values. For example, the Russian River and Estero Americano transects are the largest in cross-sectional area. Similarly, flow velocity was the greatest during wet season storm flow followed by wet season base flow and dry season base flow.

Mean fecal coliforms flux associated with suspended solids was greatest for the Russian River followed by Salmon Creek, Walker Creek, Estero Americano, and Lagunitas

Creek (Table 12). Mean *E. coli* flux associated with suspended solids differed slightly from fecal coliforms with the Estero Americano and Lagunitas Creek trading places in the order.

Table 12: Instantaneous flux of fecal coliforms and *E. coli* associated with suspended solids fraction.

	Fecal coliforms (cfu/sec)				***E. coli*(cfu/sec)**			
Estuary	mean	standard error	minimum*	maximum	mean	standard error	minimum*	maximum
Russian	11,752	428	0	77,575	8,789	337	0	74,325
Salmon	5,019	313	0	76,474	3,908	243	0	54,118
Americano	1,788	78	0	22,641	1,050	41	0	9,155
Walker	2,092	120	0	30,823	1,568	90	0	22,417
Lagunitas	1,423	53	0	22,641	1,249	51	0	9,155

Notes:
* Value for indicator bacteria in sediment sample was below detection limit resulting in a concentration value of zero and therein a load value of zero.

Table 13: Instantaneous flux of fecal coliforms and *E. coli* associated with the residual water fraction.

	Fecal coliforms (cfu/sec)				***E. coli*(cfu/sec)**			
Estuary	mean	standard error	minimum*	maximum	mean	standard error	minimum*	maximum
Russian	69,031	2,414	0	500,444	52,508	1,927	0	362,739
Salmon	31,684	1,821	0	332,760	28,220	1,649	0	320,408
Americano	78,370	4,370	0	1,319,905	81,172	5,024	0	1,546,856
Walker	34,729	1,811	0	501,872	32,914	1,997	0	558,833
Lagunitas	20,430	582	0	1,319,905	17,485	476	0	1,546,856

Notes:
* Value for indicator bacteria in sediment sample was below detection limit resulting in a concentration value of zero and therein a load value of zero.

Table 14: Instantaneous flux of fecal coliforms and *E. coli* associated with the composite water fraction.

	Fecal coliforms (cfu/sec)				***E. coli*(cfu/sec)**			
Estuary	mean	standard error	minimum*	maximum	mean	standard error	minimum*	maximum
Russian	80,783	2,814	0	551,871	61,297	2,255	0	413,899
Salmon	36,703	2,126	0	403,891	32,128	1,888	0	360,744
Americano	80,158	4,398	0	1,325,079	82,221	5,038	0	1,550,932
Walker	36,822	1,929	0	532,694	34,482	2,085	0	580,246
Lagunitas	21,783	625	0	1,325,079	18,734	523	0	1,550,932

Notes:
* Value for indicator bacteria in sediment sample was below detection limit resulting in a concentration value of zero and therein a load value of zero.

The mean flux values for residual and composite water differ from those of suspended solids across the five estuaries in one significant way. The values in the Estero Americano are as great or greater than in the Russian River (Tables 13 and 14).

The contribution that suspended solids associated fecal coliforms and *E. coli* made to the flux in composite water of these indicator bacteria ranged from approximately 2 to 15 percent (Figure 8). Suspended solids contributions were greatest in the Russian River and Salmon Creek followed by Lagunitas and Walker Creeks and Estero Americano.

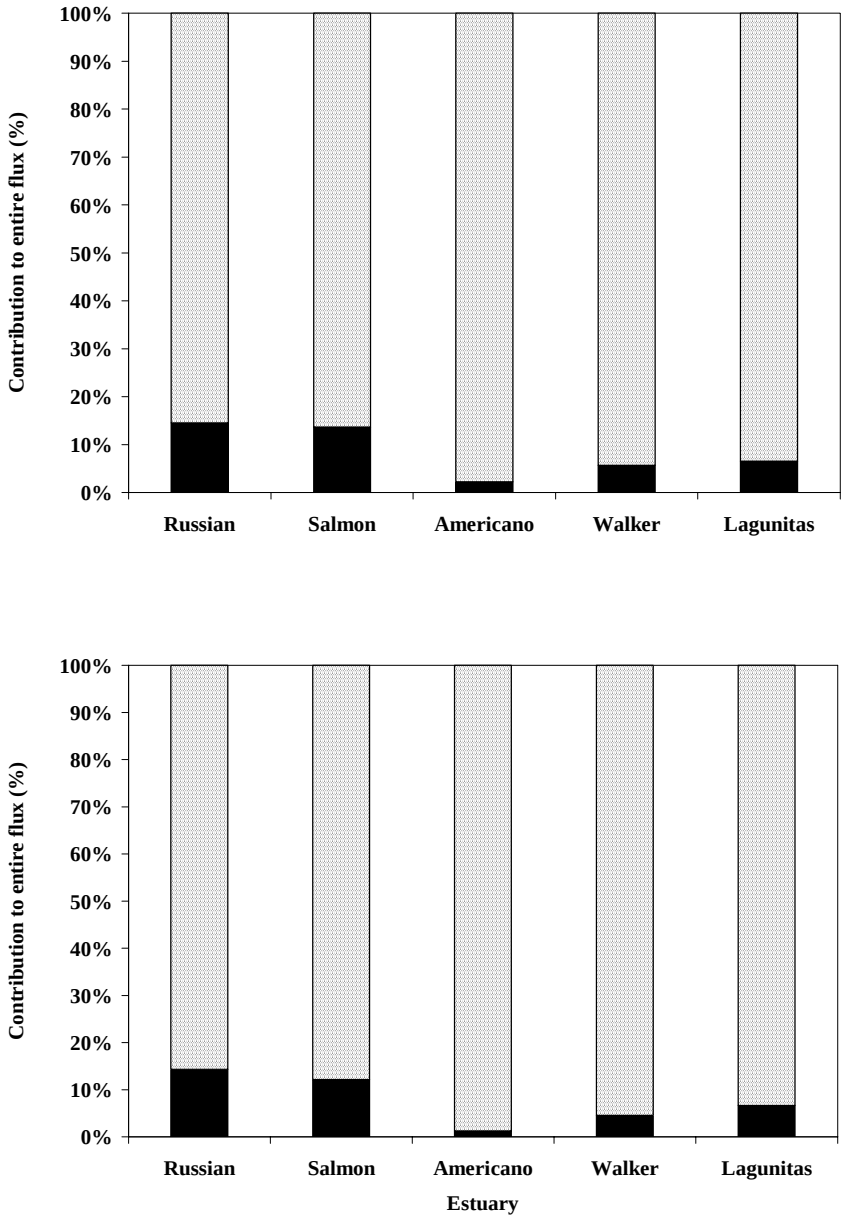


Figure 8: Contribution of suspended sediment (black) and residual water (dotted) fractions to the mean flux of fecal coliforms (upper) and *E. coli* (lower) in composite water.

Influences and Bias

In addition to the estuary-specific differences and the role of watershed-specific dynamics on indicator bacteria concentrations presented in the sedimentborne and waterborne bacteria sections, we conducted additional analysis of influences on indicator bacteria values that could bias monitoring. This included comparison of the relationships of bacteria concentrations to season, transect location, lateral position, and depth, with parallel responses of salinity and turbidity.

Season

We designated each sampling event into one of three season groups. The first is the *dry season base flow* or summer when stream discharge from the contributing watersheds is lowest. This is also when the bar-built systems typically close. The second season category is *wet season base flow*. This category captures those sampling events carried out during the winter storm season but between respective storms when winter discharge is at its lowest. The third season category was *wet season storm flow*. This category was used for sampling events conducted one to three days after winter storms, and on the falling limb of the storm hydrograph for the watershed.

Concentrations for fecal coliforms and *E. coli* were significantly greater during the wet season storm flow followed by wet season base flow and dry season base flow, respectively (Figure 9a). The one exception to this pattern was Lagunitas Creek where values for wet season base flow and storm flow where very similar (Figure 10). The response of turbidity to season paralleled that of the indicator bacteria concentrations, while salinity values demonstrated an inverse relationship. Preliminary regression analysis indicates that all of these relationships are statistically significant ($p<0.05$).

Transect

Graphical presentation of bacteria concentrations to each of the five transect locations indicates that they are greatest at transect three (Figure 9b). This is likely because of the pattern of bacteria concentrations in the Estero Americano wherein the highest concentrations were consistently at transect three (Figure 11). Regression modeling actually identified a significant increase in bacteria concentrations from transect one to transect five. Similarly salinity values decreased significantly moving inland from transect one to transect five. Turbidity values, in contrast, increased to transect four and then decreased.

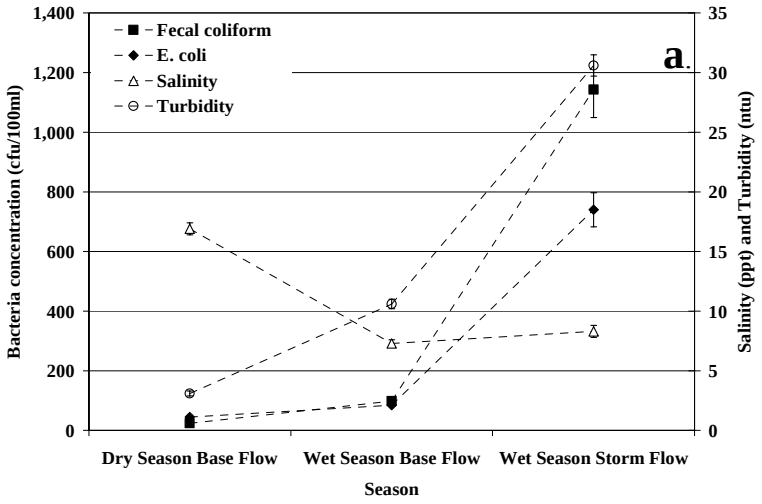
Depth

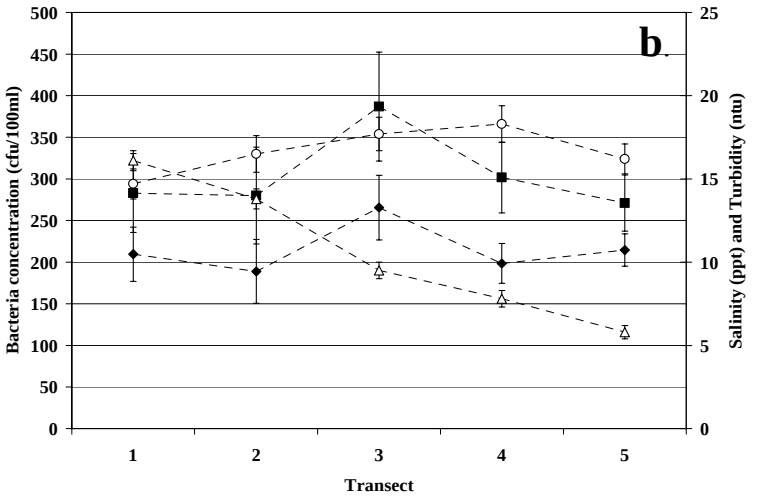
Three depth group categories were used that correspond to the depths at which water samples were collected. The *shallow* designation corresponds to samples collect 0.1 m below the water's surface. Samples collected at the mid-point of the water column were grouped into the *intermediate* category. And those samples collected at 0.1 m above the estuary bottom were grouped into the *deep* category.

Results from this analysis indicate decreasing trend in indicator bacteria concentration with depth (Figure 9c). Regression analyses also confirm this pattern is significant ($p<0.05$). In contrast, values for both salinity and turbidity increase with depth.

Sample Position

Concentrations of fecal coliforms and *E. coli* were not significantly associated with the differing sample positions of left, middle, and right.





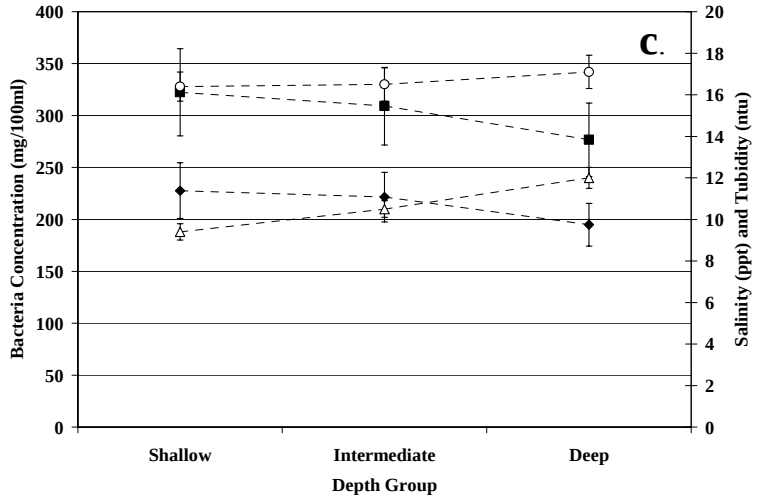


Figure 9: Relationship of composite water sample mean fecal coliforms and *E. coli* concentration, salinity and turbidity to season (a), transect (b), and depth group (c).

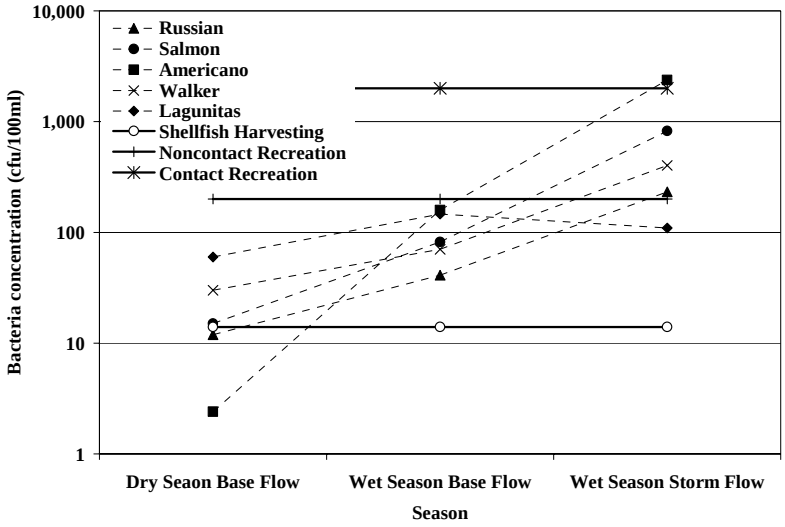


Figure 10: Concentration of fecal coliforms in composite water sample for each studied estuary grouped by season. Included are the water quality criteria for shellfish harvesting, noncontact recreation, and contact recreation.

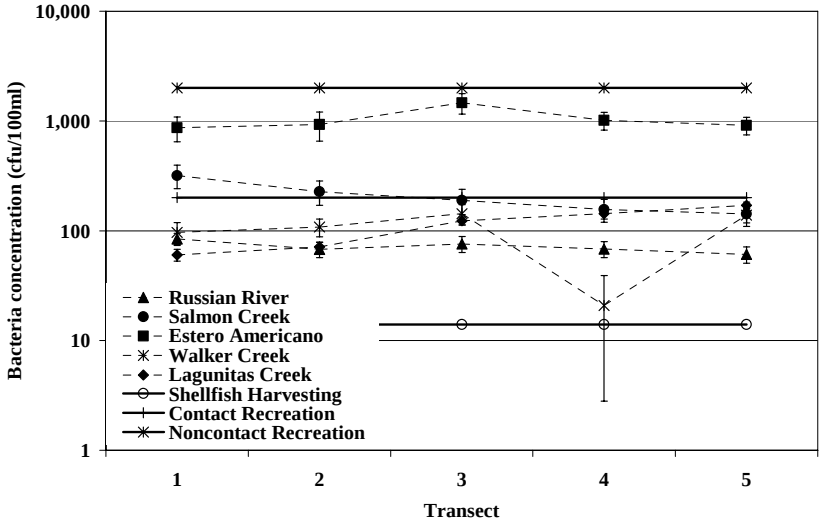


Figure 11: Concentration of fecal coliforms in composite water sample for each studied estuary grouped by transect. Included are the water quality criteria for shellfish harvesting, noncontact recreation, and contact recreation.

Bacteria Relatedness

Results

Nine hundred and seventy three isolates of commensal *E. coli* from five estuaries collected during periods of dry season base flow, winter season base flow, or winter season storm flow conditions were processed by Box-PCR. Figure 12 exemplifies the fingerprinting results for a specific collection date, and how data are generated according to the number of

pairs that match or do not match. Tables 15A through 15E show the percentage of *E. coli* whose DNA fingerprint matched another isolate of *E. coli* collected during the same day for all possible types of comparisons (e.g., *E. coli* from TSS compared to *E. coli* from water, *E. coli* from TSS compared to *E. coli* from bottom sediment, or *E. coli* from TSS compared to all other *E. coli* from TSS). A key question is whether *E. coli* contained in bottom sediments is similar or different from *E. coli* found in the water column, which itself is comprised of *E. coli* from the water fraction and/or the TSS fraction. If different, then hydrological or climatic conditions that resuspend bottom sediments into the water column may confound microbial source tracking methods. Based on the data shown in Tables 15A-E, the *E. coli* isolated from bottom sediments rarely matches the *E. coli* isolated from the water fraction or the TSS fraction (median value is 0% match), suggesting that biases as to source of *E. coli* may occur if bottom sediments are inadvertently incorporated into a water sample (turbulence from wind, tidal surge, boat anchor, etc.). Furthermore, *E. coli* transiting the fresh-to-saltwater zone rarely matched each other during the same day of collection regardless of where they were isolated from (water, TSS, sediment), indicating a high degree of genetic diversity for this waterborne population of *E. coli*.

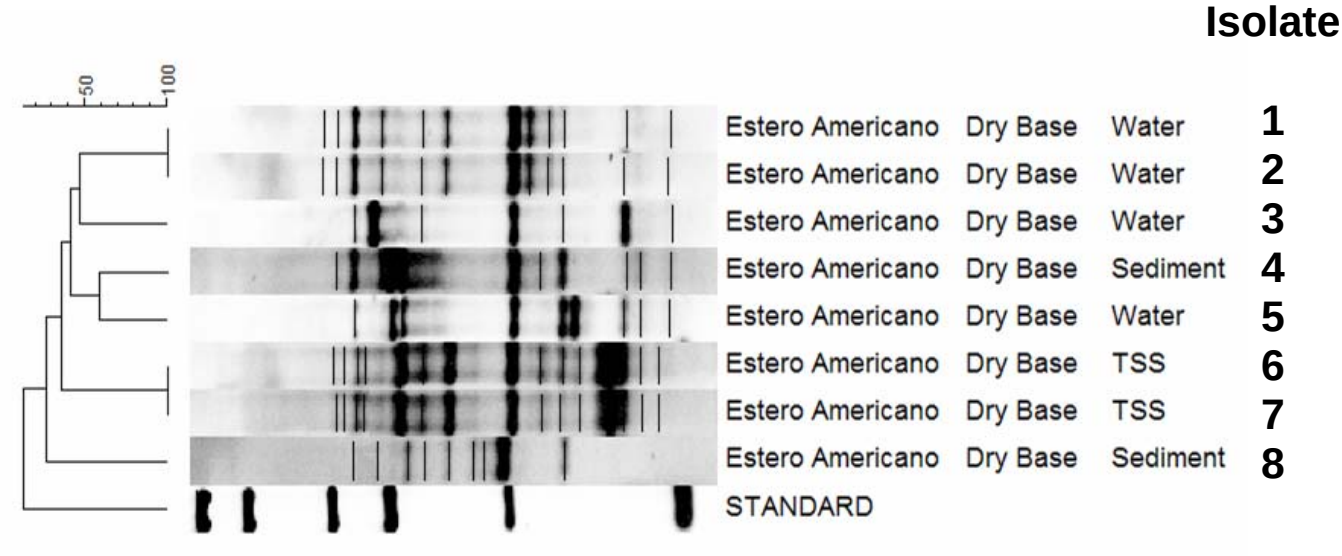


Figure 12: Box PCR DNA fingerprinting patterns of *E coli* strains from Estero Americano collection date 8/23/2004. Among the eight isolates, two different pairs of isolates were identical in the Box-PCR pattern, two from water (isolates 1 and 2) and two from TSS (isolates 6 and 7).

Tables 15A-E: Percentage of *E coli* isolates obtained from either the TSS fraction, water fraction or bottom sediments that have an identical molecular patterns to another *E coli* isolate, stratified by season (wet or dry) and streamflow (baseflow or stormflow), for each of the five estuaries in the project.

Table 15A: ESTERO AMERICANO, data from 174 *E. coli* isolates

	Dry Base			**Wet Base**			**Wet Storm**		
	# Matches[2]	Total pairs[2]	%[2]	# Matches[2]	Total pairs[2]	%[2]	# Matches[2]	Total pairs[2]	%[2]
H20/TSS[1]	0	92	0%	1	163	0.61%	3	324	0.93%
H20/Sediment[1]	0	32	0%	1	57	1.75%	0	99	0%
TSS/Sediment[1]	0	8	0%	0	51	0%	0	99	0%
H20/H20[1]	3	117	3%	2	79	2.53%	2	144	1.39%
TSS/TSS[1]	2	17	12%	1	64	1.56%	1	144	0.69%
Sediment/Sediment[1]	0	2	0%	0	6	0%	1	10	10%
TOTAL	**5**	**268**	**1.87%**	**5**	**420**	**1.19%**	**7**	**820**	**0.85%**

[1] Stratified by sampling day, DNA fingerprints of *E. coli* isolated from one matrix were either compared to *E. coli* isolated from a different matrix (e.g., *E. coli* from H2O fraction compared all other *E. coli* from TSS for that sample day and location) or to the same matrix (e.g., all *E. coli* from H2O fraction compared to each other for that sample day).

[2] Number of matches are the number of pairs of *E. coli* that have identical DNA fingerprints for the matrix combinations listed in column one; total pairs of *E. coli* are all possible pairs of *E. coli* for that sample day; % is the percentage of total pairs of *E. coli* that have identical DNA fingerprints out of all possible pairs.

Table 15B: LAGUNITAS, data from 208 *E. coli* isolates

	Dry Base			**Wet Base**			**Wet Storm**		
	# Matches	Total pairs	%	# Matches	Total pairs	%	# Matches	Total pairs	%
H20/TSS	1	245	0.41%	0	453	0%	0	72	0%
H20/Sediment	0	108	0%	1	159	0.63%	0	18	0%
TSS/Sediment	2	62	3.23%	1	153	0.65%	0	16	0%
H20/H20	2	199	1.01%	5	211	2.37%	0	36	0%
TSS/TSS	0	93	0%	1	195	0.51%	0	28	0%
Sediment/Sediment	0	10	0%	1	18	5.6%	0	1	0%
TOTAL	**5**	**717**	**0.70%**	**9**	**1189**	**0.76%**	**0**	**171**	**0%**

Table 15C: RUSSIAN, data from 205 *E. coli* isolates

	Dry Base			Wet Base			Wet Storm		
	# Matches	Total pairs	%	# Matches	Total pairs	%	# Matches	Total pairs	%
H20/TSS	1	200	0.50%	1	298	0.34%	0	441	0.0%
H20/Sediment	0	56	0.00%	0	93	0.00%	0	171	0.0%
TSS/Sediment	0	28	0.00%	0	79	0.00%	0	117	0.0%
H20/H20	2	196	1.02%	2	157	1.27%	1	312	0.32%
TSS/TSS	0	43	0.00%	1	113	0.88%	2	141	1.42%
Sediment/Sediment	0	2	0.00%	0	10	0.00%	0	18	0.0%
TOTAL	**3**	**525**	**0.57%**	**4**	**750**	**0.53%**	**3**	**1200**	**0.25%**

Table 15D: SALMON, data from 163 *E. coli* isolates

	Dry Base			Wet Base			Wet Storm		
	# Matches	Total pairs	%	# Matches	Total pairs	%	# Matches	Total pairs	%
H20/TSS	5	165	3.03%	0	317	0%	0	109	0%
H20/Sediment	0	0	NA	0	83	0%	0	45	0%
TSS/Sediment	0	0	NA	0	76	0%	0	45	0%
H20/H20	5	210	2.38%	1	146	0.68%	1	49	2.0%
TSS/TSS	1	37	2.70%	1	136	0.74%	1	61	1.63%
Sediment/Sediment	0	0	NA	0	7	0%	0	6	0%
TOTAL	**11**	**412**	**2.67%**	**2**	**765**	**0.26%**	**2**	**315**	**0.63%**

Table 15E: WALKER, data from 223 *E. coli* isolates

	Dry Base			Wet Base			Wet Storm		
	# Matches	Total pairs	%	# Matches	Total pairs	%	# Matches	Total pairs	%
H20/TSS	1	326	0.31	0	306	0.00%	0	519	0%
H20/Sediment	0	63	0%	0	63	0.00%	0	93	0%
TSS/Sediment	0	52	0%	0	61	0.00%	0	84	0%
H20/H20	2	191	1.05%	2	144	1.39%	3	297	1.01%
TSS/TSS	1	142	0.70%	0	129	0.00%	1	207	0.48%
Sediment/Sediment	0	4	0%	0	4	0.00%	0	6	0%
TOTAL	**4**	**778**	**0.51%**	**2**	**707**	**0.28%**	**4**	**1206**	**0.33%**

PUBLIC OUTREACH

We conducted public outreach through two components. At the onset of our study, we generated a project description fact sheet (Appendix C) to share with locally interested entities and agencies. This was distributed to resource conservation districts, special districts, and relevant departments in Marin and Sonoma County.

Our second outreach component included workshops and presentations to area groups to share results and discuss their implications for implementation of water quality improving practices and water quality monitoring. Combined we have held two workshops and made three additional presentations of the project results (Table 16). A copy of our flyer for the Santa Rosa, California workshop is included in Appendix D.

Table 16: Dates, descriptions, and attendance at workshops and presentations on project results.

Date	Description	Attendance
September 19, 2006	Project Workshop for the North Coast Regional Water Quality Control Board in Santa Rosa, California	33
January 16, 2007	Project Workshop for the Tomales Bay Shellfish Protection Technical Advisory Committee in Point Reyes Station, California	29
January 30, 2007	Presentation at "Cut the Crap" workshop for Santa Barbara County ranchers in Gaudalupe, California	63
January 31, 2007	Presentation at "Cut the Crap" workshop for San Luis Obispo County ranchers in Morro Bay, California	59
March 20, 2007	California Estuary Research Society Annual Meeting Presentation, Bodega Bay, California	47

CONCLUSIONS

Project Evaluation & Effectiveness

Our Project Assessment and Evaluation Plan addresses the following goals:

A. Identify the non-point source(s) and transport pathways of bacterial pollution for five estuaries in Northern California.
B. Describe the baseline water quality of estuarine environments in Northern California.
C. Describe the manner in which the project will be effective in preventing or reducing bacterial pollution and in demonstrating the desired environmental results.
D. Develop a standardized water quality monitoring protocol for bacterial pollution of California estuarine systems.

A. Identify the non-point source(s) and transport pathways of bacterial pollution for five estuaries in Northern California
This project focused on monitoring the concentration of fecal coliforms and commensal *E. coli* in water, suspended solids, and in the upper layer of the sediment underlying the water column across the fresh-to-saltwater transition zone for five estuaries. Measuring the bacterial concentration in these three fractions (water, suspended solids, sediment) allowed a better understanding of why estuaries experience elevated bacterial counts during different times of the year, as explained below.

The primary performance measures for this goal are to:
1. Identify which fraction of composite water (water or suspended solids) contains the majority of bacteria that transit the fresh-to-saltwater transition zone for northern California estuaries.
2. Characterize the magnitude and seasonal shifts in the reservoir of fecal coliforms and commensal *E. coli* in estuarine sediments.
3. Generate data that helps distinguish whether elevated bacterial counts are due to resuspension of bacterially-contaminated estuarine sediments (potential local source problem) or due to freshwater inflows contaminated by one ore more upstream terrestrial sources. Making such a distinction allows remediation efforts for improving coastal water quality to properly target where the bacterial load is coming from, either from local estuarine sediments that by some mechanism are being contaminated with bacteria, or from land uses located upstream on the watershed that are contaminating freshwater inflows that enter the estuary through rivers and streams.

In general, the project estuaries received their highest concentrations of fecal coliforms and *E. coli* during storm flow conditions of winter and spring (Lagunitas Creek was the exception). During these storm flow conditions, about 90% of the total amount of bacteria in a composite water sample were associated with the water fraction and about 10% were in the suspended solids. This ratio of 9:1 water:suspened solids of bacteria stays about the same for fecal coliforms but increases to about 25:1 (water:suspended solids) for *E. coli* counts during the dry season lower base flows. Hence, the contribution of bacterial contamination from suspended solids is much higher in wet season (winter-spring) compared to dry season. During storm flow conditions, higher concentrations of each bacterial indicator was found at

the uppermost transect (transect 5, freshwater site) compared to estuarine sites (transect 1), suggesting that the majority of bacterial contamination entering these estuaries during peak flow conditions was the result of inflows of contaminated freshwater, of which about 90% was in water fraction. Relative to the other estuarine systems in the project, Lagunitas Creek exhibited the highest counts of bacteria during summer low-flow conditions. This suggests that the source of bacteria for Lagunitas Creek during the dry season is not the result of a source that relies on precipitation and saturated soils to create overland flow conditions, but is instead a source of bacteria that is directly discharged into the creek. Mean counts of fecal coliforms were on average substantially higher in the upstream, freshwater transect compared to estuarine sampling site. Extending the sampling network to sites further upstream may readily identify the point or non-point source of these bacteria given the likely proximity of this bacteria source to Lagunitas Creek.

A year-round reservoir of fecal coliforms and *E. coli* was identified in estuarine sediments, with the highest concentrations typically observed in wet weather or winter. A variety of publications have indicated that in tropical-like conditions, bacterial indicators can replicate in the warm sediments when an appropriate mix of nutrients are present to support replication. Although we cannot prove or disprove this assertion, it is unlikely that the cold wintertime storm flow conditions associated with these higher bacterial counts in estuarine sediments are the result of localized, indigenous bacteria replicating on sediment grain surfaces. Instead, we assert that the bacterial increases that occur this time of year in sediment may be the result of bacterial deposition either from bacterial filtration as water flows through the hyporheic zone and/or gravitational settling of bacteria attached to sediment particles such as clays or silts, with the originating source of these bacteria being up in the watershed. Interestingly, results from the DNA fingerprinting indicate that very few of the *E. coli* captured in the water column are identical to that those isolates cultured from bottom sediments. This leads us to speculate that either the *E. coli* located within estuarine bottom sediments are independent from the *E. coli* transiting the estuary in the water column and whose source is somewhere upstream, or, if most of the *E. coli* do in fact originate from sources upstream of the estuary, that high levels of genetic diversity for this population of *E. coli* results in very low probabilities of a DNA match.

B. Describe the baseline water quality of estuarine environments in Northern California
We have provided one of the most thorough datasets on the concentration of fecal coliforms and *E. coli* for these five estuaries along their fresh-to-saltwater transition zone. The reported means for bacterial concentration have been by generated for five different Northern California estuaries during both base flow and storm flow conditions, with samples taken along a detailed 3-by-5 point grid extending from predominately pure saltwater upstream to sites that are predominately freshwater. In addition, these mean values for bacterial indicators are derived from taking three water samples along the length of the water column.

Primary performance measures for this goal are:
1. Quantify the concentration of fecal coliforms and *E. coli* in water and sediment along the fresh-to-saltwater transition zone for five estuaries in northern California.
2. Develop a database from performance measure (1) that will allow the RWQCB to determine if the ambient water quality during storm flow or base flow conditions is meeting the standard for the listed beneficial uses.

In general, mean fecal coliforms concentrations were above water quality criteria for shellfish harvesting in all three seasons. It is important to point out that the majority of our sampling locations were upstream and not in shellfish harvesting areas. Mean values in all five estuaries were close to shellfish harvesting criteria in the dry season base flow season. Mean concentrations for fecal coliforms grouped by season were above the criteria for noncontact recreation in all estuaries during the wet season storm flow with the exception of Lagunitas Creek. The values for Estero Americano exceeded the criteria for contact recreation during the wet season storm flow.

Comparing mean bacteria concentrations grouped by transect to beneficial use water quality criteria indicate that Estero Americano waters at all five transects were above shellfish harvesting and noncontact recreation criteria. This is also true for values at Lagunitas Creek transects one and two. Values for the remaining four estuaries at all five transects were between the criteria for contact recreation and shellfish harvesting, with the exception of Lagunitas Creek.

To our knowledge there are no established regulatory standards for maximum allowable concentrations of fecal coliforms and *E. coli* in sediment for streams or estuaries. We determined that in the upper 15 cm of sediment there is on average about 5 to 13 cfu of bacteria per gram wet-weight of sediment. Depending on the estuary, this can translate to bacterial loads as high as $1H10^6$ and even $1H10^7$ cfu per square meter, which when extrapolated to an entire estuarine mud flat at low tide, would be a sizable load of bacterial pollution were it to be disturbed or mobilized. These values for bacterial loads in sediment should be considered a lower estimate given that we measured only that fraction of bacteria that were reversibly attached and thereby able to be eluted from the sediment matrix given our analytical method of moderate agitation on a wrist shaker. Enteric bacteria such as *E. coli*, under certain environment conditions, are know to join biofilms and when later detached may exist as large, complex fragments of colonies of mixed species of bacteria that our method of membrane filtration would have enumerated as a single colony forming unit. With this caveat in mind, and given the large surface area of estuarine sediment exposed to turbulent conditions induced by either storm flow conditions in winter or when a windward shoreline is exposed to wind waves at lower tides, it is conceivable that bacterial indicators can detach from the sediment matrix and elevate levels of bacteria in the water column in localized areas. Interestingly, there was a mean 10-fold increase in the concentration of bacteria in sediment going from dry season base flow to wet season storm flow conditions. One might expect that the conditions for bacterial replication in estuarine sediment would be more prevalent in summer compared to winter, suggesting that the source of bacteria driving these higher concentrations during winter are from contaminated freshwater inflows and not bacterial replication of local, indigenous bacteria surviving in the sediment. If this were the case, then one might have expected more matches among the 787 pairs of *E. coli* (H2O versus sediment or TSS versus sediment) during wet storm-flow conditions, but our study found that none of these 787 pairs of isolated bacteria from the same day of collection were identical to each other (Tables 15A-15E).

C. Describe the manner in which the project will be effective in preventing or reducing bacterial pollution and in demonstrating the desired environmental results

The methods we are using to assist RWQCB and allied groups to prevent or reduce bacterial pollution is to generate high quality monitoring data that informs these groups as to major transport pathways, possible major sources of bacterial indicators (local sediment, upstream land uses), and to conduct workshops for these groups on these same topics.

Moreover, remediation efforts to restore water quality need to distinguish whether the source of elevated bacteria is attributable to the local estuarine environment or is the result of upstream terrestrial sources such as animal agriculture and pasture runoff. Failure to resolve this distinction could substantially reduce the effectiveness of TMDL plans or water quality projects designed to reduce pathogen loading, and thereby delay the restoration of beneficial uses of California's bays, estuaries, and near-shore marine environments.

Primary performance measures for this goal are:
1. Identify major transport pathways being utilized to deliver bacterial loads to estuarine systems.
2. Generate data that helps distinguish whether elevated bacterial counts are due to resuspension of bacterially-contaminated estuarine sediments or due to freshwater inflows contaminated by one ore more upstream terrestrial sources.
3. Conduct workshops for RWQCBs that cover project conclusions and recommendations for how best to monitor these complex systems to detect trends in degradation or improvement in microbial water quality.

The conclusion stated in Section A above (*Identify the non-point source(s) and transport pathways of bacterial pollution for five estuaries in Northern California*) presents our conclusion that contaminated freshwater inflows that enter the estuary, especially during storm flow conditions, is the primary transport pathway for delivering bacterial pollution to these systems. A small percentage of bacteria are in the suspended solids of the water column, reaching a peak in concentration and also a peak in the proportion of the overall load of waterborne bacteria during wet season storm flow conditions. This is not surprising given the ability of precipitation to erode soil and other terrestrial surfaces and the stream's turbulence associated with the rising limb of the storm hydrograph to generate substantial increases in suspended solids, some of which likely originate from terrestrial locations on the watersheds. Furthermore, elevated bacterial counts experienced in the winter and early springtime for most of these estuaries appears to be from upstream land use activities and not the result of resuspension of contaminated estuarine sediments. The exception is Lagunitas Creek, where there appears to be a source of fecal coliforms in summer with direct hydrological connection to the creek or one of its tributaries given the relatively high levels of bacteria present in the freshwater sampling sites for this system. Alternatively, the tidal regime and high residence time of water in the lower portion of Tomales Bay and Lagunitas Creek may be concentrating bacteria. As stated above, carefully extending the sampling network to sites further upstream may readily identify the point or non-point source of these bacteria given the likely proximity of this bacterial loading source to Lagunitas Creek.

We have conducted two workshops and made three additional presentations through which we have shared the results of this project and their implications for estuary monitoring and management to improve water quality. A total 62 participants attended our workshops and 169 attendees were present for our presentations at the other three presentations. Specific information shared included summaries of the overall results. This included a description of estuary sediment as a bacterial reservoir, as well as the identification of bacteria associated with the water fraction as the greatest contributor to composite water sample bacteria concentrations. Additionally, we provided an explanation of the need to account for season, transect location, and depth in addition to measuring environmental parameters like salinity in water quality monitoring programs.

D. Develop a standardized water quality monitoring protocol for bacterial pollution of California estuarine systems

This monitoring project was motivated in part by our concern that ambient water quality monitoring may be performed in a manner that inadvertently creates an upward or downward bias in bacterial enumerations. For example, if estuarine sampling is to be done on foot and is located at a boat ramp or other such site, does sampling close to the shoreline bias the bacterial counts due to shallower conditions and possibly excessive amounts of suspended solids or resuspended bottom sediments in the sample? Does sampling too far upstream properly reflect microbial water quality conditions down in the estuary, or vice versa? Does saltwater intrusion at an upstream sampling due to high tidal conditions bias bacterial enumeration? It is these types of questions that motivate this goal.

Primary performance measures for this goal were:
1. Identify factors that substantially influence or bias the concentration of indicator bacteria from its central tendency.
2. Conduct workshops for RWQCBs that cover project conclusions and recommendations for how best to monitor these complex systems to detect trends in degradation or improvement in microbial water quality.

The primary spatial factors that we examined for their ability to influence or bias bacterial indicator counts were transect location along the fresh-to-saltwater transition zone, depth of sample, lateral position relative to the shoreline. In general, incoming tidal conditions, low summer streamflow conditions, sampling near the bottom of the water column, or locating a sampling site in a predominately saline location (e.g., transect 1 or 2 down in the estuary) resulted in lower mean counts for fecal coliforms and commensal *E. coli* compared to the alternative condition (outgoing or ebb tide, winter or spring stormflows, sampling in the mid to upper reaches of the water column, and sampling at the upper freshwater transect sites). For the analyses conducted to date, all things being equal, sampling lateral to the midline of the channel nearer to the shoreline did not influence the mean count of fecal coliforms or commensal *E. coli* compared to the alternative condition of sampling in the middle of channel. Based on the data shown in Tables 15A-E, the *E. coli* isolated from bottom sediments rarely matches the *E. coli* isolated from the water fraction or the TSS fraction (median value is 0% match), suggesting that biases as to source of *E. coli* may occur if bottom sediments are inadvertently incorporated into a water sample during turbulent conditions (wind, tidal surge, boat anchor, etc.).

This information was presented to 169 representatives of resource agencies, local government departments, and volunteer groups at two organized workshops and three additional presentations.

Next Steps

The University of California Cooperative Extension and UC Davis will continue to conduct research and extension activities on how to reduce waterborne microbial pathogens in northern California's estuaries, bays, and near-shore environments. Bacterial contamination of Tomales Bay is a primary threat to the commercial and recreational shellfish beds present in the Bay, resulting in the California Regional Water Quality Control Board developing strict total maximum daily loads for reducing waterborne pathogens in the Bay.

Planned research projects include generating a better understanding of the key processes (tidal, climate, etc.) that govern the rate of dispersion of freshwater bacterial plumes discharging into the Bay and identifying key environmental predictors that signal when these bacterial contaminants are present or absent in cultured or wild shellfish of Tomales Bay.

APPENDIX A

References

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APPENDIX B

Estuary Sample Location Map Data

Sample Transect GPS Coordinates

Creek Name	Site #	Landmark	East-GPS D.D.	East-GPS UTM	North-GPS D.D.	North-GPS UTM
Estero Americano	1	Downstream of first bend or upstream of last bend	-122.9958143	10-5-00-367	38.2992328	42-38-962
Estero Americano	2	Downstram of last broad reach, upstream of last broad reach	-122.9883338	10-5-01-021	38.3070733	42-39-958
Estero Americano	3	Upstream of divided channel next to blind and bench	-122.9660843	10-5-02-781	38.3149370	42-40-553
Estero Americano	4	Bank of willows	-122.9579739	10-5-03-675	38.3156283	42-40-730
Estero Americano	5	Bay tree downstream right, before going into narrows.	-122.9435978	10-5-04-932	38.3124679	42-40-499
Lagunitas	1	Channel narrows in line with bivalve	-122.8346791	10-5-14-496	38.0917413	42-15-801
Lagunitas	2	Upstream of start of levee	-122.8263111	10-5-15-232	38.0810939	42-14-621
Lagunitas	3	Pool half between transects 2 and 4	-122.8201618	10-5-15-774	38.0732164	42-13-748
Lagunitas	4	White house pool	-122.8192742	10-5-15-853	38.0632737	42-12-645
Lagunitas	5	Downstream of green bridge	-122.8051690	10-5-17-091	38.0649937	42-12-794
Russian	1	Downstream of boat ramp	-123.1189308	10-4-89-623	38.4495913	42-55-695
Russian	2	Powerline and fences downstream left and outcrop downstream right	-123.1142763	10-4-90-028	38.4485145	42-55-380
Russian	3	Downstream of powerline / upstream of cattle pastures (left) and downstream of pastures/ upstream of outcrop (right)	-123.1090425	10-4-90-483	38.4372630	42-54-131
Russian	4	Upstream of houses and downstream of outcrop (right)	-123.0964205	10-4-91-340	38.4386800	42-54-287
Russian	5	Willow Creek confluence below house in line with outcrop, on other side highway / River Road	-123.0969416	10-4-91-480	38.4428343	42-54-748
Salmon	1	Below dock	-123.0635838	10-4-94-444	38.3508905	42-44-543
Salmon	2	Between bridges	-123.0609462	10-4-94-674	38.3517587	42-44-639
Salmon	3	Upstream of bend	-123.0598514	10-4-94-770	38.3545156	42-44-945
Salmon	4	Upstream of bend and bedrock lined outside bank	-123.0572864	10-4-94-994	38.3555460	42-45-590
Salmon	5	Ausprey nest	-123.0570595	10-4-94-140	38.3576368	42-45-291
Walker	1	Rock outcrop and east of bend but downstream of island	-122.9277503	10-5-06-060	38.2141128	42-29-368
Walker	2	Powerline	-122.9229463	10-5-06-485	38.2201573	42-30-039
Walker	3	Upstream of old bridge	-122.9200419	10-5-06-905	38.2226429	42-30-486
Walker	4	At fishing parking lot	-122.9163693	10-5-07-232	38.2273900	42-30-842
Walker	5	Upstream of confluence for Keyes and Walker Creeks	-122.9121388	10-5-07-689	38.2330907	42-31-474

APPENDIX C

Bacterial Sources and Transport in Marin and Sonoma County Estuaries

David J. Lewis, UCCE Watershed Management Advisor
E. Robert Atwill, UCCE Specialist, DVM, Ph.D.

Overview

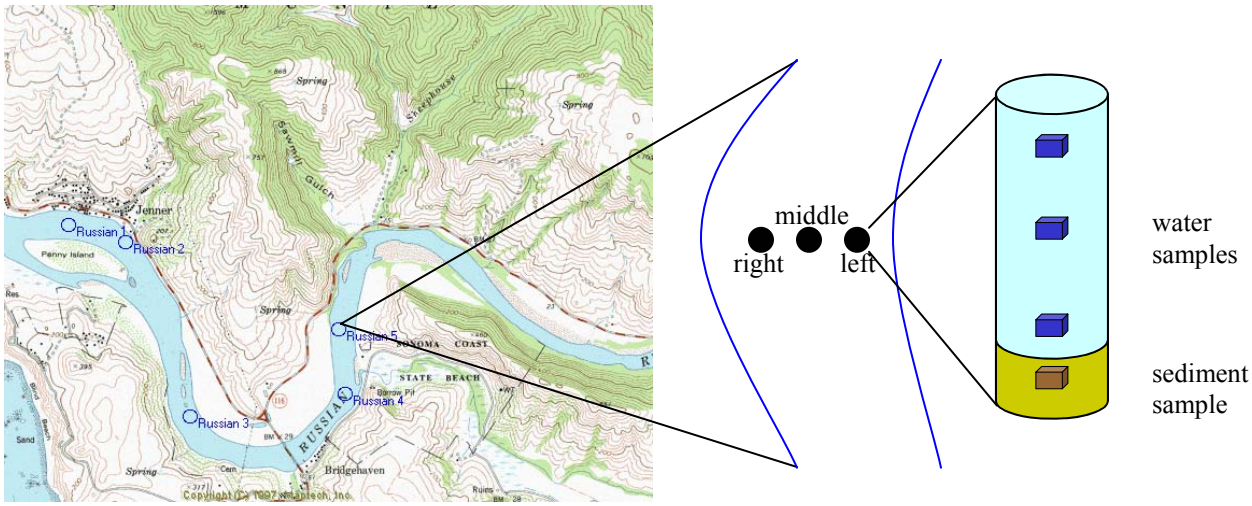
Beneficial uses of California's bays, estuaries, and near-shore marine environments include shellfish harvesting, swimming, and boating. These beneficial uses are vulnerable to elevated bacterial levels in winter stream flow and suspension of bay or estuarine sediments by tidal currents, stream flow, and wind generated waves. It is very important that water quality monitoring be able to clearly identify sources and reservoirs of bacterial contamination so that remediation efforts such as Total Maximum Daily Load (TMDL) plans and on-farm management practices can effectively reduce pollution of coastal waters. More specifically, when estuaries experience elevated counts of fecal coliforms and *E. coli* during base- or storm flow conditions, monitoring protocols need to be able to determine if the elevated bacterial counts are due to suspension of estuarine sediments or freshwater inflows from upstream runoff.

The goal of our study is to develop a comprehensive understanding of these different bacterial sources and develop a water quality monitoring protocol for quantifying sources and transport processes of microbial contaminants (pathogens) in the estuaries. To achieve this goal we are conducting water and sediment sampling and analysis from August 2004 to July 2005 in the Russian River, Salmon Creek, Estero Americano, Walker Creek, and Lagunitas Creek estuaries. We hope this summary provides you with some background information regarding our research project and answers any questions that you may have.

Why were these five estuaries selected? These five estuaries represent a variety of environmental conditions across the coast of Marin and Sonoma Counties. In a general sense, they have similar climate, precipitation, and hydrology. Specifically, this similarity is the result of the Mediterranean climate in California with cool wet winters and dry hot summers. As a result, all five estuaries experience an in flow of freshwater during the winter, followed by an introduction of salt water in the summer. The five estuaries differ in size, area of contributing watershed and with regard to land use including agriculture, urbanization, and recreation. These differences and similarities will provide us with a good context for understanding how these factors interact with bacteria movement in estuaries.

APPENDIX C

Where are samples being collecting? We will collect water and sediment samples from each estuary once a month for ten months. Each estuary will be sampled through a series of five transects. Each transect consists of three sampling locations, right, middle, and left facing downstream. At each location, we will collect three water samples at one foot below the water surface, half the depth of the water column, and one foot above the estuary bottom. A sediment sample will be collected from the estuary sediments at each location.



Map of the Russian River Estuary with the five sampling transect locations identified. Each transect consists of three sampling locations from which three water samples and one sediment sample will be collected.

How will the results from this research be used? The results of this work will be shared with local land owners and managers, resource conservation districts, water quality regulatory staff (e.g., RWQCBs), conservation organizations, watershed groups, and non-profit watershed entities. In addition, we will develop a monitoring protocol to be shared at workshops planned for the Spring of 2006.

What are fecal coliforms and *E. coli*? Bacteria are part of our world and environment, existing in most substances and on most surfaces including our skin. Some of the bacteria that are of significant concern to human health are those of fecal origin. These bacteria and the associated pathogens have the potential to cause disease in human. Indicator bacteria are used as analytical tools to assess this risk of exposure. Fecal coliforms and *E.coli* are two of these indicator bacteria.

Why not conduct research on a specific pathogen or group of pathogens? Testing for a specific pathogen is very expensive and difficult, so water quality investigators often use these more common non-pathogenic bacteria to indicate the potential presence of these pathogens.

How can you tell one bacteria from another? There is increasing ability through the development of new laboratory methods to identify different strains of bacteria from another. These methods rely on genetic differences between bacterial communities. We will be using a method referred to as BOX-PCR to generate a fingerprint of selected individuals from each E.

coli community (water and sediment). This method of DNA fingerprinting targets interspersed conserved repetitive DNA elements present in multiple copies throughout the E. coli genome. Amplifying the distinct genomic regions located between these repetitive DNA elements generates the distinctive pattern that distinguishes one strain or community from another.

Is sediment really a source of bacteria? Recent research has documented that sediment can be a medium on which bacteria can survive and be transported through a watershed. One of the goals of this project is to measure the amount of fecal coliforms and *E. coli* contained in the bottom sediments of estuaries. Large storm events and wave action have the potential to suspend these sediments and elevate bacteria concentrations in these waters.

(Study is funded by the Costa-Machado Water Act of 2000 (Proposition 13) under contract with the California State Water Resources Control Board and North Coast Regional Water Quality Control Board)

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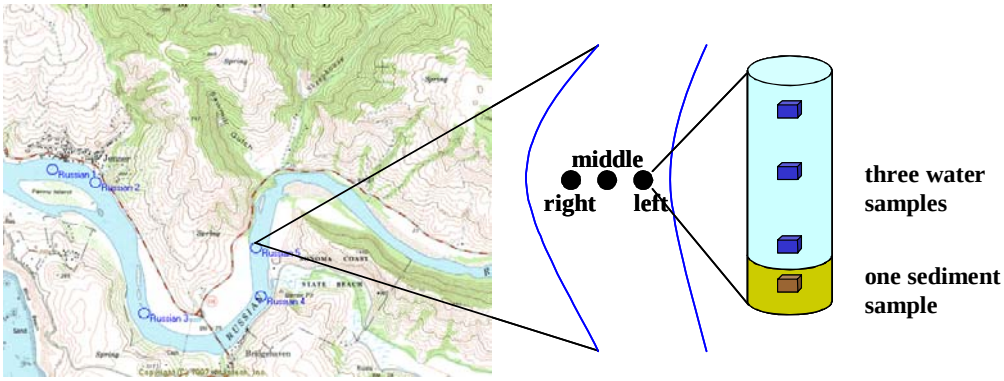
E. Robert Atwill is the Environmental Animal Health Specialist for the University of California Cooperative Extension and the School of Veterinary Medicine, Davis campus.

APPENDIX D

Monitoring Bacterial Sources and Transport in Five Northern California Estuaries

North Coast Regional Water Quality Control Board, DCJ Room
Santa Rosa, California
Tuesday September 19, 2006
9:00 am – 12:00 pm

Please join us for a workshop on bacteria research in five Marin and Sonoma County estuaries. We sampled water, suspended solids, and sediment across the freshwater-saltwater transition zone at each estuary ten times from August 2004 to June 2005. Samples were analyzed for fecal coliform and *E. coli*, DNA fingerprinting is also being used to differentiate bacterial populations in estuary sediment, suspended solids, and water fractions. Our results provide water quality sampling and monitoring programs with a context for the interaction between sediment and freshwater sources of bacteria, as well as salinity and seasonal influences on bacterial levels. We look forward to discussing with you our results, the relationship between sediment and water column bacteria, and methods for statistical analysis.



Map of the Russian River Estuary with the five sampling transect locations. Each transect consists of three sampling locations (right, middle, and left) from which three water samples and one sediment sample were collected.

Presenters: E. Robert Atwill, UCCE Specialist, DVM, Ph.D.
David J. Lewis, UCCE Watershed Management Advisor

Please **RSVP** to Kathy Perry at 707-565-2621 by Monday, September 4, 2006 so we can plan accordingly.

Directions to workshop location are available at http://www.waterboards.ca.gov/northcoast/contact.html

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Workshop made possible through support from the State Water Resources Control Board and the Costa-Machado Act of 2000.