RUSSIAN RIVER PATHOGEN TMDL MONITORING PILOT PROJECT:

A SUMMARY REPORT TO THE NORTH COAST REGIONAL WATER QUALITY CONTROL BOARD

06-428-110 PROJECT REPORT

Prepared By:

Joshua H. Viers, Fraser M. Shilling, Michael L. Johnson, Lizabeth Bowen, Rachel A Hutchinson, Henry Calanchini, Anja Wehrman, and Heidi Schott

University of California, Davis

September 2009

Acknowledgements

Portions of this work were funded by a North Coast Regional Water Quality Control Board grant (SWRCB# **06-428-110**).

Please cite this report as follows:

Viers, JH, FM Shilling, ML Johnson, L Bowen, RA Hutchinson, H Calanchini, A Wehrman, and H Schott. 2009. Russian River Pathogen TMDL Monitoring Pilot Project: a Summary Report to the North Coast Regional Water Quality Control Board. University of California, Davis. 156 ppd.

Table of Contents

List of Figures

List of Tables

Executive Summary

We present here a summary of a pilot project to investigate pathogens in the tributaries of the Lower Russian River. We developed a weight-of-evidence approach and alternative methods to model potential sources of pathogenic bacteria and to assess their relative relationship to results of ongoing fecal indicator bacteria monitoring programs. We determined that pathogenic bacteria are pervasive, persistent, and overwhelmingly human in origin.

We used a weight-of-evidence approach to identify and quantify fecal contamination, potential sources, and trends. Foremost is the use of real time quantitative polymerase chain reaction ("DNA fingerprinting"), coupled with stable isotope analysis of nitrate, traditional fecal indicator bacteria, and spatial land use analyses. We focused on the winter storm period or wet season, with sampling activity spanning from December 19, 2008 to May 15, 2009. From our sampling strategy covering a range of hydrologic conditions and differing by dominant land uses, we were able to separate samples into three broad periods of precipitation magnitude, and three broad classes of land uses. For each of these separate and in combination, we analyzed sample values for underlying correlative drivers of contamination.

We have every reason to believe other sites identified by Regional Board staff as exceeding recommended limits on fecal indicator bacteria are as contaminated as any site sampled in our pilot study. No data have been presented to us to suggest that source and level of impairment would differ from those surveyed and presented here. In effect, the Lower Russian River watershed is heavily polluted by human waste under a wide variety of hydrological conditions. We should caution that these methods do not attempt to identify or quantify specific disease causing pathogens or their pathogenicity; however, our proxies do quantify the relative potential for many important pathogens that emanate from human (e.g., , *Cryptosporidium* spp.) and cattle (*Escherichia coli* 0157:H7) feces. We conclude by suggesting that long term strategies for monitoring of pathogens in the Lower Russian River watershed be sufficiently robust in the frequency and location, but also opportunistic, to capture episodic events as warranted.

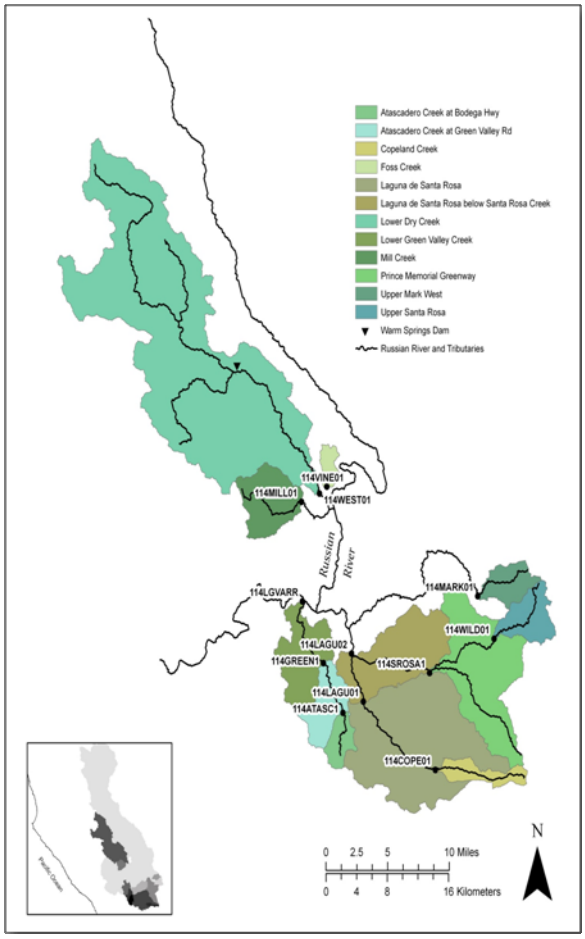
I. Introduction

The purpose of this pilot project, conducted over the months of December 2008 – May 2009 was: 1) to evaluate emerging techniques for identifying and quantifying fecal indicator bacteria; 2) to document spatial and temporal trends in fecal indicator bacteria, as related to winter storm water pulses; and 3) to provide the North Coast Regional Water Quality Control Board (NCRWQCB) recommendations toward improving detection, quantification, monitoring, and remediation of pathogens in impaired waterbodies throughout the Region.

The summary below describes sampling of fecal indicator bacteria (FIB) on 8 occasions over a 6 month period using standard methods (see Quality Assurance Project Plan, submitted to NCRWQCB, and included in Appendix H) and evaluated in conjunction with several emerging techniques to elucidate potential FIB sources, trends, and outcomes. These techniques include real-time quantitative polymerase chain reaction (rt-qPCR), stable isotope analysis of nitrate (SIA), and spatio-temporal land use analysis within a geographical information system (GIS).

Pilot Study Location

The pilot study was located in the lower Russian River watershed, within Sonoma County and included the cities of Santa Rosa and Healdsburg. Prior to the winter storm sampling period, we identified several potential sampling locations distributed throughout the lower Russian River watershed to cover a wide range of land uses, stream course sizes and drainage areas, in addition to accounting for nestedness (to evaluate potential cumulative impacts), previously known pathogen problems, and any confounding gaps in historical data. After field evaluation and consultation with NCRWQCB staff, we selected 12 locations to use in the pilot project (Table 1) based on a rationale described in Appendix H.



Due to the listing of the Lower Russian River for Pathogen impairment as per Section 303(d) of the federal Clean Water Act, we understood there to be a number of potential sources for fecal indicator bacteria, not limited to human activities, septic and/or sewer failure, wild animals, and animal agriculture. This understanding was due to a conventional assessment of surrounding land uses, exceptionally high FIB readings in certain locations, previously published material and personal observation (Figure 1). The spatial distribution of the chosen sites covered dominant land uses, drainage areas, and geography (Figure 2).

Figure 1 Land uses in the study area include crop and animal agriculture, urban and exurban residential areas, and open spaces.

Figure 2 Map of Project Area and Sample Locations

Table 1 List of Pilot Project Sample Locations with corresponding Regional Board identifiers and coordinate locations.

RRPPID	Site	RBID	Existing	Latitude (DD)	Longitude (DD)
114GREEN1	Atascadero Creek at GVR	RBATA001	Regional Board	38.44431	-122.877
114ATASC1	Atascadero Creek at Bodega Hwy	RBATA004	Regional Board	38.39747	-122.8482
114COPE01	Copeland Creek	RBCOP001	Regional Board	38.34312	-122.71196
114VINE01	Foss Creek	RBFOS001	Regional Board	38.61111	-122.87188
114LAGU01	Laguna de Santa Rosa	RBLAG005	Regional Board	38.4079267	-122.81807
114SROSA1	Prince Memorial Greenway	RBSRC004	Regional Board	38.4348139	-122.71968
114WILD01	Upper Santa Rosa	UCDRRPP001	New Site	38.4667194	-122.62166
114MARK01	Upper Mark West	CCMWC004	CCWI	38.5070844	-122.64615
114WEST01	Lower Dry Creek	UCDRRPP002	New Site	38.6045739	-122.88254
114MILL01	Mill Creek	CCMIL001	CCWI	38.5965139	-122.90969
114LAGU02	Laguna de Santa Rosa downstream of Confluence	114LAGU02	Regional Board	38.4515976	-122.83453
114LGVARR	Lower Green Valley Creek	CCGVC00	CCWI	38.5023613	-122.90854

II. Methods & Results

Monitoring stations were sampled eight times between December 19th, 2008 (2008-12-19) and May 15th, 2009 (2009-05-15), capturing a range of hydrological conditions during the winter storm pulse period (Figure 3). For each sampling event, excluding two dates[1], all 12 monitoring stations were visited. All samples were properly collected, stored, and processed according to the Quality Assurance Procedure Program (QAPP) prior to delivery to Brelje & Race Labs[2] by UC Davis Aquatic Ecosystems Analysis Laboratory (AEAL)[3] (Appendix H). At least one blank and one duplicate sample were collected during each sampling event. At each monitoring station sampled during a particular sampling event, Surface Water Ambient Monitoring Program (SWAMP) data forms were completed and entered into a Microsoft Access database (copies of these data were also forwarded to the NCRWQCB for input into the state-wide SWAMP database). For additional information regarding field conditions during collection, please see Appendix A.

In addition to surface water samples delivered to Brelje & Race Labs, samples were transported to UC Davis for rt-qPCR analysis by AEAL, and SIA by the UC Davis Stable Isotope Facility[4]. Additional field methods included mapping each site, and describing site conditions (see Appendices A & G). At each location, a coordinate pair was determined and stored using a global position system (GPS); each site's position was then entered into the GIS to be used in spatial analysis (see Section C below).

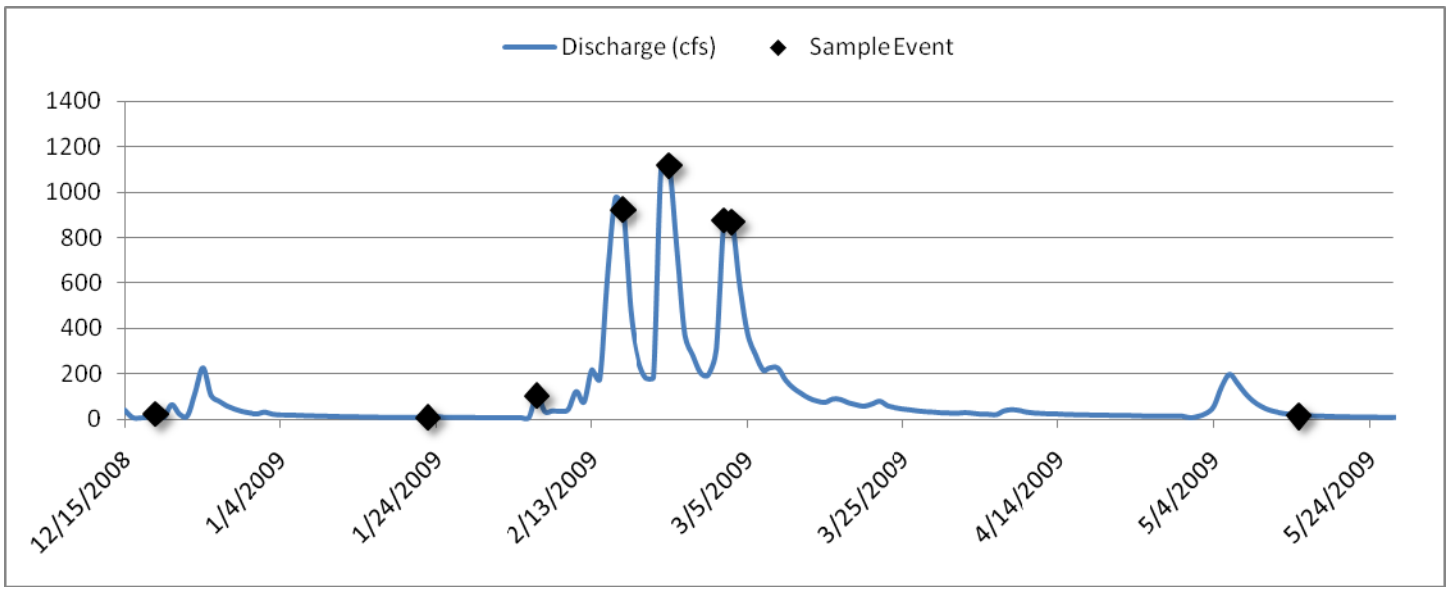


Figure 3 Hydrograph of 2008-2009 water year for period of sampling showing sample events by date. Discharge (cfs) is a composite of two gages (USGS 11466200 & 11465750) as a relative proxy for hydrological processes found in Lower Russian River tributaries.

A. Pathogens: *E. coli* & *Enterococcus* spp.

The normative FIB monitoring program for water quality monitoring entities in and around the project area regularly rely on Colilert® and Enterolert® quantitray methodologies for detection and quantification. While the intent of this pilot project was not to reproduce analyses that would normally be included in the regular monitoring of NCRWQCB waterbodies that use such data, we have included here an overview of results to show the spatial variability and temporal trends of our wet season storm event sampling effort.

---

[1] One sample (114WILD01) was not collected on 2009-01-23 (see Appendix A), and five locations were not sampled on 2009-02-17 due to lab[2] inabilities.

[2] Brelje & Race Labs: http://www.brlabsinc.com/

[3] UC Davis AEAL: http://aeal.ucdavis.edu/

[4] http://stableisotopefacility.ucdavis.edu/

A1. Pathogen Methods

Pathogen samples (*E. coli*, *Enterococcus* spp., and Total Coliform) were collected via grab sample, stored on ice, and delivered to the Brelje & Race Lab for analysis (see QAPP for specific method details). The samples were diluted by 10mL or 100mL, depending whether or not appropriate values were detectable in earlier sampling event(s) (note: the first sampling event, 2008-12-19 was diluted at 10mL as no previous data were available). After laboratory procedures, data were reported to UC Davis, entered into a Microsoft Access database, quality controlled for potential errors, and statistically analyzed in the statistical software JMP 8 (SAS, Cary, NC) [5]. Additional field data were collected, such as water temperature, pH, and dissolved oxygen, as described in the QAPP (Appendix H), and are briefly discussed below.

A2. Pathogens Results

Environmental data were analyzed as a normative or standard part of the quality assurance portion of our data preparation and analysis procedures. Trends in water quality were in accordance with *a priori* expectations; for example, water temperatures warmed in the latter half of our sampling period (Figure 4). Our control sites (114MARK01, 114MILL01, 114WILD01) were consistently of better water quality than all other sites, including cooler water temperatures ($p < 0.0003$), higher dissolved oxygen ($p < 0.0001$), and less acidic ($p < 0.0001$) as adjusted for date. See Appendix B for detailed environmental data.

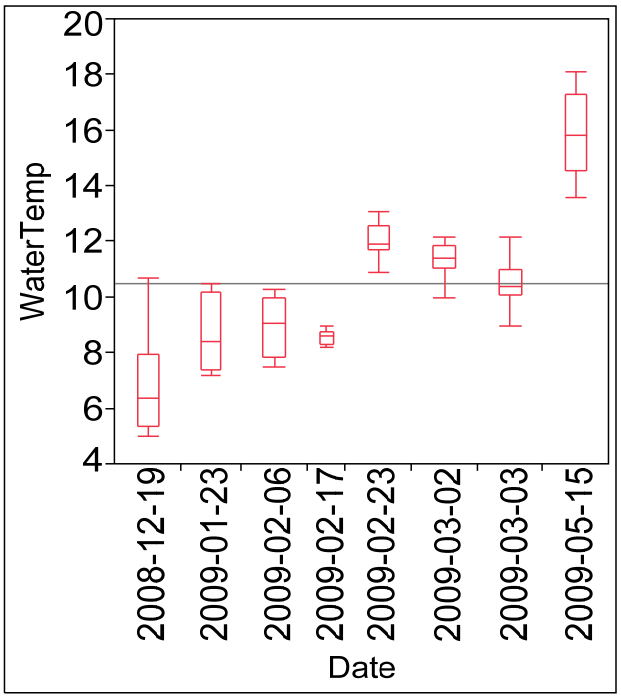


Figure 4 Water temperature (°C) trends by date.

Pathogenic FIB values were evaluated for exceedances, frequencies, and trends using a variety of statistical techniques. Geometric means were calculated for *E. coli* and *Enterococcus* data for the following periods: December – January, January – February, and February – March. Each period combined samples from dates taken within the two months indicated. Because our procedures were designed to capture flow variability, the total number of days for each period varied. The December-January values included only two sample events; the other periods each had five sample events within 30 days (2009-1-23 : 2009-2-23 and 2009-2-6 : 2009-3-3). Thus, for the *E. coli* and *Enterococcus* spp. measures shown in Figure 5 and Figure 6, respectively, the charted values represent geometric means for that period indicated, with values shown in comparison to the geometric mean of all samples pooled across locations, geometric means pooled within each sample location (or grand geometric mean), and recommended numeric limits. Only the grand geometric mean includes the 2009-5-19 sample. The recommended numeric limits from the California Department of Public Health (formerly known as Department of Health Services or DHS) reference the Guidance for Fresh Water Beaches (DHS 2007 [6]) and its Appendix B US EPA Guidance for Recreational Waters and Beaches (US EPA 2000 [7]). Although we show only the single day value thresholds as recommended numeric limits for illustrative purposes, the 30 day thresholds are roughly 50% lower in magnitude.

The three semi-natural controls sites (114MARK01, 114MILL01, 114WILD01) were consistently at the lower end of impairment. Of the twenty total samples taken at these three sites, ten samples (50%) were in exceedance for *E. coli*; however, sixteen samples (80%) were in exceedance for *Enterococcus* spp. All *Enterococcus* spp. exceedances were

[5] http://www.jmp.com/

[6] http://www.cdph.ca.gov/HealthInfo/environhealth/water/Documents/Beaches/DraftGuidanceforFreshWaterBeaches.pdf

[7] http://www.cdph.ca.gov/HealthInfo/environhealth/water/Documents/Beaches/AppendixB.pdf

within an order of magnitude of the DHS limit of 61 MPN/100mL, with maxima of 738 MPN/100mL at 114WILD01 and 945 MPN/100mL at 114MARK01, both on 3/2/2009. Each measure was at least 50% less the following day. In effect, these sites were the least impaired from FIBs as measured using standard monitoring protocols.

The most problematic sites from elevated *E. coli* include 114LAGU01, in Sebastopol, and 114VINE01, in Healdsburg. The geometric mean for all *E. coli* samples taken was 1934.8 MPN/100mL and 1936.8 MPN/100mL respectively. For five samples in a 30-day period, these geometric means were more than twice as high during January – February. Other sites with exceptionally high *E. coli* samples include 114ATASC1, 114SROSA1, and 114LAGU02. The non-control sites averaged above limit detections for 79% of all samples for *E. coli*. There was one site that exceeded recommended *E. coli* limits for 100% of the samples taken: 114SROSA1. The maximum detected *E. coli* measure was 48,840 MPN/100mL at 114LAGU01 on 2/17/2009.

Similar to *E. coli*, the most problematic sites for *Enterococcus* spp. were 114LAGU01 and 114VINE01 with geometric means of 913.9 MPN/100mL and 926.6 MPN/100mL for all samples respectively. Again each site had five samples with 30-day periods at 2-3 times these values. Although the control sites exceeded *Enterococcus* spp. limits 80% of the time, the non-control sites were in exceedance 89% of the time, with three sites 100% of the time: 114COPE01, 114LAGU01, and 114VINE01. The maximum detected *Enterococcus* spp. measure was 10910 MPN/100mL at 114LAGU01 on 2/23/2009.

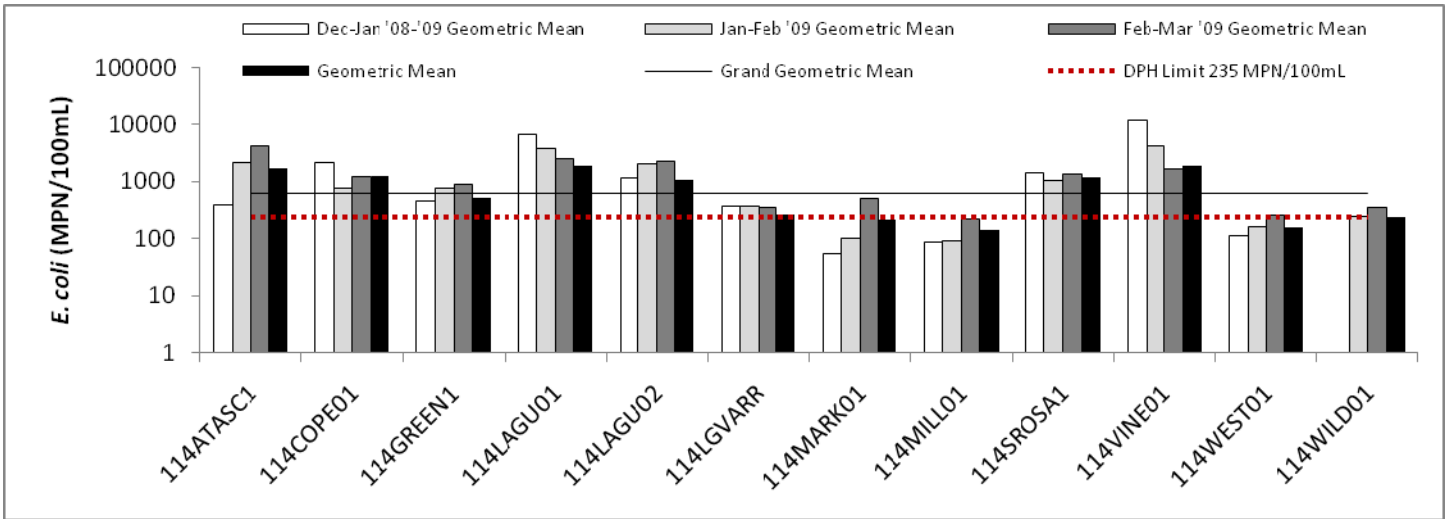


Figure 5 Geometric means calculated for *E. coli* samples collected throughout the 2008-09 pilot study. Geometric means were commonly above the numeric limit of 235 MPN/100mL.

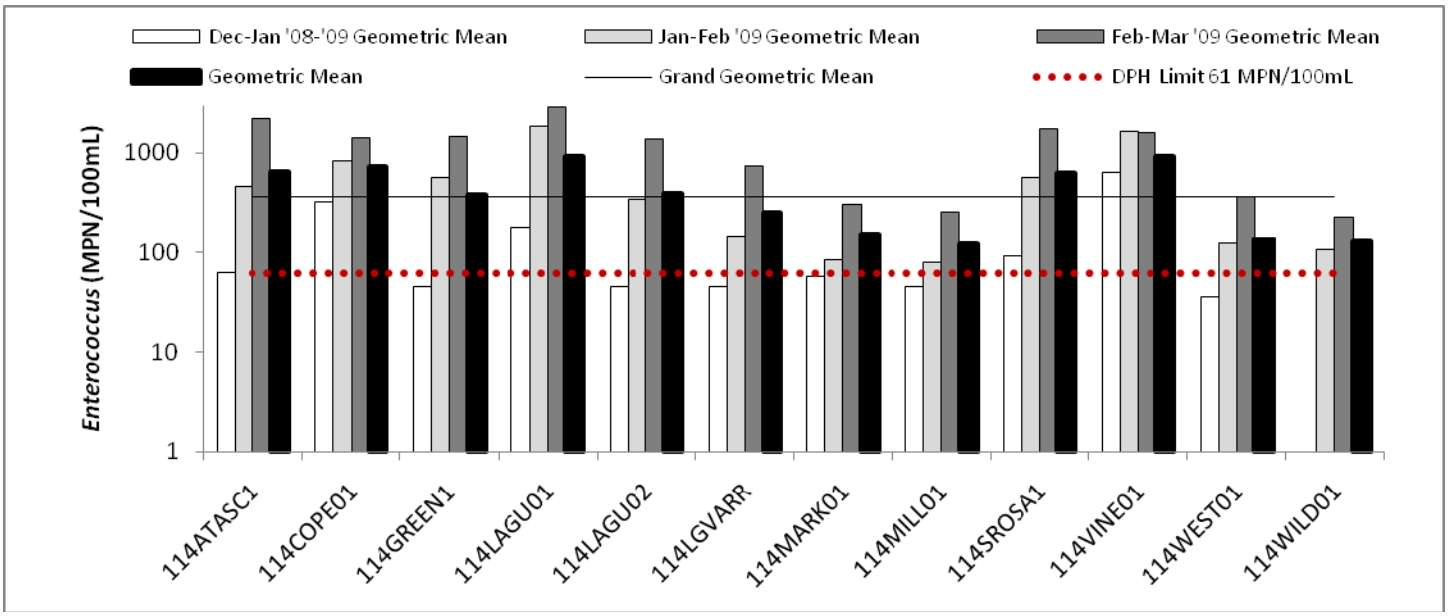


Figure 6 Geometric means calculated for *Enterococcus* spp. samples collected throughout the 2008-09 pilot study. Geometric means were commonly above the numeric limit of 61 MPN/100mL.

Temporal trends were largely consistent with hydrologic conditions and time of year, and relative magnitudes were synchronous (Figure 7). When examining hydrologic phase (i.e., antecedent "small" storms, storm pulses, and recession flows), there are significant differences in FIBs. For *Enterococcus* spp., after excluding all samples below detection limits (n=17) and $\log_{10}$ transforming to achieve a normal distribution, we compared means adjusted for significance for all pairwise combinations (Tukey-Kramer Honestly Significant Difference [8]). The storm pulse *Enterococcus* spp. samples were greater than antecedent storms ($p < 0.0001$), which were greater than recession flows ($p=0.0111$). Similarly we examined the role of semi-natural watershed controls, which are the three headwater sites dominated by forest cover. They are considered "controls" from a statistical perspective because they can be used to control for baseline levels of naturally occurring FIBs from wildlife and soil biota. They are considered "semi"-natural in that there has been both historical and contemporary human disturbance, and certain land uses such as residential homes, grazing, and timber removal continue to happen across these lands. Thus some levels of impairment are expected, but they provide the best baseline condition within the study watershed. In this case, the semi-natural watershed controls which were significantly lower across all dates ($p=0.0049$), and by dominant land use category, where semi-natural was significantly less impaired than urban ($p=0.0114$) and agricultural ($p=0.0216$), but no differences were found between urban and agricultural sites ($p=0.98$). We found no differences between sites located in separate drainages (i.e., Laguna de Santa Rosa, Atascadero, Dry Creek). For *E. coli*, we conducted a similar analysis to compare means across all dates, excluding all detection limited samples, $\log_{10}$ transformed for normality, and adjusted for multiple comparisons. Our study control sites had significantly fewer *E. coli* than other sites ($p=0.0046$). These *E. coli* samples were significantly lower during the recession flow phase than both the storm pulse phase ($p < 0.0001$) and antecedent storm phase ($p<0.0001$), which were not different from each other ($p=0.77$). As with *Enterococcus* spp., *E. coli* samples taken from semi-natural sites were significantly lower than both urban ($p=0.0001$) and agricultural ($p=0.0064$) sites, which were not different from each other ($p=0.43$). Source drainage had no role in sample differences.

[8] http://en.wikipedia.org/wiki/Tukey's_range_test

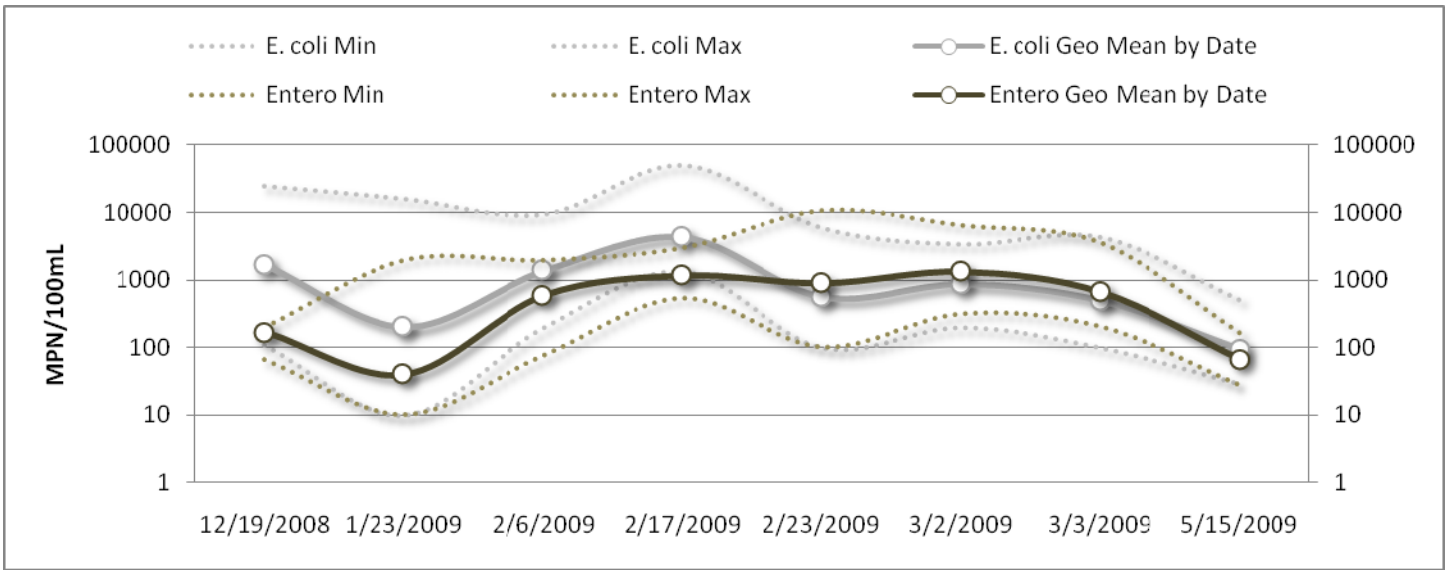


Figure 7 Temporal trends of FIBs for dates sampled.

B. Antecedent Precipitation & Flow Analyses

Understanding the relationship between precipitation, flow, and FIB is a critical component of monitoring and containment. Conceivably, if relationships exist between winter storm conditions and FIB conditions, they can not only identify trigger points for monitoring activity, but also help in load allocation determinations through historical trend analysis or serve as a benchmark for future load reductions. This latter point, which is in effect site indexing, could serve as an important strategy going forward for improving water quality in the basin more generally.

B1. Antecedent Precipitation & Flow Methods

Daily flow and precipitation data for selected stations were downloaded for the time period of this study (Dec '08 –May '09). Flow data were obtained from four USGS gages[9] and precipitation data were obtained for 18 weather stations[10] (Table E1), seven of which were located near RRPP sampling locations, and the others on a wide periphery to capture variations in precipitation due to proximity to the Pacific Ocean and variable elevation. All data were entered into the project's database and analyzed in JMP 8. The flow gage on Dry Creek (USGS 11465350), associated with the 114WEST01 site, was not used as it records only flows below 200 cfs, leaving three USGS gages for comparative purposes. We used standard GIS methods to interpolate daily precipitation across the project area for all sampling dates and each preceding two days, except for the last sampling date (2009-05-15) which was a dry season event (i.e., no active storm pulse). Thus seven sampling dates were used in the analyses presented here. We used inverse distance weighting (IDW[11]) in ArcGIS 9.3 Geostatistical Analyst (ESRI, Redlands, CA), which is an interpolation technique, to create precipitation layers at 10m resolution by assigning values for unknown cells from known neighboring values. IDW weights neighboring values higher (i.e., the closer the neighbor is to the cell to be assigned, the more influence it carries) as opposed to linear interpolation.

Comparison of Precipitation to Flow

We compared average precipitation, as estimated from the IDW method for each cumulative watershed, to gauged flow data at three locations. While this correlative method does not account for spatial variability in land cover (e.g., capture

[9] http://waterdata.usgs.gov/nwis/rt

[10] http://www.wunderground.com/weatherstation/index.asp

[11] http://en.wikipedia.org/wiki/Inverse_distance_weighting

losses due to evapotranspiration) or potential temporal lags (i.e., flashiness due to impervious cover), the method is easily conducted.

Comparison of Precipitation to FIB

We correlated FIB sample values and IDW watershed averages of estimated precipitation for one day (day of sample) and three day antecedent sum (i.e., 2 days prior to and the day of sample).

B2. Antecedent Precipitation & Flow Results

The IDW precipitation estimation for one day and three day antecedent sums compared well to gauged daily discharge at three USGS gauged locations for the 7 sampling periods. Using a natural log-log regression, both day of event and three day antecedent values were highly predictive. The IDW precipitation for the Copeland Creek site (114COPE01) – a smaller urban headwater catchment – resulted in an adjusted R^2 of 0.74 (p=0.0081), for the Santa Rosa Creek site – a mid-course urban watershed – resulted in adjusted R^2 of 0.59 (p=0.03), and for the Laguna de Santa Rosa site (114LAGU02) – a larger mixed land use watershed with low gradient – resulted in an adjusted R^2 of 0.42 (p=0.07). Results of the antecedent condition assessment (i.e., three day mean discharge vs. three day summed precipitation averaged over the cumulative watershed) exhibited higher explanatory (R^2) and significance (p) values (Figure 8, see also Appendix E).

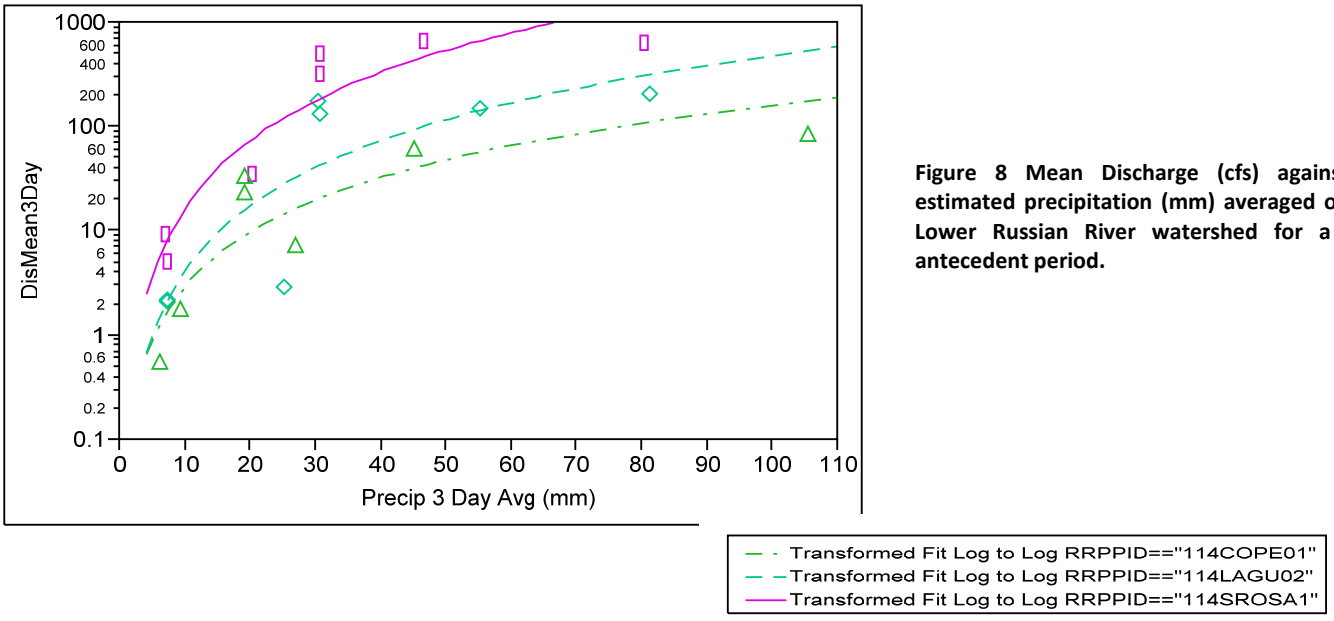


Figure 8 Mean Discharge (cfs) against IDW estimated precipitation (mm) averaged over the Lower Russian River watershed for a 3 day antecedent period.

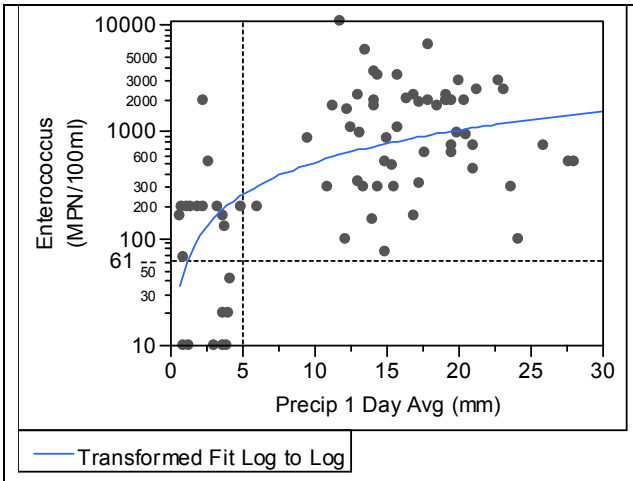
Precipitation estimates were positively and significantly correlated with FIB values (Table 2). Using the non-parametric Spearman rank correlation, which doesn't carry assumptions regarding the normality of distribution, and excluding any values at minimum or maximum detection limits, both one day and three day precipitation estimates were highly correlated with FIB values (note: all values were natural log transformed).

Table 2 Spearman rank correlations between IDW estimated precipitation and sampled FIB values.

Variable	by Variable	Spearman ρ	Prob>|ρ|
LN(1DayPrecip mm)	LN(ECOLI)	0.32	0.017
LN(1DayPrecip mm)	LN(ENTERO)	0.40	0.002
LN(3DayPrecip mm)	LN(ECOLI)	0.32	0.016
LN(3DayPrecip mm)	LN(ENTERO)	0.40	0.002

The most elucidating of the relationships between FIBs and precipitation is *Enterococcus* spp. As shown in Table 3, regressions of *Enterococcus* against precipitation resulted in significantly positive relationships that each accounted for over one-third of the observed variance. In effect, a phase change is grossly apparent at 5 mm for 1 day precipitation and 10 mm for 3 day precipitation to elevate sample values above the CDPH recommended standard of 61 MPN/100ml (Table 3; Appendix E). Above those levels of precipitation, all observed *Enterococcus* values were above the CDPH standard. Based on fitted relationships for each regression, however, *Enterococcus* would exceed the recommended limit at 1.2 mm for one day precipitation and 3.5 mm for three day precipitation, suggesting that even minor amounts of precipitation mobilize *Enterococcus* into receiving waters.

Table 3 Comparison of 1 and 3 day antecedent rainfall to all *Enterococcus* spp. observations. There is a 5mm 1 day precipitation threshold, below which some *Enterococcus* spp. are below the 61 MPN/100mL limit. Similarly, there is a 10mm 3 day precipitation threshold, below which some *Enterococcus* spp. are below the 61 MPN/100mL limit. We consistently modeled the relationship between precipitation and observed Enterococcus spp. levels.



Transformed Fit LN to LN

LN(Enterococcus (MPN/100ml)) = 3.9675696 + 0.9939001*LN(Precip 1 Day Avg (mm))

Summary of Fit

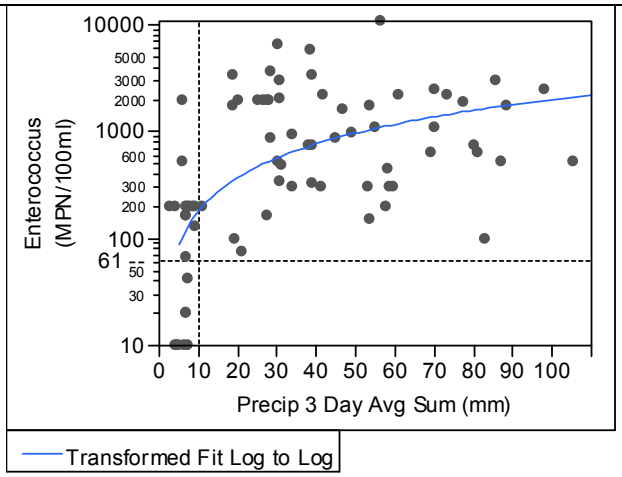
RSquare	0.367947
RSquare Adj	0.359631
Root Mean Square Error	1.329221
Mean of Response	6.155339
Observations (or Sum Wgts)	78

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	78.17007	78.1701	44.2432
Error	76	134.27899	1.7668	Prob > F
C. Total	77	212.44906		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	3.9675696	0.36171	10.97	<.0001*
LN(Precip 1 Day Avg (mm))	0.9939001	0.149424	6.65	<.0001*



Transformed Fit LN to LN

LN(Enterococcus (MPN/100ml)) = 2.809244 + 1.0420307*LN(Precip 3 Day Avg Sum (mm))

Summary of Fit

RSquare	0.376533
RSquare Adj	0.368329
Root Mean Square Error	1.320163
Mean of Response	6.155339
Observations (or Sum Wgts)	78

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	79.99400	79.9940	45.8989
Error	76	132.45506	1.7428	Prob > F
C. Total	77	212.44906		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.809244	0.516022	5.44	<.0001*
LN(Precip 3 Day Avg Sum (mm))	1.0420307	0.153808	6.77	<.0001*

On a site by site basis, *Enterococcus* spp. was consistently modeled as a function of antecedent precipitation conditions (Appendix E); however, the form of the model was not always consistent. While *E. coli* was occasionally modeled well with antecedent conditions, it is clear that other factors such as seasonality, longevity and land uses would be more definitive from a source-response perspective. For example, four sites (114MILL01, 114SROSA1, 114LGVARR,

114LAGU01) had no relationship between *E. coli* and precipitation. Seven sites were adequately modeled for *E. coli* concentrations as a function of antecedent precipitation using natural log-linear transformations. The 114WEST01 site (114WEST01) was likely confounded by releases from Warm Springs Dam. In a visual example, *E. coli* response is shown as 2nd degree polynomial fit to 1 Day precipitation in Figure 9. In this case, higher precipitation, overland flow, and potential upstream dam releases, lowered the observed *E. coli* counts. With regards to *Enterococcus* spp. concentrations, best fitted models were inconsistent in form, transformation, or antecedent period (Table 4), although each sampling site did exhibit some statistical relationship with antecedent precipitation conditions. In other words, there was no one consistent statistical model for *Enterococcus* spp. and precipitation across all sites (i.e., single mathematical formula relating these data to each other at each site), though each site did exhibit consistent responses within sites. For example (see Table 4), three sites exhibited strong relationships for both 1 Day and 3 Day antecedent values (114VINE01, 114WEST01, 114 SROSA1), while all other sites showed statistical strength for only 1 Day precipitation. In other words, observed values of Enterococcus spp increase with increasing precipitation on a 1 Day basis; it appears that as total precipitation is accumulated over a 3 day period, however, this response diminishes. An illustrative example is provided in Table 5, where *Enterococcus* spp. concentrations are regressed against antecedent precipitation at 1 Day and 3 Day periods 114SROSA1. The 3 day antecedent precipitation show that with increasing precipitation, and hence overland flow, the magnitude of *Enterococcus* spp. decreases. It is important to keep in mind that these are concentration based responses (MPN/100ml) to log transformed precipitation (i.e., exponentially increasing water volume), meaning that the total load of *Enterococcus* spp. in the receiving water is much higher with increasing precipitation.

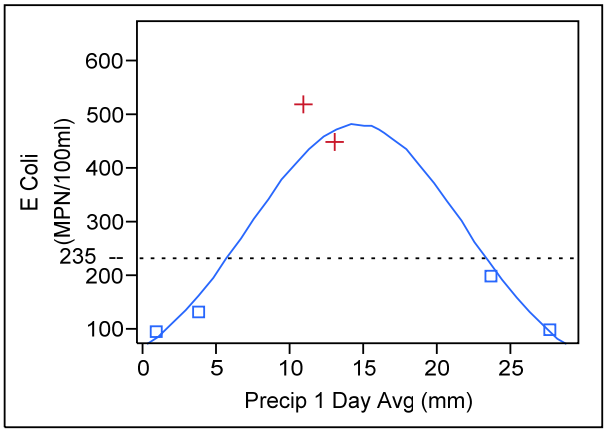


Figure 9 Polynomial fit between *E. coli* and estimated antecedent 1 day rainfall-runoff potential.

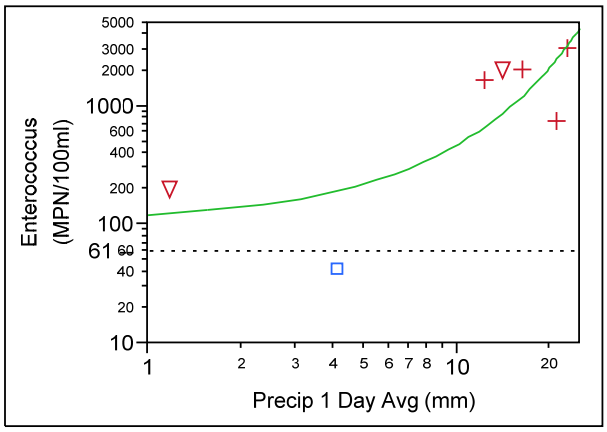
Table 4 Best regression model fits between *Enterococcus* spp. and estimated antecedent precipitation (details in Appendix E).

Site	Best *Enterococcus* spp. Precipitation Model	Competing *Enterococcus* spp. Precipitation Model
114ATASC1	3 Day Log Quadratic	
114COPE01	1 Day Log Quadratic	
114GREEN1	1 Day Log Linear	
114LAGU01	1 Day Log Linear	
114LAGU02	1 Day Log Linear	
114LGVARR	3 Day Linear	
114MARK01	1 Day Log Linear	
114MILL01	1 Day Log Quadratic	
114SROSA1	1 Day Log Linear	3 Day Log Quadratic

Site	Best *Enterococcus* spp. Precipitation Model	Competing *Enterococcus* spp. Precipitation Model
114VINE01	1 Day Log Linear	3 Day Log Linear
114WEST01	1 Day Linear	3 Day Linear
114WILD01	1 Day Log Linear	

Table 5 Example of regression models for *Enterococcus* spp. concentrations against estimated antecedent precipitation at 1 Day and 3 Day periods. (Note change in model form).

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114SROSA1



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 4.6403182 + 0.1510259*Precip 1 Day Avg (mm)

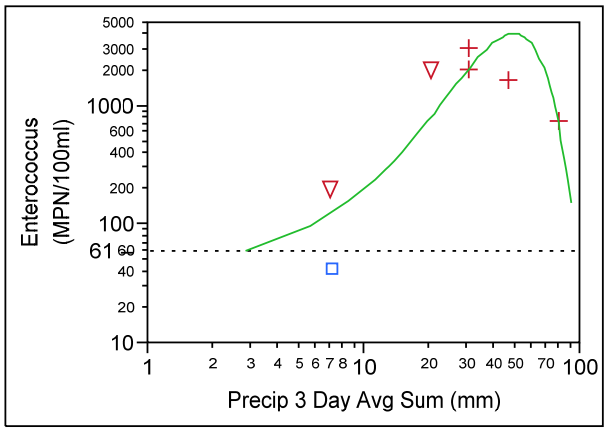
Summary of Fit

RSquare	0.606759
RSquare Adj	0.52811
Root Mean Square Error	1.073115
Mean of Response	6.617626
Observations (or Sum Wgts)	7

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	8.884217	8.88422	7.7148
Error	5	5.757878	1.15158	**Prob > F**
C. Total	6	14.642095		0.0390*

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 3 Day Avg Sum (mm) RRPPID=114SROSA1



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 3.5642776 + 0.1933456*Precip 3 Day Avg Sum (mm) - 0.0019666*Precip 3 Day Avg Sum (mm)^2

Summary of Fit

RSquare	0.771834
RSquare Adj	0.65775
Root Mean Square Error	0.913898
Mean of Response	6.617626
Observations (or Sum Wgts)	7

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	3.5642776	0.902149	3.95	0.0168*
Precip 3 Day Avg Sum (mm)	0.1933456	0.053927	3.59	0.0231*
Precip 3 Day Avg Sum (mm)^2	-0.001967	0.000609	-3.23	0.0320*

C. GIS Analysis and Land Use Contributions

C1. GIS Analysis and Land Use Contributions Methods

We used a standard framework for our GIS analysis to evaluate potential land use contributions to observed FIB concentrations. The framework consisted of establishing a series of spatial sampling units based on a 10m digital elevation model (DEM[12]). All analyses were conducted in ArcGIS (v. 9.3 ESRI, Redlands, CA) using standard algorithms[13] such as FLOWDIRECTION, FLOWACCUMULATION, and WATERSHED.

[12] http://seamless.usgs.gov/index.php

For each sampling station, we created a sub-watershed and a cumulative watershed using the flow direction and flow accumulation surfaces derived from the DEM. One half of the sampling stations have no upstream sampling stations; thus, the sub-watershed delineation is the same as the cumulative one. Secondly, we buffered stations by 500, 1000, 2000, and 4000 m and intersected with their sub-watersheds to create sub-watershed scale buffer zones (see for example Figure 12). These buffered zones were intersected with: (1) the City of Santa Rosa septic or sewer layer, (2) the Department of Water Resources (DWR) land cover (1999), (3) 2000 census data processed by CalFire, (4) Sonoma County Parcel Data, and (5) Sonoma County Urban Service Areas.

C2. GIS Analysis and Land Use Contributions Results

We analyzed land use data in several ways: 1) broad land use designations using principal components analysis of percent land cover over the entire watershed; 2) a nested approach of concentric buffered contributing areas within each watershed and percent land cover to help determine any spatial dependencies; 3) influence of dairies based on DWR designations; and 4) influence of sewer-septic systems based on City of Santa Rosa GIS layers.

Land Use Designations

Dominant land-use types were determined by performing a principal components analysis (PCA[14]) on land use categories (e.g. "Urban", "Developed", "Natural") and estimates of human population density (Appendix F). The PCA takes a large number of correlated variables and transforms them into fewer uncorrelated variables. The results of which, called components, can be further analyzed to determine which of the original variables best describe the new component. PCA was used here to minimize multicollinearity[15]. The first two PCA components accounted for 92% of the variation and were deemed sufficient to separate sites by three dominant land uses: Agriculture, Semi-Natural, and Urban, with four sites per type. As shown Appendix F, the statistical results indicate that measures of percent urban land use drove scores of the first PCA component, and percent agriculture largely drove the second component. In other words, the other independent variables, such as population density, housing unit density, parcel density, and percent urban service area, were significantly correlated with percent urban, thus percent urban was chosen to represent this axis.

Given the land use composition for each site, it is clear in the PCA plot of the first two components (as shown in Figure 10) that semi-natural sites are well clustered, and there exists some relative cross-over between sites containing both urban and agricultural land uses on the first component. However, the two most closely positioned sites designated as Urban and Agriculture in Figure 10 are located on Laguna de Santa Rosa and thus are hydrologically connected and geographically close. The relative land use contributions become more apparent in Figure 11, which is a ternary plot that shows the relative contribution from three variables, or percent land uses here, and the total for each site (sum of three axes) equates to 100%. The land use designations, such as Urban, Semi-natural, and Agriculture, follow the designations from accompanying GIS data. For example, Urban is often composed of commercial, industrial, and residential land uses. Semi-natural is often composed of natural vegetation, such as forest, and Agriculture includes agricultural activities, such as row crops and dairies. Agriculture for this study should not be viewed in isolation; rather it used here as some inter-mix of crop and animal agriculture with residences and open areas interspersed.

---

[13] http://webhelp.esri.com/arcgisdesktop/9.3/index.cfm?TopicName=Delineating_watersheds

[14] http://en.wikipedia.org/wiki/Principal_components_analysis

[15] http://en.wikipedia.org/wiki/Multicollinearity

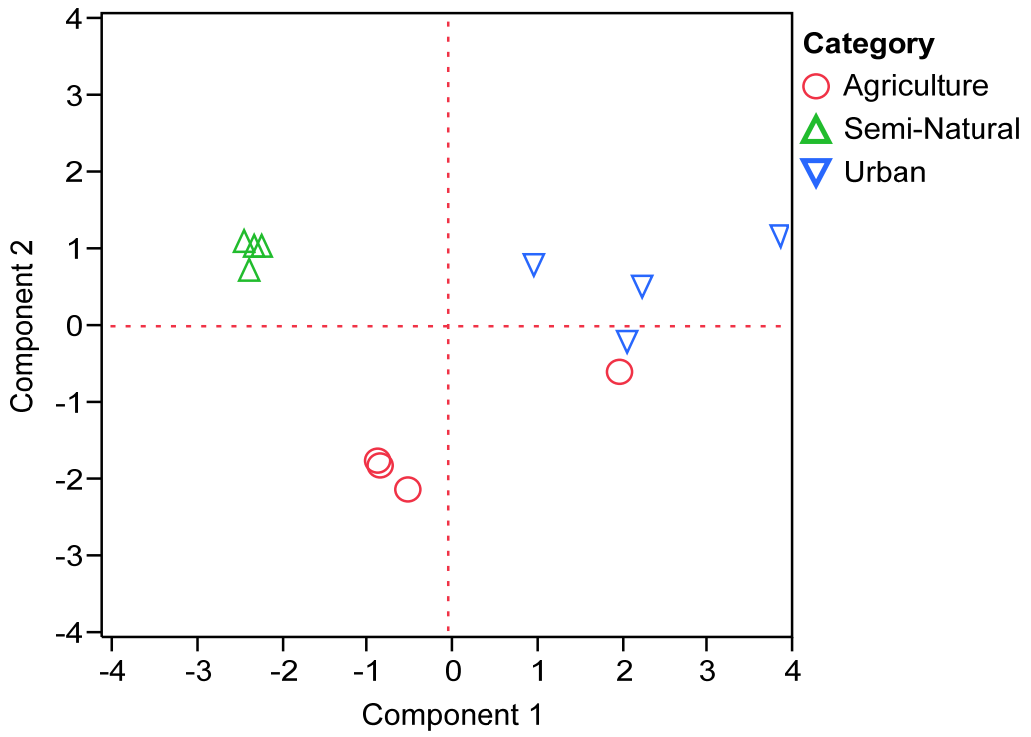


Figure 10 Plot of Principal Component Analysis scores by land use designations (see Appendix F for details).

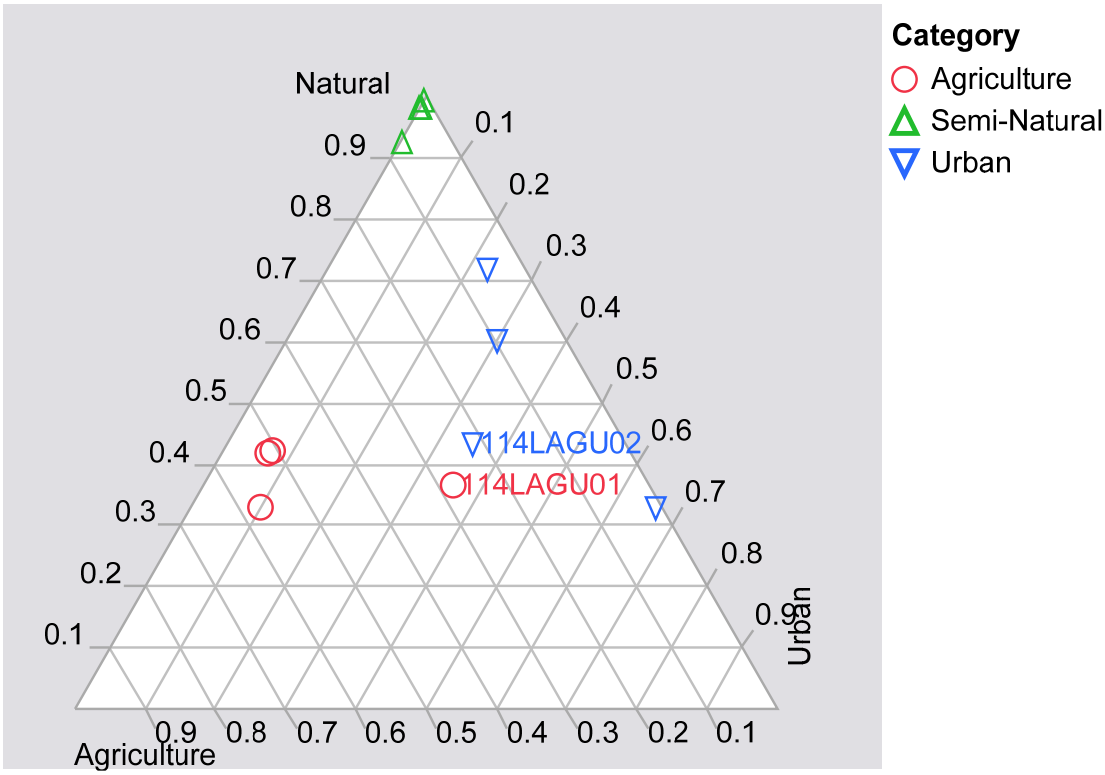


Figure 11 Ternary plot of percent land uses by sample site and designation. The two Laguna de Santa Rosa sites were very similar in composition.

The actual characterization of each site is dependent upon local and regional land uses and hydrological processes. Thus, we used a combination of scales to determine each site's land use category and to establish other designations, such as dominant watershed, and watershed position. In the Lower Dry Creek case, it has the largest drainage area, but is also hydrologically altered in that its flows are regulated by Warm Springs Dam. For this reason, we have also identified which sites are hydrologically dependent, defined as being within the same drainage or tributary watershed (e.g., Atascadero Creek, Laguna de Santa Rosa) or are independent (i.e., Northern drainages) (Table 6). We selected three semi-natural sites of the four designated to serve as study controls and to establish relative background rates: 114MILL01, 114MARK01, and 114WILD01 (as shown for Role in Table 6). We omitted the 114WEST01 site from serving as a semi-natural control because the flow is impaired by Warm Springs dam, disconnecting it from the dominant forest cover in the upper watershed, and the close proximity of vineyards to the waterbody below the dam.

Table 6 Study Sites by Dominant Land Use Category as determined by Principal Components Analysis

RRPPID	Site	Category	Drainage	Role
114GREEN1	Atascadero Creek at GVR	Agriculture	Atascadero	
114ATASC1	Atascadero Creek at Bodega Hwy	Agriculture	Atascadero	
114COPE01	Copeland Creek	Urban	Laguna Rosa	
114VINE01	Foss Creek	Urban	Northern	
114LAGU01	Laguna de Santa Rosa	Agriculture	Laguna Rosa	
114SROSA1	Prince Memorial Greenway	Urban	Laguna Rosa	
114WILD01	Upper Santa Rosa	Semi-Natural	Laguna Rosa	Control
114MARK01	Upper Mark West	Semi-Natural	Laguna Rosa	Control
114WEST01	Lower Dry Creek *	Semi-Natural	Northern	
114MILL01	Mill Creek	Semi-Natural	Northern	Control
114LAGU02	Laguna de Santa Rosa downstream of Confluence	Urban	Laguna Rosa	
114LGVARR	Lower Green Valley Creek	Agriculture	Atascadero	

Nested Watershed Approach

We chose to use a nested approach to determine relative effects of land use activities on waterbodies, meaning that we delineated all contributing drainage area to each sample location and segmented it into catchments 500m, 1000m, 2000m, and 4000m from the location (Figure 12). We also segmented by sub-watershed, meaning that drainages were divided at each upstream sample location if present. This nested approach has been used for variety of applications, including the identification of land use impacts on salmon habitat (Viers 2008). Each watershed segment was intersected with maps of land uses and analyzed for relative relationship to observed FIB values. There are several such land use maps, each with data integrity issues to be considered such as intent, scale or resolution, date of publication et cetera. We chose the land use data published by the California Department of Water Resources (DWR) because of its focus on agriculture and because it is field verified. However, we do note that the rapidity of urbanization in Sonoma County outpaces most if not all efforts to map it; thus the level of urbanization is likely greater than approximations used in this analysis.

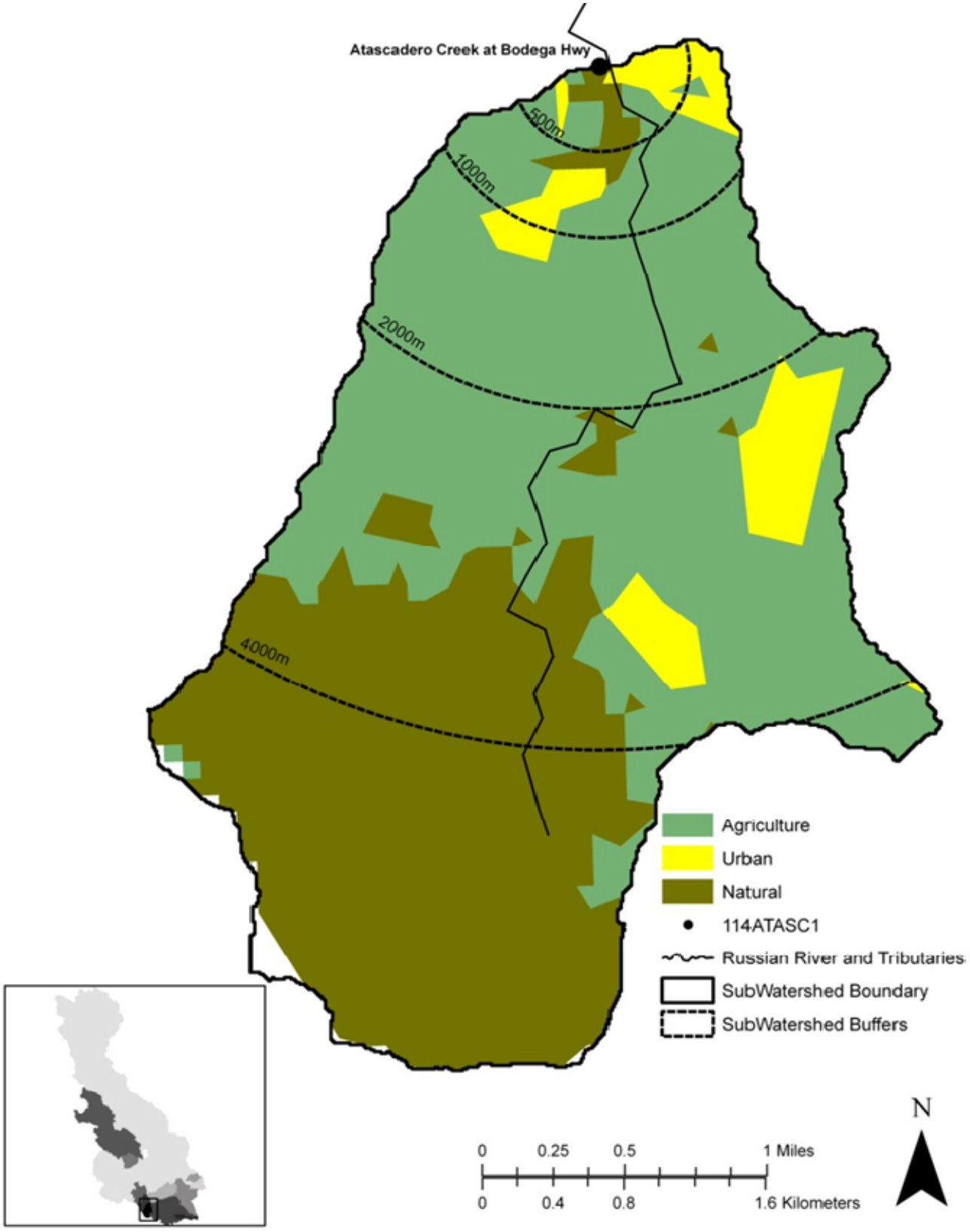


Figure 12 Map of Atascadero Creek at Bodega Highway (114ATASC1) showing nested segmentation of watershed and general land use categories.

DWR land uses

Farmsteads, dairies, poultry farms, and livestock feedlots did not make up a significant proportion of most sub-watersheds, as determined by the Department of Water Resource land use data. However, these components can still be considered a contributing factor to watershed impairment. Both of the Laguna de Santa Rosa sites (114LAGU01 & 114LAGU02) have a higher concentration of dairies than the other ten sub-watershed buffer zones. Without specific information about animal husbandry practices, these potential sources of FIB were captured in a broad class of animal agriculture, which included pasture land in addition to areas specifically designated for specific uses. We combined the following land use classes:

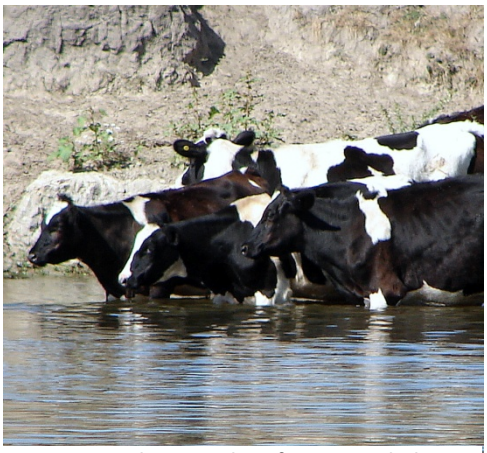


Figure 13 Photograph of potential bovine sources in the Laguna de Santa Rosa between sites 114LAGU01 and 114LAGU02.

- *Animal Agriculture*: [S] "Semi-agriculture" such as Farmsteads, livestock feed lots, Dairies, Poultry Farms; [P] "Pasture"; and [I] "Idle"
- *Crop Agriculture*: [D] "Deciduous Fruits and Nuts"; [F] "Field Crops"; [G] "Grain"; [T] "Truck/Nursery/Berry"; and [V] "Vineyards"
- *Urban*: [U] "Urban"; [UC] "Commercial"; [UI] "Industrial"; [UR] "Residential"; and [UV] "Urban Vacant"

Sewer –Septic Land Uses

We were able to identify 6 of the 12 monitoring stations with some portion of their sub-watershed covered by the City of Santa Rosa's sewer – septic GIS layer. For each portion of the sub-watershed covered by the layer, we correlated percent septic with housing unit density (US Census HU100), population density (US Census POP100), SCWA's urban service area (USA), and Sonoma County's parcel density (number of parcels per km^2). All were highly correlated (Table 7). After having reviewed these data, we felt that parcel density was the best land use predictor to use as a surrogate for sewer and septic, albeit with caveats.

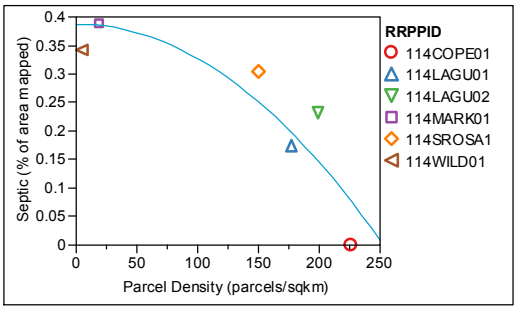


Figure 14 Relationship between percent septic and parcel densities for six sites partially covered by City of Santa Rosa's Sewer-Septic GIS.

For example, the regression in Figure 14 (R^2 = 0.76; p = 0.01) indicates that percent septic becomes asymptotic at around ~40%, and it is very likely that this should approach ~100% septic in some small drainages. Further, at very high parcel densities (such as 114VINE01), percent septic can go negative. Thus this relationship should be used with caution. However, because parcel density is readily calculable and ostensibly regularly updated by County authorities, we felt that short of actual data designating septic for all other areas, parcel density was the best proxy.

Table 7 Pearson correlation coefficients between different proxies of septic use.

	Septic	HU100	POP100	USA	Parcel Density
Septic	1.0000	-0.8149	-0.8294	-0.7133	-0.8256
HU100_DEN	-0.8149	1.0000	0.9907	0.9769	0.9915
POP100_DEN	-0.8294	0.9907	1.0000	0.9796	0.9958
USA	-0.7133	0.9769	0.9796	1.0000	0.9781
Parcel Density	-0.8256	0.9915	0.9958	0.9781	1.0000

Human population density data was compared with *E. coli* and *Enterococcus* values for each of the sub-watershed buffer zones. While there was no overall trend linking human population density to *E. coli* values, *E. coli* showed a positive relationship to population density for some, but not all, data collection dates. Interestingly, population density appeared to have the strongest correlation to the first flush (12/17/2008), mid-season (3/2/2009), and late season (5/15/2009) data collection points. The *E. coli* levels on other dates (i.e. 1/23/2009) appeared to have little to no correlation to human population densities.

D. Stable Isotope Sampling

D1. Stable Isotope Methods

The use of stable isotope analysis (SIA) has been largely restricted to terrestrial systems, although recently it has been applied to watershed sciences and hydrology (Kendall and McDonnell 1998). Based on the discussion of Kendall & McDonnell (1999), as shown in Figure 15 below, we contracted the Stable Isotope Facility at UC Davis to process water grab samples taken during the pilot study to evaluate nitrate for relative source differences in oxygen ($\delta^{18}O$ or heavy to light oxygen isotopic ratio relative to standard) and nitrogen ($\delta^{15}N$ or heavy to light nitrogen isotopic ratio relative to standard) as per standard methods (Casciotti et al. 2002; Sigman et al. 2001).

Isotopic values are noted as delta (δ) per mil (‰) with relative respect to international standards:

Where R is the $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratio for either the sample or the standard (i.e., $\delta^{15}N$ is relative to atmospheric air and $\delta^{18}O$ is relative to ocean water).

The primary outcome of this SIA pilot is the ability to relate other constituents (i.e., FIB, DNA) to their respective relative position or Kendall territories; for example, samples with $\delta^{18}O$ measures above 15 ‰ are largely runoff processes as, samples with $\delta^{15}N$ measures below 5 ‰ are typically ammonium from *in situ* processes such as wastewater treatment, and samples with $\delta^{15}N$ measures above 5 ‰ are manure and septic waste. These territories serve as a diagnostic source assessment methodology.

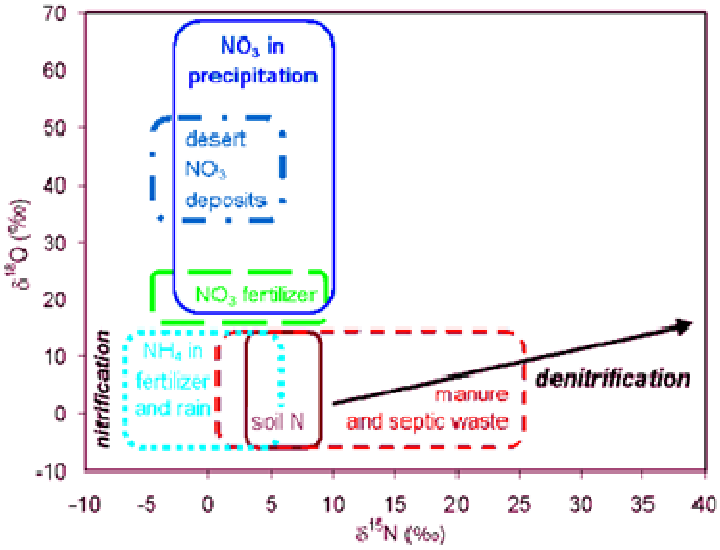


Figure 15 Diagram of $\delta^{18}O$ to $\delta^{15}N$ stable isotope ratios with regards to sources of nitrogen (from Kendall 1999 [16])

D2. Stable Isotope Results

Of 109 processed samples (see Appendix C for all results), 17 were excluded from this analysis because of the unreliability of the results due to low measurable nitrate or because they were duplicates or blanks. Those that had nitrate levels below SIA detection were either from 114MILL01, 114WILD01, or 114MARK01 (each is a semi-natural control site), or in one instance from 114LGVARR on 2008-12-18. We are continuing to work with the Stable Isotope Facility to determine if samples flagged for low nitrate values can be used in future analyses.

Of the remaining 92 samples analyzed for $\delta^{18}O$ and $\delta^{15}N$, the results were fairly consistent with expectations with both isotopic values ranging from near zero (0 ‰) to near 40 ‰ (Table 8); $\delta^{18}O$ samples registered a mean of 7.5 ‰ and median of 5.8 ‰ and the $\delta^{15}N$ samples registered a mean of 7.4 ‰ and median of 6.7 ‰. As with other analytical studies, all events were taken during measurable precipitation events except for the May 15th date (2009-05-15).

N Rows	Min(d18O)	Mean(d18O)	Median(d18O)	Max(d18O)
92	-0.16	7.52	5.83	39.1
N Rows	**Min(d15N)**	**Mean(d15N)**	**Median(d15N)**	**Max(d15N)**
92	1.72	7.38	6.66	36.7

Table 8 Ranges of SIA Values.

Individual sites were evaluated for the range of SIA conditions. Two sites upstream of Mill Creek were checked with independent samples, taken above the tributary junction, to determine if storm water was mixing downstream, as field observations noted a visual flow separation in turbidity. These sites (114MILLUS, 114WALLCRK) were not significantly different from the other Mill Creek samples ($p > 0.05$), and were pooled for subsequent analyses. A wide range of values for $\delta^{18}O$ were observed at urban locations (114COPE01, 114VINE01, 114SROSA1) with less consistency below 15 ‰, while $\delta^{15}N$ showed less variability and more consistency above 5 ‰ (Figure 16). In other words, the high observed variability (range of ~35 ‰) in $\delta^{18}O$ for urban sites is indicative of flashy hydrographs driven by large areas of impervious surface as $\delta^{18}O$ per mil values above 15 ‰ are from generally from atmospheric source waters. In contrast, the $\delta^{15}N$ showed less variability (range of ~12 ‰) and were centered on or about 8 ‰, which is borderline for septic and sewer denitrification. The general ratios of $\delta^{18}O$ to $\delta^{15}N$ were distributed in accordance with expected values from catchment

[16] http://wwwrcamnl.wr.usgs.gov/isoig/isopubs/itchfig16-9.html

hydrology (Kendall and McDonnell 1998), with the greatest density of observations less than 15 ‰ for $\delta^{18}O$ and more than 5 ‰ for $\delta^{15}N$. In accordance with other studies on microbial denitrification of nitrate in stream waters, our results suggest that most samples are in a zone of microbial denitrification (Figure 17), which became more pronounced during the storm pulse hydrologic phase (Figure 18) suggesting that surface and subsurface runoff processes experience substantial *in situ* denitrification. Few samples exhibited ratio values indicative of atmospheric derived N or from applied fertilizer (< 5 ‰).

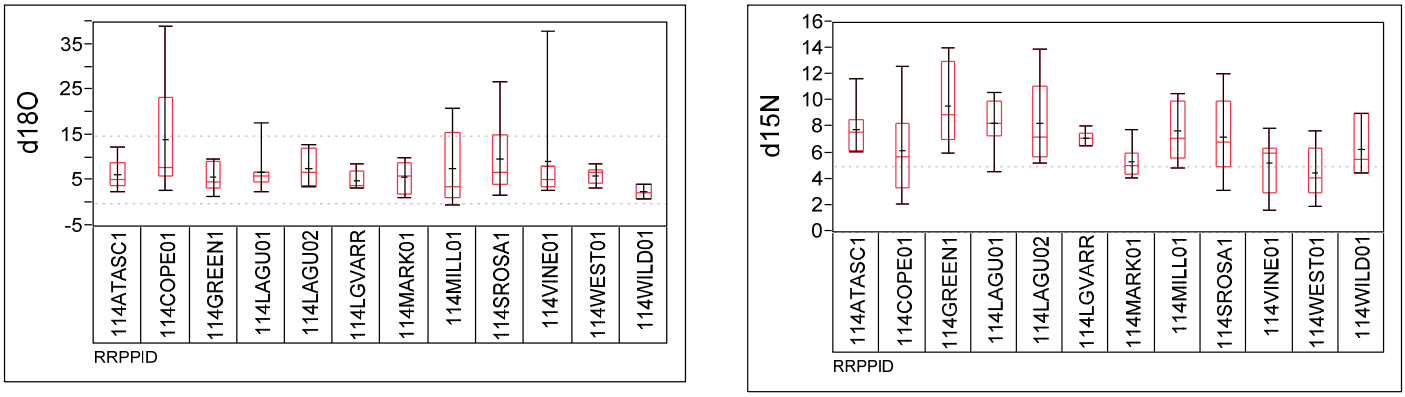


Figure 16 Variability Charts for SIA by Site (δ18O (left) and δ15N (right)).

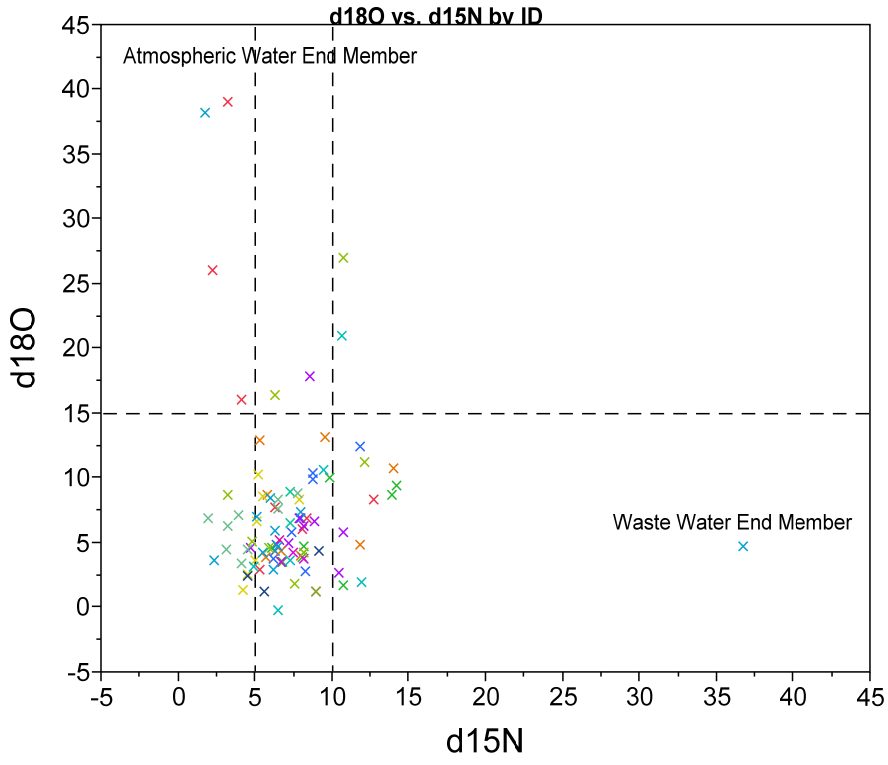


Figure 17 Relationship between $\delta^{18}O$ and $\delta^{15}N$ for Pilot SIA with generalized Kendall territories (Kendall and McDonnell 1998). Dashed lines for $\delta^{18}O$ indicate approximate breakline for atmospheric water (> 15 ‰) and for $\delta^{15}N$ indicate approximate breakline for partial soil nitrogen and manure / septic source waters (> 5 ‰) and exclusive manure / septic source waters (> 10 ‰).

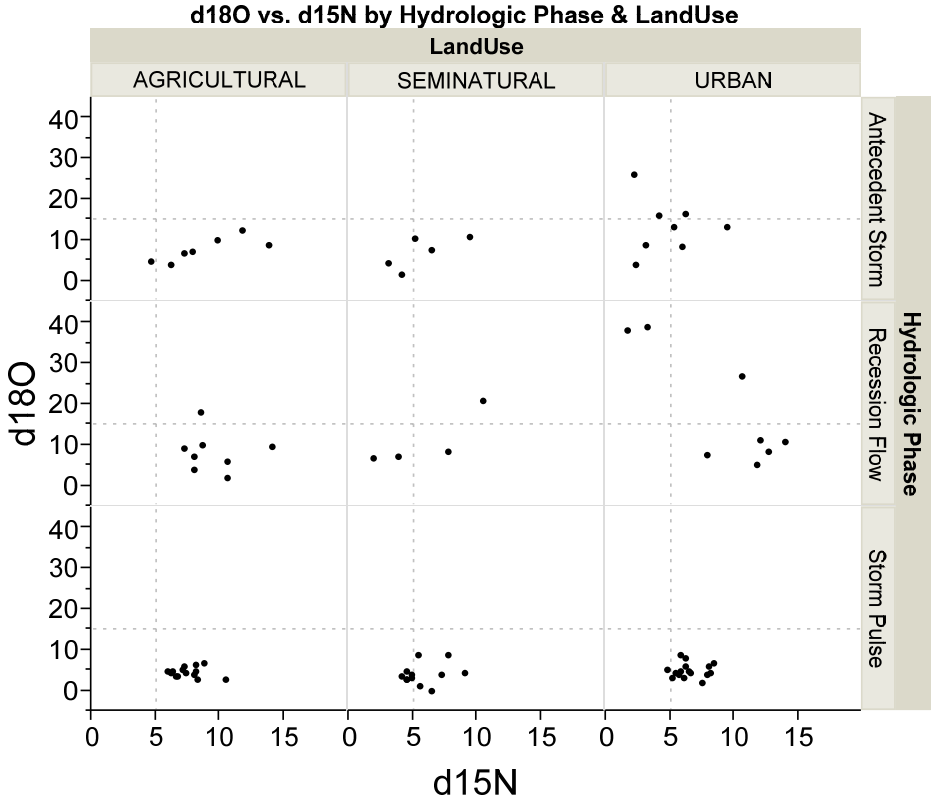


Figure 18 Stable Isotope ratios by Hydrologic Phase by dominant Land Use category. Dashed lines for $\delta^{18}O$ indicate approximate breakline for atmospheric water (> 15 ‰) and for $\delta^{15}N$ indicate approximate breakline for partial soil nitrogen and manure / septic source waters (> 5 ‰).

E. DNA

E1. DNA Rationale

There are numerous methods available to track sources of microbial contamination (or microbial source tracking – MST). There are numerous discussions of the methods as well as the pros and cons involved in their uses (e.g., Domingo et al. 2007; Field and Samadpour 2007), which are summarized in Table 3-1 of the US EPA Microbial Source Tracking Guide (US EPA 2005) and Field and Samadpour (2007). MST techniques fall into two main categories; library dependent and library independent. Library dependent techniques are divided into phenotypic (measurement of a trait, e.g. antibiotic resistance) and genotypic methods (measure genetic variability in some way). Library dependent methods require the development of a "signal" from known sources in the watershed. These signals can be pattern of antibiotic resistance of various bacterial isolates, patterns of carbon utilization, or patterns of genotypic variation in known isolates (e.g. genetic fingerprints). All techniques require substantial investment in library development and can be very efficient if the library is sufficiently large. Library independent methods involve using genetic material to identify specific bacterial isolates from known hosts. Sequences from specific genes in known hosts (e.g. humans, cats, cows, birds) are isolated from sample water to identify the sources of fecal contamination. With the appropriate technique, the amount of genetic material can be quantified to obtain an estimate of the amount of fecal material in the sample. All techniques have advantages and disadvantages enumerated in Table 3-2 of the EPA MST Guide Document (2005), and many are evolving rapidly promising faster and more efficient microbial source tracking in the future.

The technique selected for this study was real time - quantitative Polymerase Chain Reaction (rt-qPCR) of Bacteroides DNA. This technique allows the identification of specific hosts, and in this study, we focused on Bacteroides from human and bovine intestinal systems as those taxa were identified as the most probable sources of fecal contamination. In addition, the method for identifying these two sources is well established in the literature and required a minimal amount of development. The primary development involved establishing a calibration curve to create the basis for the

quantification of DNA in the samples. Using relationships established in the literature linking number of copies of DNA in the sample to the mass of fecal material, an estimate of the amount of fecal material in a sample can be generated.

This study focused on identifying bacteria from the genus Bacteroides because these bacteria are found as a normal component of the intestinal fauna of all warm-blooded animals and are voided in feces. It is estimated that these bacteria compose as much as 50% of the fecal material produced by animals (Lamendella et al. 2006). These bacteria are not *E. coli* nor are they related to *E. coli*. *Bacteroides* and *E. coli* both inhabit the intestinal tracts of animals and are voided together in fecal material. A major difference between the two is that *Bacteroides* are obligately anaerobic, i.e. they cannot live for any length of time in the presence of oxygen and they cannot reproduce in the presence of oxygen. Field et al. (2003) in a recent review indicated that Bacteroides can persist up to 14 days at temperatures of 4°C and only 4-5 days at temperatures of 14°C.

E. coli are not obligately anaerobic and may persist in the presence of oxygen in the environment for periods of time after being voided. They are also known to reproduce in the environment and can be mobilized from the sediment into the water column (see review in Field et al. 2003). The conditions under which these processes occur are not well understood and will require additional research. However, detection of *E. coli* indicates that fecal contamination may have occurred in the past, but the contamination may have occurred weeks or even months prior to sampling. Detection of *Bacteroides* indicates recent contamination by fecal material.

E2. DNA Methods

DNA was extracted from the samples using the QiaAmp DNA Mini Kit (Qiagen, Valencia, California) following manufacturer's guidelines for the buccal swab spin protocol. Final extraction volume was 100ul.

Real-time PCR assays were performed in an ABI 7300 (Applied Biosystems, Carlsbad, California) and consisted of the human-associated (HuBac), and bovine-associated (BoBac) assays (Layton et al. 2006). HuBac and BoBac forward and reverse primers were used without probes for consistency with SYBR Green technology. All real-time PCR assays were performed using Power SYBR Green Master Mix (Applied Biosystems, Carlsbad, California), with 15 pmol of primer. PCR assays were run with two sample types. First plasmid DNA containing 16S rRNA genes from *Bacteroides* (Blue Heron Biotechnology, Bothell, Washington) were run as standards using 10-fold dilutions of the plasmid ranging from 300,000 copies to 30 copies per PCR. Second, DNA extracted from filtered water samples was added in 10ul volumes to the PCRs. PCR amplification protocols consisted of two minutes at 50^{o}C, followed by 10 minutes at 95^{o}C, and 40 cycles of 95^{o}C for 15 seconds, and 60^{o}C for one minute (Human) or 57^{o}C for one minute (Bovine). Reaction specificity was monitored by melting curve analysis using a final data acquisition phase of 95^{o}C for 15 seconds, 60^{o}C for one minute, and 95^{o}C for 15 seconds and verified by direct sequencing of randomly selected amplicons.

The threshold cycle (C_T) value for all measurements was determined as the cycle at which fluorescence reached 5 standard deviations above the background, averaged over 5 cycles collected within the first 15 cycles of PCR amplification. For all PCR runs, standards, negative controls (no DNA), and samples were run in duplicate. Gene copies were calculated from standard curves based on the log transformation of known concentrations versus the threshold cycle.

Field duplicates and field blanks were collected at 5% each of the total samples as described in the QAPP. Laboratory blanks were run with each batch as a negative control, and all samples were run in duplicate, i.e. for each sample, two Human *Bacteroides*, and two Bovine *Bacteroides* were run.

E3. DNA Results

Forty four samples including blanks and field duplicates were used to quantify human and bovine Bacteroides. There was a wide range in the of number of copies of human and bovine DNA in the source water with estimates ranging from

nondetect to over 4.6 x 10^7 copies per 10 µL DNA. The mean concentration of human Bacteroides across all samples and dates was just over 5 x 10^6 copies of human DNA and 4 x 10^4 copies of bovine DNA. For comparison, Layton et al. (2006) found 10^5 to 10^6 copies per nanogram of DNA extracted from raw fecal material. The amount of human Bacteroides was usually 2 – 4 orders of magnitude greater than the amount of bovine Bacteroides.

Sampling dates within months were combined into groups and an Analysis of Variance performed using the natural logarithm of either HuBac or BoBac as the response variable (duplicates and blanks removed from the analysis). There were significant differences between dates in the amount of human Bacteroides in the system and no significant differences among dates in the amount of bovine Bacteroides (Table 9). The results were similar when examining differences among sites as there were no significant differences in bovine Bacteroides across sites and significant differences among sites in human Bacteroides (Table 10).

Table 9. Analysis of Variance Table for Bacteroides by Date.

HuBac						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
A: Date	4	294.37	73.59	4.68	0.004350	0.9144
S	32	503.16	15.72			
Total (Adjusted)	36	797.54				
Total	37					
BoBac						
Source						
Term						
A: Date	4	91.59	22.89	1.46	0.236674	0.4008
S	32	501.11	15.65			
Total (Adjusted)	36	592.70				
Total	37					

Table 10. Analysis of Variance Table for Bacteroides by Site.

BoBac						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
A: Site	10	128.39	12.83	0.69	0.723563	0.282646
S	30	556.45	18.54			
Total (Adjusted)	40	684.84				
Total	41					
HuBac						
Source						
Term						
A: Site	10	565.18	56.51	3.21	0.0064	0.94749
S	30	527.87	17.59			
Total (Adjusted)	40	1093.05				
Total	41					

The differences in the human Bacteroides signal across dates reflect relatively low human Bacteroides counts in March relative to December, February or April. January counts were slightly lower but not significantly so. The low count in the human Bacteroides signal in March occurred during the third major storm within a short period of time. However, the low counts in January occurred during low flow with no antecedent storm(s). When sites were categorized into urban, semi-natural, or agricultural, there were no differences among sites in the amount of bovine Bacteroides but significant differences in the amount of human Bacteroides across categories (Table 11). For bovine Bacteroides, there were very slight differences between urban and agricultural land use categories and a somewhat larger amount of

bovine Bacteroides in the semi-natural land use class compared to either of the other two categories. For human Bacteroides, there were only slight differences between the urban and agricultural land use classes in the human Bacteroides signal, but both were significantly larger than the signal from the semi-natural land use category.

Table 11. Analysis of Variance Table for Bacteroides by Land Use Category.

BoBac						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
A: Category	2	41.25	20.62	1.22	0.307129	0.249645
S	38	643.59	16.93			
Total (Adjusted)	40	694.84				
Total	41					
HuBac						
Source						
Term						
A: Category	2	211.56	105.78	4.56	0.016786	0.741002
S	38	881.49	23.19			
Total (Adjusted)	40	1093.05				
Total	41					

Seurinck et al. (2005) estimated the number of copies of Bacteroides per gram of fecal material from raw human feces and from samples collected from raw sewage. Assuming the amplification efficiencies are similar across the two studies, our estimates of the amount of fecal material in the samples collected during this study range from 1.9×10^{-6} – 1.7×10^{-7} grams per liter of water to 3 - 0.3 g/L. The upper estimates for the fecal material are skewed by two samples in which an extremely large number of copies were detected. Without those values, the estimates are 0.16 – 0.015 gm fecal material/L which is still quite large. The elevated Bacteroides counts were both from May samples in two subwatersheds. Both sets of estimates are very large and indicate relatively direct movement of fecal material to the surface water from either leaky sewer lines or failing septic systems.

Table 12. Estimated amount of fecal material in the sample.

Site	Number of copies HuBac	Grams human feces per liter[1]	Grams human feces per liter[2]
114ATASC1	ND	0	0
114ATASC1	ND	0	0
114MARK01	ND	0	0
114MARK01	ND	0	0
114MILL01	ND	0	0
114COPE01	29	1.88E-06	1.69E-07
114SROSA1	2553	0.000166	1.49E-05
114WILD01	2662	0.000173	1.55E-05
114MARK01	3567	0.000231	2.08E-05
114ATASC1	19580	0.00127	0.000114
114MILL01	33277	0.002158	0.000194
114WEST01	36968	0.002397	0.000215
114WEST01	82262	0.005334	0.000479
114LAGU02	86642	0.005618	0.000504
114VINE01	171343	0.011111	0.000998
114LAGU01	417768	0.02709	0.002432
114LGVARR	489424	0.031737	0.00285
114LGVARR	619465	0.040169	0.003607
114LAGU02	656911	0.042597	0.003825
114LAGU01	824996	0.053497	0.004803

Site	Number of copies HuBac	Grams human feces per liter[1]	Grams human feces per liter[2]
114VINE01	871277	0.056498	0.005073
114VINE01	1293260	0.083861	0.00753
114COPE01	1348260	0.087428	0.00785
114LAGU01	1504170	0.097538	0.008758
114ATASC1	1548850	0.100435	0.009018
114LAGU02	1597330	0.103579	0.0093
114WEST01	1706500	0.110658	0.009936
114LAGU01	2318370	0.150335	0.013499
114SROSA1	2623790	0.17014	0.015277
114WEST01	2801220	0.181645	0.01631
114GREEN1	2819460	0.182828	0.016416
114LAGU01	3492950	0.2265	0.020337
114SROSA1	3641310	0.236121	0.021201
114VINE01	4161720	0.269867	0.024231
114ATASC1	4653910	0.301783	0.027097
114COPE01	8125350	0.526888	0.047309
114SROSA1	12042000	0.780863	0.070114
114GREEN1	12581600	0.815853	0.073255
114VINE01	13930100	0.903297	0.081107
114LAGU02	31928700	2.070415	0.185902
114GREEN1	46505300	3.015634	0.270773
Avg all samples minus ND		0.297103	0.026677
Average all samples minus ND and largest two values[3]		0.16499	0.014814

[1]Based on estimate of copies per gram of human feces provided in Seurinck et al. (2005).
[2]Based on estimates of copies per gram of raw sewage provided in Seurinck et al. (2005).
[3]Two largest samples are 114LAGU02 and 114GREEN1.

III. Synthesis

Land Use & FIBs

Multivariate analyses of the FIBs indicated strong effects of hydrology and land use. For *E. coli*, precipitation, urban development, and animal agriculture were consistent predictors of elevated FIB conditions. At the most local scale, modeled as 500m drainage from a sample location (see hydrologic buffer section), the strongest model, as measured by the highest coefficient of determination (R^2) and lowest Akaike Information Criterion (AIC[17]), a score that favors parsimonious statistical models, was increasing percent Urban land use and increasing precipitation (3 day maximum) resulted in the highest *E. coli* $\log_{10}$ values (R^2 = 0.34, AIC = 159). By increasing scale, new patterns emerged. At 1000m drainage, four terms became important: increasing 3 day maximum precipitation, increasing Urban land use, increasing Dairy, and decreasing Animal Agriculture resulted in the highest *E. coli* (R^2 = 0.39, AIC = 156). While dairies are included within animal agriculture, the latter indicates the amount of pasture and potential buffering from filter strips. However, at 2000m and 4000m drainages, this trend is reversed with Animal Agriculture becoming positively correlated with increasing *E. coli* levels as well as increasing parcel density playing a more significant role in high *E. coli* readings. These coarser spatial models did not add appreciably to model determination. Animal agriculture changed at the subwatershed scale, with a negative association. However at the coarsest model, the entire cumulative watershed for

[17] http://en.wikipedia.org/wiki/Akaike_information_criterion

each location, the best model included only the 3 day maximum precipitation and parcel density; as each variable increased, so did the *E. coli* log_{10} values (R^2 = 0.37, AIC = 154), which was also the best two parameter model at all scales. The best mixed model combining all scales is provided in Table 13, which had parcel density within 500m and Animal Agriculture within 2000m, in addition to heavy precipitation, as the dominant significant factors in elevated *E. coli* log_{10} values.

Table 13 Multivariate regression of Log_{10} *E. coli* at all spatial scales.

Summary of Fit

RSquare	0.399936
RSquare Adj	0.377982
Root Mean Square Error	0.566664
Mean of Response	2.78901
Observations (or Sum Wgts)	86

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	17.549210	5.84974	18.2174
Error	82	26.330854	0.32111	**Prob > F**
C. Total	85	43.880064		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1.6832556	0.162717	10.34	<.0001*
lnPpt3DayMax	0.221238	0.041543	5.33	<.0001*
b0500parcelden	0.0012547	0.000291	4.31	<.0001*
b2000dwrAnimalAg	2.1895047	0.72387	3.02	0.0033*

For *Enterococcus* spp., we again determined the strongest model as measured by the highest coefficient of determination (R^2) and lowest AIC score for FIB samples log_{10} transformed for normality assumptions. We excluded 17 samples due to detection limits. At the most local scale (within 500m drainage), the best model indicated that increasing percent Urban land use and 3 day maximum precipitation were the driving factors behind elevated *Enterococcus* spp. (R^2 = 0.53, AIC 107). At 1000m drainage, three terms produced the best model: increasing 3 day maximum precipitation, increasing Urban land use and increasing Animal Agriculture (R^2 = 0.58, AIC = 104). At 2000m the model was reduced to maximum 3 day precipitation and increasing percent Urban cover (R^2 = 0.55, AIC = 107), and at 4000m the best model adds increasing percent Animal Agriculture (R^2 = 0.56, AIC = 107). At the subwatershed scale, the model is again reduced to two terms, maximum 3 day precipitation and increasing Urban cover (R^2 = 0.51, AIC = 112), whereas at the cumulative watershed scale, it is the combination of maximum 3 day precipitation, increasing Crop Agriculture, and increasing parcel density that drives elevated *Enterococcus* spp. log_{10} sample values (R^2 = 0.55, AIC = 112). The best two parameter model was maximum 3 day precipitation and percent Urban cover within 1000m (R^2 = 0.55, AIC = 106). The best mixed model combining all scales is show in Table 14, which had percent Animal Agriculture within 1000m and percent Urban cover within 1000m, in addition to heavy precipitation, as the dominant significant factors in elevated *Enterococcus* spp. log_{10} values.

Table 14 Multivariate regression of *Enterococcus* spp. log10 against land uses at multiple scales.

Summary of Fit

RSquare	0.576632
RSquare Adj	0.558224
Root Mean Square Error	0.470787
Mean of Response	2.584448
Observations (or Sum Wgts)	73

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	20.829486	6.94316	31.3262
Error	69	15.293210	0.22164	Prob > F
C. Total	72	36.122696		<.0001*

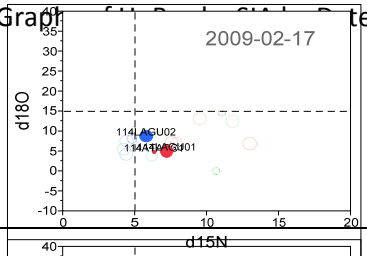
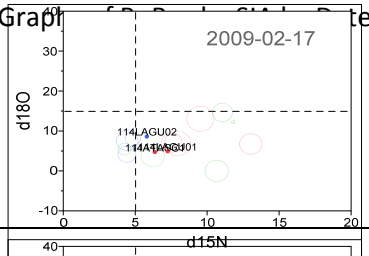
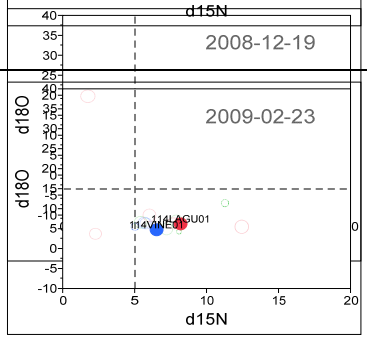
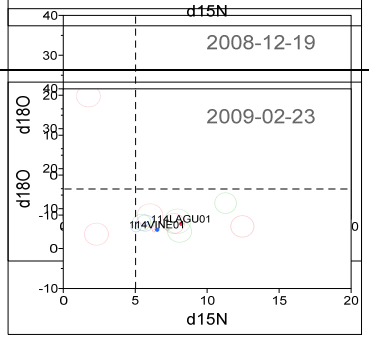
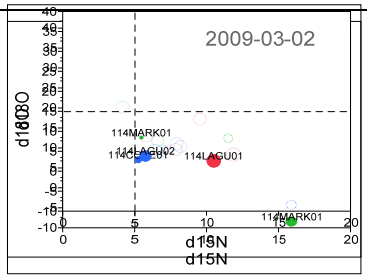
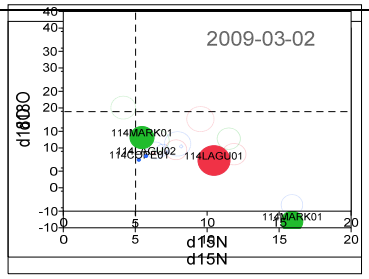
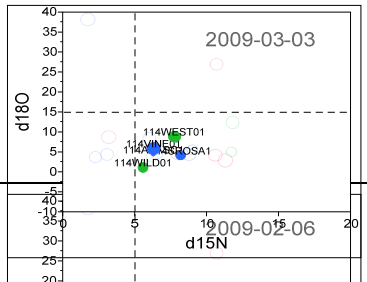
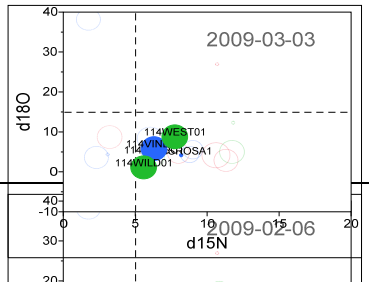
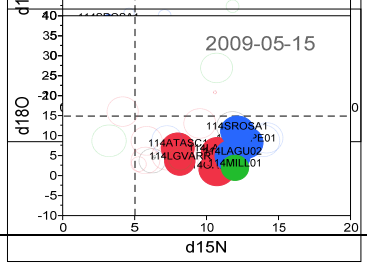
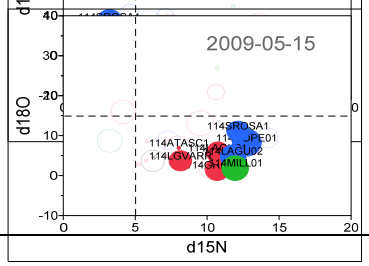
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1.4267295	0.13183	10.82	<.0001*
lnPpt3DayMax	0.2931355	0.035034	8.37	<.0001*
b1000dwrAnimalAg	1.692423	0.833021	2.03	0.0460*
b1000dwrUrban	0.5673291	0.143108	3.96	0.0002*

DNA & Stable Isotopes

The most integrative and novel aspect of our weight-of-evidence approach is through the combination of SIA and DNA techniques to determine source of water (SIA) and source of bacterial DNA through time. We analyzed the position of SIA samples as a function of the $\delta^{18}O$:$\delta^{15}N$ ratio (i.e., Kendall territories) in relation to the HuBac and BoBac magnitude for each of our seven sample periods. An interesting trend emerges, which basically can be described as two distinct hydrologic periods: storm water pulse and flow recession. The SIA analysis revealed that during the storm water pulse period, sources of nitrate found in samples were a mix of atmospheric water (i.e., rainfall runoff), and both ammonized and denitrifying sources, indicating *in situ* or localized hydrological processes. Few samples were in the "NO3 from fertilizer territory", and any fertilizer sources derived from manure should be sterile (i.e., no detection of *Bacteroides*, which is anaerobic). Similarly, any excess soil nitrogen should be free from *Bacteroides*, which are short lived and anaerobic. Thus, the ammonized and denitrifying waters are from some combination of wastewater treatment effluent, sewer, septic, or manure. From the DNA analysis, 5 of 41 samples were non-detects for human sourced *Bacteroides*; in other words, human signatures were detected in 88% of samples. In only one case was bovine *Bacteroides* higher than human and sites with repeat samples during the storm water pulse period do not have consistent magnitudes, indicating episodic entry of manure into receiving waters. Elevated levels of human *Bacteroides*, however, were consistently found *in situ* (i.e., localized denitrification) with the exception of Foss Creek (114VINE01), which had both a high human *Bacteroides* signal and an atmospheric water source. The flow recession period, which can only be characterized by the May 15 samples, showed the greatest concentration of high magnitude HuBac and elevated nitrate with microbial denitrification (median = 11.25‰ $\delta^{15}N$; SD = 1.8‰ $\delta^{15}N$).

Table 15 Synthesis of PCR Results by SIA Results.

Graph of HuBac by SIA by Date	Circle sizes by log$_e$ HuBac (left) and log$_e$ BoBac (right) scaled independently (median HuBac:BoBac ratio is 1281:1); δ18O on Y axis, δ15N on X axis; labels indicate sample for that date. Source codes of the area equivalent to sewer influent for HuBac and manure slurries for BoBac: Blue = Urban, Red = Agriculture, Green = Seminatural	Graph of BoBac by SIA by Date
	Only three samples were processed for this date. All three were non-detects for BoBac. Atascadero Creek (114ATASC1) was a non-detect for HuBac. The upper and lower Laguna de Santa Rosa sites (114LAGU01, 114LAGU02) had moderate HuBac values (10^5 100 mL^{-1}) in the mixed SIA territory.	
	High levels of HuBac at all sites ($1.3 – 12.5 \times 10^6$ 100 mL^{-1}), mostly in manure/septic nitrate territory with the exception of Copeland Creek (114COPE01), which likely has some storm water mixing. The two Laguna de Santa Rosa sites also had moderately high BoBac values ($1.1 – 2.8 \times 10^4$ 100 mL^{-1}), which were an order of magnitude higher than 114COPE01 and 114VINE01. The other two sites had non-detects for BoBac (114ATASC1 and 114GREEN1).	
	Very high HuBac values ($>10^6$ 100 mL^{-1}) in the storm water runoff (114COPE01, 114LAGU02), lower (114LAGU01) and the highest BoBac values (114GREEN1). This site (114GREEN1) also had very high HuBac (2.3×10^6 100 mL^{-1}), clearly with septic contamination. Mark West Creek (114MARK01) having moderately low HuBac values and very low nitrate levels (i.e., negative d18O) but clear septic signal. BoBac values were very low, and present at all sites but 114SROSA1.	
	We evaluated five sites, with two sites having non-detects for BoBac (114ATASC1, 114SROSA1) and the other three registering less than 10^4 copies 100mL^{-1}. The Foss Creek site (114VINE01) was high in HuBac (1.2×10^6 100 mL^{-1}), while 114ATASC1 was a non-detect for HuBac. Three of four sites were in the ammonia SIA territory, with Santa Rosa Creek (114SROSA1) having very high HuBac values (12.0×10^6 100 mL^{-1}) and the other two sites are very near Healdsburg (114WEST01, 114VINE01), indicating possible wastewater releases and storm water mixing. BoBac was present at all sites but one (114WEST01), with moderately low readings.	
	All six of the samples taken on this date contained detectable HuBac. The HuBac values represented the highest average human *Bacteroides* detected (12.1×10^6 100 mL^{-1} average) and most STA values were clearly in septic territory. The most exceptional sites were lower Laguna de Santa Rosa (114LAGU02) and Atascadero Creek at Green Valley Road (114GREEN1), with HuBac values of 31.9×10^6 100 mL-1 and 46.5×10^6 100 mL^{-1} respectively.	

	While 114COPE01 had the highest detected BoBac (7.8×10^4 100 mL^{-1}), the 114ATASC1 site was a non-detect.	

Multivariate Human *Bacteroides* Analysis

We conducted a multivariate analysis of human *Bacteroides* (Log_e HuBac) to determine what primary factors were driving elevated fecal contamination. Partition analysis, also known as classification and regression trees, choose the most parsimonious solution with recursive splits (i.e., the first split of the data has the greatest explanatory power; each subsequent group is split again with the variable having the greatest explanatory variable for that group). Using dominant land use category, hydrologic phase, major drainage, and stable isotope values, our partition analysis resulted in high explanatory power ($R^2 = 0.66$) and results that corroborate our other observations. Each split resulted in either 3 or 4 samples, mean Log_e HuBac counts are provided in Table 16, leaves show in Figure 19 and model performance in Figure 20. This analysis revealed that during storm pulses, sites in either agricultural or semi-natural settings that had $\delta^{15}N$ values less than 6.5‰ (i.e., ammonium and soil N) had the least fecal contamination (e^1.96). There was a marked jump to the next highest group which was characterized by either recession flows or antecedent storms in the semi-natural sites of the northern portion of the study site (i.e., 114MILL01 and 114WEST01). Storm pulses with $\delta^{15}N$ values less than 6.5‰ in urban settings were the next highest, followed by similar storm pulses on urban and semi-natural lands but with $\delta^{15}N$ values greater than 6.5‰.

At the other end of the spectrum, the samples with the highest fecal contamination (e^15.8) were in the Atascadero drainage, had $\delta^{15}N$ values greater than 10.7‰ during low flow conditions (i.e., recession and antecedent conditions). Of the four samples in this group, three were at 114GREEN1 and the other from 114ATASC1. There were two non-detects of bovine *Bacteroides* (the other two samples were less than 2500/100mL), and a mean $\delta^{15}N$ of 12.6‰, clearly indicating one or more failed septic systems above 114GREEN1. With very similar high values were urban sites within the greater Laguna de Santa Rosa drainages during recession flows (e^15.8), which were only slightly less during antecedent conditions (e^14.9). There were three sites and seven samples in this group (114COPE01, 114LAGU02, 114SROSA1), but two of the samples were likely confounded by wastewater releases, as their $\delta^{15}N$ values were less than 4.1‰ indicating high ammonium with very high fecal counts (114COPE01 on 5/15/2009 and 114SROSA on 2/6/2009). The other five samples had $\delta^{15}N$ values greater than 9.5‰, indicating either failed septic or faulty sewer.

Table 16 Multivariate partition analysis of Log_e HuBac.

Partition Leaf Label	Mean Log_e Hu Bac	Samples (n)
Hydrologic Phase (Storm Pulse) & d15N<6.50239725 & Land Use (AGRICULTURAL, SEMINATURAL)	1.97180215	4
Hydrologic Phase (Recession Flow, Antecedent Storm) & Drainage (Northern Russian) & Land Use(SEMINATURAL)	8.41940592	3
Hydrologic Phase (Storm Pulse) & d15N<6.50239725 & Land Use (URBAN)	10.5596829	4
Hydrologic Phase (Storm Pulse) & d15N>=6.50239725 & Land Use (URBAN, SEMINATURAL)	10.9469364	3
Hydrologic Phase (Recession Flow, Antecedent Storm)^& Land Use (SEMINATURAL, AGRICULTURAL) & Drainage (Laguna Rosa)	12.4899235	3
Hydrologic Phase (Recession Flow, Antecedent Storm)^& Land Use (SEMINATURAL, AGRICULTURAL) & Drainage (Atascadero) & d15N<10.6843456	12.9182833	4
Hydrologic Phase (Storm Pulse) & d15N>=6.50239725 & Land Use (AGRICULTURAL)	13.7407314	3
Hydrologic Phase (Recession Flow, Antecedent Storm) & Drainage (Northern Russian) & Land Use (URBAN)	14.5808101	3
^& Drainage (Atascadero, Laguna Rosa) & Land Use (URBAN) & Hydrologic Phase (Antecedent Storm)	14.900694	3
^&Drainage (Atascadero, Laguna Rosa) & Land Use (URBAN) & Hydrologic Phase (Recession Flow)	15.7693751	4
Hydrologic Phase (Recession Flow, Antecedent Storm)^& Land Use (SEMINATURAL, AGRICULTURAL) & Drainage(Atascadero) & d15N>=10.6843456	15.7769758	4

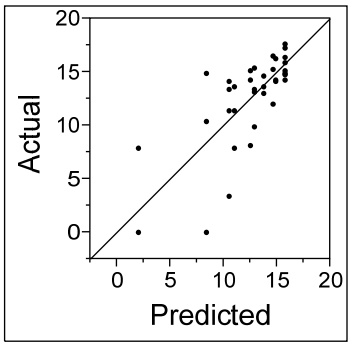


Figure 20 Log_e HuBac multivariate model, actual vs. predicted.

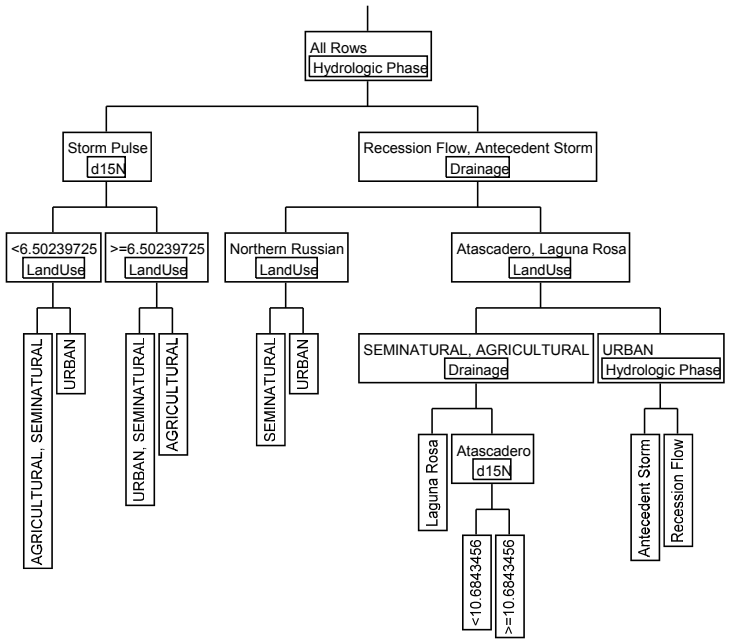


Figure 19 Partition leaves of Log_e HuBac multivariate model.

IV. Discussion

Our wet season pilot study found elevated FIBs throughout the study area and across the time period of study. While our intent was to determine if contaminated storm waters were correlated with specific land uses, our weight-of-evidence approach resulted in a more comprehensive understanding. Whereas semi-natural land uses, and in particular the study control sites, were consistently of better water quality (i.e., lower water temperature, higher dissolved oxygen, lower levels of FIBs, and less fecal contamination), the other land uses were more context dependent to determine differences in elevated FIBs and fecal contamination. Foremost, human *Bacteroides* was exceptionally elevated in both agricultural and urban settings during low flow conditions, often greater than 10^6 DNA copies / 100mL, which approximates typical sewage influent (Kephart and Bushon 2009). Human *Bacteroides* was often greater than 3 orders of magnitude higher than bovine *Bacteroides* except for some large storm events. Further, neither *E. coli* nor *Enterococcus* spp. measures correlated with human *Bacteroides*, except for in semi-natural settings. Our stable isotope analysis suggested that nitrate was often from a mixed source of soil nitrogen (transforming from ammonium to nitrate via soil media) or outright from either manure / sewage or septic. The former (manure) can be largely dismissed as the elevated nitrate was uncorrelated with bovine *Bacteroides*. The multivariate land use models we developed consistently showed that measures of humans in close proximity (500m – 2000m), as either parcel, housing, or population density, were strong predictors of elevated fecal bacteria and microbial denitrifying nitrate. A composite of these trends are contained in Figure 21.

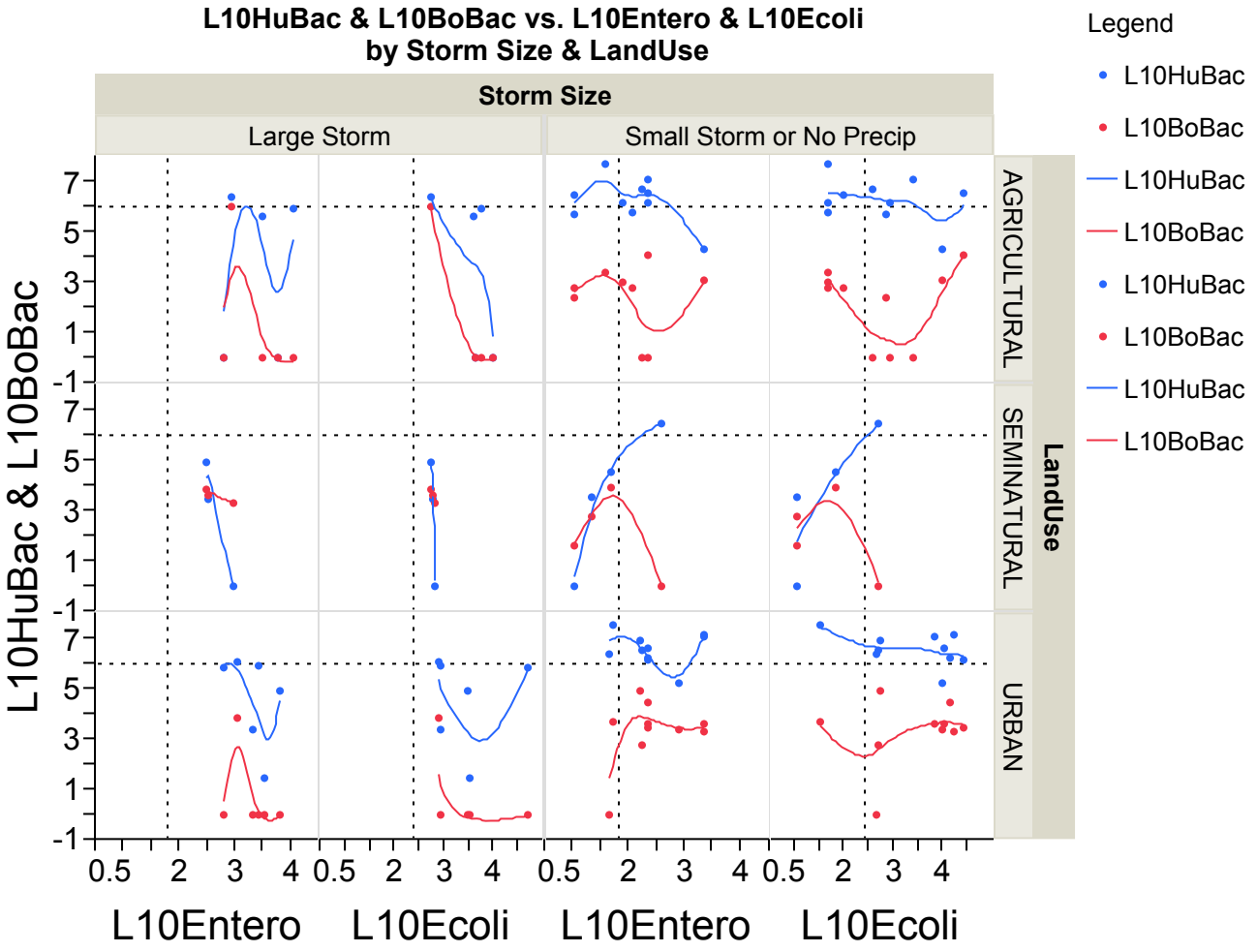


Figure 21 ***Bacteroides*** **from human and bovine sources (copies of DNA / 100mL) in comparison to** ***E. coli*** **and** ***Enterococcus*** **spp. as a function of hydrologic phase and land uses. Log_{10} values greater than 6.0 are equivalent to sewage influent (humans) or manure slurries (cattle). Dotted vertical lines indicate CDPH recommended limits for fecal indicator bacteria (61 MPN/100mL for** ***Enterococcus*** **spp., and 235 MPN/100mL for** ***E. coli*****).**

V. Summary

Our weights of evidence approach toward understanding the source and magnitude of potential pathogen impairment in the tributaries of the lower Russian River revealed elevated fecal contamination throughout the study area. We determined that pathogenic bacteria are pervasive, persistent, and overwhelmingly human in origin under a wide range of hydrological and land use conditions. Future monitoring efforts will need to combine a regular monitoring framework that is both high density in the number of sites and high frequency in the rate of sampling, with opportunistic sampling that uses emerging techniques and is focused on problematic sites or flow conditions. Efforts to remediate conditions will need to leverage existing efforts to improve flows, increase riparian cover, and limit direct discharges. Further, direct investment in improved septic technology for many of the landowners adjacent to tributaries throughout the watershed will ultimately be necessary to reduce loads below their present levels.

VI. Literature Cited

Casciotti KL, Sigman DM, Hastings MG, Bohlke JK, Hilkert A. 2002. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal Chem 74(19): 4905-4912.

Domingo JWS, Bambic DG, Edge TA, Wuertz S. 2007. Quo vadis source tracking? Towards a strategic framework for environmental monitoring of fecal pollution. Water Research 41(16): 3539-3552.

Field KG, Bernhard AE, Brodeur TJ. 2003. Molecular approaches to microbiological monitoring: Fecal source detection. Environ Monit Assess 81(1-3): 313-326.

Field KG, Samadpour M. 2007. Fecal source tracking, the indicator paradigm, and managing water quality. Water Research 41(16): 3517-3538.

Johnson, ML, Bowen, L, Turner, MA. 2006. East San Joaquin Water Quality Coalition Bacterial Source Identification Study. Final Report to the East San Joaquin Water Quality Coalition, (52 pp).

Johnson, ML, Bowen, L, Turner, MA, Chu, L. 2007. San Joaquin County and Delta Water Quality Coalition Bacterial Source Identification Study. Final Report to the San Joaquin County and Delta Water Quality Coalition, (38 pp).

Kendall C, McDonnell JJ. 1998. Isotope tracers in catchment hydrology. Amsterdam ; New York: Elsevier.

Kephart CM, Bushon RN. 2009. Results from a Microbial Source-Tracking Study in the Portage River Watershed, 2008. (2008, ed). Reston, Va.: U.S. Geological Survey, 1 online resource (iv, 14 p.).

Sigman DM, Casciotti KL, Andreani M, Barford C, Galanter M, Bohlke JK. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem 73(17): 4145-4153.

US EPA. 2005. Microbial source tracking guide document. Cincinnati, Oh.: United States Environmental Protection Agency. Office of Research and Development.

Viers JH. 2008. Objective classification of Navarro River salmon habitat: a watershed-based critical habitat case study. Aquat Conserv 18(2): 147-162.

VII. Appendix

Appendix A Sampling Event Observations

19 December 2008

The following is a brief summary of the weather conditions and observations made prior to and during the first RRPP sampling event on Friday, December 19, 2008.

After receiving less than 0.1" of rain during the first thirteen days of December, 0.49" of rain were recorded in Santa Rosa from Dec 14-15. No rain was recorded for December 16-17. A second weather system moved in on Thursday, December 18 dropping 0.17" of rain from 14:00-20:00. Rainfall began again around midnight on the 18th. Over the course of the next four hours approximately 0.23" of rain was recorded until the storm had passed through around 04:00 on December 19. Runoff sampling began that morning with the first sample collected at Copeland Creek at 08:20 and the last sample collected at 16:10 at Prince Memorial Greenway.

Runoff was observed in the form of cloudy water at the first two sites visited that day; Copeland and Foss creeks. Runoff was not visible in terms of water clarity at the next three sites visited (Lower Dry Cr, Mill Cr, Lower Green Valley Cr). However, Wallace Creek, a tributary entering Mill Creek approximately 20' upstream of the sample collection point, was a milky green color. The Mill Creek sampling location is an existing NCRWQCB monitoring site. The water at this location is too close to the confluence of the two streams to be considered well-mixed. This may explain the large difference in Total Coliform counts between the primary and duplicate samples collected at this site; 4,611 MPN vs 10,462 MPN. Other observations of note at the aforementioned sites were the presence of toilet paper in the bushes around the stream at Lower Dry Creek with signs of human encampment, and human feces visible within 15' of the water's edge and a pair of underwear in the water, both approximately 40' upstream of the sample collection point on Mill Creek.

The Laguna de Santa Rosa downstream of the confluence with Santa Rosa Creek (114LAGU02), called Santa Rosa Flood Canal at this location in the DeLorme Atlas-Gazeteer, is a difficult site to access. Guerneville Road (Hwy 116) in the area of the Laguna carries a lot of traffic including big-rigs and has no shoulder for parking. We had not been to this site before but managed to find just enough room to park at the intersection with Timberhill Road approximately 250m west of the Laguna. The target lat-longs provided by the NCRWQCB would have us sampling on the south side of Guerneville Road. Access to the target location did not seem possible in the dense riparian zone along the Laguna. Instead we collected the sample on the north side of the highway at these coordinates: N 38.45229 W -122.83495. The target coordinates are N 38.4515972 W -121.834722. Both sites are downstream of the confluence with the Santa Rosa Flood canal. The water in the Laguna was a murky brown with no visible flow. However, due to the wide, deep profile of the stream channel a small amount of flow would not be noticeable. I took two photos at this site with the second photo (114LAGU02_2008_12_19_2) showing what I believed at the time to be the Santa Rosa Flood Canal. After viewing an aerial photo I realize now that I was really only looking at a water filled depression.

Atascadero Creek at Green Valley Road also exhibited no visible flow; the water was murky green with low dissolved oxygen content.

The Laguna de Santa Rosa (114LAGU01) east of Sebastopol at Laguna State Park was a murky brown with no visible flow, although it is a wide and deep channel at this point. Numerous dog droppings were visible around the pathway above the Laguna.

Atascadero Creek at Bodega Hwy (114ATASC1) was a cloudy green color with no visible flow.

The water was colorless at both Upper Mark West Creek and Upper Santa Rosa Creek however, both streams were running at about 3-4 cfs on this day when they had been dry during the November 13 scouting trip. The water in these streams may have consisted primarily of groundwater recharge from the December 14-15 rainfall. However, I suspect that some runoff from the early morning rain was still present when we sampled these sites at 14:50 and 15:30, respectively.

Prince Memorial Greenway was flowing at about 10 cfs. The water had a slight yellow tint with excellent clarity. It was hard to say how much runoff was present here.

23 January 2009

Saturday, January 24 would have been the ideal day to perform our sampling but the analytical lab was unable to accommodate that schedule on short notice. Even so, looking at the Quantitative Precipitation Forecast (QPF) it appeared that we may have a steady light rainfall beginning early in the morning of January 23 and extending into the evening which most likely would have generated same-day runoff. However, all that changed overnight when the system shifted south as has seemed to be the pattern recently. The QPF was not updated until after 08:00 Friday when we were already onsite and ready to begin. After some extended consultation with Josh Viers, we decided to proceed with the sampling event and use the data as the" wet season baseline". The rain did finally begin to fall lightly at 13:30 and continued through the last of our sites two hours later.

Due to the delay in getting started we were unable to visit the last site, Upper Santa Rosa Creek, and still make the 16:00 deadline set by the lab for sample delivery. We were able to visit all of the other sites though. Here are some observations (if a site is not listed there was nothing unusual to report):

Copeland Creek: had standing water but did not appear to be flowing. The water was not colored by runoff. There was an oily sheen on the water surface.

Mill Creek: low and clear. There was some rusty colored flocculent along the edges of Mill Creek and Wallace Creek near the confluence of the two.

Laguna de Santa Rosa below confluence with Santa Rosa Creek: collected a 300ml lab duplicate sample to be split into two parts in the lab. This will give us an idea of the lab's precision.

Atascadero Creek at Bodega Highway: There was no observed flow. As I waded into the stream a strong sulfur odor was released from the approximately 12" of benthic sediment due to anaerobic decomposition.

Upper Mark West Creek: There was maybe 0.3 cfs which must have been groundwater recharge from the little bit of rain we have had this winter since this stream was dry in October.

6 February 2009

By 05:00 on 6 February about 0.3" of rain had fallen in the Santa Rosa area during the previous 24 hours. By 10:00 the 24 hour total was up to 0.7". Runoff was evident at every site we visited. Here are some general observations:

Both Copeland and Foss Creek had 3-4x more flow in them than during the previous sampling event on 23 January.

At the Laguna de Santa Rosa the water level was maybe 1.5' higher than on 23 January and for the first time stream flow was evident. In the 15-20 minutes we spent at this site the water level rose an additional 2" and began to flow over the bank and drain back into a low lying area.

The water level in Atascadero Creek at Green Valley Road did not appear to be any higher than during the 23 January event and, as in the two previous events, the water did not appear to be flowing. There was however some localized runoff occurring as indicated by a light brown plume of water mixing with the darker brown water of the stream.

In Atascadero Creek at Bodega Hwy there was noticeable flow unlike the two previous events and, like the last event, a strong sulfide odor was released from the benthic sediments when I waded in.

For the first time in our four visits (including site scouting) the direction of flow was noticeable in the Laguna de Santa Rosa near Sebastopol, but just barely.

Both of the control sites, Upper Mark West and Upper Santa Rosa Creek had moderate flow with a very slight tint of color indicating overland runoff.

When we dropped off the samples at the lab we were given the results from the previous sampling event. Based on those we asked that the lab use the same dilution factors they had in the previous event with the exception of Foss Creek where we asked them to dilute the *Enterococcus* by a factor of 1:100. The sample dilutions for this event will be: *E. coli* + Total coliform samples 1:100 for Copeland Cr, the two Laguna sites and Santa Rosa Creek at Prince Memorial Greenway; 1:10 for all other sites. *Enterococcus* 1:100 for Foss Creek and 1:10 for all other sites.

17 February 2009

We sampled the tail end of the big storm that came through northern California last weekend. What I am calling "the big storm" began Saturday evening, February 14 and continued until about 16:00 on Tuesday, February 17. There were some breaks as long as four hours in between rainfall so perhaps, technically, this was more than one storm event. The storm was preceded by several days of minimal rainfall early in the week and then a moderate rain event on Thursday, February 13. Here are some 24-hour rainfall totals for Santa Rosa as recorded on WeatherUnderground.com (keep in mind that these are from a single station and that there was most likely some regional variation within the watershed):

Date	precip (in)
2/7-9	0.11
2/10	0.34
2/11	0.14
2/12	0.06

2/13	0.62
2/14	0.16
2/15	1.41
2/16	0.94
2/17	0.78

Ideally we would have sampled on Sunday, February 15, however, the analytical lab was unable to accommodate that schedule. Going into the weekend our plan was to sample on Wednesday, February 18, because the forecast showed a new system arriving early this week. Once that storm system disappeared we changed tacks and sampled on the 17th. Unfortunately the lab was unable to fully accommodate us without more notice. They agreed to take seven samples for each analysis. Josh Viers came up with the following site priority list for this event: Copeland Creek, Foss Creek, both Laguna sites, both Atascadero Creek sites and Santa Rosa Creek at Prince Memorial Greenway. We did not collect any quality control samples for the *E. coli* or *Enterococcus* analyses due to the limited number of samples the lab would accept. We did collect QCs for the other Bacteroidales and stable isotope samples. The following is a summary of observations from 17 February:

In the Santa Rosa watershed It rained steadily throughout the morning and up until about 16:00. All streams were high and off-color with runoff. Drains and ditches alongside roads were all carrying substantial runoff.

Both Copeland and Foss Creeks were high and moving at a good clip. I estimated both of them to be running somewhere around 20 cfs.

The Laguna de Santa Rosa was flooded downstream of the confluence with Santa Rosa Creek (114LAGU02). Water was running through the riparian vegetation from the bankfull channel to about 75 meters back into the floodplain on the west side of the channel. I waded to about waist deep to collect the samples and I was still 40-50 meters away from the edge of the channel. I estimated the flow at greater than 200 cfs.

The Laguna de Santa Rosa at Laguna State Park was high enough that I was unable to wade in to collect the sample. I took a grab sample from the bank but outside of the current. Still, the water appeared to be well-mixed throughout the channel.

Atascadero Creek at Green Valley Road was flooded. The staff gage there read 9.25'; it was about 3.5' during the previous event. The stream was over its banks and spread far into the floodplain. The hiking trail about 120 meters east of the stream was under a foot of standing water. Atascadero Creek at Bodega Avenue was slightly flooded with the water a couple of feet into the sloping floodplain.

Santa Rosa Creek at Prince Memorial Greenway was very high and fast. I estimated the flow at greater than 200cfs.

When we delivered the samples to the analytical lab we asked to see the results from the previous event. A report had not yet been prepared but we were able to view the raw results. Based on those results we asked the lab to dilute all

the *Enterococcus* samples at 1:100. We also had them dilute the *E. coli* samples for Copeland Cr, the two Lagunas and Santa Rosa Cr at Prince Memorial at 1:100 and all other sites at 1:10.

23 February 2009

Heidi Schott and I completed another round of sampling today. We squeezed in all 12 sites plus we grabbed a couple of extra stable isotope samples; one from Mill Cr above the confluence of Wallace Cr and one from Wallace Cr.

It rained steadily all day, ranging from light to moderate intensity. The Russian watershed is thoroughly saturated from the 4" + of rain that has fallen since Saturday. Weather Underground lists the rainfall total for 22 February at 2.47" in the Santa Rosa area. All of the tributaries we sampled today were high and muddy.

Copeland Creek was actually a bit lower today than it was on Tuesday, 17 February. Foss Creek, both Atascadero Cr sites and Santa Rosa Cr at Prince Memorial Greenway all appeared to be at about the same levels as they were on Tuesday, 17 February.

Lower Dry Creek was up into its floodplain and running > 200cfs. The water was dirty unlike every other event where it has been clear due to the Warm Springs Dam upstream.

Mill Creek was raging at maybe 90 cfs. I had to collect the sample along the left bank about 150-200m downstream of the confluence with Wallace Cr. Normally I wade across the channel from above the confluence and then walk down to about the same place but this time I had to sneak through a hole in someone's fence to gain safe access. I estimated Wallace Cr to be flowing at 65-70 cfs. The water in Wallace Cr was darker than that of Mill Cr. Another small tributary that I had not noticed before (because it was dry) was also entering Mill Cr about 40m downstream of the confluence with Wallace Cr. It was running at maybe 2 cfs. The primary samples from Mill Creek were collected downstream of the two confluences. The water is not well-mixed here but it was the best we could do without getting swept away.

Lower Green Valley Creek was never been much more than knee deep during previous sampling events. Today it was literally 15' or more deep at the location where I normally collect the sample. The sampling location is in a deep narrow cut about 50m upstream of where the creek empties into the Russian River. Because the Russian is swollen from runoff right now the sampling location is completely flooded. We collected the sample about 100m upstream of the usual location due to better access to the moving water. I estimated the flow from 20-50 cfs although it was hard to say due to the flooding and my uncertainty of the flooded channel contour.

Both Laguna de Santa Rosa sites were flooded noticeably more than they were on Tuesday, 17 February. Once again the sample from the site downstream of Santa Rosa Creek was collected high in the floodplain.

The staff gage at Atascadero Creek at Green Valley Road read 8.55'.

Both Mark West Cr and Upper Santa Rosa Creek were flowing at or above 20 cfs. Both streams were a light brown but nowhere near as turbid as any of the other creeks.

We had the lab run all samples at 1:100 dilutions after looking at the results from last Tuesday's sampling. I don't recall any specific numbers but there were highly elevated levels of bacteria in all of the samples.

2-3 March 2009

This sampling event was done across two days to provide a better picture of extended runoff conditions. The five day period from 24-28 February only recorded a total of 0.22" of rain in the Santa Rosa area with 0.20" of that falling on 25 February (www.wunderground.com). Beginning around 22:00 on 28 February and continuing through 1 March a low pressure system moved into the Santa Rosa area bringing a total of 1.05" of rain in a 24 hour period (wunderground.com). A light rain fell through the morning on 2 March until around 13:00. The rainfall increased to a steady rate the morning of 3 March. Rainfall totals for those two days were 0.36" & 0.52" on March 2&3, respectively.

Overland runoff was observed at all sites in the form of cloudy water. The stream levels in Copeland and Foss Creeks were noticeably less than during the 23 February event.

The water level in Lower Dry Creek was noticeably higher on 3 March than it had been the day before.

The water in Mill Creek did not appear to be well-mixed. Our 2 March sample consisted mostly of Wallace Creek water due to the access issue described in the 23 February sampling notes. On 3 march we collected our samples above the confluence with Wallace Creek in order to capture a more representative sample of Mill Creek.

The staff gage at Atascadero Creek at Green Valley Road read 6.1' compared to 8.55' on 23 February. The Atascadero Creek at Bodega Hwy site was noticeably higher during the second day of the sampling event.

I estimated the flow in Laguna de Santa Rosa downstream of the confluence with Santa Rosa Creek at somewhere between 50-200 cfs and only around 5-20 cfs at the upper Laguna de Santa Rosa site.

Upper Mark West Creek was flowing between 5-20 cfs while the Upper Santa Rosa Creek was running in the 20-50 cfs range. In lower flows these two tributaries seem to carry an equal amount of water.

15 May 2009

This sampling event was intended as a "dry season" event and was preceded by eight days of warm-hot, dry weather. The previous rainfall of any significance was the four day period of 1-4 May, 2009 when a combined total of 2.80" of rain was recorded in the Santa Rosa area (www.wunderground.com). The weather on 15 May was hot, dry and pleasant.

Both Copeland and Foss Creek had less than 1 cfs of flow. Lower Dry Creek was running clear at around 35 cfs.

At the Laguna de Santa Rosa downstream of the confluence with Santa Rosa Creek the aquatic macrophytes *Lemna* and *Ludwigia* were observed growing along the edges of the slow moving channel indicating warmer water and increased sunlight.

No flow was observed in Atascadero Creek at Green Valley Road. The flow was less than 1 cfs in Atascadero Creek at Bodega Highway.

The flow was moderate (5-20 cfs) in both Mark West and upper Santa Rosa creeks.

No other noteworthy observations were made.

Appendix B Pathogen Data

*100ml lab dilution, default lab dilution is 10ml
†levels exceed recommended CDPH limit for single event samples: *E. coli* ≥235 MPN/100mL, Total Coli: ≥10,000 MPN/100mL, and *Enterococcus* ≥61 MPN/100mL

http://www.cdph.ca.gov/HealthInfo/environhealth/water/Documents/Beaches/DraftGuidanceforFreshWaterBeaches.pdf

RRPPID Date	Time	*E. coli*	*E. coli* Duplicate	*E. coli* Blank	Total Coliform	Total Coliform Duplicate	*Enterococcus*	*Enterococcus* Duplicate	*Enterococcus* Blank
114ATASC1									
19-Dec-08	14:00	794†			>24192†		>200.5†		
23-Jan-09	13:20	183	228		1354	3076	20	31	
06-Feb-09	11:00	8664†			>24192†		>2005†		
17-Feb-09	11:40	9804†			>24192†		640*†		
23-Feb-09	12:20	1460*†			7630*		1780*†		
02-Mar-09	11:50	2650*†			38730*†		3440*†		
03-Mar-09	10:50	4190*†			41060*†		5910*†		
15-May-09	12:30	344.8†			>2419.2		165.2†		
114COPE01									
19-Dec-08	8:20	>24192†			>24192†		>200.5†		
23-Jan-09	8:40	200*			1690*		531†		
06-Feb-09	6:50	2620*†			18600*†		>2005†		
17-Feb-09	9:30	1600*†			15530*†		530*†		
23-Feb-09	8:00	410*†			4570*		870*†		
02-Mar-09	8:20	3310*†			3310*		3440*†		
03-Mar-09	7:40	410*†			4500*		1780*†		
15-May-09	8:00	488.4†			>2419.2		144.5†		

114GREEN1									
19-Dec-08	12:40	2359†			>24192†		>200.5†		<10
RRPPID Date	**Time**	***E. coli***	***E. coli* Duplicate**	***E. coli* Blank**	**Total Coli**	**Total Coli Duplicate**	***Enterococcus***	***Enterococcus* Duplicate**	***Enterococcus* Blank**
23-Jan-09	11:30	86		<10	4611		<10		<10
06-Feb-09	10:20	1012†			>24192†		>2005†		
17-Feb-09	11:20	2481†			>24192†		2220*†		
23-Feb-09	11:50	1450*†			16160*†		2220*†		
02-Mar-09	11:20	1350*†			36540*†		2220*†		
03-Mar-09	10:30	100*			2810*		310*†		
15-May-09	12:00	42.6			>2419.2		34.4		
114LAGU01									
19-Dec-08	13:30	24192†			>24192†		>200.5†		
23-Jan-09	12:40	1890*†			3590*		164†		
06-Feb-09	11:20	4710*†			15000*†		>2005*†		
17-Feb-09	12:00	4080*†			24000*†		3060*†		
23-Feb-09	12:40	5760*†			16070*†		10910*†		
02-Mar-09	12:10	520*†			13540*†		870*†		
03-Mar-09	11:10	1750*†			20140*†		3640*†		
15-May-09	13:00	43.2			>2419.2		69.7†		
114LAGU02									
19-Dec-08	12:00	12997†			>24192†		>200.5†		
23-Jan-09	12:00	100*	<100*	<10*	840*	310*	<10	10	<10

06-Feb-09	10:00	2780*†			6310*		>2005†		
17-Feb-09	11:00	48840*†			>241192*†		640*†		
23-Feb-09	11:30	1340*†			8620*		1110*†		

RRPPID Date	Time	*E. coli*	*E. coli* Duplicate	*E. coli* Blank	Total Coli	Total Coli Duplicate	*Enterococcus*	*Enterococcus* Duplicate	*Enterococcus* Blank
02-Mar-09	11:00	2980*†			72700*†		6590*†		
03-Mar-09	10:10	100*			4500*		530*†		
15-May-09	11:10	29.4			1732.9		50.4		
114LGVARR									
19-Dec-08	11:20	211			6867		200.5†		
23-Jan-09	11:00	630†			816		10		
06-Feb-09	9:20	197			2359		164†		
23-Feb-09	11:00	410*†	970*†		8650*	7760*	1920*†	1780*†	
02-Mar-09	10:30	410*†		<100*	9070*		990*†		100*†
03-Mar-09	9:50	410*†	100*		3540*	4190*	990*†	310*†	
15-May-09	10:30	44.1	62.4		2419.2	2419	109.1†	69.7†	
114MARK01									
19-Dec-08	14:50	305†			8664		165.2†		
23-Jan-09	14:40	<10			3654		20		
06-Feb-09	13:00	201			4611		99†		
23-Feb-09	14:10	481†			5172		306†		
02-Mar-09	13:30	663†	624†		9208	7701	945†	560†	
03-Mar-09	12:20	959†			4360		306†		
15-May-09	15:20	108.1			1986.3		73.8†		
114MILL01									
19-Dec-08	10:10	749†	836†	<10	4611	10462†	>200.5†	>200.5†	<1
23-Jan-09	10:10	10			413		<10		

06-Feb-09	8:30	749†	565†		9804	8664	478†	406†	

RRPPID Date	Time	*E. coli*	*E. coli* Duplicate	*E. coli* Blank	Total Coli	Total Coli Duplicate	*Enterococcus*	*Enterococcus* Duplicate	*Enterococcus* Blank
23-Feb-09	10:00	<100*			740*		100*†		
02-Mar-09	9:40	336*†			6488*		453*†		
03-Mar-09	9:10	100*			1970*		200*†		
15-May-09	9:40	60.1			1732.9		45.3		
114SROSA1									
19-Dec-08	16:10	5172†			>24192†		>200.5†		
23-Jan-09	14:10	410*†			740*		42		
06-Feb-09	12:00	6160*†			43520*†		>2005†		
17-Feb-09	12:40	1350*†			19890*†		750*†		
23-Feb-09	13:00	310*†			2490*		1640*†		
02-Mar-09	12:40	2010*†			34480*†		3060*†		
03-Mar-09	11:40	850*†			11120*†		2070*†		
15-May-09	13:30	461.1†		<1.0	2419.2		165.2†		<1.0
114VINE01									
19-Dec-08	9:20	9208†			>24192†		>200.5†		
23-Jan-09	9:30	15531†			>24192†		>2005†		
06-Feb-09	7:40	9139†			>24192†		738*†		
17-Feb-09	10:10	2723*†	2909*†		19863*†	17329*†	1780*†	1500*†	
23-Feb-09	9:00	850*†	740*†		10760*†	8130*	2540*†	2380*†	
02-Mar-09	9:10	860*†			12740*†		2540*†		
03-Mar-09	8:30	740*†			6270*		1110*†		
15-May-09	8:50	102.2			>2419.2		144.5†		

Environmental Data Collected for each Sampling Event by Site

Site ID	Date	Water Temp °C	Air Temp °C	pH	Specific Conductivity µs/cm	O_2 mg/L	O_2 %
114ATASC1							
	12/19/2008	5	6.1	7.09	311	6.71	56.4
	1/23/2009	7.2	12	7.5	402	6.49	61.8
	2/6/2009	8.3	10.9	7.9	265	8.69	78.3
	2/17/2009	8.6	12.4	7.08	213	10.72	93.7
	2/23/2009	11.8	13.3	7.58	210	9.73	90.1
	3/2/2009	11.4	13.6	7.4	201		
	3/3/2009	10	13.9	6.9	196	9.8	88.7
	5/15/2009	13.9	24.9	6.91	426	3.38	36.1
114COPE01							
	12/19/2008	8.8		7.02	124	7.53	67.1
	1/23/2009	9.7	10.8	7.43	617	3.07	28.3
	2/6/2009	10.3	15.3	7.07	119	7.95	75.6
	2/17/2009	8.3		7.21	175	10.07	86.8
	2/23/2009	10.9	20	7.25	228	8.85	81.9
	3/2/2009	11.7		7.35	175	9.5	89.6
	3/3/2009	10.3	12.1	7.3	223	9.47	86.3
	5/15/2009	16	19.8	7.3	662	3.64	40.5
114GREEN1							
	12/19/2008	5.3	8	5.99	415	3.97	34.9
	1/23/2009	7.3	12.4	7.35	371	2.97	26.3
	2/6/2009	8	10.3	8.51	333	4.89	42.6
	2/17/2009	8.2	12	6.7	194	8.03	68.6
	2/23/2009	12	13.9	6.67	172	6.31	58.6
	3/2/2009	11.4	12.1	6.87	229		
	3/3/2009	11	14.6	6.56	206	5.51	51.3
	5/15/2009	15.3	22.4	6.64	334	1.41	16.3
114LAGU01							
	12/19/2008	6.7	9.8	7.2	385	7.14	62.9
	1/23/2009	8.7	13.9	8.04	422	7.54	68.4
	2/6/2009	9.7	10.7	8.45	192	9.09	82.6
	2/17/2009	8.8	11.9	6.99	190	6.79	56.7
	2/23/2009	13.1	13	7.32	178	5.92	55.9
	3/2/2009	11.9	12.2	7.38	376		
	3/3/2009	11.5	12.8	7	205	5.87	54.6
	5/15/2009	18.1	21.9	7.2	448	5.75	66.3
114LAGU02							
	12/19/2008	7.3	11.1	7.61	336	8.97	78.2
	1/23/2009	8.4	12.1	8.36	488	7.67	70.7

2/6/2009	9.9	9.7	9.23	397	8.17	76.3
2/17/2009	8.6	13.8	7.14	226	7.28	67

Site ID	Date	Water Temp °C	Air Temp °C	pH	Specific Conductivity µs/cm	O_2 mg/L	O_2 %
	2/23/2009	11.7	14.7	7.23	162	6.68	64.1
	3/2/2009	12.2	12.7	7.24	166		
	3/3/2009	12.2	11.2	6.96	181	5.06	47.6
	5/15/2009	17.5	24.3	6.96	358	1.62	17.4
114LGVARR							
	12/19/2008	5.6		7.5	367	10.94	88.5
	1/23/2009	7.4	10.8	7.53	380	9.98	89.6
	2/6/2009	7.5	9.7	8.17	341	10.1	86.5
	2/23/2009	11.7	13.2	6.94	181	8.25	76.1
	3/2/2009	11.2	13.8	7	198	8.53	81.3
	3/3/2009	11	11	6.85	178	8.59	79.3
	5/15/2009	15.3	22.1	7.32	301	7.3	78.3
114MARK01							
	12/19/2008	5.8	3.7	7.93	456	11.09	92.7
	1/23/2009	7.9	12.6	8.81	440	9.12	84.1
	2/6/2009	7.8	12	9.1	395	10.58	93
	2/23/2009	11	14.1	9.35	181	11.02	98.8
	3/2/2009	10	15.3	7.98	160		
	3/3/2009	9	13.6	7.95	178	10.64	94.8
	5/15/2009	15.7		8.23	409	7.94	89.2
114MILL01							
	12/19/2008	5	6	7.66	223	11.04	87.9
	1/23/2009	7.4	9.8	7.79	206	10.48	95.5
	2/6/2009	7.7	9	7.95	190	10.54	91.8
	2/23/2009	11.7	13.7	7.29	106	10.12	94.2
	3/2/2009	11	13.3	7.4	130		
	3/3/2009	10.5		7.13	146	10.49	95.1
	5/15/2009	14.3	17.3	7.69	214	8.89	91.6
114SROSA1							
	12/19/2008	7.8	8	7.7	243	10.48	90.1
	1/23/2009	10.3	11.9	8.56	443	11.21	100
	2/6/2009	10	11.3	8.16	141	9.96	97.6
	2/17/2009	8.6	13.8	7.49	151	11.04	96.2
	2/23/2009	13.1	13.6	7.75	162	10.19	95.5
	3/2/2009	11.7	13.8	7.7	149		
	3/3/2009	10.4	15.1	7.48	158	10.38	94.5
	5/15/2009	17.7	19.5	8.32	413	11.86	131.9
114VINE01							
	12/19/2008	8	11	7.36	223	9.04	77.8
	1/23/2009	10.2	11.4	7.36	443	6.82	62
	2/6/2009	10	11.7	7.24	137	9.08	84.1

Site ID	Date	Water Temp °C	Air Temp °C	pH	Specific Conductivity µs/cm	O_2 mg/L	O_2 %
	2/17/2009	9		7.11	176	9.91	87
	2/23/2009	12.7	17.7	6.98	148	9.1	85.3
	3/2/2009	12.1	14.1	7.1	172	9.25	87.7
	3/3/2009	10.3	11.1	7.06	156	9.76	89.4
	5/15/2009	16.5		7.31	502	4.92	54.7
114WEST01							
	12/19/2008	10.7	8.4	7.5	188	8.89	81.2
	1/23/2009	10.5	10.1	7.67	191	10.33	96.5
	2/6/2009	10.2	10	7.78	175	9.78	88
	2/23/2009	12.3	14.3	7.24	144	9.36	88.3
	3/2/2009	11.3	13.6	7.32	156	9.74	90.8
	3/3/2009	10.4	10	7.27	164	10.1	91.4
	5/15/2009	13.6	15.1	7.66	184	9.1	90.7
114WILD01							
	12/19/2008	6.1	6.7	8.34	476	11.11	93.9
	2/6/2009	8.5	11.2	9.22	438	10.46	94.8
	2/23/2009	12.3	13.7	8.98	175	10.48	97.5
	3/2/2009	10.7		8.08	169		
	3/3/2009	9.7	11.4	8	188	10.8	97.3
	5/15/2009	16.7		8.39	403	8.34	89.6

Appendix C Stable Isotope Data Summary

Sample key:

primary sample: sample time is rounded to nearest ten minutes

field blank: has a one minute offset from primary sample time

field duplicate: has a three minute offset from primary sample time

Samples denitrified with either *P. aureofasciens* for 18O or *P. chlororaphis* for 15N.

Sample size was either 1 mL or 5 mL depending on nitrate concentration (determined from 1st analysis run).

The 'area' is the integrated signal from N_2O

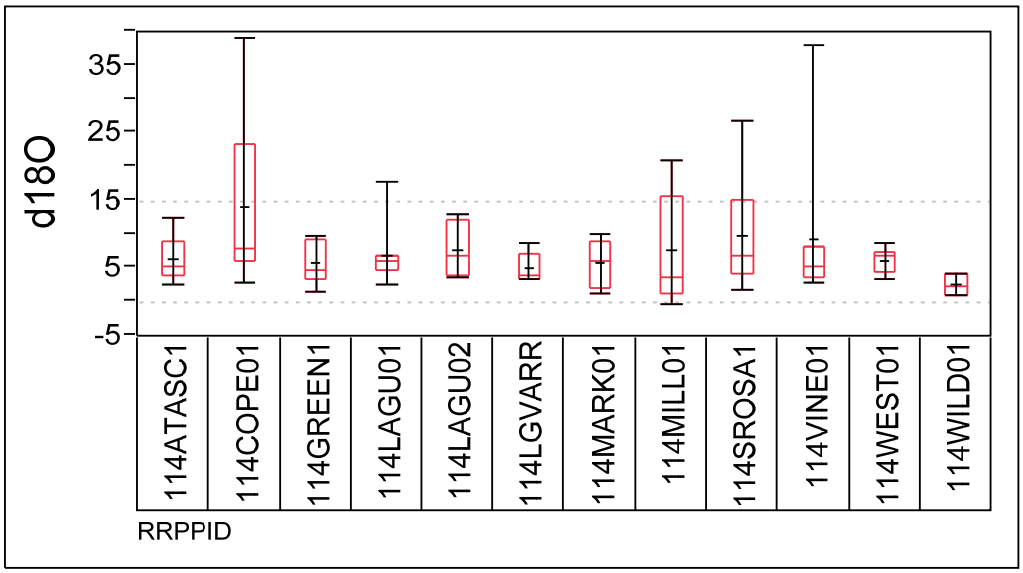
					P. aureofasciens		*P. chlororaphis*	
sample	ID	Date	time	mL sample	area	$d^{18}O$	Area	$d^{15}N$
1	114COPE01	12/19/2008	8:20	1	14.943	16.05	19.687	4.14
2	114VINE01	12/19/2008	9:20	1	15.2	8.44	19.459	6.00
3	114WEST01	12/19/2008	9:40	5	5.667	7.65	6.558	6.45
4	114MILL01	12/19/2008	10:10	5	0.628	-16.59	4.266	12.98
5	114MILL01	12/19/2008	10:11	5	0.651	-19.12	4.938	12.03
6	114MILL01	12/19/2008	10:13	5	0.629	-16.59	4.533	12.14
7	114LGVARR	12/19/2008	11:20	5	0.773	-10.21	4.383	11.34
8	114LAGU02	12/19/2008	12:00	1	9.382	13.15	13.687	9.50
9	114GREEN1	12/19/2008	12:40	1	9.055	8.75	12.49	13.89
10	114LAGU01	12/19/2008	13:30	1	24.267	6.95	25.612	7.89
11	114ATASCI	12/19/2008	14:00	5	29.871	12.48	30.511	11.78
12	114MARK01	12/19/2008	14:50	5	11.526	10.27	11.729	5.19
13	114WILD01	12/19/2008	15:30	5	0.837	-19.12	5.278	9.75
14	114SROSA1	12/19/2008	16:10	5	24.34	16.43	25.253	6.24
73	114COPE01	1/23/2009	8:40	1	9.951	39.10	10.65	3.21
74	114VINE01	1/23/2009	9:30	1	16.493	38.23	18.318	1.72
75	114WEST01	1/23/2009	9:50	5	15.895	6.89	14.665	1.95
76	114MILL01	1/23/2009	10:10	5	9.3	21.01	10.481	10.58
77	114LGVARR	1/23/2009	11:00	5	9.123	8.96	9.725	7.24
78	114GREEN1	1/23/2009	11:30	5	11.571	9.48	7.373	14.15
79	114GREEN1	1/23/2009	11:31	5	0.769	-22.52	0.843	29.72
80	114LAGU02	1/23/2009	12:00	5	8.264	10.84	9.335	14.02
81	114LAGU01	1/23/2009	12:40	5	29.759	17.85	33.48	8.53
82	114ATASC1	1/23/2009	13:20	1	12.957	9.97	15.06	8.71
83	114ATASC1	1/23/2009	13:23	1	12.647	10.43	14.185	8.77
84	114SROSA1	1/23/2009	14:10	5	16.303	27.04	18.128	10.67
85	114MARK01	1/23/2009	14:40	5	1.217	-8.35	1.186	15.86
15	114COPE01	2/6/2009	6:50	1	12.068	26.10	13.178	2.19
16	114VINE01	2/6/2009	7:40	1	23.802	3.72	26.622	2.28
17	114WEST01	2/6/2009	8:00	5	16.082	4.46	17.537	3.06
18	114WEST01	2/6/2009	8:03	5	15.224	6.30	15.613	3.21
19	114MILL01	2/6/2009	8:30	5	5.636	10.63	5.375	9.46
20	114LGVARR	2/6/2009	9:20	5	8.47	6.58	9.763	7.24
21	114LAGU02	2/6/2009	10:00	5	40.675	12.96	43.12	5.29
22	114GREEN1	2/6/2009	10:20	5	11.265	10.02	12.195	9.82
23	114ATASC1	2/6/2009	11:00	1	12.279	3.83	14.333	6.18

24	114LAGU01	2/6/2009	11:20	1	13.249	4.68	14.514	4.67
25	114SROSA1	2/6/2009	12:00	1	13.013	8.76	15.397	3.18
26	114MARK01	2/6/2009	13:00	5	2.573	1.33	3.416	4.18
27	114WILD01	2/6/2009	13:30	5	0.728	-14.07	2.45	8.03
28	114WILD01	2/6/2009	13:31	5	0.763	-23.76	3.088	-10.46
86	114COPE01	2/17/2009	9:30	1	22.014	7.76	22.842	6.22
87	114VINE01	2/17/2009	10:10	1	18.04	4.29	19.162	5.45
88	114VINE01	2/17/2009	10:13	1	19.294	6.99	19.869	5.11
89	114LAGU02	2/17/2009	11:00	1	11.801	8.79	12.222	5.80
90	114GREEN1	2/17/2009	11:20	1	24.942	4.68	26.243	6.03
91	114ATASC1	2/17/2009	11:40	1	45.653	4.86	46.795	6.33
92	114LAGU01	2/17/2009	12:00	1	49.258	5.05	51.631	7.20
93	114SROSA1	2/17/2009	12:40	1	26.806	5.18	28.042	4.79
94	114SROSA1	2/17/2009	12:41	5	0.601	-31.81	1.385	3.11
43	114COPE01	2/23/2009	8:00	1	20.593	6.08	22.564	8.02
44	114VINE01	2/23/2009	9:00	1	14.399	4.74	16.241	6.50
45	114VINE01	2/23/2009	9:03	1	15.303	4.70	16.067	36.66
46	114WEST01	2/23/2009	9:30	5	26.195	3.45	26.447	4.13
47	114WEST01	2/23/2009	9:31	5	1.153	-31.81	2.201	12.39
48	114MILL01	2/23/2009	10:00	5	7.195	3.72	7.833	7.23
49	114WALLCRK	2/23/2009	10:00	5	4.551	1.30	4.284	8.90
50	114MILLUS	2/23/2009	10:00	5	9.655	5.28	9.544	6.61
51	114LGVARR	2/23/2009	11:00	1	17.447	3.60	18.72	6.65
52	114LAGU02	2/23/2009	11:30	1	14.95	4.37	17.694	6.63
53	114GREEN1	2/23/2009	11:50	1	16.302	3.50	19.021	6.80
54	114ATASC1	2/23/2009	12:20	1	37.514	5.80	40.341	7.33
55	114LAGU01	2/23/2009	12:40	1	21.074	6.38	24.125	8.14
56	114SROSA1	2/23/2009	13:00	1	21.062	4.58	22.41	5.86
57	114MARK01	2/23/2009	14:10	5	32.506	2.59	35.63	4.52
58	114WILD01	2/23/2009	14:40	5	15.192	2.52	16.604	4.50
29	114COPE01	3/2/2009	8:20	1	8.922	2.97	10.34	5.24
30	114VINE01	3/2/2009	9:10	1	13.128	3.01	13.909	6.15
31	114WEST01	3/2/2009	9:20	5	18.597	4.55	18.767	4.51
32	114MILL01	3/2/2009	9:40	5	3.434	-0.16	2.768	6.50
33	114LGVARR	3/2/2009	10:30	1	11.491	4.22	11.932	7.41
34	114LGVARR	3/2/2009	10:31	5	1.136	0.78	1.296	8.62
35	114LAGU02	3/2/2009	11:00	1	14.119	3.88	14.861	5.71
36	114GREEN1	3/2/2009	11:20	1	10.544	4.80	12.138	8.13
37	114ATASC1	3/2/2009	11:50	1	20.974	2.85	21.807	8.27
38	114LAGU01	3/2/2009	12:10	1	33.129	2.70	34.378	10.46
39	114SROSA1	3/2/2009	12:40	1	11.887	1.90	15.865	7.53
40	114MARK01	3/2/2009	13:30	5	13.417	8.58	13.561	5.45
41	114MARK01	3/2/2009	13:33	5	15.753	6.72	16.209	5.06
42	114WILD01	3/2/2009	14:00	5	6.123	4.39	6.15	9.09
95	114COPE01	3/3/2009	7:40	1	17.711	6.89	19.086	8.38
96	114VINE01	3/3/2009	8:30	1	10.695	5.94	11.72	6.29
97	114WEST01	3/3/2009	8:40	5	6.643	8.87	7.048	7.74
98	114WEST01	3/3/2009	8:43	5	15.801	8.39	16.968	6.43
99	114MILL01	3/3/2009	9:10	5	11.391	3.18	9.727	4.88
100	114WALLCRK	3/3/2009	9:10	5	3.036	1.32	4.281	8.95

101	114LGVARR	3/3/2009	9:50	1	13.324	3.57	13.826	6.66
102	114LAGU02	3/3/2009	10:10	1	12.716	3.99	13.207	7.95
103	114GREEN1	3/3/2009	10:30	1	12.231	3.93	11.736	8.03
104	114ATASC1	3/3/2009	10:50	1	20.962	4.44	22.133	6.25
105	114LAGU01	3/3/2009	11:10	1	21.21	6.64	21.619	8.88
106	114SROSA1	3/3/2009	11:40	1	12.074	4.30	13.079	8.14
107	114MARK01	3/3/2009	12:20	5	21.566	3.71	20.897	4.99
108	114WILD01	3/3/2009	12:40	5	10.241	1.21	9.837	5.55
109	114WILD01	3/3/2009	12:41	5	2.467	-26.91	1.342	10.15
59	114COPE01	5/15/2009	8:00	1	28.771	8.32	32.302	12.71
60	114VINE01	5/15/2009	8:50	1	17.02	7.42	17.744	7.97
61	114WEST01	5/15/2009	9:10	5	11.271	7.12	11.387	3.88
62	114MILL01	5/15/2009	9:40	5	2.197	1.97	1.17	11.92
63	114LGVARR	5/15/2009	10:30	5	12.401	3.81	14.112	8.11
64	114LAGU02	5/15/2009	11:10	5	4.689	4.91	5.375	11.79
65	114GREEN1	5/15/2009	12:00	5	3.283	1.70	6.783	10.68
66	114ATASC1	5/15/2009	12:30	1	9.322	6.96	10.615	7.99
67	114LAGU01	5/15/2009	13:00	5	14.285	5.85	15.161	10.71
68	114SROSA1	5/15/2009	13:30	5	19.871	11.23	18.367	12.11
72	114SROSA1	5/15/2009	13:31	5	0.767	-15.33	1.095	13.34
69	114WILD01	5/15/2009	14:30	5	0.629	-29.06	1.268	8.42
70	114WILD01	5/15/2009	14:33	5	2.2	-7.10	1.353	-1.79
71	114MARK01	5/15/2009	15:20	5	15.791	8.42	13.674	7.85

The following variability charts and accompanying tables detail the distribution of SIA sample values for all samples and for pooled samples by site, including the mean, standard deviation, standard error, 95% confidence intervals, minimum value, maximum value, and number of observations.

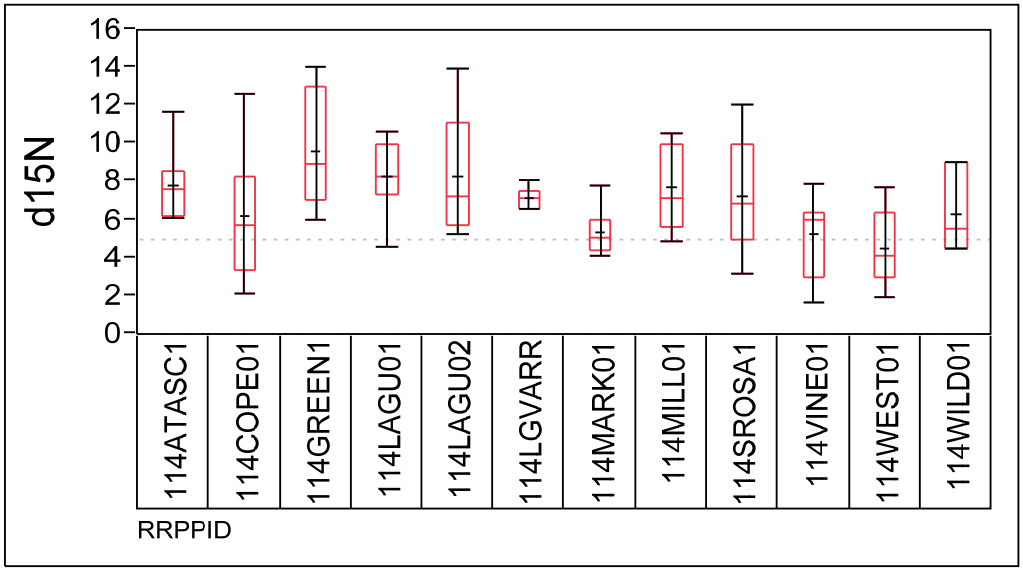
Variability Chart for d18O



Variability Summary for d18O

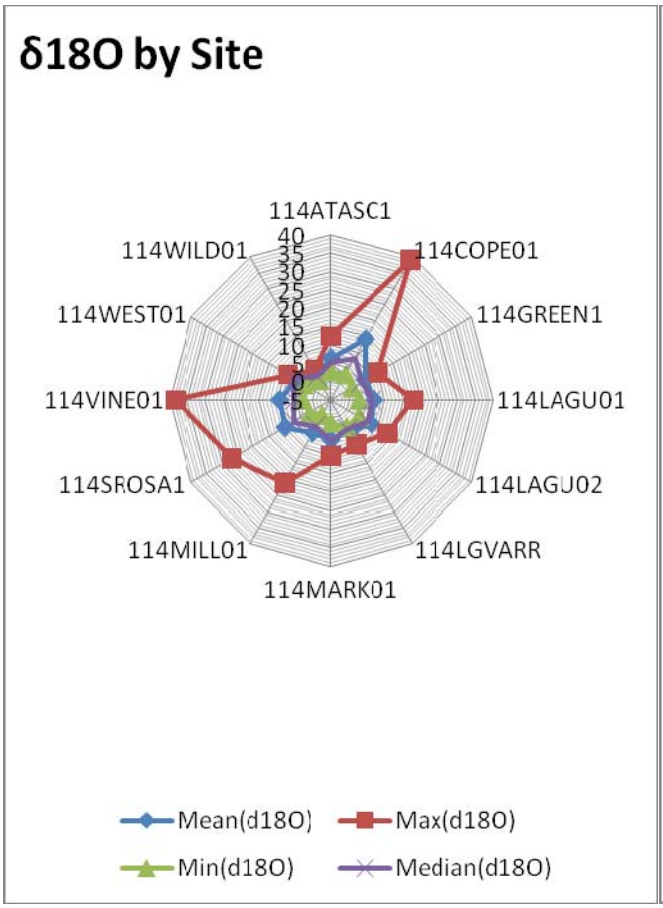
	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%	Minimum	Maximum	Observations
d18O	7.718959	6.939548	0.761714	6.203666	9.234251	-0.15689	39.09625	83
RRPPID[114ATASC1]	6.398929	3.29275	1.164163	3.646121	9.151737	2.848304	12.4835	8
RRPPID[114COPE01]	14.15758	12.45903	4.404933	3.741571	24.5736	2.965055	39.09625	8
RRPPID[114GREEN1]	5.858668	3.114278	1.101064	3.255066	8.46227	1.703973	10.01943	8
RRPPID[114LAGU01]	7.013491	4.585414	1.621189	3.179989	10.84699	2.696789	17.84729	8
RRPPID[114LAGU02]	7.861715	4.059092	1.435106	4.468229	11.2552	3.881723	13.14786	8
RRPPID[114LGVARR]	5.122453	2.197448	0.897104	2.816373	7.428533	3.570282	8.95953	6
RRPPID[114MARK01]	5.81701	3.721889	1.519455	1.911127	9.722893	1.325631	10.27134	6
RRPPID[114MILL01]	7.67485	8.421157	3.766056	-2.7814	18.1311	-0.15689	21.00667	5
RRPPID[114SROSA1]	9.92736	8.326122	2.943729	2.966548	16.88817	1.896739	27.0372	8
RRPPID[114VINE01]	9.474847	11.76416	4.159257	-0.36023	19.30993	3.011316	38.22922	8
RRPPID[114WEST01]	6.142169	1.993026	0.753293	4.298928	7.985411	3.44587	8.870264	7
RRPPID[114WILD01]	2.708869	1.596194	0.921563	-1.2563	6.674034	1.212983	4.389323	3

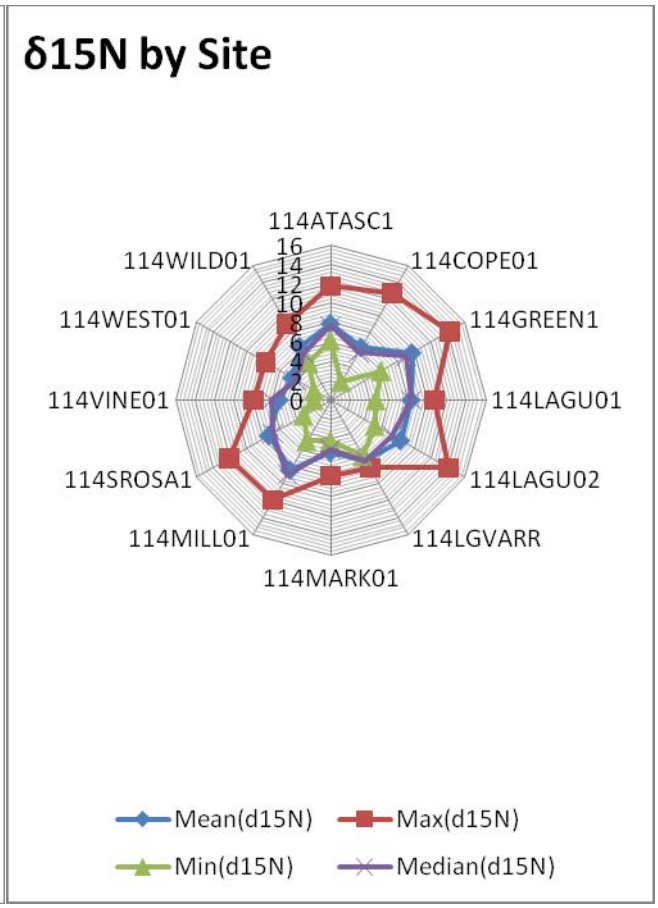
Variability Chart for d15N



Variability Summary for d15N

	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%	Minimum	Maximum	Observations
d15N	7.102416	2.726691	0.299293	6.507027	7.697806	1.717241	14.14517	83
RRPPID[114ATASC1]	7.854145	1.862893	0.658632	6.296727	9.411562	6.179151	11.77857	8
RRPPID[114COPE01]	6.264135	3.395134	1.200361	3.425731	9.102538	2.187492	12.71401	8
RRPPID[114GREEN1]	9.690333	3.05699	1.080809	7.134625	12.24604	6.03123	14.14517	8
RRPPID[114LAGU01]	8.310176	1.906471	0.674039	6.716326	9.904025	4.66897	10.71101	8
RRPPID[114LAGU02]	8.335244	3.190902	1.128154	5.667582	11.0029	5.29105	14.02116	8
RRPPID[114LGVARR]	7.216951	0.541754	0.22117	6.648415	7.785487	6.646939	8.109169	6
RRPPID[114MARK01]	5.363369	1.30281	0.53187	3.996153	6.730585	4.175449	7.851606	6
RRPPID[114MILL01]	7.729784	2.290546	1.024363	4.885696	10.57387	4.88177	10.5796	5
RRPPID[114SROSA1]	7.315926	2.970978	1.050399	4.832126	9.799726	3.179152	12.11241	8
RRPPID[114VINE01]	5.293857	2.163427	0.764887	3.485186	7.102527	1.717241	7.970808	8
RRPPID[114WEST01]	4.531277	1.973911	0.746068	2.705715	6.35684	1.952483	7.737521	7
RRPPID[114WILD01]	6.380088	2.404374	1.388166	0.40729	12.35288	4.498295	9.088796	3


δ18O by Site
114ATASC1
114COPE01
114GREEN1
114LAGU01
114LAGU02
114LGVARR
114MARK01
114MILL01
114SROSA1
114VINE01
114WEST01
114WILD01
40
35
30
25
20
15
10
5
0
Mean(d18O)
Max(d18O)
Min(d18O)
Median(d18O)


δ15N by Site
114ATASC1
114COPE01
114GREEN1
114LAGU01
114LAGU02
114LGVARR
114MARK01
114MILL01
114SROSA1
114VINE01
114WEST01
114WILD01
16
14
12
10
8
6
4
2
0
Mean(d15N)
Max(d15N)
Min(d15N)
Median(d15N)

AGRICULTURAL,Antecedent Storm

Date	RRPPID
2008-12-19	114ATASC1
2008-12-19	114GREEN1
2008-12-19	114LAGU01
2009-02-06	114ATASC1
2009-02-06	114GREEN1
2009-02-06	114LAGU01
2009-02-06	114LGVARR

AGRICULTURAL,Recession Flow

Date	RRPPID
2009-01-23	114ATASC1
2009-01-23	114GREEN1
2009-01-23	114LAGU01
2009-01-23	114LGVARR
2009-05-15	114ATASC1
2009-05-15	114GREEN1
2009-05-15	114LAGU01
2009-05-15	114LGVARR

AGRICULTURAL,Storm Pulse

Date	RRPPID
2009-02-17	114ATASC1
2009-02-17	114GREEN1
2009-02-17	114LAGU01
2009-02-23	114ATASC1
2009-02-23	114GREEN1
2009-02-23	114LAGU01
2009-02-23	114LGVARR
2009-03-02	114ATASC1
2009-03-02	114GREEN1
2009-03-02	114LAGU01
2009-03-02	114LGVARR
2009-03-03	114ATASC1
2009-03-03	114GREEN1
2009-03-03	114LAGU01
2009-03-03	114LGVARR

SEMINATURAL,Antecedent Storm

Date	RRPPID
2008-12-19	114MARK01
2008-12-19	114WEST01
2009-02-06	114MARK01
2009-02-06	114MILL01
2009-02-06	114WEST01

SEMINATURAL,Recession Flow

Date	RRPPID
2009-01-23	114MILL01
2009-01-23	114WEST01
2009-05-15	114MARK01
2009-05-15	114WEST01

SEMINATURAL,Storm Pulse

Date	RRPPID
2009-02-23	114MARK01
2009-02-23	114MILL01
2009-02-23	114WEST01
2009-02-23	114WILD01
2009-03-02	114MARK01
2009-03-02	114MILL01
2009-03-02	114WEST01
2009-03-02	114WILD01
2009-03-03	114MARK01
2009-03-03	114MILL01
2009-03-03	114WEST01
2009-03-03	114WILD01

URBAN,Antecedent Storm

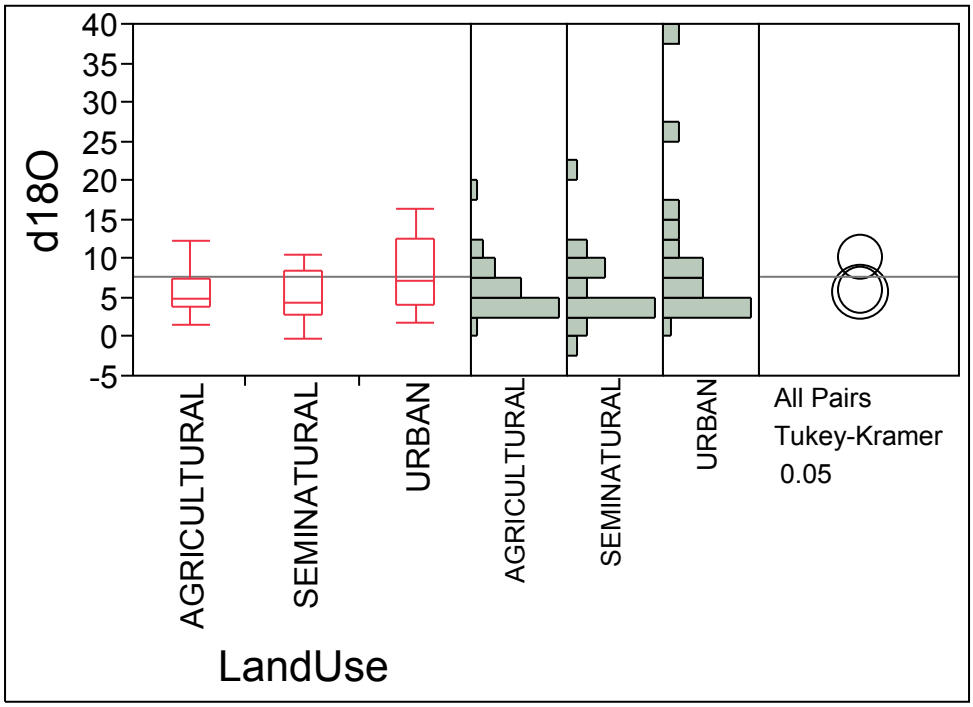
Date	RRPPID
2008-12-19	114COPE01
2008-12-19	114LAGU02
2008-12-19	114SROSA1
2008-12-19	114VINE01
2009-02-06	114COPE01
2009-02-06	114LAGU02
2009-02-06	114SROSA1
2009-02-06	114VINE01

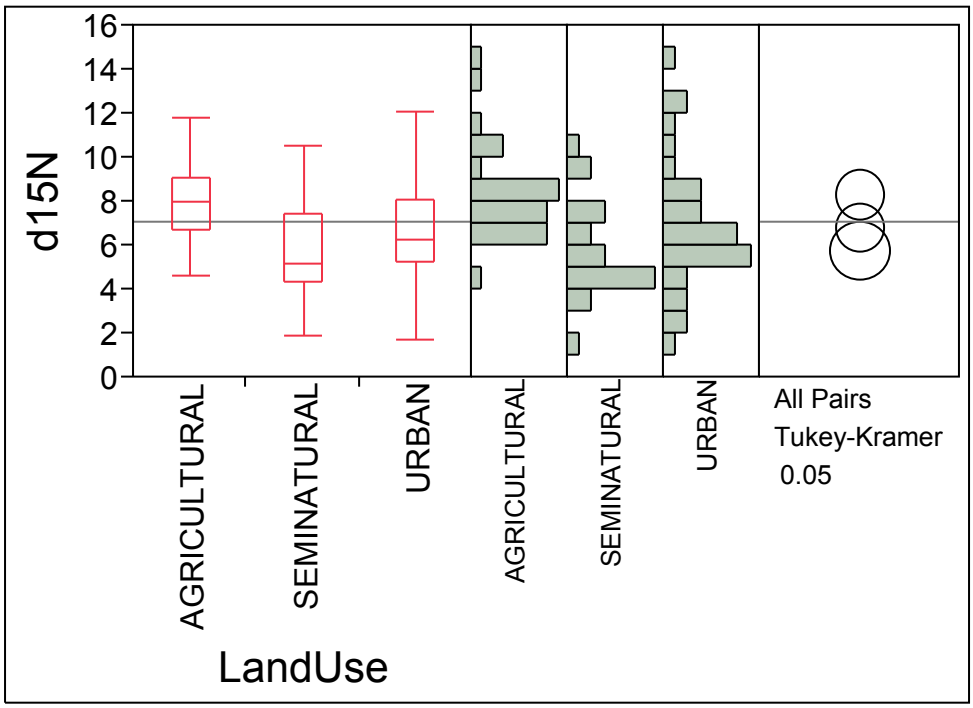
URBAN,Recession Flow

Date	RRPPID
2009-01-23	114COPE01
2009-01-23	114LAGU02
2009-01-23	114SROSA1
2009-01-23	114VINE01
2009-05-15	114COPE01
2009-05-15	114LAGU02
2009-05-15	114SROSA1
2009-05-15	114VINE01

URBAN,Storm Pulse

Date	RRPPID
2009-02-17	114COPE01
2009-02-17	114LAGU02
2009-02-17	114SROSA1
2009-02-17	114VINE01
2009-02-23	114COPE01
2009-02-23	114LAGU02
2009-02-23	114SROSA1
2009-02-23	114VINE01
2009-03-02	114COPE01
2009-03-02	114LAGU02
2009-03-02	114SROSA1
2009-03-02	114VINE01
2009-03-03	114COPE01
2009-03-03	114LAGU02
2009-03-03	114SROSA1
2009-03-03	114VINE01


d18O
40
35
30
25
20
15
10
5
0
-5
AGRICULTURAL
SEMINATURAL
URBAN
AGRICULTURAL
SEMINATURAL
URBAN
All Pairs
Tukey-Kramer
0.05
LandUse


d15N
16
14
12
10
8
6
4
2
0
AGRICULTURAL
SEMINATURAL
URBAN
AGRICULTURAL
SEMINATURAL
URBAN
All Pairs
Tukey-Kramer
0.05
LandUse

Appendix E Antecedent Precipitation & Flow Analyses

Table E1.

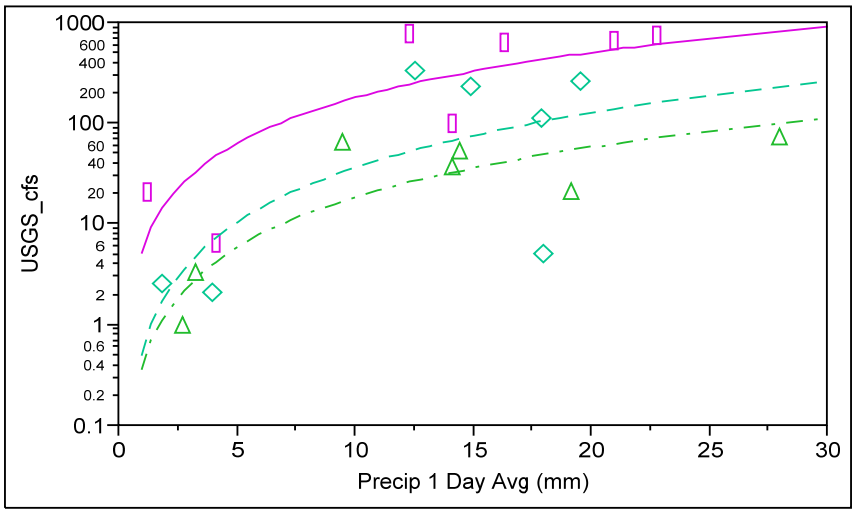
This table shows the 18 weather stations used to develop inverse distance weighted (IDW) interpolated precipitation surfaces for each sampling station watershed.

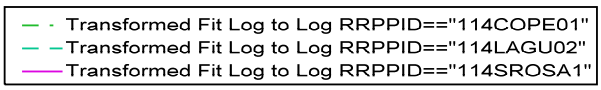
Wunderground Stations used in IDW Analysis			
WeatherID	**Lon DD**	**Lat DD**	**Nearby RRPP Stations**
KCAASTI1	-122.963	38.781	
KCAHEALD5	-122.947	38.649	
KCAHEALD6	-122.862	38.641	114VINE01
KCAPETAL8	-122.632	38.248	
KCAROHNE1	-122.676	38.341	114COPE01
KCASAINT2	-122.281	38.505	
KCASANTA105	-122.615	38.364	
KCASANTA126	-122.727	38.429	114SROSA1
KCASANTA127	-122.63	38.468	114WILD01
KCASEBAS10	-122.84	38.392	114ATASC1, 114LAGU01, 114LAGU02
KCASEBAS12	-122.887	38.462	114GREEN1
KCAVALLE14	-122.904	38.348	
MAS725	-122.319	38.329	
MBBYCA	-123.070	38.320	
MBNVC1	-123.349	38.987	
MC1766	-122.683	38.534	

Wunderground Stations used in IDW Analysis			
WeatherID	**Lon DD**	**Lat DD**	**Nearby RRPP Stations**
MHWKC1	-122.837	38.735	
MRODCA	-122.96	38.51	114LGVARR

E. Statistical Results

Bivariate Fit of USGS_cfs By Precip 1 Day Avg (mm)





Transformed Fit LN to LN RRPPID=="114COPE01"

LN(USGS_cfs) = -0.874945 + 1.6522477*LN(Precip 1 Day Avg (mm))

Summary of Fit

RSquare	0.783551
RSquare Adj	0.740261
Root Mean Square Error	0.848952
Mean of Response	2.89902
Observations (or Sum Wgts)	7

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	13.045111	13.0451	18.1001
Error	5	3.603594	0.7207	**Prob > F**
C. Total	6	16.648704		0.0081*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.874945	0.943318	-0.93	0.3962
LN(Precip 1 Day Avg (mm))	1.6522477	0.38836	4.25	0.0081*

Fit Measured on Original Scale

Sum of Squared Error	4719.362
Root Mean Square Error	30.722506
RSquare	0.066257
Sum of Residuals	7.8103244

Transformed Fit LN to LN RRPPID=="114LAGU02"

LN(USGS_cfs) = -0.554872 + 1.8071911*LN(Precip 1 Day Avg (mm))

Summary of Fit

RSquare	0.514366
RSquare Adj	0.417239
Root Mean Square Error	1.775668
Mean of Response	3.558934
Observations (or Sum Wgts)	7

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	16.697710	16.6977	5.2958
Error	5	15.764983	3.1530	**Prob > F**
C. Total	6	32.462692		0.0697

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.554872	1.909461	-0.29	0.7830
LN(Precip 1 Day Avg (mm))	1.8071911	0.785303	2.30	0.0697

Transformed Fit LN to LN RRPPID=="114SROSA1"

LN(USGS_cfs) = 1.7749172 + 1.4883015*LN(Precip 1 Day Avg (mm))

Summary of Fit

RSquare	0.659874
RSquare Adj	0.591849
Root Mean Square Error	1.260979

Mean of Response	5.110141
Observations (or Sum Wgts)	7

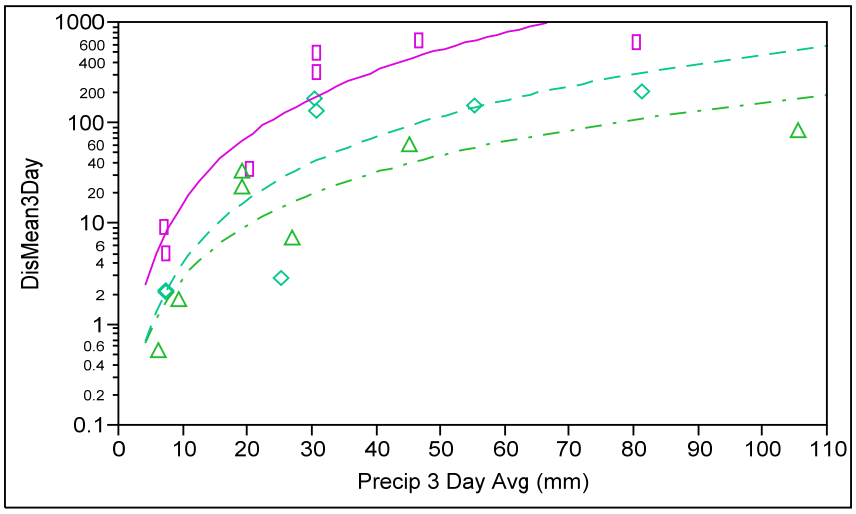
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	15.424332	15.4243	9.7004
Error	5	7.950335	1.5901	**Prob > F**
C. Total	6	23.374667		0.0264*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1.7749172	1.172125	1.51	0.1904
LN(Precip 1 Day Avg (mm))	1.4883015	0.477854	3.11	0.0264*

Bivariate Fit of DisMean3Day By Precip 3 Day Avg Sum (mm)



Transformed Fit LN to LN RRPPID=="114COPE01"

LN(DisMean3Day) = -2.896157 + 1.7330429*LN(Precip 3 Day Avg Sum (mm))

Summary of Fit

RSquare	0.770592
RSquare Adj	0.72471
Root Mean Square Error	0.986382
Mean of Response	2.469261
Observations (or Sum Wgts)	7

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	16.340887	16.3409	16.7952
Error	5	4.864750	0.9729	**Prob > F**
C. Total	6	21.205637		0.0094*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-2.896157	1.361263	-2.13	0.0867
LN(Precip 3 Day Avg Sum (mm))	1.7330429	0.42288	4.10	0.0094*

Transformed Fit LN to LN RRPPID=="114LAGU02"

LN(DisMean3Day) = -3.300345 + 2.0624142*LN(Precip 3 Day Avg Sum (mm))

Summary of Fit

RSquare	0.719274
RSquare Adj	0.663128
Root Mean Square Error	1.317204
Mean of Response	3.296542
Observations (or Sum Wgts)	7

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	22.227305	22.2273	12.8109
Error	5	8.675129	1.7350	**Prob > F**
C. Total	6	30.902435		0.0159*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-3.300345	1.909155	-1.73	0.1444
LN(Precip 3 Day Avg Sum (mm))	2.0624142	0.576216	3.58	0.0159*

Transformed Fit LN to LN RRPPID=="114SROSA1"

LN(DisMean3Day) = -2.15114 + 2.1594524*LN(Precip 3 Day Avg Sum (mm))

Summary of Fit

RSquare	0.886202
RSquare Adj	0.863443
Root Mean Square Error	0.774933
Mean of Response	4.628695
Observations (or Sum Wgts)	7

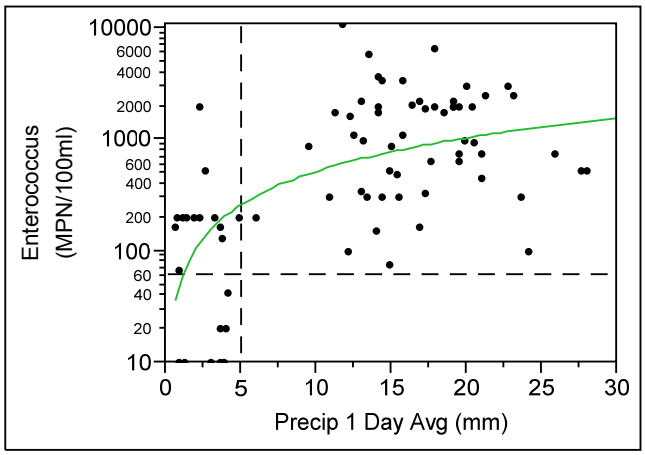
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	23.382833	23.3828	38.9376
Error	5	3.002606	0.6005	**Prob > F**
C. Total	6	26.385439		0.0015*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-2.15114	1.125299	-1.91	0.1141
LN(Precip 3 Day Avg Sum (mm))	2.1594524	0.346066	6.24	0.0015*

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm)



Transformed Fit Ln to Ln

Ln(Enterococcus (MPN/100ml)) = 3.9675696 + 0.9939001*Ln(Precip 1 Day Avg (mm))

Summary of Fit

RSquare	0.367947
RSquare Adj	0.359631
Root Mean Square Error	1.329221
Mean of Response	6.155339
Observations (or Sum Wgts)	78

Analysis of Variance

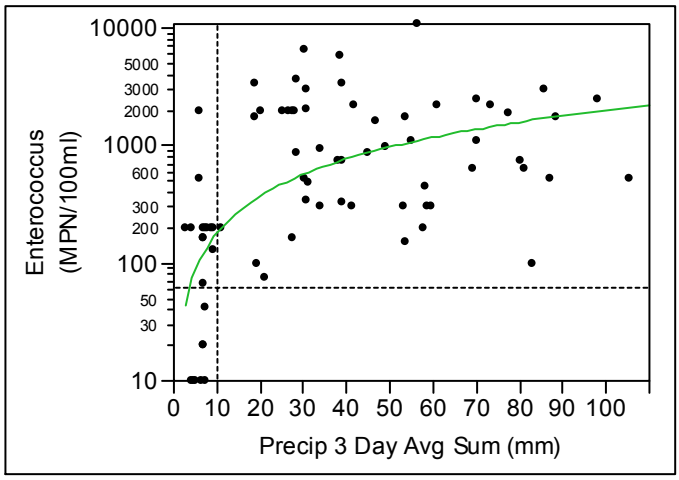
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	78.17007	78.1701	44.2432
Error	76	134.27899	1.7668	**Prob > F**
C. Total	77	212.44906		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	3.9675696	0.36171	10.97	<.0001*
Ln(Precip 1 Day Avg (mm))	0.9939001	0.149424	6.65	<.0001*

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 3 Day Sum of Avg (mm)

Sample values were regressed against the Inverse Distance Weighted watershed average of precipitation summed over the 3 day antecedent period (i.e.,2 days prior to and the day of sample).



Transformed Fit Log to Log

Transformed Fit Ln to Ln

Ln(Enterococcus (MPN/100ml)) = 2.809244 + 1.0420307*Ln(Precip 3 Day Avg Sum (mm))

Summary of Fit

RSquare	0.376533
RSquare Adj	0.368329
Root Mean Square Error	1.320163
Mean of Response	6.155339
Observations (or Sum Wgts)	78

Analysis of Variance

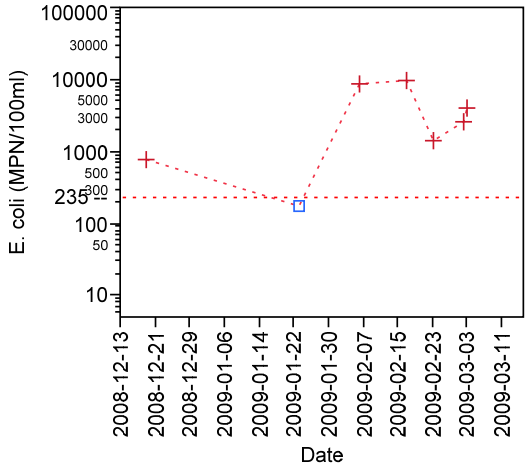
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	79.99400	79.9940	45.8989
Error	76	132.45506	1.7428	**Prob > F**
C. Total	77	212.44906		<.0001*

Parameter Estimates

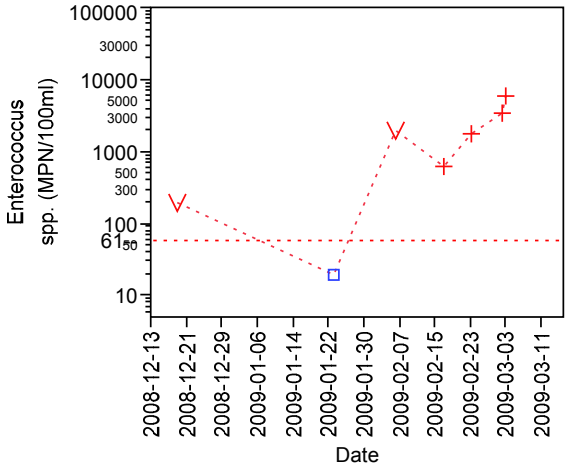
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.809244	0.516022	5.44	<.0001*
Ln(Precip 3 Day Avg Sum (mm))	1.0420307	0.153808	6.77	<.0001*

NOTE: All Red X's (x) with corresponding dates indicate excluded data points. Plus symbols (+) are above California Department of Public Health recommended limits for single day samples, open boxes (□) are below this limit. Triangles indicate detection limits in quantification method (▼upper : ▲ lower).

114ATASC1

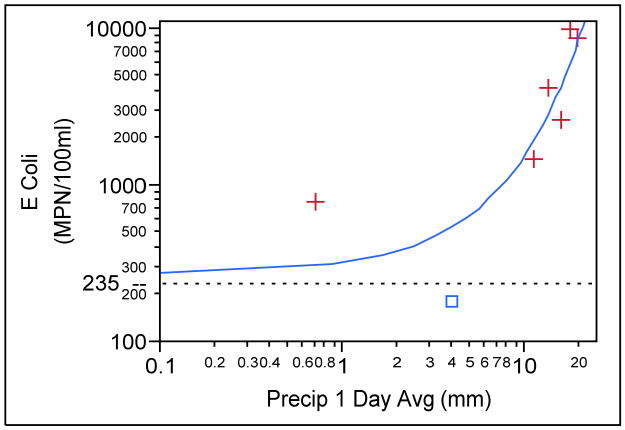


Plot for RRPPID=114ATASC1



Plot for RRPPID=114ATASC1

Bivariate Fit of E Coli (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114ATASC1



Transformed Fit Log

Log(E Coli (MPN/100ml)) = 5.5967479 + 0.1757283*Precip 1 Day Avg (mm)

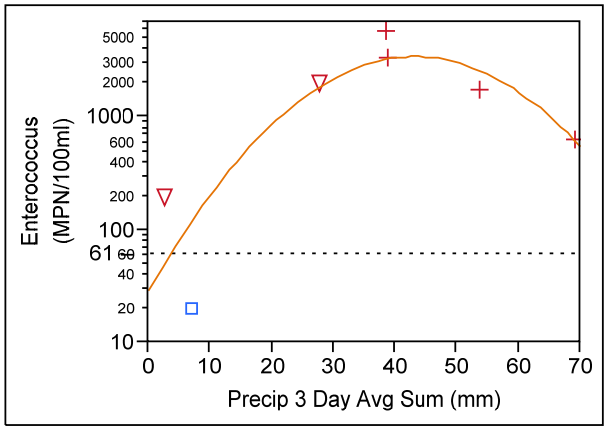
Summary of Fit

RSquare	0.766501
RSquare Adj	0.719802
Root Mean Square Error	0.746885
Mean of Response	7.664716
Observations (or Sum Wgts)	7

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	9.156005	9.15600	16.4134
Error	5	2.789187	0.55784	Prob > F
C. Total	6	11.945192		0.0098*

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 3 Day Avg Sum (mm) RRPPID=114ATASC1



Transformed Fit Log

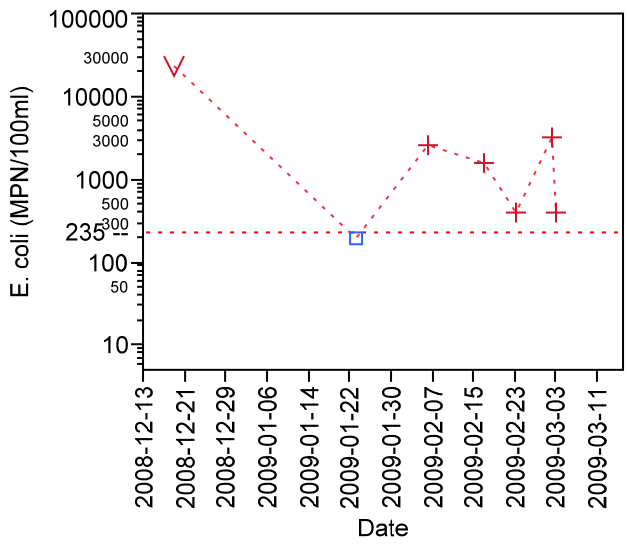
Log(Entero (MPN/100ml)) = 3.3478231 + 0.2222763*Precip 3 Day Avg Sum (mm) - 0.0025742*Precip 3 Day Avg Sum (mm)^2

Summary of Fit

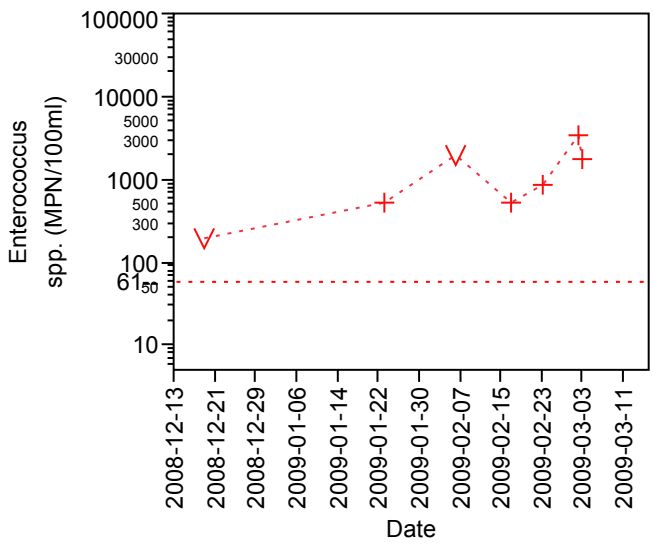
RSquare	0.758321
RSquare Adj	0.637482
Root Mean Square Error	1.183639
Mean of Response	6.667273
Observations (or Sum Wgts)	7

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	3.3478231	1.042364	3.21	0.0325*
Precip 3 Day Avg Sum (mm)	0.2222763	0.068679	3.24	0.0318*
Precip 3 Day Avg Sum (mm)^2	-0.002574	0.000965	-2.67	0.0560

114COPE01

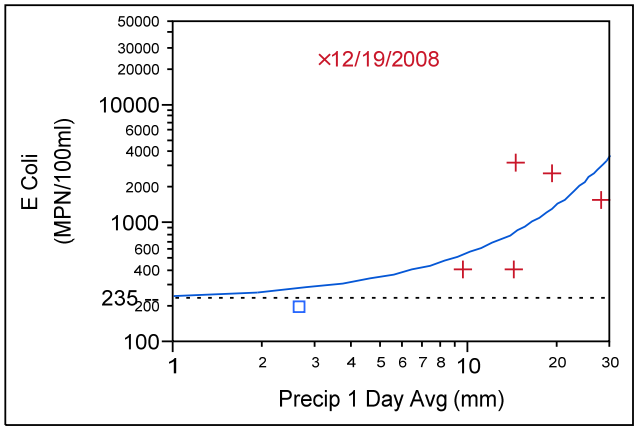


Plot for RRPPID=114COPE01



Plot for RRPPID=114COPE01

Bivariate Fit of E Coli (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114COPE01



Transformed Fit Log

Log(E Coli (MPN/100ml)) = 5.4074273 + 0.09392*Precip 1 Day Avg (mm)

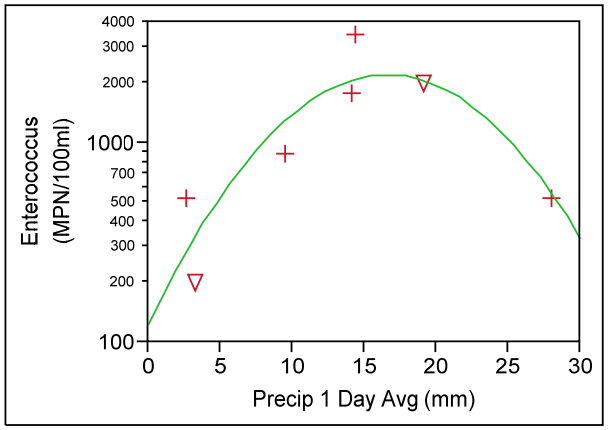
Summary of Fit

RSquare	0.489107
RSquare Adj	0.361384
Root Mean Square Error	0.922616
Mean of Response	6.780671
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3.2596931	3.25969	3.8294
Error	4	3.4048818	0.85122	Prob > F
C. Total	5	6.6645749		0.1220

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114COPE01



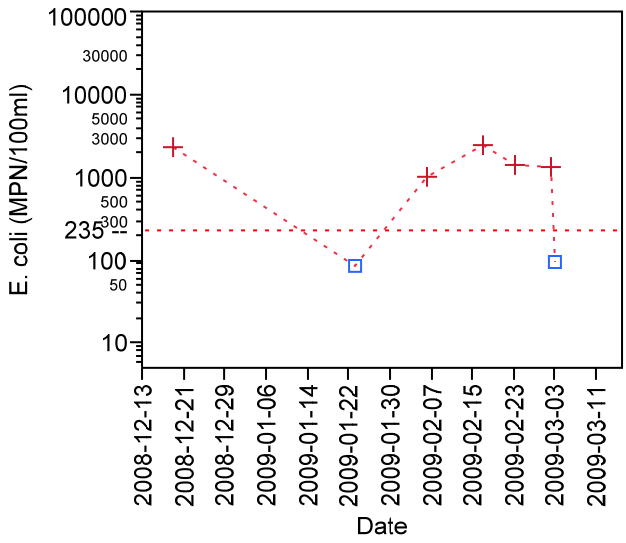
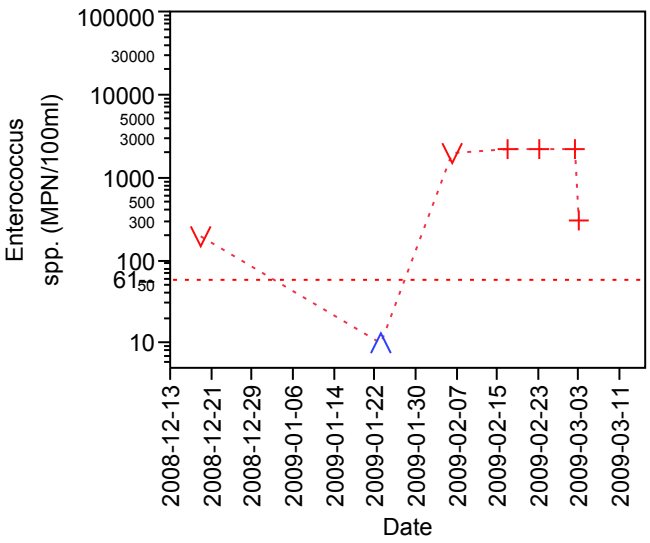
Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 4.8200185 + 0.3461562*Precip 1 Day Avg (mm) - 0.0104536*Precip 1 Day Avg (mm)^2

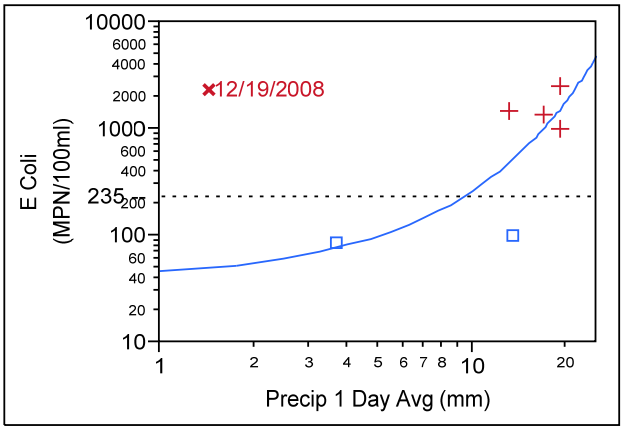
Summary of Fit

RSquare	0.809144
RSquare Adj	0.713717
Root Mean Square Error	0.522386
Mean of Response	6.835063
Observations (or Sum Wgts)	7

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	4.8200185	0.539868	8.93	0.0009*
Precip 1 Day Avg (mm)	0.3461562	0.08502	4.07	0.0152*
Precip 1 Day Avg (mm)^2	-0.010454	0.002799	-3.74	0.0202*

114GREEN1	
	
Plot for RRPPID=114GREEN1	Plot for RRPPID=114GREEN1

Bivariate Fit of E Coli (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114GREEN1



Transformed Fit Log

Log(E Coli (MPN/100ml)) = 3.6272957 + 0.1937229*Precip 1 Day Avg (mm)

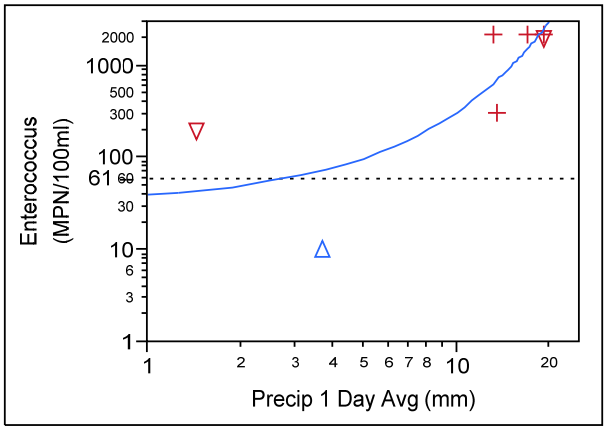
Summary of Fit

RSquare	0.590172
RSquare Adj	0.487715
Root Mean Square Error	1.047373
Mean of Response	6.380466
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	6.318878	6.31888	5.7602
Error	4	4.387959	1.09699	**Prob > F**
C. Total	5	10.706837		0.0744

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114GREEN1



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 3.452709 + 0.2293873*Precip 1 Day Avg (mm)

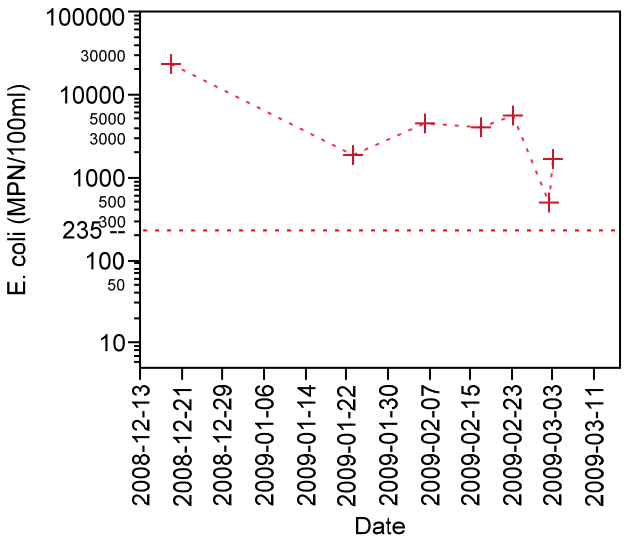
Summary of Fit

RSquare	0.651236
RSquare Adj	0.581483
Root Mean Square Error	1.318503
Mean of Response	6.293809
Observations (or Sum Wgts)	7

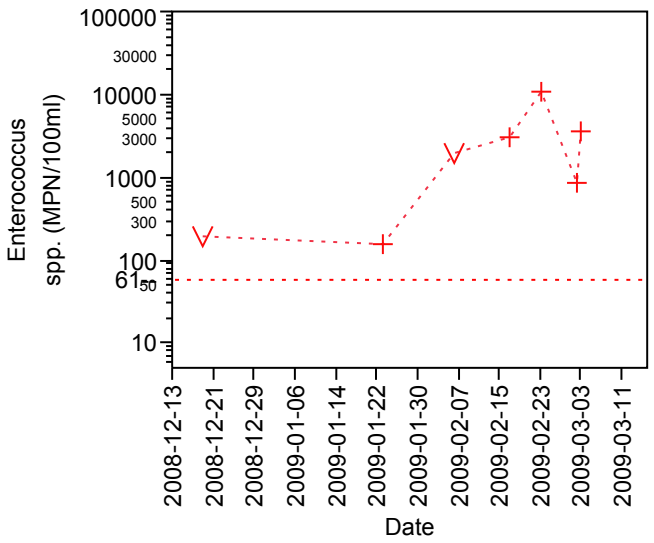
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	16.230759	16.2308	9.3363
Error	5	8.692255	1.7385	**Prob > F**
C. Total	6	24.923014		0.0282*

114LAGU01



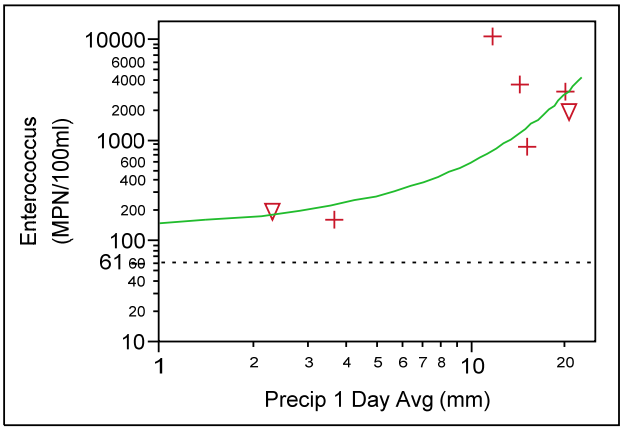
Plot for RRPPID=114LAGU01



Plot for RRPPID=114LAGU01

No Significant Relationship with 1 Day or 3 Day Rainfall-Runoff

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114LAGU01



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 4.8778904 + 0.1553267*Precip 1 Day Avg (mm)

Summary of Fit

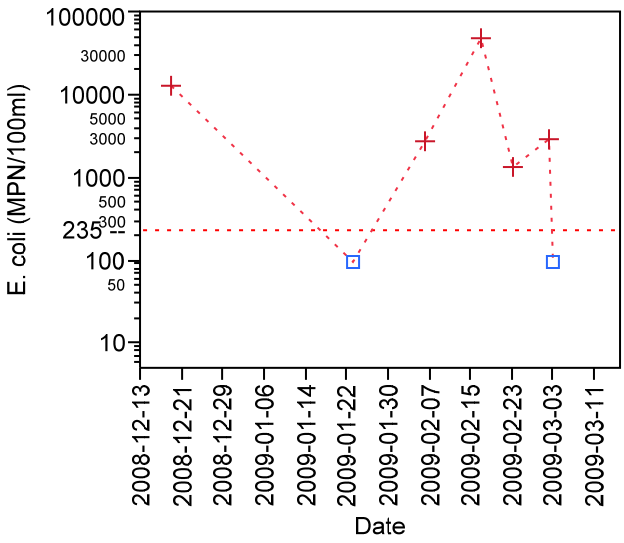
RSquare	0.808628
RSquare Adj	0.760785
Root Mean Square Error	0.665181
Mean of Response	6.832664
Observations (or Sum Wgts)	6

Analysis of Variance

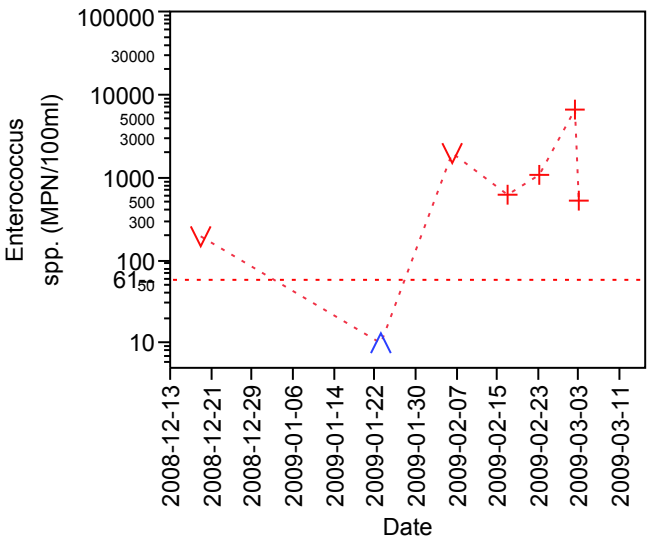
Source	DF	Sum of Squares	Mean Square	F Ratio

	Model	1	7.4784447	7.47844	16.9017
	Error	4	1.7698642	0.44247	**Prob > F**
	C. Total	5	9.2483089		0.0147*

114LAGU02

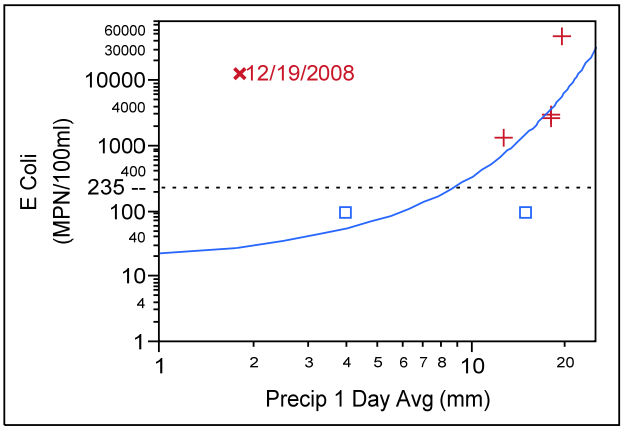


Plot for RRPPID=114LAGU02



Plot for RRPPID=114LAGU02

Bivariate Fit of E Coli (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114LAGU02



Transformed Fit Log

Log(E Coli (MPN/100ml)) = 2.8128099 + 0.3030318*Precip 1 Day Avg (mm)

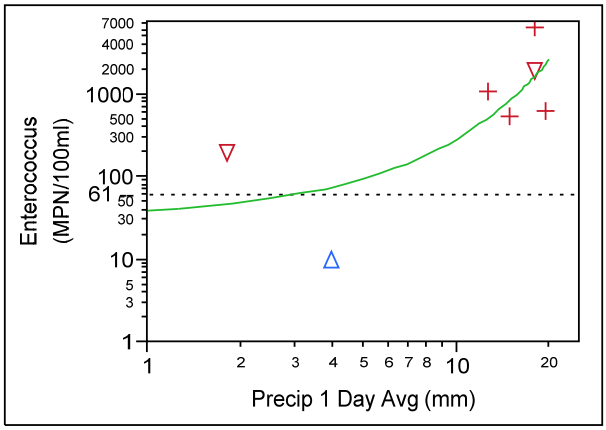
Summary of Fit

RSquare	0.545309
RSquare Adj	0.431636
Root Mean Square Error	1.770354
Mean of Response	7.189492
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	15.035109	15.0351	4.7972
Error	4	12.536608	3.1342	**Prob > F**
C. Total	5	27.571717		0.0937

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114LAGU02



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 3.4484399 + 0.2215952*Precip 1 Day Avg (mm)

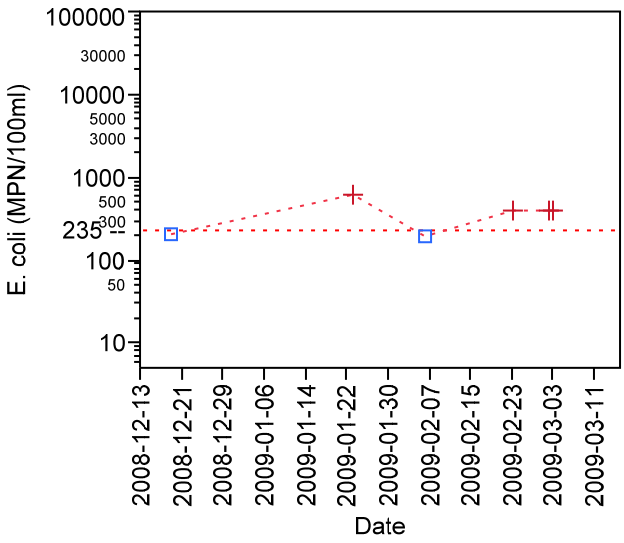
Summary of Fit

RSquare	0.580492
RSquare Adj	0.496591
Root Mean Square Error	1.460229
Mean of Response	6.249153
Observations (or Sum Wgts)	7

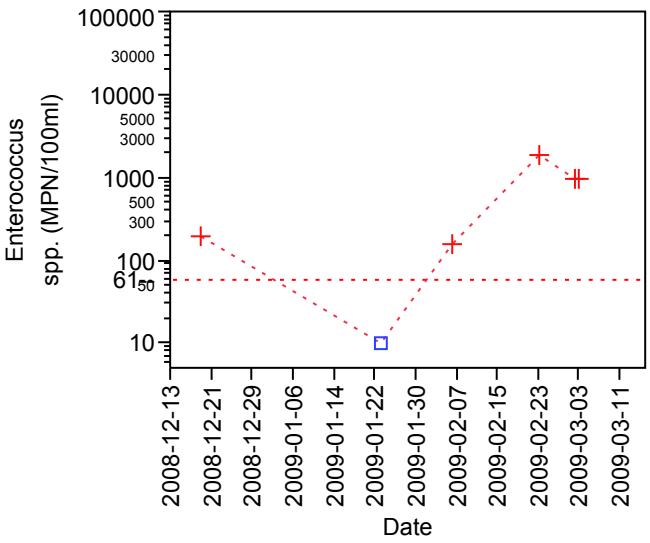
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	14.752600	14.7526	6.9187
Error	5	10.661350	2.1323	**Prob > F**
C. Total	6	25.413950		0.0465*

114LGVARR



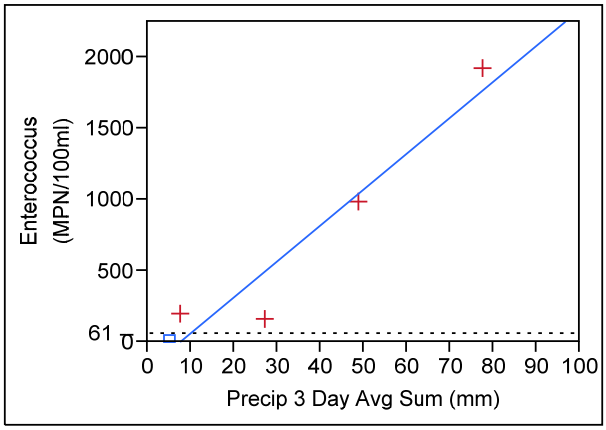
Plot for RRPPID=114LGVARR



Plot for RRPPID=114LGVARR

No Significant *E. Coli* Relationship with 1 Day or 3 Day Rainfall Estimates

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 3 Day Avg Sum (mm) RRPPID=114LGVARR



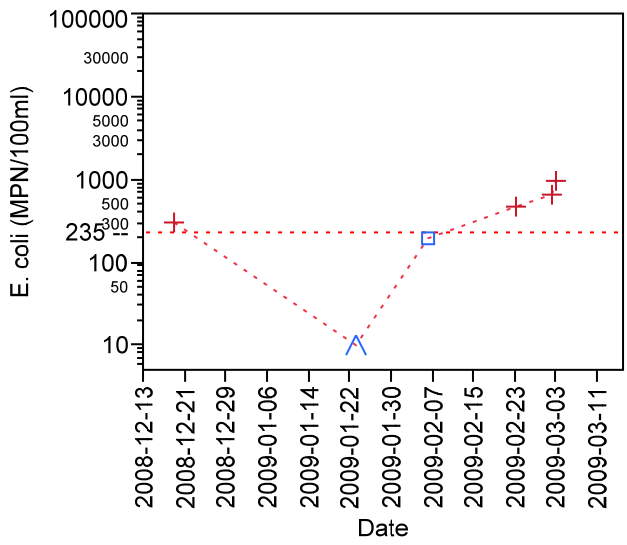
Linear Fit

Enterococcus (MPN/100ml) = -193.1464 + 25.293803*Precip 3 Day Avg Sum (mm)

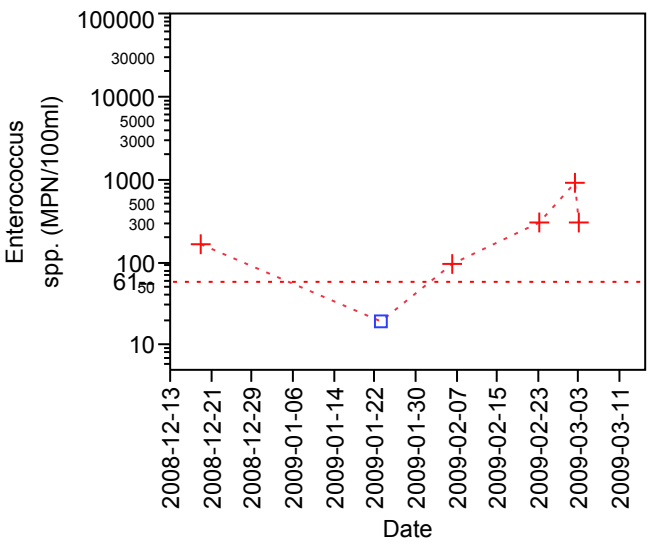
Summary of Fit

RSquare	0.929985
RSquare Adj	0.912481
Root Mean Square Error	216.1457
Mean of Response	712.3333
Observations (or Sum Wgts)	6

114MARK01

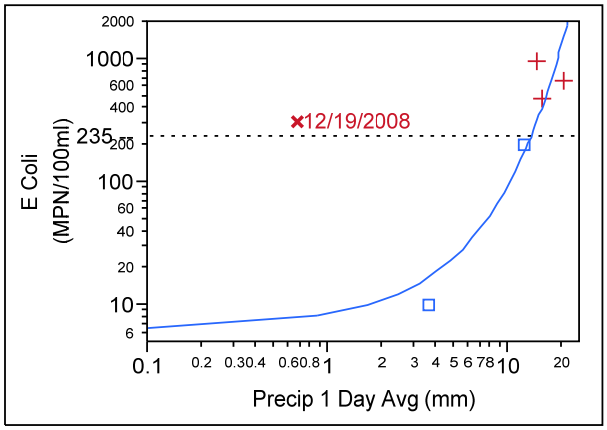


Plot for RRPPID=114MARK01



Plot for RRPPID=114MARK01

Bivariate Fit of E Coli (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114MARK01



Transformed Fit Log

Log(E Coli (MPN/100ml)) = 1.8555439 + 0.2697476*Precip 1 Day Avg (mm)

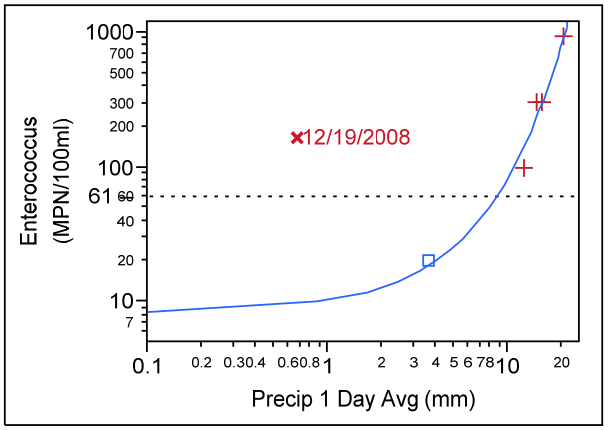
Summary of Fit

RSquare	0.821169
RSquare Adj	0.761558
Root Mean Square Error	0.898828
Mean of Response	5.428885
Observations (or Sum Wgts)	5

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	11.129202	11.1292	13.7756
Error	3	2.423678	0.8079	**Prob > F**
C. Total	4	13.552879		0.0340*

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114MARK01



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 2.0904705 + 0.2330623*Precip 1 Day Avg (mm)

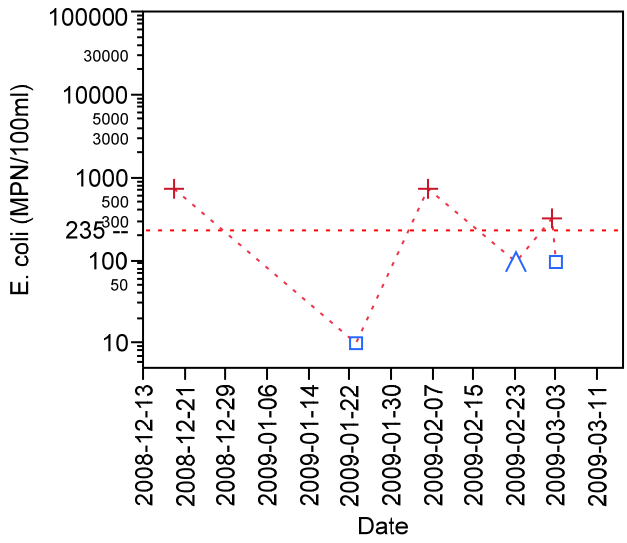
Summary of Fit

RSquare	0.977758
RSquare Adj	0.970344
Root Mean Square Error	0.250989
Mean of Response	5.177841
Observations (or Sum Wgts)	5

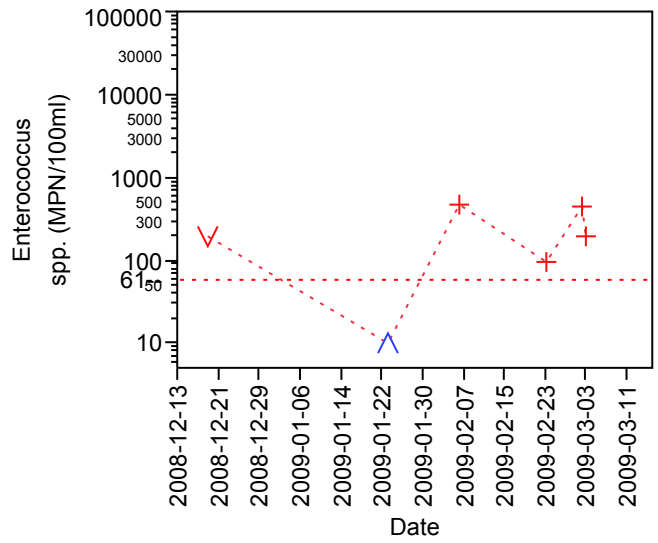
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	8.3079291	8.30793	131.8813
Error	3	0.1889865	0.06300	**Prob > F**
C. Total	4	8.4969156		0.0014*

114MILL01



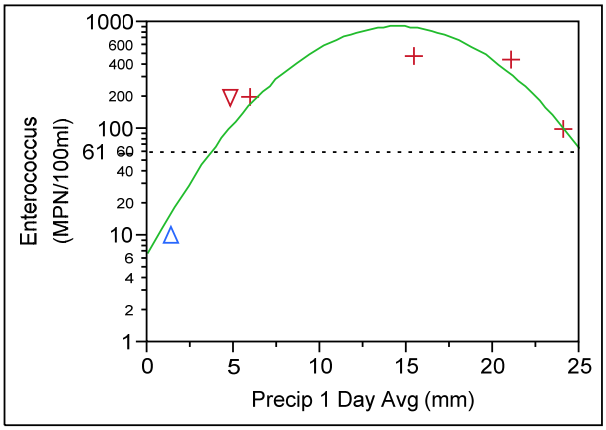
Plot for RRPPID=114MILL01



Plot for RRPPID=114MILL01

No Significant *E. Coli* Relationship with 1 Day or 3 Day Rainfall Estimates

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114MILL01



Transformed Fit Log

Log(Entero (MPN/100ml)) = 1.9331312 + 0.6777666*Precip 1 Day Avg (mm) - 0.0235088*Precip 1 Day Avg (mm)^2

Summary of Fit

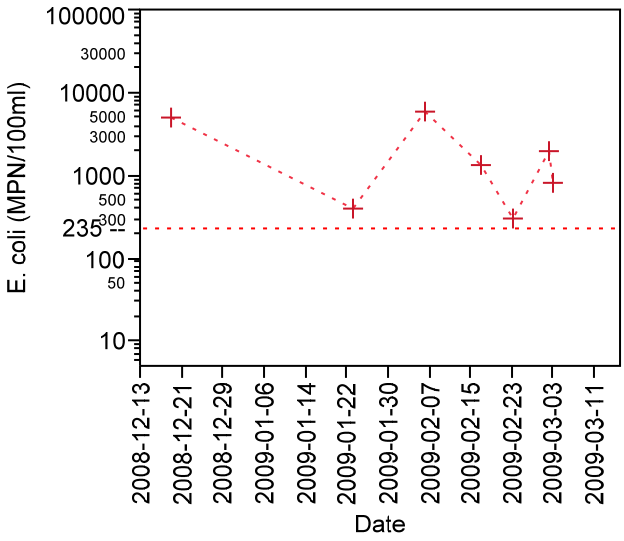
RSquare	0.886121
RSquare Adj	0.810202
Root Mean Square Error	0.622725
Mean of Response	4.964982
Observations (or Sum Wgts)	6

Analysis of Variance

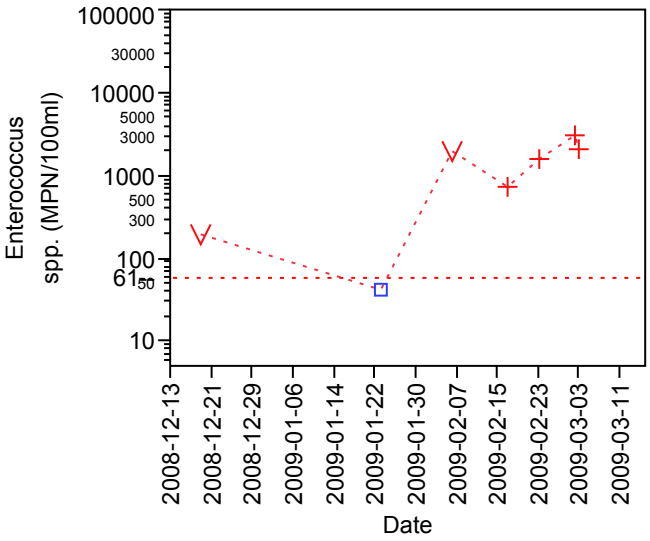
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	9.052412	4.52621	11.6719

	Error	3	1.163359	0.38779	**Prob > F**	
	C. Total	5	10.215771		0.0384*	
	Term		**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
	Intercept		1.9331312	0.677431	2.85	0.0649
	Precip 1 Day Avg (mm)		0.6777666	0.150719	4.50	0.0205*
	Precip 1 Day Avg (mm)^2		-0.023509	0.005788	-4.06	0.0269*

114SROSA1



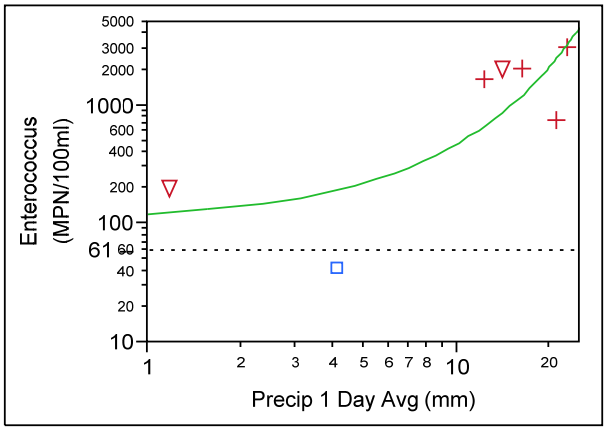
Plot for RRPPID=114SROSA1



Plot for RRPPID=114SROSA1

No Significant *E. Coli* Relationship with 1 Day or 3 Day Rainfall Estimates

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114SROSA1



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 4.6403182 + 0.1510259*Precip 1 Day Avg (mm)

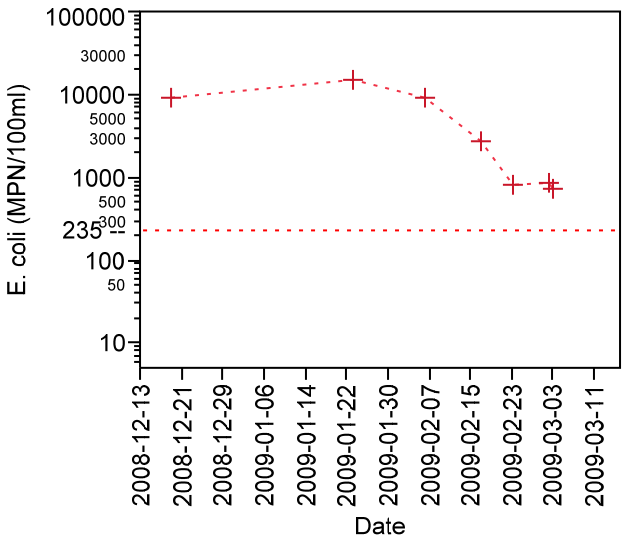
Summary of Fit

RSquare	0.606759
RSquare Adj	0.52811
Root Mean Square Error	1.073115
Mean of Response	6.617626
Observations (or Sum Wgts)	7

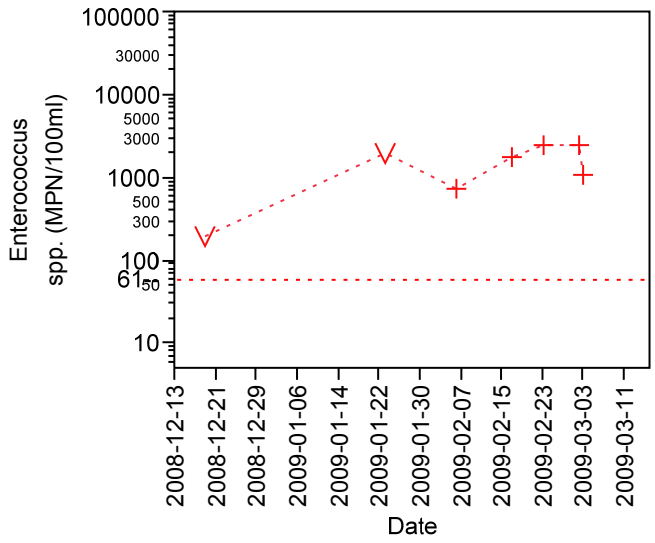
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	8.884217	8.88422	7.7148
Error	5	5.757878	1.15158	**Prob > F**
C. Total	6	14.642095		0.0390*

114VINE01



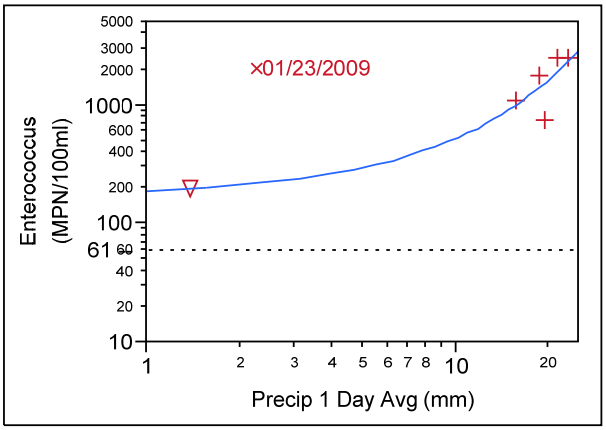
Plot for RRPPID=114VINE01



Plot for RRPPID=114VINE01

No Significant *E. Coli* Relationship with 1 Day or 3 Day Rainfall Estimates

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114VINE01



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 5.128612 + 0.1138263*Precip 1 Day Avg (mm)

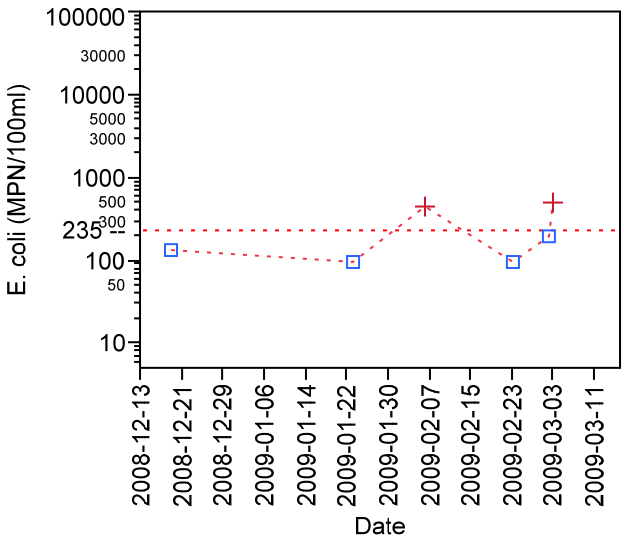
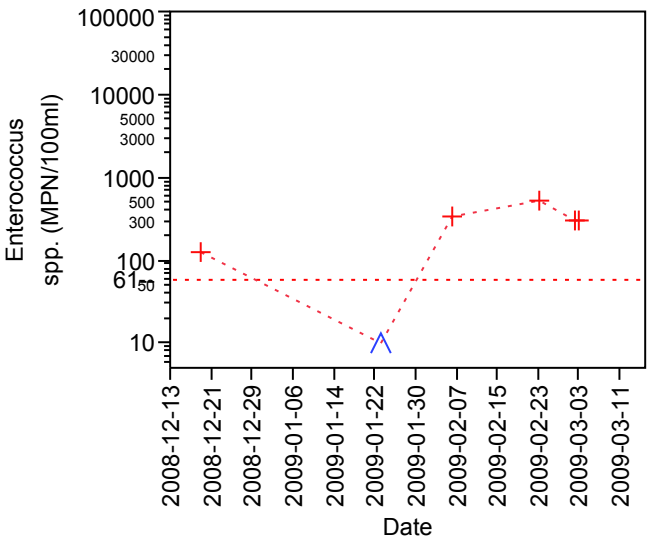
Summary of Fit

RSquare	0.848236
RSquare Adj	0.810295
Root Mean Square Error	0.422159
Mean of Response	7.013097
Observations (or Sum Wgts)	6

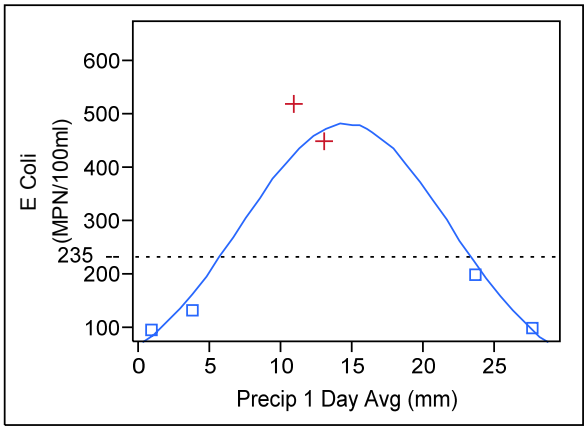
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio

	Model	1	3.9843724	3.98437	22.3567
	Error	4	0.7128715	0.17822	**Prob > F**
	C. Total	5	4.6972439		0.0091*

114WEST01	
	
Plot for RRPPID=114WEST01	Plot for RRPPID=114WEST01

Bivariate Fit of E Coli (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114WEST01



Transformed Fit Log

Log(E Coli (MPN/100ml)) = 4.2234275 + 0.2702829*Precip 1 Day Avg (mm) - 0.0093356*Precip 1 Day Avg (mm)^2

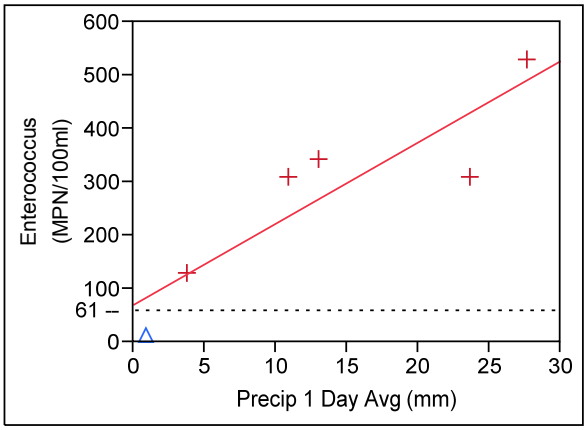
Summary of Fit

RSquare	0.960101
RSquare Adj	0.933502
Root Mean Square Error	0.190505
Mean of Response	5.291092
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	2.6199617	1.30998	36.0954
Error	3	0.1088767	0.03629	**Prob > F**
C. Total	5	2.7288384		0.0080*

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114WEST01



Linear Fit

Enterococcus (MPN/100ml) = 69.229792 + 15.238499*Precip 1 Day Avg (mm)

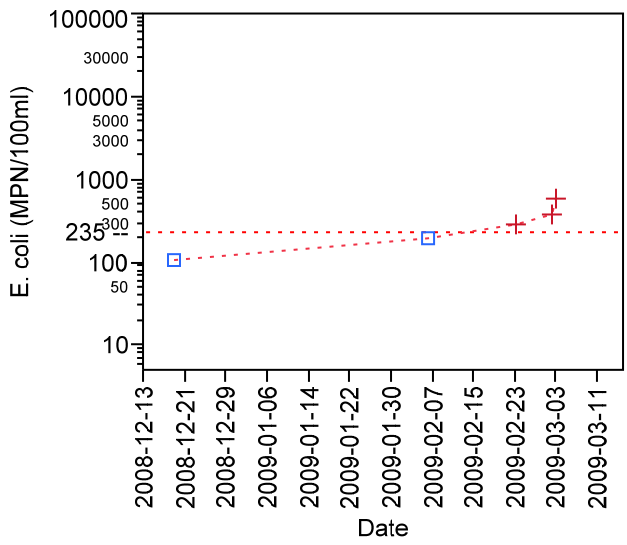
Summary of Fit

RSquare	0.800203
RSquare Adj	0.750254
Root Mean Square Error	90.35413
Mean of Response	272.3333
Observations (or Sum Wgts)	6

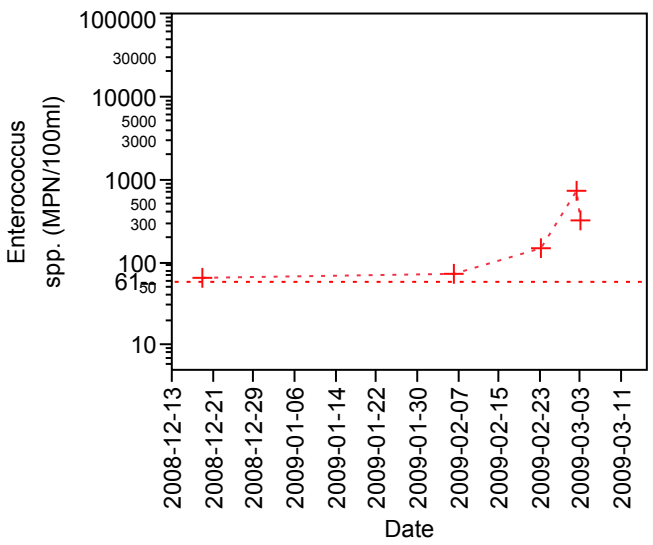
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	130787.86	130788	16.0203
Error	4	32655.47	8164	**Prob > F**
C. Total	5	163443.33		0.0161*

114WILD01

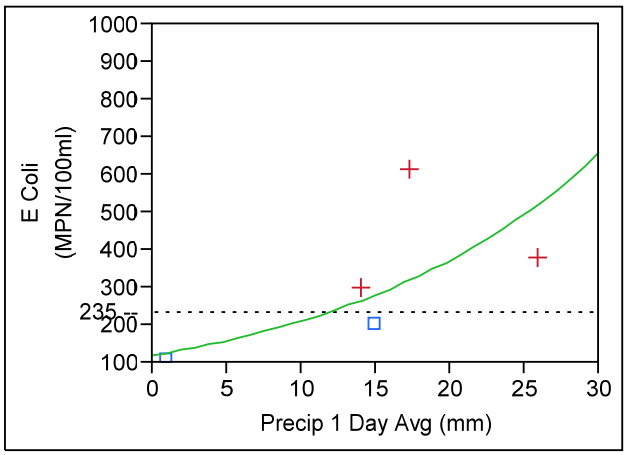


Plot for RRPPID=114WILD01



Plot for RRPPID=114WILD01

Bivariate Fit of E Coli (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114WILD01



Transformed Fit Log

Log(E Coli (MPN/100ml)) = 4.7862998 + 0.0568062*Precip 1 Day Avg (mm)

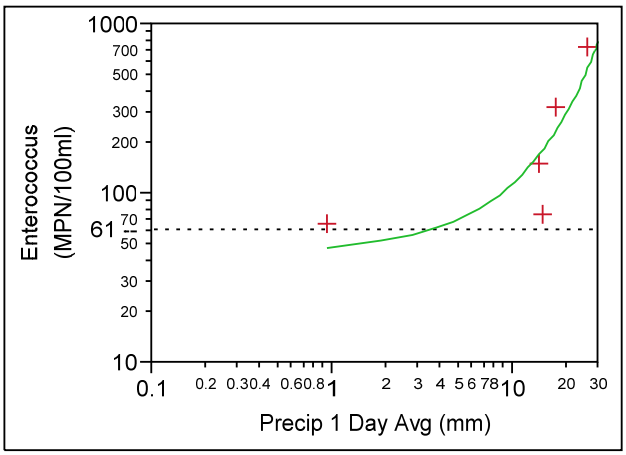
Summary of Fit

RSquare	0.607487
RSquare Adj	0.476649
Root Mean Square Error	0.471911
Mean of Response	5.613513
Observations (or Sum Wgts)	5

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.0340097	1.03401	4.6431
Error	3	0.6681013	0.22270	**Prob > F**
C. Total	4	1.7021109		0.1202

Bivariate Fit of Enterococcus (MPN/100ml) By Precip 1 Day Avg (mm) RRPPID=114WILD01



Transformed Fit Log

Log(Enterococcus (MPN/100ml)) = 3.7726219 + 0.096681*Precip 1 Day Avg (mm)

Summary of Fit

RSquare	0.723129
RSquare Adj	0.630838
Root Mean Square Error	0.61827
Mean of Response	5.180493
Observations (or Sum Wgts)	5

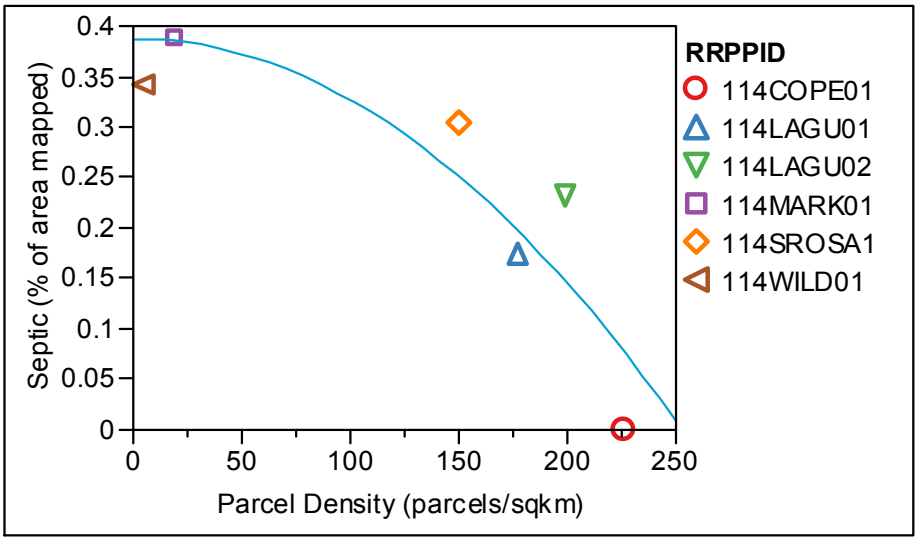
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	2.9951286	2.99513	7.8354
Error	3	1.1467739	0.38226	**Prob > F**
C. Total	4	4.1419025		0.0679

Appendix F Land Use Analyses

Statistical Results

Bivariate Fit of Septic By Parcel Density (#/sqkm)



Transformed Fit to Square

Transformed Fit to Square

Septic = 0.3876964 - 6.0775e-6*Square(Parcel Density (#/sqkm))

Summary of Fit

RSquare	0.810124
RSquare Adj	0.762654
Root Mean Square Error	0.06852
Mean of Response	0.24083
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.08012693	0.080127	17.0663
Error	4	0.01878012	0.004695	**Prob > F**
C. Total	5	0.09890705		0.0145*

Parameter Estimates

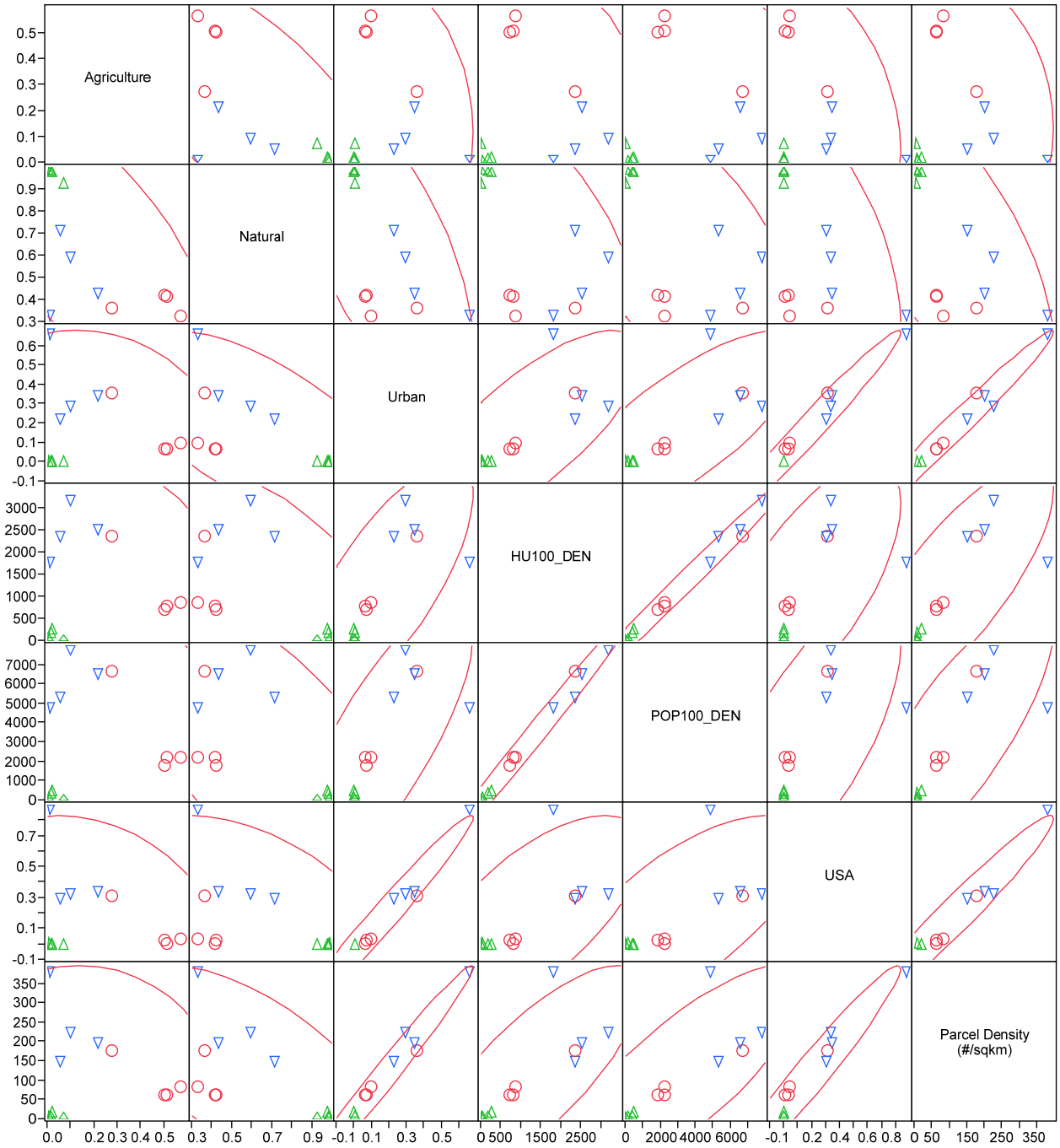
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.3876964	0.045237	8.57	0.0010*
Square(Parcel Density (#/sqkm))	-6.077e-6	1.471e-6	-4.13	0.0145*

Multivariate Sub-watershed Land Use Correlations

	Agriculture	Natural	Urban	HU100_DEN	POP100_DEN	USA	Parcel Density (#/sqkm)
Agriculture	1.0000	-0.6792	-0.1428	-0.0140	0.0253	-0.2876	-0.1179
Natural	-0.6792	1.0000	-0.6292	-0.5473	-0.6012	-0.4998	-0.6394
Urban	-0.1428	-0.6292	1.0000	0.7420	0.7739	0.9793	0.9847
HU100_DEN	-0.0140	-0.5473	0.7420	1.0000	0.9935	0.6701	0.7828
POP100_DEN	0.0253	-0.6012	0.7739	0.9935	1.0000	0.6882	0.8022
USA	-0.2876	-0.4998	0.9793	0.6701	0.6882	1.0000	0.9708
Parcel Density (#/sqkm)	-0.1179	-0.6394	0.9847	0.7828	0.8022	0.9708	1.0000

The correlations are estimated by REML method.

Scatterplot Matrix



Pairwise Correlations on Land Use Variables

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
POP100_DEN	HU100_DEN	0.9935	12	0.9760	0.9982	<.0001*
Parcel Density (#/sqkm)	Urban	0.9847	12	0.9446	0.9958	<.0001*
USA	Urban	0.9793	12	0.9256	0.9944	<.0001*
Parcel Density	USA	0.9708	12	0.8963	0.9920	<.0001*

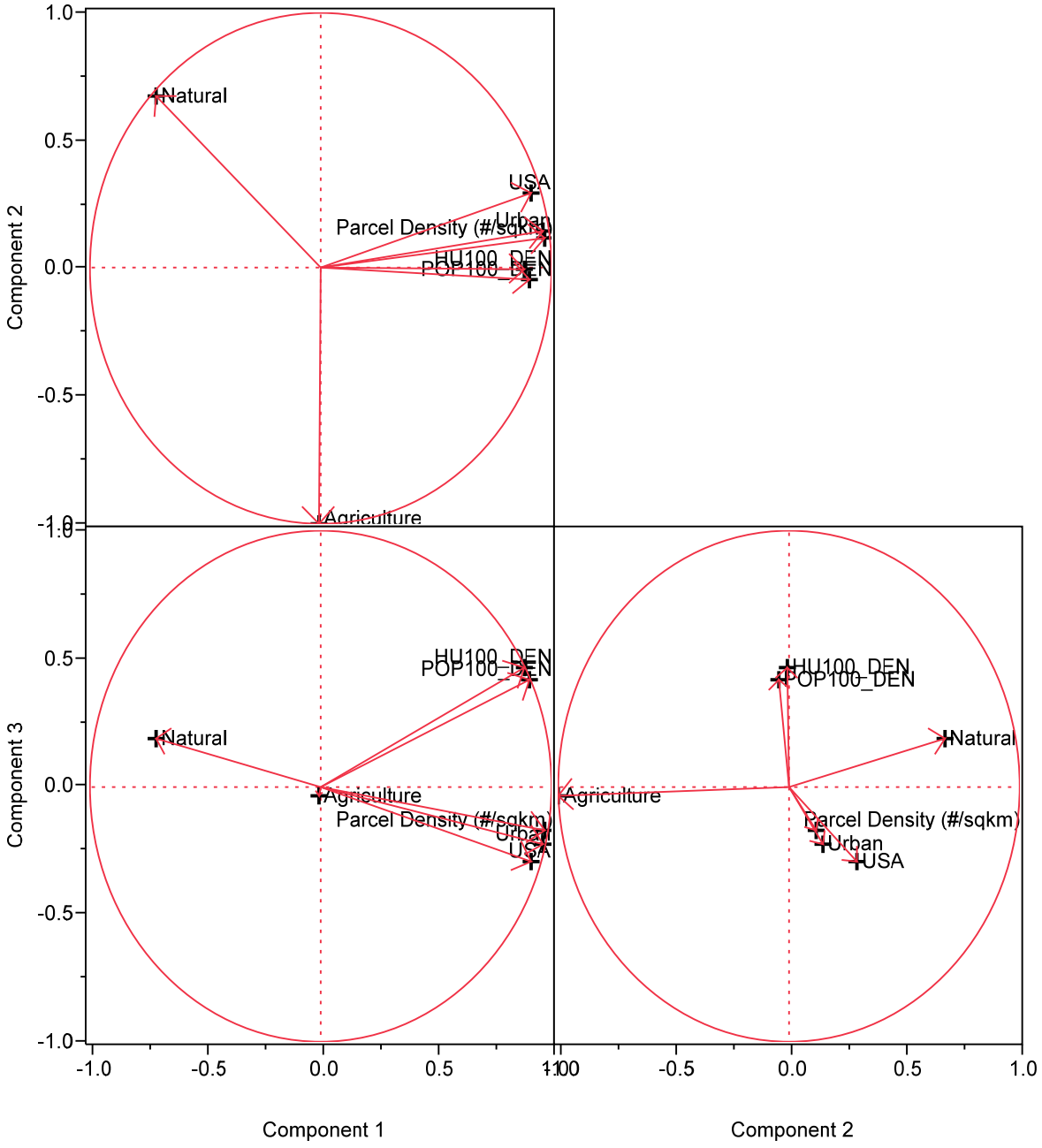
Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
Parcel Density (#/sqkm)	POP100_DEN	0.8022	12	0.4231	0.9423	0.0017*
Parcel Density (#/sqkm)	HU100_DEN	0.7828	12	0.3793	0.9361	0.0026*
POP100_DEN	Urban	0.7739	12	0.3599	0.9333	0.0031*
HU100_DEN	Urban	0.7420	12	0.2928	0.9229	0.0057*
USA	POP100_DEN	0.6882	12	0.1890	0.9048	0.0133*
Natural	Agriculture	-0.6792	12	-0.9016	-0.1726	0.0151*
USA	HU100_DEN	0.6701	12	0.1564	0.8985	0.0171*
Parcel Density (#/sqkm)	Natural	-0.6394	12	-0.8876	-0.1034	0.0252*
Urban	Natural	-0.6292	12	-0.8839	-0.0866	0.0284*
POP100_DEN	Natural	-0.6012	12	-0.8737	-0.0416	0.0387*
HU100_DEN	Natural	-0.5473	12	-0.8532	0.0388	0.0655
USA	Natural	-0.4998	12	-0.8344	0.1040	0.0980
USA	Agriculture	-0.2876	12	-0.7395	0.3429	0.3647
Urban	Agriculture	-0.1428	12	-0.6624	0.4696	0.6579
Parcel Density (#/sqkm)	Agriculture	-0.1179	12	-0.6480	0.4891	0.7151
POP100_DEN	Agriculture	0.0253	12	-0.5567	0.5906	0.9379
HU100_DEN	Agriculture	-0.0140	12	-0.5832	0.5644	0.9655

Principal Components / Factor Analysis

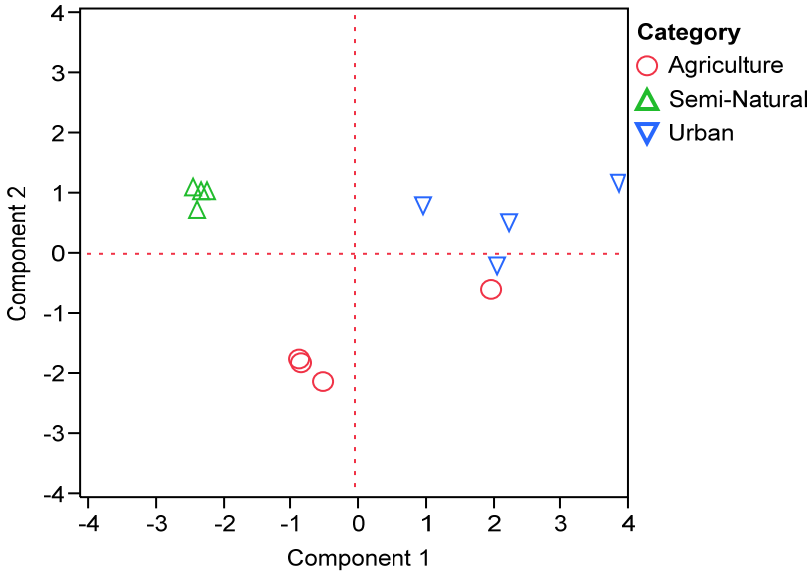
Principal Components: on Correlations

Number	Eigenvalue	Percent	Percent	Cum Percent
1	4.8078	68.683		68.683
2	1.5817	22.595		91.278
3	0.5877	8.396		99.674
4	0.0183	0.261		99.935
5	0.0043	0.061		99.997
6	0.0002	0.003		100.000
7	0.0000	0.000		100.000

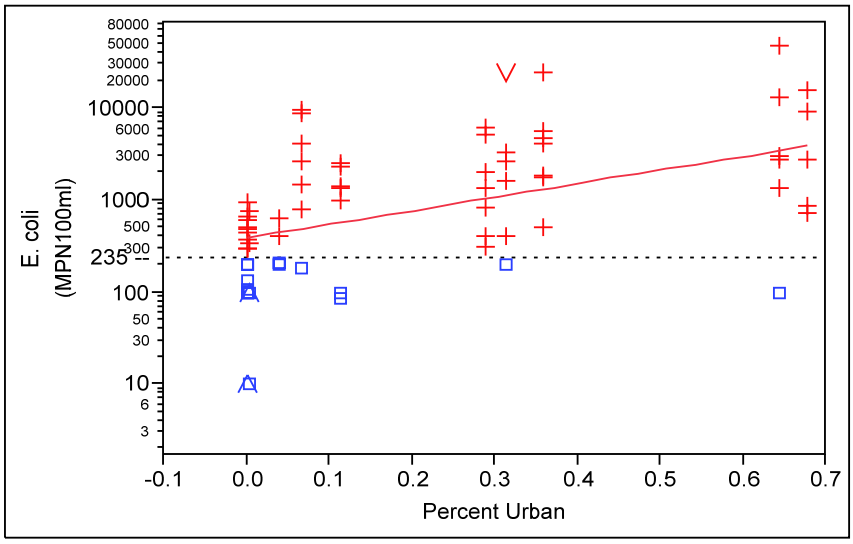
Loading Plot



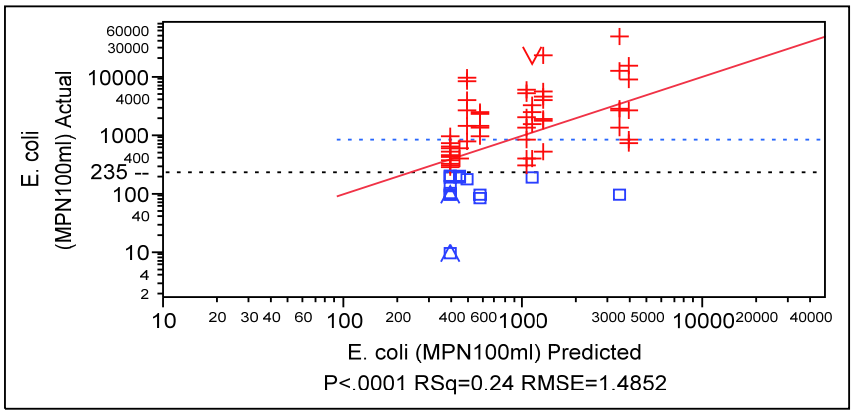
Score Plot


Category
Agriculture
Semi-Natural
Urban
Component 2
Component 1
4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4

Response Log(E. coli (MPN/100ml))
Whole Model
Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.235558
RSquare Adj	0.2255
Root Mean Square Error	1.485189
Mean of Response	6.727686
Observations (or Sum Wgts)	78

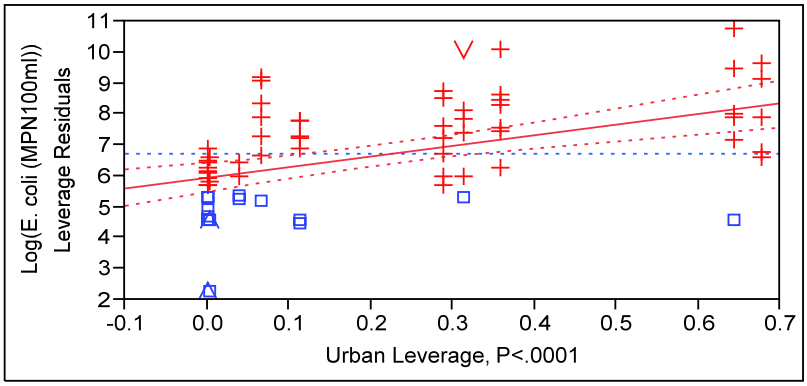
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	51.65712	51.6571	23.4189
Error	76	167.63970	2.2058	**Prob > F**
C. Total	77	219.29681		<.0001*

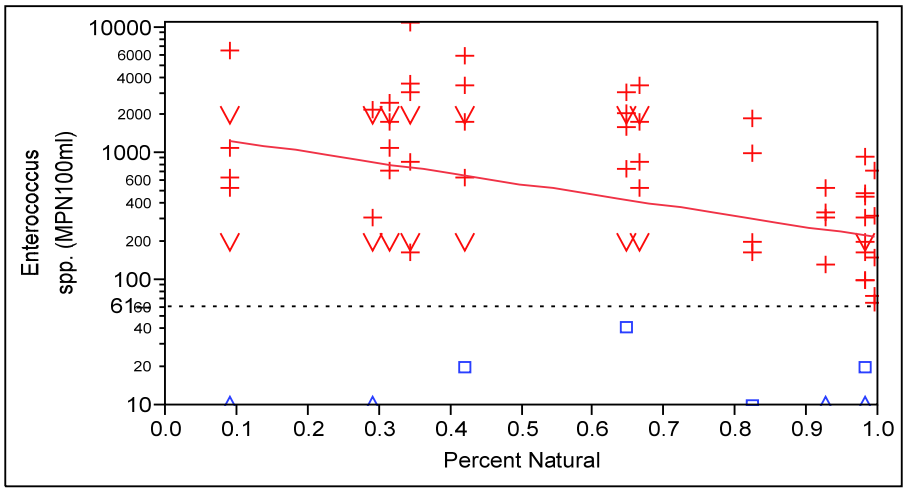
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5.9664549	0.230267	25.91	<.0001*
Urban	3.3938212	0.701303	4.84	<.0001*

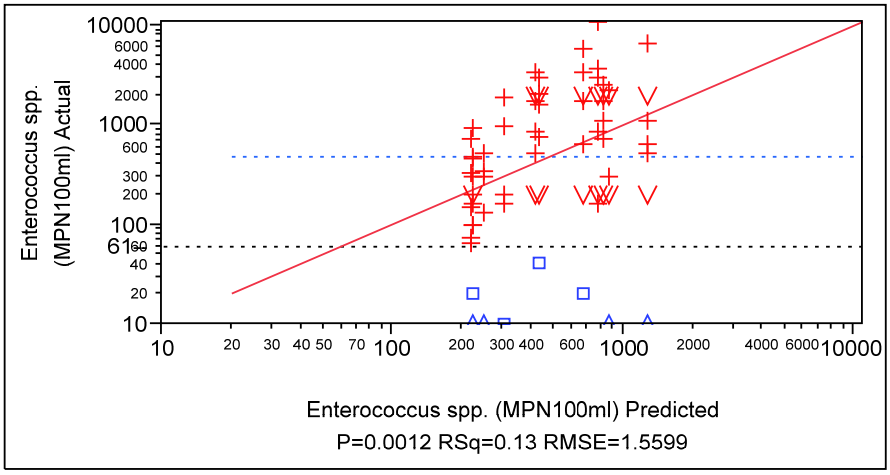
Urban
Leverage Plot



Response Log(Enterococcus spp. (MPN100ml)) Dummy=1
Whole Model
Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.129541
RSquare Adj	0.118088
Root Mean Square Error	1.559893
Mean of Response	6.155339
Observations (or Sum Wgts)	78

Analysis of Variance

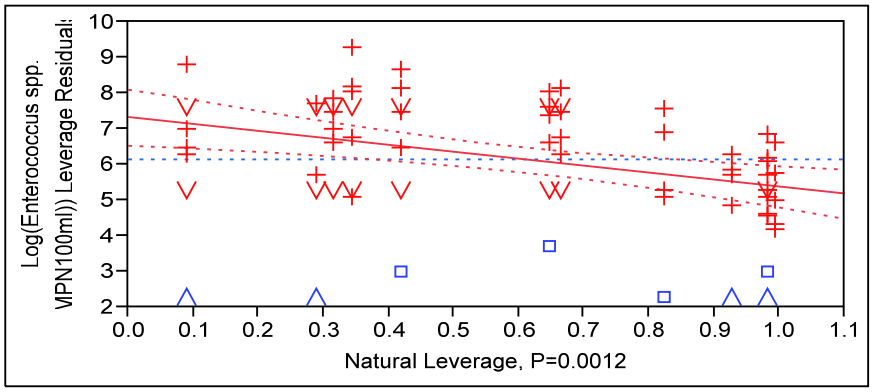
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	27.52092	27.5209	11.3103
Error	76	184.92814	2.4333	**Prob > F**
C. Total	77	212.44906		0.0012*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	7.3192654	0.388554	18.84	<.0001*

Natural	-1.947226	0.579001	-3.36	0.0012*

Natural
Leverage Plot



Appendix G Monitoring Station Vegetation Descriptions

Methods follow the California Native Plant Society rapid field assessments, with vegetative cover estimated from published cover class diagrams (see http://cnps.org/cnps/vegetation/pdf/percent_cover_diag-cnps.pdf).

Copeland Creek

The Copeland Creek site is dominated by non-native privets (Ligustrum sp.) in the over story that are less than 5 meters tall and have a diameter at breast height (DBH) between 1-6". California bay (*Umbellularia californica*) is also common in the over story and Himalayan blackberry (*Rubus discolor*) is the dominant species in the understory (10% cover). There are also a few non-native, horticultural species at this site, including *Cotoneaster* sp. Evidence of last season's horsetail (*Equisetum* sp. (*telmateia*?)) and Harding grass (*Phalaris aquatica)* were present in low densities. Total Vegetative Cover: 25%

Foss Creek

The Foss Creek site is heavily disturbed with a mowed grass lawn extending up to the west bank of the creek. There are horticultural (likely planted) non-native bulbs and weedy herbaceous species (mint (*Mentha spicata*), umbrella sedge (*Cyperus eragrostis*), etc.) along the waters' edge. At this site the overstory is scarce with scattered box elder (*Acer negundo*) and willow (*Salix* sp.) trees (8.5m tall, DBH between 1-6"). These tree species may have been planted or are the only trees remaining in the riparian zone after heavy disturbance from bridge building and landscaping. Himalayan blackberry (*Rubus discolor*) is dense on the east bank but is absent and likely managed for on the grassy west bank. North of the sampling site the vegetation is more intact and has a higher cover of willow species. Total Vegetative Cover: 30%

Lower Dry Creek

The over story at Lower Dry Creek is dominated by mature alder's (*Alnus rhombifolia*) and walnut's (*Juglans hindsii*) that are between 14-16m tall and have DBH's between 11-24". This site has a mid-level tree/shrub layer that includes willows (*Salix* sp.; 5-10m tall) and elderberry's (*Sambucus* sp.; 3m tall). Beneath these two tree strata, the dense understory is dominated by non-natives including the highly invasive periwinkle (*Vinca major)* and Himalayan blackberry (*Rubus discolor*). Total Vegetative Cover: 45%

Mill Creek

Mill Creek is dominated by coast redwood (*Sequoia sempervirens*; 36m tall) in the overstory and California bay (*Umbellularia californica*; 10-15m tall) and *Cotoneaster* sp. (7m tall) in the lower tree strata. The understory includes sword fern (*Polystichum* sp.), periwinkle (*Vinca major*), both native blackberry (*Rubus ursinus*) and Himalayan blackberry (*Rubus discolor*), Santa Barbara sedge (*Carex barbarae)*, and a non- native ivy. Total Vegetative Cover: 50%

Lower Green Creek

The Lower Green Valley Creek site is dominated by coast redwood (*Sequoia sempervirens*) and California bay (*Umbellularia californica*) in the highest and middle strata, respectively. This site is multi-layered, with smaller mature trees growing under a canopy of larger trees (in this case coast redwood). The understory in dominated by Himalayan blackberry (*Rubus discolor;* 30% cover), grape (*Vitus californica*) climbing the bay trees, and a small amount of periwinkle (*Vinca major*). Total Vegetative Cover: 55%

Laguna de Santa Rosa at Confluence

The Laguna de Santa Rosa confluence site contains a few scattered willows (*Salix* sp.) and box elders (*Acer* sp.) that range in height from 9.94m to 16.07m and range in DBH from around 10" to 20". The understory is dominated by Himalayan blackberry (*Rubus discolor*). The path and road leading up to the sampling site is dominated by other non-native forbs and grasses, including bristly ox tongue (*Picris echioides)* and Bermuda grass (*Cynodon dactylon*). Total Vegetative Cover: 65%

Atascadero Creek at Green Valley Road

Atascadero Creek at Green Valley Road is dominated by willows (*Salix* sp.) that are about 8m tall and have a DBH between 6-11". Other tree species, including alder (*Alnus rhombifolia*), walnut (*Juglans hindsii)* and oak (*Quercus garryana*), are scattered throughout the landscape. The understory is dominated by Himalayan blackberry (*Rubus discolor*) and a variety of other non-native species, including periwinkle (*Vinca major).* Total Vegetative Cover: 60%

Atascadero Creek at Bodega Highway

Atascadero Creek at Bodega Highway has very low tree cover (3%) consisting of willow (*Salix sp.;* 5.26m tall), alder (*Alnus rhombifolia*), and walnut (*Juglans hindsii*; 15.23m tall). The site is dominated by Himalayan Blackberry (*Rubus discolor*; 70%). Total Vegetative Cover: 75%

Laguna de Santa Rosa

There are many different over story tree species at the Laguna de Santa Rosa, including walnut (*Juglans sp.*), two species of oak (*Quercus agrifolia* and *Quercus garryana*), box elder (*Acer negundo*), and a few species of willow (*Salix sp.*) ranging from 5m to 15m tall. Tree cover comprises about 15% of the total cover for this site. In the shrub layer, Himalayan blackberry (*Rubus discolor*) was dominant and makes up 45% of the total cover. The herbaceous layer contained very few species at the time of sampling. Total Vegetative Cover: 60%

Prince Memorial Greenway

Prince Memorial Greenway is dominated by young alders (*Alnus rhombifolia*) and willows (*Salix sp.*) that are between 5m to 10m tall and have a DBH between 1-6". The site is restricted by a cement walkway and appears to be actively managed both through plantings and invasive species control. Total Vegetative Cover: 35%

Upper Mark West

The overstory at Upper Mark West includes two species of oaks (*Quercus garryana:* 15% cover and *Quercus agrifolia:* 5% cover), California bay (*Umbellularia californica*), madrone (*Arbutus menziesii),* and non-native privet (*Ligustrum sp.*). The tree species range from 4.2m tall near the bank to 12.76m tall as you move away from the channel. The understory includes California wild rose (*Rosa californica)*, goldenback fern (*Pentagramma triangularis*), *Lonicera sp.,* periwinkle (*Vinca major*)*,* and a few other native and non-native species. Total Vegetative Cover: 45%

Upper Santa Rosa Creek

Upper Santa Rosa Creek contains a large amount of alder (*Alnus rhombifolia;* 13.69m tall to 18.9m tall) and oak (*Quercus garryana*) in the tallest strata and willow (*Salix* sp.; 2.22m tall) in the secondary strata. There is one giant California bay (*Umbellularia californica*) at this site that is about 23m tall and has a DBH >24 inches. The understory is a mix of Himalayan blackberry (*Rubus discolor*), horsetail (*Equisetum* sp., last season's), *Polypodium* sp., etc. There is a small patch or giant reed (*Arundo donax)* at this site. Total Vegetative Cover: 45%

Appendix H Quality Assurance Project Plan

UNIVERSITY OF CALIFORNIA, DAVIS

BERKELEY ● DAVIS ● IRVINE ● LOS ANGELES ● MERCED ● RIVERSIDE ● SAN DIEGO ● SAN FRANCISCO ● SANTA BARBARA ● SANTA CRUZ

JOHN MUIR INSTITUTE OF THE ENVIRONMENT

ONE SHIELDS AVENUE

DAVIS, CALIFORNIA 95616-8576

QUALITY ASSURANCE PROJECT PLAN

(QAPP)

RUSSIAN RIVER PATHOGEN PROJECT

SWRCB AGREEMENT 06-428-110

Prepared By

Aquatic Ecosystems Analysis Laboratory

March 2009

Approval Signatures

North Coast Regional Water Quality Control Board (NCRWQCB)

Title:	Name:	Signature:	Date:
Project and Grant Manager	Matt St. John		

Information Center for the Environment (ICE), University of California, Davis

Title:	Name:	Signature:	Date:
Contractor Project Manager	Josh Viers		
Contractor Project Manager	Fraser Shilling		

Aquatic Ecosystems Analysis Laboratory (AEAL), University of California, Davis

Title:	Name:	Signature:	Date:
Contractor Project Supervisor	Henry Calanchini		
Contractor Quality Assurance Officer	Zephyr Papin		

Brelje and Race, Santa Rosa, CA

Title:	Name:	Signature:	Date:
Lab Quality Assurance Officer	Ann Hill		

Title:	Name:	Signature:	Date:
Lab Quality Assurance Officer	David Harris		

Table of Contents

LIST OF TABLES & FIGURES

LIST OF APPENDICES

Personnel Responsibilities

Sample collection will be performed by the Aquatic Ecosystems Analysis Laboratory (AEAL) of University of California, Davis. Sample analysis for *Bacteroidales* and stable isotopes will be performed by the AEAL. Sample analysis for *E-coli* and *Enterococcus* will be performed by Brelje and Race Laboratories in Santa Rosa, CA. The primary project personnel include a project and grant manager of the North Coast Regional Water Quality Control Board (NCRWQCB); a contractor project manager, project supervisor and quality assurance officer/lab manager from UC Davis; and a laboratory quality assurance officer from Brelje and Race.

NCRWQCB Project and Grant Manager role:
Matt St. John of the NCRWQCB is the Project and Grant Manager of the Russian River Pathogen Project. He is responsible for ensuring completion of work by all involved parties and for reviewing and approving payment for work performed by the grantee in accordance with the terms of the grant agreement.

Contractor Project Manager role:
Josh Viers and Fraser Shilling are the Contractor Project Managers. They will be responsible for all aspects of the project including project design, monitoring site selection, organization of field staff, scheduling of sampling days and interactions with the analytical laboratories and the Grant Manager.

Contractor Project Supervisor role:
Henry Calanchini is the Project Supervisor. The Project Supervisor will assist the Project Manager by training and supervising all monitoring staff and contributing to the monitoring program report.

Contractor Quality Assurance Officer role:
Zephyr Papin is the AEAL Quality Assurance Officer. Zephyr's role is to establish the quality assurance (QA) and quality control (QC) procedures found in the project QAPP as part of the sampling and field analysis. Zephyr will also work with laboratory personnel by communicating all QA and QC requirements contained in the project QAPP and resolving any issues in regards to meeting these requirements. Zephyr will also review the DNA data and will be responsible for the quality control for the *Bacteroidales* analyses.

Laboratory Quality Assurance Officer role:
Ann Hill is the Quality Assurance Officer for Brelje & Race Laboratories. Brelje & Race will perform the *E-coli* and *Enterococcus* analyses. David Harris is the Quality Assurance Officer for the UC Davis Stable Isotope Facility. Ann and David will maintain all records associated with the receipt and analyses of those samples and will verify that the measurement process is "in control" (i.e., all specified data quality objectives were met or acceptable deviations explained) for each batch of samples before proceeding with analysis of a subsequent batch.

Table 1. Personnel responsibilities

Name	Organizational Affiliation	Title	Contact Information
Matt St. John	North Coast Regional Water Quality Control Board	Grant Manager	Ph: (707) 570-3762 Fax: (707) 523-0135 mstjohn@waterboards.ca.gov
Josh Viers	Information Center for the Environment, UC Davis	Contractor Project Manager	Ph: (530) 754-5061 jhviers@ucdavis.edu
Fraser Shilling	Information Center for the Environment, UC Davis	Contractor Project Manager	Ph: (530) 752-7859 fmshilling@ucdavis.edu
Henry Calanchini	Aquatic Ecosystems Analysis Laboratory, UC Davis	Contractor Project Supervisor	Ph: (530) 297-4684 Fax: (530) 757-3498 hjcalanchini@ucdavis.edu
Zephyr Papin	Aquatic Ecosystems Analysis Laboratory, UC Davis	Quality Assurance Officer	Ph: (530) 297-4684 Fax: (530) 757-3498 zmpapin@ucdavis.edu
Anne Hill	Brelje and Race Laboratories, Inc	Lab Quality Assurance Officer	Ph: (707) 544-8807 office Fax: (707) 544-5736 office Home: (707) 823-9210 Cell: (707) 975-2839 brlabs@sbcglobal.net
David Harris	UC Davis Stable Isotope Facility	Lab Quality Assurance Officer	Ph: (530) 754-7517 office Fax: (530) 752-4361 office dharris@ucdavis.edu

Problem Definition / Background

Problem statement:

Contact recreation (REC-1) is the primary waterway use that is affected by the presence of high concentrations of fecal organisms in the lower Russian River and its tributaries. Shellfishing (SHELL) is also an impaired beneficial use for this waterbody. The numeric standards for recreational contact must be met by any means available to the North Coast Regional Water Quality Control Board (Regional Board) for waters within the Basin. The Russian River and several major tributaries are used for contact recreation and at certain times of the year, exceed these numeric standards. Those exceedances have resulted in an impaired waterbody listing under Section 303(d) of the federal Clean Water Act, and initiation of Total Maximum Daily Load (TMDL) development for the Russian River mainstem and Santa Rosa Creek.

Decisions or outcomes:

The goal of the project is to design and pilot a forensic monitoring and evaluation program for the Russian River watershed to identify: 1) potential primary sources of pathogenic fecal bacteria (e.g., dairies, municipal waste/runoff, wildlife and recreational sites), 2) potential secondary sources (e.g., benthic sediments, periphyton, side channels, stock ponds), and 3) contributing factors (e.g., agricultural fertilizer, managed flows, water temperature, available organic material). The proposed evaluation program is designed to help resolve spatio-temporal issues, problems with source identification, potential load allocations, and factors that contribute to in-stream exacerbation or remediation of the problem. The program is designed to support TMDL planning and implementation at the North Coast Regional Water Quality Control Board.

Water quality or regulatory criteria:
The U.S. Environmental Protection Agency (EPA) uses *E. coli* as an "indicator organism" to monitor for bacteria. Indicator organisms have been used for more than a century to help identify where fecal contamination has occurred and, therefore, where disease-causing microbes may be present. Although the presence of these organisms generally does not cause illness directly, they have characteristics that make them good indicators that fecal contamination has occurred and that harmful pathogens may be in the water. The US EPA recommended acceptable level for *E. coli* in freshwater at 235MPN/100mL. The recommended acceptable level for Total Coliform is 10,000 MPN/100ml. The bacteria objective for SHELL is a fecal coliform concentration throughout the water column not exceeding 43/100 ml for a 5-tube decimal dilution test or 49/100 ml when a 3-tube decimal dilution test is used.

Project / Task Description

Work statement and produced products:
This project will monitor for *Bacteroidales*, *E. coli* and *Enterococcus* at twelve sites in the Russian River Basin. In addition, water will be collected at all sites, during each event, and preserved for later analysis of primary heavy isotopes from nitrate (N, O) and dissolved organic carbon (C) using Stable Isotope Analysis (SIA). Monitoring will encompass winter storm events and monthly non-storm events from November 2008 to May 2009. The number of sampling events will be dependent upon available funds. Monitoring sites were selected based on the results of previous studies.

Other work products are:

- Monitoring Program Overview
- Draft Summary of Data Analysis
- Summary of Data Analysis
- Addition of Spatial Data into ICE's Russian River GIS

Constituents to be monitored and measurement techniques:
Bacteroidales will be examined by the Aquatic Ecosystems Analysis Laboratory using species-specific markers. The markers used will be avain (all), canid (all), bovine and human. The protocol is listed in Appendix 4 of the QAPP. The MDL of the analytical method is 10 gene copies/TaqMan. The RL will be determined after the completion of each sample run.

E. coli will be quantified by Brelje & Race Laboratories in Santa Rosa utilizing the Quantitray procedure. The quantitation range for this procedure is 1 – 2419.2 MPN/100ml. The Practical Quantification Limit (PQL) is 1 MPN/100mL. A Method Detection Limit (MDL) is not applicable.

Enterococcus will be quantified by Brelje & Race Laboratories in Santa Rosa utilizing the Enterolert test kit. The Practical Quantification Limit (PQL) is 1 MPN/100mL. A Method Detection Limit (MDL) is not applicable.

Stable Isotope Analysis of oxygen from nitrate in water samples will be performed by the UC Davis Stable Isotope Facility using the denitrifier method (Casciotti, et al. 2002). Stable isotope analysis may also be performed on biological material including riparian and in-stream vegetation, and tissues from aquatic snails and mussels.

Monitoring will also consist of field measurements for pH, electrical conductivity and water temperature using an Oakton pH/Con10 pH/Conductivity/Temperature meter and for dissolved oxygen using an Accumet AP74 Dissolved Oxygen Meter (Appendices 6 & 7). Data will be consistent with field sheets provided by Regional Board staff (Appendix 8).

Project schedule

Table 2. Project schedule timeline

Activity	Date		Deliverable	Deliverable Due Date
	Anticipated Date of Initiation	Anticipated Date of Completion		
Bacterial monitoring	Dec 2008	May /2009	NA	NA
Sample analysis	Dec 2008	May 2009	Results in Excel format	May 2009
Draft results report	May 2009	June 2009	Draft report	06/30/2009
Final results report	June 2009	July 2009	Final report	07/31/2009

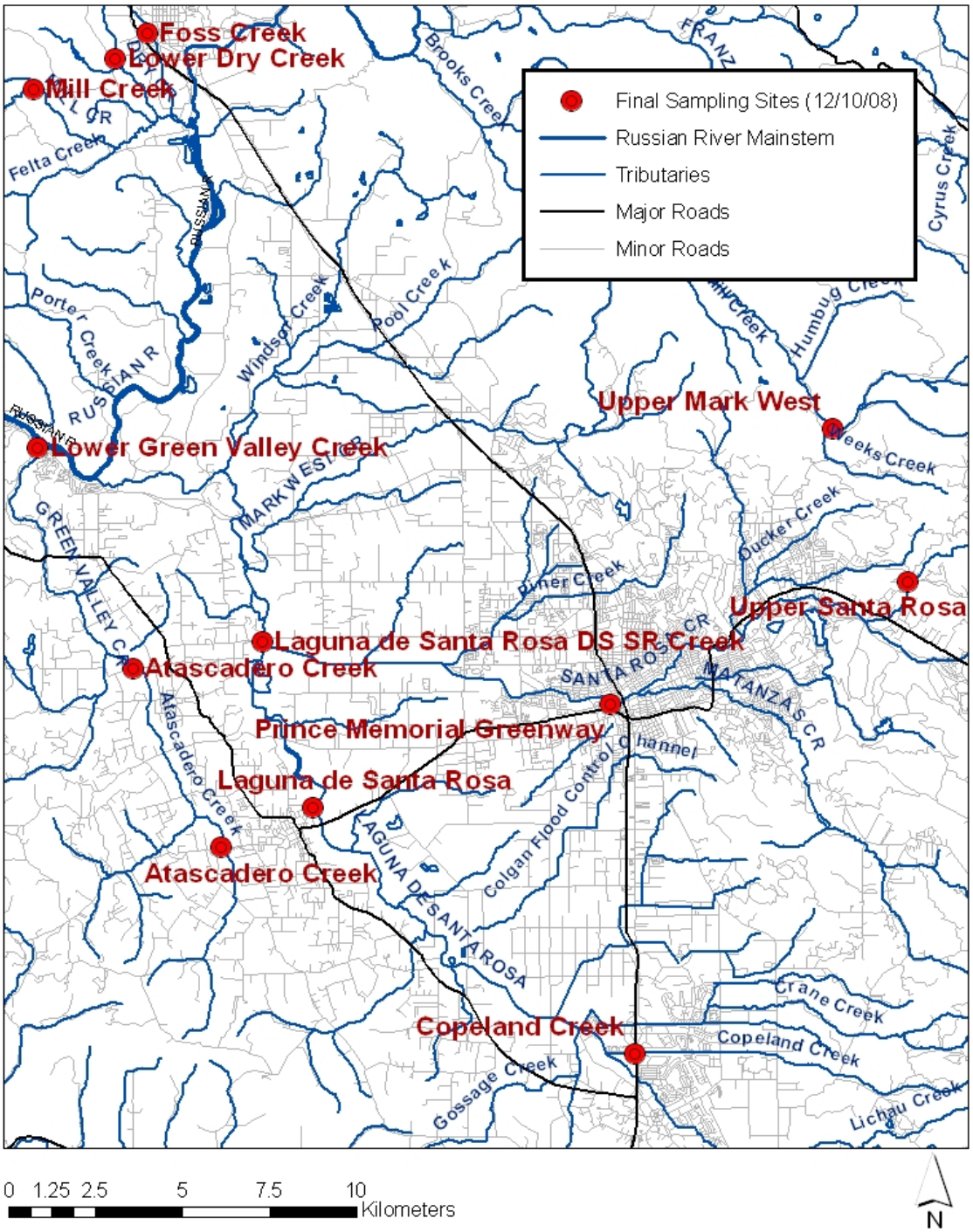
Geographical setting

Table 3. Table of the 12 Sampling Locations for the Russian River Pathogen Project

Station Name	Purpose	SWAMP Station Code	Target Lat	Target Long
Atascadero Creek at Green Valley Rd	Mixed	114GREEN1	38.444309	-122.877000
Atascadero Creek at Bodega Hwy	Agricultural	114ATASC1	38.397469	-122.848200
Copeland Creek	Urban	114COPE01	38.343119	-122.711960
Foss Creek	Urban	114VINE01	38.611109	-122.871880
Laguna de Santa Rosa	Mixed	114LAGU01	38.407926	-122.818068
Laguna de Santa Rosa downstream of confluence with Santa Rosa Creek	Mixed	114LAGU02	38.4515972	-122.834722
Prince Memorial Greenway	Urban	114SROSA1	38.434813	-122.719683
Upper Santa Rosa	Urban	114WILD01	38.466719	-122.621656
Upper Mark West	Semi Natural	114MARK01	38.512367	-122.637522
Lower Dry Creek	Agricultural	114WEST01	38.604573	-122.882535
Mill Creek	Semi Natural	114MILL01	38.596513	-122.909694
Lower Green Valley Creek at River Rd	Mixed	114LGVARR	38.50233	-122.90838

Figure 1. Map of the 12 Sampling Locations for the Russian River Pathogen Project

Russian River Watershed Sample Locations



Documents and Records

Table 4. Document and record retention, archival and disposition information

	Identify Type Needed	Retention	Archival	Disposition
Sample Collection Records	Chain of Custody	Original with analyzing laboratories	Copies with AEAL and the NCRWQCB	Stored at AEAL for 5 years, permanently at NCRWQCB
Field Records	Field Sheet	AEAL	AEAL	Stored at AEAL for 5 years
Analytical Records	Analyses Result Reports	Brelje and Race, UC Davis Stable Isotope Facility	Copies to AEAL and NCRWQCB	Stored permanently at NCRWQCB
Database	Access database	ICE	NCRWQCB	Stored permanently at NCRWQCB
Final Report	PDF or Word document	AEAL	Copy to NCRWQCB	Stored permanently at NCRWQCB

Sampling Process Design

A total of twelve monitoring sites were chosen to reflect a mix of land uses, watershed position, potential cumulative effects, spatial coverage, as well as previous monitoring activity. There are presently two semi-natural controls (Upper Mark West Creek and Mill Creek), two agriculturally dominated reaches (Upper Atascadero Creek and Lower Dry Creek), four urban (Copeland and Foss Creeks, Upper Santa Rosa Creek and the downstream Prince Memorial Greenway), and four mixed land uses (Laguna de Santa Rosa and downstream of Santa Rosa Creek, and lower portions of Green Valley and Atascadero Creeks). Please see the initial findings of Shilling et al. 2008 for a discussion of previous monitoring results.

Sampling Methods

The samples will be collected as grab samples from approximately midstream and below the surface following the SOPs in Appendices 2 & 3 of this QAPP. If the water levels won't allow wading midstream, the sample will be collected from the bank or by using a pole sampler.

Strictly following the sample procedure will assure that the samples will be collected consistently between locations and by different sampling teams. The sampling container will only be used once during a collection run.

Sample Handling Custody

Once sample containers are labeled, they are filled with environmental (sample) water and stored on ice during transport to the laboratory. Samples to be analyzed for *E. coli* and *Enterococcus* will be delivered to Brelje and Race Laboratories, Inc. in Santa Rosa. To avoid contamination during transport the sample bottles will be in a sealed plastic bag placed on ice inside a cooler. The holding time for the *E. coli* samples is 6 hours.

Samples for *Bacteroidales* will be filtered in the field immediately after collection. The sample filter will be placed in a labeled vial partially filled with the preservative RNAlater, stored in a cooler on dry ice and delivered to the analytical lab. At the analytical lab the sample will be stored in a -80C freezer until analysis is performed. See *Bacteroidales* Genetic Profiling SOP; Appendix 4 for details.

Samples collected for stable isotope analysis will be placed in sealed containers and stored on ice until arriving at the AEAL where they will be stored in a -30°C freezer until ready for analysis at the UC Davis Stable Isotope Facility.

Each sample will be documented on a chain of custody form at the time of collection. The chain of custody will remain with the samples at all times. When the samples are delivered to the lab, the sampler will relinquish custody by signing the appropriate space on the chain of custody form. The lab attendant will accept custody by signing the appropriate space on the chain of custody form. The lab attendant will make a copy of the chain of custody form and give it to the sampler for filing at the AEAL office. The original chain of custody form will stay with the analyzing laboratory; a copy will be filed at the main. Appendix 1 contains an example of the chain of custody form that will be used for this project.

Analytical Methods

Table 5. Field analytical methods

Analyte	Laboratory / Organization	Project Quantification Limit (units, wet or dry weight)	Analytical Method	
			Analytical Method/ SOP	Modified for Method yes/no
pH	Field monitoring by AEAL field staff	±0.01 pH	Appendix 6	None
Electrical Conductivity	Field monitoring by AEAL field staff	0.01 mS	Appendix 6	None
Temperature	Field monitoring by AEAL field staff	0.1ºC	Appendix 6	None
DO	Field monitoring by AEAL field staff	0.01mg/L	Appendix 7	None

Quality Control

Table 6. Data quality objectives for *E. coli* and *Enterococcus* analyses

QA Procedure	Parameter	Frequency	Criterion	Corrective Action
Field Blanks	Contamination	1 per sampling day	Absence or < (sample value ÷ 5)	Examine field log. Identify contamination source. Qualify data as needed
Field Duplicate	Verification	1 per sampling day	Presence/Absence (consistency between duplicates)	Repeat batch analysis
Lab Duplicate	Precision[1]	One per batch of samples	$R_{log} \leq 3.27 * \text{mean } R_{log}$	Recalibrate and reanalyze
Negative Control Samples	Contamination	1 per culture medium or reagent lot	<RL	Identify Source. Clean equipment and prepare new media Re-examine negative control
Positive Control Samples	Assay function	1 per culture medium or reagent lot	≥RL	Identify and correct problem. Re-examine positive control.
Assess percent of data successfully collected	Data completeness	1 per planned sample event	90%	Reschedule sample events as necessary or appropriate.

Notes: RL=Reporting Limit

(1) R_{log} is the absolute difference between algorithms of coliform counts for duplicate analyses. The mean R_{log} is determined by performing duplicate analyses on the first 15 positive samples analyzed for each matrix type.

Table 7. Field Quality Control for *E. coli* and *Enterococcus* analyses

Field QC	Frequency	Acceptance Limits
Field Blanks (*E. coli* and *Enterococcus*)	1 per sampling day	Absence
Field Duplicates (*E. coli* and *Enterococcus*)	1 per sampling day	Relative Percent Difference

		(RPD) of ≤ 25% of environmental sample

Table 8. Data quality objectives for *Bacteroidales* analyses

QA Procedure	Parameter	Frequency	Criterion	Corrective Action
Field Blanks	Contamination	1 per sampling day	Absence	Examine field log. Identify contamination source. Qualify data as needed
Field Duplicate	Verification	1 per batch	Presence/Absence (consistency between duplicates)	Repeat batch analysis Identify Source.
Negative Control Samples	Contamination	1 per batch	Absence	Repeat batch analysis. If necessary, replace reagents.
Positive Control Samples	Assay function	1 per batch	Presence	Identify and correct problem. Repeat batch analysis.

Table 9. Field QC for *Bacteroidales* analyses

Field QC	Frequency	Acceptance Limits
Field Blanks	1 per sampling day	Absence
Field Duplicate Pairs	1 per sampling day	Relative Percent Difference (RPD) of ≤ 25% of environmental sample

Data Management

AEAL and Brelje & Race Laboratory Data Management

AEAL staff will maintain the database and all project records in AEAL custody. AEAL project data is stored on a secure server with a four-partition memory so that if any single memory partition fails it can be rebuilt from the remaining three.

Field data sheets are returned to AEAL after the sampling event and filed in the AEAL office. Field data including field descriptions and water quality parameters are entered electronically into the database and double-checked by a second person.

Copies of the chain of custody forms will also be filed at the AEAL. Sample results from the analyzing UCD laboratory will be sent to the AEAL lab via electronic data deliverables (EDD). Brelje and Race will send results as a hard copy by mail. After data entry or data transfer procedures are completed for each sample event, data will be inspected for data transcription errors and corrected as appropriate.

UC Davis Stable Isotope Facility (UCDSIF) Data Management

Raw and preliminary calculated isotope analysis data are generated on the instrument control computer (Windows XP) by the instrument control software. These files are simple ASCII and are automatically duplicated in such a way that the analytical result can be 'replayed' if needed. The files are backed up to a central server daily. The server is automatically backed up daily. At this point there are 6 copies of the raw data.

This preliminary data is loaded into an Excel spreadsheet for processing and error checking by UCDSIF staff. Further error checking is performed by a senior staff member before emailing out the data as an Excel spreadsheet. The Excel spreadsheet data is stored and backed up by the server. If necessary the entire process of data reduction can be regenerated from one of the backup copies of the raw data.

Reports to Management

Reports will be issued according to the following table.

Table 9. Reports to management

Type of Report	Projected Delivery Dates(s)	Person(s) Responsible for Report Preparation	Report Recipients
Draft Pathogen Source Analysis Report	6/30/2009	Josh Viers, Fraser Shilling, Michael Johnson	NCRWQCB
Final Pathogen Source Analysis Report	7/31/2009	Josh Viers, Fraser Shilling, Michael Johnson	NCRWQCB

Final Report

The project manager will prepare a final report of the results of this study and the activities used to generate them. The report will be delivered to the NCRWQCB no later than July 31, 2009. The elements described below will be addressed and included in the report:

- Description of the project including the number of samples, analyses, completeness and any significant problems or occurrences that influence data use
- The QA/QC activities performed during this project
- QC sample results, type and number of samples including the results that did not meet the project objectives and the impact on usability.
- Tables of analytical results

Sources Cited

Measurement of the Oxygen Isotopic Composition of Nitrate in Seawater and Freshwater Using the Denitrifier Method K. L. Casciotti,,, D. M. Sigman,, M. Galanter Hastings,, J. K. Böhlke, and, A. Hilkert Analytical Chemistry **2002** 74 (19), 4905-4912

Shilling et al., 2008, **http://rrpp.ice.ucdavis.edu/files/rrpp/Russian_River_Pathogen_Project_Report.pdf**

Appendix 1. Chain of Custody Form

REQUEST FOR ANALYSIS AND CHAIN OF CUSTODY RECORD

Sampler	Send Results To	Matt St. John	Lab Number
Address UC Davis 1490 Drew Ave Suite 150		Henry Calanchini hjcalanchini@ucdavis.edu	Field Number
City Davis Zip 95616 CA			Lab Storage

Ice Chest Temp at Log-in:	Analysis Requested>>>		Fecal coliform	*E. coli*											Others (specify)			# of containers			
	Collection																				
Sample Identification	Date	Time																Plastic	Glass	Vial	Whirlpak
114GREEN1	12/23/2008	9:00	x	x														2			
114ATASC1	12/23/2008	9:30	x	x														2			
114COPE01	12/23/2008	10:00	x	x														2			
114MINE01	12/23/2008	11:30	x	x														2			
114LAGU01	12/23/2008	12:40	x	x														2			
114LAGU02	12/23/2008	14:00	x	x														2			

Comments / Special Instructions

Samples Reliquished By (Signature)	Print Name	Date	Received By (Signature)	Print Name	Date

Appendix 2. SOP for *Bacteroidales* sample collection and filtering process

Sample collection and filtering protocol for *Bacteroidales* in surface water

(A. Wehrmann and L. Chu, August 2006)

1. Supplies:

Filtering supplies:

1. 2ml cyrotubes (Fisher # 05-669-64; Externally threaded Cryovials 2.0ml self-standing 100/pack)
2. RNAlater (Ambion # 7021; 500ml)
3. Each cryotube is filled with 1.5 ml RNAlater in the lab before sampling
4. Sharpie markers to label cryotubes
5. Scotch tape to cover the writing (so writing won't run)
6. Nitrile gloves
7. Vacuum pump set up plus car power inverter
8. Filter forceps (at least 2 pairs) (Fisher # 09-753-50; Fisherbrand filter forceps)
9. Filters (Pall Gelman Science Inc. supor 0.2µm strl. 47mm 200/pack – Fisher no longer carriers them, but VWR does)
10. 3 plastic 50ml conical vials for cleaning the filter forceps – one for 20% bleach solution, one for 95% ethanol, and one for deionised water

Water collection supplies:

1. Vials to collect 50 (100ml) water for Bacterioides (we used Sarstedt 50ml plastic conical vials)
2. Sampling pole with an adjustable "claw" on the end that can hold the conical vials

Supplies for cleaning filtering equipment:

1. Large plastic container (e.g.Nalgene) – for holding 20% bleach solution – for soaking (glass) filtering equipment in
2. Carboy of DI water (to rinse the glassware with)
3. Spray bottle with 95% Ethanol (easy to cover glassware with ethanol using a spray bottle)
4. Kimwipes (to dry glassware after spraying with ethanol)
5. Ziplocs (to store glassware pieces between sites)

Miscellaneous:

1. Freezer box to hold the vials containing the filter
2. Cooler with Dry ice (so that samples can be kept frozen in the field)

2. Collecting the sample

Use a clean pair of nitrile gloves to collect one (two) 50ml conical vials with the sampling pole filling the vial to the 50mL line. Try to collect the water in an area of the stream that seems representative for the water body and where there is flow, if conditions are safe.

3. Filtering process

1. Use a pair of nitrile gloves for the entire process, but put on a new pair before the glass cleaning process.

2. Water sampling and filtering
    a. Make sure the cryotube is labeled with the Site Code, Sample Date, and Sample Time. Also record the sample time on the "sample tracking" sheet. Cover the writing with Scotch tape (so the writing won't run)
    b. Tube and cap should also have the corresponding number on it (see sample tracking sheet)
    c. Put on a clean pair of nitrile gloves
    d. Assemble the water filtration set-up as shown
        i. Set-up the three conical vials filled with 20% bleach, DI water, and 95% ethanol to sterilize the

filter forceps (solutions should be changed as needed, but at least renewed before a new sampling day)
    ii. Clean a pair of filter forceps – rinse briefly in bleach solution, DI water and then with 95% ethanol..
    iii. Use a Kimwipe to wipe forceps off after rinsing in the ethanol
    iv. Using the clean filter forceps, place a filter (grid-side up) on the filter plate
e. Filter the sample water through the filter set-up (if the water is very murky and the filtering process is taking a very long time, only use 50ml of water and make a note on the field sheet and the tracking sheet)
f. Clean the filter forceps if they get in contact with anything (rinse briefly in bleach solution, DI water, ethanol and dry with Kimwipe)
g. Unclamp the filter flask and the filter plate. Place the filter flask down (anywhere since it is now dirty and needs to be cleaned)
h. Using 2 pairs of clean filter forceps, fold the filter in half and then in half again. Roll up and put into a prepared sample vial (prepared with 1.5 ml of RNAlater) - use only cleaned forceps to touch the filter at any time
i. Cleaning the filter set-up
    i. Put on a fresh pair of nitrile gloves before cleaning glassware
    ii. Soak the filter flask in the large Nalgene container (containing 20% bleach solution) at least for 2 minutes.
    iii. Rinse off with DI (from the DI carboy)
    iv. Spray with 95% Ethanol thoroughly inside and outside (from spray bottle)
    v. Wipe off with Kimwipes
    vi. Put into clean Ziploc bag and wrap in bubble wrap (new Ziploc bags every day for both the filtering glass wear)
    vii. Repeat with any other glassware
j. Put the sample vial into the appropriate freezer box
k. Put the freezer box of completed samples into the cooler with dry ice

3. Use DI water for all Field Blank samples
    a. For a site with a Field Blank, we filter blank water first and then rest the filter flask on a clean Kimwipe in between the blank sample and the environmental sample
    b. We then reassemble the filter set-up with a new filter (without cleaning it in between filtering the DI water and the environmental sample)

4. Fill out field sheets and a COC, and write the sample number on the field sheet.

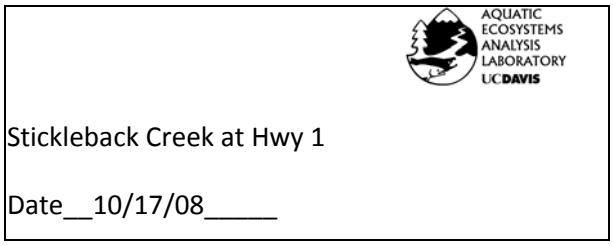
5. Sign COC and put it in the -30 freezer in Room 114 (weight them underneath the freezer box full of samples)

6. BACK IN DAVIS: put the Bacterioides sample vials into the freezer box that is in the -30° freezer in Room 114

Appendix 3: Standard Operating Procedure for the Collection of Surface Water Samples for Bacterial Analysis

1. Labeling the sample bottle

- Use pre-printed labels for each site. The label should include the site name, ID number, date, sample time, and your initials
- Complete the printed label with an extra-fine-point Sharpie. Cover the entire label with a piece of clear tape to prevent peeling
- Use 24-hour military time for the sample time; round to the nearest 10 minutes. For example: a sample collected at 09:52 would have the sample time on the label and Chain of Custody (COC) form rounded off to 09:50; a sample collected at 09:57 would be rounded up to 10:00; 09:55 would also be rounded up to 10:00. Use the following format for the date: mm/dd/yy



2. Check the Quality Control (QC) Schedule to see if a QC sample is scheduled for the site

If so, label an additional sample bottle according to the instructions in Step 5 below. Read the QC sampling procedure before sampling.

3. Fill out Field Sheet at each sampling site

Sampling Information

- Sampling Type is already filled out. Add sampler initials

- Sample Bottle: we will use sealed 100ml plastic bottles containing a powdered preservative. Sample bottles will be supplied by Brelje and Race Laboratories.
- Sampling Method: all samples are grab

Sample Collected

- If a QC sample is scheduled, place a check beside the type of QC sample
- Sampling Time: Record rounded sample time (see above)

Field Measurements

Use Oakton and Accumet multi-parameter meters and DO meters; follow calibration procedures outlined in user's manual prior to collection of first sample each day.

- BANK SAMPLE: measure directly from stream's edge

At the end of the day replace storage solution in the probe cap

Note anything significant or unusual under Observations on the field sheet; for example waste disposal, discolored water, foam on water surface, dead fish, etc.

Original field sheets stay with UC Davis in a prepared folder in the AEAL office.

4. Collecting the sample

Always wear clean gloves during sampling procedure!

100ml plastic bottle:

a) Place the bottle in the pole sampler if needed, otherwise perform grab sample by hand

b) Wearing clean gloves remove the quality control seal from the neck of the bottle

c) Fill the bottle to the 100ml fill-line. If you overfill it is OK to pour off until you reach the fill-line. **If you pour off too much, do not add water back into the bottle; begin again with a new bottle.**

d) Put container into Ziplock bag to prevent ice from contaminating the sample.

e) Place Ziplock into a cooler with ice.

The last thing to do before filling any sample bottle, regardless of method, is to remove the lid. The first thing to do after filling any sample bottle, regardless of method, is to replace the lid. If you have more than one sample bottle to fill, remove each lid just prior to filling the bottle.

5. If scheduled collect a quality control sample

View the QC Schedule to find out which type of QC sample you should collect that day

-- Field duplicate:

a) Collect both samples simultaneously. If using a pole sampler place two bottles in the sampler.
b) Mark the sampling time of the duplicate sample by adding **3 minutes** to the time of the environmental sample (e.g. environmental sample collected at 14:00 then duplicate time is 14:03). **Do not** indicate *duplicate* on the label or on the COC!

-- Blank sample:

Do not indicate blank on label or on COC. Time offset: add **1 minute** to the time of the environmental sample (e.g. environmental sample collected at 14:00 then blank time is 14:01).

BANK SAMPLE

Fill one 1L bottle with deionized water for the blank

Whoever did not fill out the field sheet and COC should double check all of the recorded times for completeness and error at the end of the sampling day

Check ice level

The temperature of the ice chest should be around 4°C. Make sure to add ice if necessary.

6. Deliver samples within specified hold-time

7. Complete Chain of Custody form

Complete a Chain of Custody form for each sampling day.

- The original COC's for will stay with the analytical lab. Be sure to have receiving person make you a copy of the COC. Upon return to the Davis place our copy of the COC in the prepared folder at the AEAL office.

Sample transfer between field staff and laboratory is documented by **signing and dating** "relinquished by" and "received by" blocks whenever sample possession changes. The document must have both your signature **and** a signature from the receiving lab.

Appendix 4. Standard Operating Procedure for Genetic Profiling of Bacteroidales

Bacteroides Genetic Profiling S.O.P for Real-Time PCR

9/23/2008

Part I. DNA Extraction

Part II. A) PCR Sample Preparation

B) 7300 System Software Run Setup

Part I. DNA Extraction

Items needed:

- General PPE
- Pipettes, p1000, p100, p20 w/ respective sterile tips
- 2.0 ml and 1.5 ml sterile (autoclaved) microcentrifuge tubes
- Qiagen MinElute Gel Extraction kit
- Water bath at 56^{o}C
- 100% EtOH
- Sterilizing solutions- 20% Bleach, ddH_2O, and 100% EtOH
- Forceps
- PBS pH 7.4

1. Open microcentrifuge tube and unfold filter using sterile forceps and then refold the filter so that the inside, which contains bacteria, will now be on the outside and place into a 2ml microcentrifuge tube.
   **make sure to sterilize forceps between each sample*
2. Add 250µl of PBS to sample along with 20µl of Proteinase K
3. Repeat steps 1 and 2 for all samples
4. Add 500 µl of Buffer AL to the sample and vortex for 15s.
5. Incubate at 56^{o}C for 10 min and quick spin.
6. Add 500µl of 100% EtOH and vortex/quick spin
7. Add 700µl of mixture from step 6 to the QIamp Spin Column, which should be within a clean microcen. tube.
8. Spin at 8000 rpm for 1 min.
9. Place spin column in new microcen. tube and add the remaining solution from step 6 and repeat step 8

10. Add 500µl of buffer AW1 and centrifuge at 8000 rpm for 1 min. Place Spin Column in a clean 2ml tube and discard filtrate collection tube
11. Add 500µl of buffer AW2 and spin at full speed for 4 min
12. Place the Spin Column in a clean 1.5 ml tube (not provided in kit) and discard collection tube. Add 50µl of buffer AE and spin for 1 min at 8000rpm.
13. To the same spin column, add another 50µl of AE buffer, making sure to use the same 1.5ml collection tube as in step 12.
14. Store the eluate in the -20^{o}C fridge

Part II. A) PCR Sample Preparation

Items needed:

- General PPE
- Pipettes, p100, p20, p2 w/ their corresponding sterile tips
- Real-Time Thermal Cycler
- Power SYBR green PCR Master Mix
- Molecular Grade Water
- 1.5 ml sterile microcentrifuge (autoclaved) tubes
- 96 well PCR plate (non-fast)
- Optic PCR plate film
- Ice bucket w/ ice

1. Thaw all materials including PCR Master Mix, H_2O, extracted DNA, positive control (196B for HuBac and 186 for BoBac and AvBac) and primers.
2. When an individual item is thawed, vortex/quick spin, and immediately place in ice.
   **Note, It is imperative that the Taq is kept cold at all times*

3. Calculate master mix depending on total samples to be run including PC and NC plus one: n+PC+NC+1, where n=number of DNA samples.
4. Refer to the Matrix presented below when calculating reagents and add to sterile microcentrifuge tube in the order as listed.
   **Note, Take appropriate steps to ensure reagent contamination does not occur*

Primer Series, ie. HuBac	Amount per 20µl Rxn	Multiple needed,ie for 10 samples 10+PC+NC+1=**13**

H_2O	11.25	111.25
PCR Master Mix	12.5	12.5
F_{primer}	0.125	1.25
R_{primer}	0.125	1.25

5. Once master mix is made, vortex/quick spin
6. Pipette 24µl of the master mix into an appropriately labeled PCR tube
7. Pipette 1µl of template DNA(or water for blank) into the assigned PCR plate well containing the master mix
8. Trombone the solution within the well to mix
9. Cover PCR plate with optic film and seal
10. Place in thermal cycler and run appropriate program (see Part II B)

Part II. B) 7300 System Software Run Setup

1. Open 7300 Software on Desktop
2. Create a new document
    a. Within new document wizard, only change plate name
3. Select appropriate detectors for the plate (i.e. HuBac if using HuBac primers)
4. Highlight areas of plate that correspond to the locations of the sample wells being used for current run
5. Add dissociation state, change default volume from 50µl to 25µl, and run samples with default settings* after saving run setup.

* Default PCR Conditions:

Step 1 50^0C for 2 min

Step 2 95^0C for 10 min

Step 3 95^0C for 15 sec

Step 4 60^0C for 1 min

Appendix 5. Brelje and Race Standard Operating Procedure for Chromogenic Substrate Coliform Test

I. Scope and Application

A. Introduction

The chromogenic substrate test utilized hydrolysable substrates for the simultaneous detection of enzymes of total coliform bacteria and *Escherichia coli.* This technique for the coliform group is defined as all bacteria possessing the enzyme ß-D-galactosidase. *Escherichia coli* are defined as bacteria possessing the enzyme ß-glucouronidase. The test can be used in either a presence-absence or quantitative format.

B. Detection Limits

<1.0 MPN is the lower limit for this method using 100mL of sample.

C. Working Range

If quantitative format is used (using commercially available Quanti-tray © or individual screw cap test tubes) the range extends to >2419.2.

D. Summary of Methods

Pre-weighed enzymatic substrate is added aseptically to 100mLs of sample in a sterile bottle. Sample is capped and mixed thoroughly to dissolve. Samples are incubated at 35 ± 0.5° C for a minimum of 24 to a maximum of 28 hours.

E. Sample Collection, Preservation, and Holding Times

Samples are collected in sterile transparent, non-fluorescent containers with sodium thiosulfate powder, provided by the container manufacturer. Hold temperatures of all samples is to be below 10° C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 24 hours.

II. Quality Assurance

A. Equipment

Same as for multiple tube fermentation method standard operating procedure.

B. Media

A commercial substrate reagent is purchased for manufacturer, such as Colilert from IDEXX. From each lot of media purchased, three containers are filled with sterile DI water and a pre-weighed packet of media is added. These are then inoculated with *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. These controls are then incubated at 35 ± 0.5° C for a minimum of 24 to a maximum of 28 hours. They are read and recorded on QC sheets.

C. Sample Bottles

Refer to the SOP for the multiple tube fermentation, with the exception that the bottle is checked for auto-fluorescence under a UV light.

D. Duplicates

At least one duplicate run every day or with every batch.

E. Completed Test

To establish the presence of coliform bacteria and to provide QC data, use the completed test on 10% of positive confirmed samples.

1. 0.1 mL positive sample pippeted into 10mL Trytophan Broth. Incubate at 44.5° C for 24 ± 2 hours.
2. After 24 hours inoculate 10 mL Koser Citrate medium from Trytophan Broth. Incubate K.C. medium at 35° C for 48 ± 3 hours. Turbidity indicates positive results for *Klebsiella pneumoniae*.
3. After inoculating K.C. tube, pipette 0.1 mL Kovac's reagent into Trytophan tube. A red color band on top of the media indicated positive results for *E. coli*. Use a control of *E. coli* and *Klebsiella pneumoniae*.

III. Procedure

A. Disinfectant

Spray down counter with disinfectant before starting and after finishing tests.

B. Test

Aseptically add pre-weighed enzymatic substrate to 100mL of sample in the appropriate container. Aseptically cap and mix thoroughly to dissolve. Incubate at 35 ± 0.5° C for a minimum of 24 to a maximum of 28 hours.

C. Interpretation

1. Total Coliform Bacteria

After 24-28 hour incubation, examine container for color change. A distinct chromogenic response (yellow color) is a positive reaction for total coliform. Compare each sample against the color comparator available from the manufacturer of the media. If the color intensity is greater than, or equal to that of the comparator, total coliforms are present. Samples are negative or absent if no color is observed. If the chromogenic response is questionable after 24 hours, incubate up to an additional 4 hours. If the chromogen intensifies, the sample is total – coliform positive; if it does not, the sample is negative.

2. Escherichia coli

Examine positive total coliform samples for fluorescence using a long wavelength (366nm) ultraviolet (6 watt bulb). Compare each sample against the reference comparator. The presence of fluorescence is a positive test for *E. coli*. If fluorescence is questionable, incubate for an additional 4 hours; intensified fluorescence is a positive result.

3. Reporting

If performing an MPN procedure, calculate the MPN value for total coliform and *E. coli* from the number of positive cells (Quanti-tray) from charts provided by the media manufacturer. If using the presence-absence procedure, report results as present or absent per 100mL sample.

Appendix 6. Oakton Portable Waterproof pH/CON 10 Meter Calibration Standard Operating Procedure

OAKTON PORTABLE WATERPROOF PH/CON 10 METER CALIBRATION STANDARD OPERATING PROCEDURE

JMIE January 2004

Purpose: This standard operation procedure (SOP) provides a detailed description for the calibration of the OAKTON Portable Waterproof pH/CON 10 Meter (Model #35630-02)

Note: All calibrations used pH/conductivity/temperature probes designed for the OAKTON Portable Waterproof pH/CON 10 Meter (Model #35630-02) only.

Step 1: Reset pH and conductivity to the factory defaults.

To reset pH, make sure the meter is in pH mode, then:

1.) While in measurement mode, press CAL/MEAS and hold for 3 seconds.
2.) The meter will prompt RST in the upper display and CAL in the lower display.
3.) Press enter to reset the meter to its factory defaults. The screen will flash all characters, then return to measurement mode once the meter is reset.

To reset conductivity, make sure the meter is in conductivity mode, and then follow steps 1-3 above.

Step 2: Preparing the pH/CON meter for calibration.

1.) Remove the protective rubber cap of the probe before calibration.
2.) Wet the probe in tap water for 10 minutes before calibrating or taking readings to saturate the pH electrode surface and minimize drift.

Step 3: 3-point (OAKTON pH 4.00, 7.00 and 10.00) pH calibration.

1.) If necessary, press the MODE key to select pH mode. The pH indicator appears in the upper right hand corner of the display.
2.) Rinse the probe thoroughly with de-ionized water or a rinse solution. Do not wipe the probe; this causes a build-up of electrostatic charge on the glass surface.
3.) Dip the probe into the calibration buffer. The end of the probe must be completely immersed into the sample. Stir the probe gently to create a homogenous sample.
4.) Wait for the measured pH value to stabilize. The READY indicator will display when the reading stabilizes.
5.) Press CAL/MEAS to enter pH calibration mode. The primary display will show the measured reading, while the smaller secondary display will indicate the pH standard buffer solution. Scroll up or down until the secondary display value is the same as the pH buffer value you are using (pH 4.00, 7.00 or 10.00).

6.) Wait for the measured pH value to stabilize. The READY indicator will display when the reading stabilizes.
7.) After the READY indicator turns on, press ENTER to confirm calibration. A confirming indicator (CON) flashes and disappears. The meter is now calibrated at the buffer indicated in the secondary display.
8.) The secondary display automatically scrolls to the next buffer calibration option. Scroll up or down to select the next buffer value you want to calibrate (pH 4.00, 7.00 or 10.00).
9.) Rinse the probe with de-ionized water or a rinse solution, and place it in the next pH buffer.
10.) Follow steps 5-8 for additional calibration points.
11.) When calibration is complete, press CAL/MEAS to return to pH measurement mode.

Note: If the selected buffer value is not within +/-1.00 pH from the measured value: the electrode and buffer icon blink and the ERR annunciator appears in the lower left corner of the display. These indicators also flash if the buffer used in not the same as the buffer value on the secondary display.

Step 4: Conductivity Calibration

1.) Pour out two separate portions of the calibration standard and one of deionized water into separate clean containers. Choose a calibration solution value that is approximately 2/3 the full-scale value of the measurement range (e.g. in the 0 to 1999 μS range, use a 1413 μS solution for calibration). A 447 μS standard solution is generally adequate in this study.
2.) If necessary, press the MODE key to select the Conductivity Mode. The μS or mS indicator will appear on the right side of the display.
3.) Rinse your probe with deionized water, then rinse the probe in one of the portions of calibration standard.
4.) Immerse the probe into the second portion of calibration standard. The meter's autoranging function selects the appropriate conductivity range (four ranges are possible). Be sure to tap the probe to remove air bubbles. Air bubbles will cause errors in calibration.
5.) Wait for the reading to stabilize. The READY indicator lights when the reading is stable.

6.) Press the CAL/MEAS key. The CAL indicator appears above the primary display. The primary display shows the factory default and the secondary display shows the temperature.
7.) Scroll up or down to the value of your conductivity standard. Press and hold the scroll keys to go faster. The meter automatically compensates for temperatures using a factor of 2.00% per C.
8.) Press the ENTER key to confirm calibration. Upon calibration, the CON indicator appears briefly. The meter automatically switches back into Measurement mode. The display now shows the calibrated, temperature compensated conductivity value.
9.) For calibration in other ranges (Maximum: 4 ranges) repeat steps 1 through 9 with the appropriate calibration standards.

Note: if the calibration value input into the meter is different from the factory default value displayed by more than 30%, the ERR annunciator appears in the lower left corner of the display. Clean probe with alcohol. Verify that your calibration standard is fresh and accurate.

*Steps were transposed from the OAKTON Portable Waterproof pH/CON 10 Meter (Model #35630-02) manual of operating instructions (68X230403 rev2 01 / 02).

Appendix 7. Accumet AP74 Dissolved Oxygen Meter Calibration and Operation Standard Operating Procedure

CALIBRATION INSTRUCTIONS FOR

ACCUMET AP74 DISSOLVED OXYGEN METER

TO CALIBRATE 100% SATURATION

1. **Rinse the probe well with DI water or rinse solution.** For best accuracy, wrap the end of the probe in a damp cloth. Do not touch the membrane.

2. **Press the MODE key** to select % saturation mode.

3. **Press the CAL key**. The CAL indicator will appear above the primary display. The primary display shows the current values of the measurement and the secondary display will show 100.0.

4. **Hold the probe in the air (or in damp cloth).** Wait for the reading to stabilize. If the ready indicator feature is enabled, it will appear when the ready is stable.

5. **Press the ENTER key.** The meter automatically calibrates to 100.0% air saturation and returns to Measurement mode.

Note: The reading in the primary display in step 3 must read at 50% or above for the calibration to work correctly. Whenever an error occurs during calibration, the ERR indicator appears in the lower left hand corner of the display.

TEMPERATURE CALIBRATION [***CALIBRATE ONLY IF YOU SUSPECT DRIFT THAT MAY HAVE OCCURRED OVER A LONG PERIOD OF TIME OR IF YOU HAVE A REPLACEMENT PROBE**]

1. Switch the meter on. Press MODE to select mg/1(ppm) Measurement mode.

2. **Press the CAL/MEAS key to enter mg/1(ppm) calibration mode.** The CAL indicator will appear above the primary display.

3. **While in mg/1(ppm) calibration mode, press the MODE key to enter temperature calibration mode**. The primary display shows the last set of temperature reading and the secondary display shows you the factory default temperature value.

4. **Compare the primary display reading to a NIST-traceable thermometer or another thermometer known to be accurate.**

5. **Press the up and down arrow keys** to adjust the primary display reading to agree with your temperature standard.

6. **Press the ENTER key** to confirm temperature calibration and return to Measurement mode.

Note: To exit from Temperature calibration mode without confirming calibration, DO NOT press ENTER in step 6. Press CAL/MEAS instead.

Appendix 8. Field Sheet

Russian River Pathogen Project Field Data Sheet (UC Davis)					
StationID:	Date (mm/dd/yyyy): / /				
Sampling Crew:	ArrivalTime:	DepartureTime:		Sample Time:	
Field Observations		WADEABILITY: YES / NO			WIND DIRECTION (from):
DOMINANTSUBSTRATE:	Concrete,Cobble,Gravel,Sand,Mud,Other_____,unk				
SITE ODOR:	None,Sulfides,Sewage,Petroleum,Mixed,Other_____	SKY CODE:		Clear, Partly Cloudy, Overcas	
OTHERPRESENCE:	Vascular,Nonvascular,OilySheen,Foam,Trash,Other_____	PRECIPITATION:		None, Foggy, Drizzle, Rain, S	
WATERODOR:	None, Sulfides, Sewage, Petroleum, Mixed, Other_____	PRECIPITATION (last 24 hrs):		Unknown, <1	
WATERCLARITY:	Clear (see bottom), Cloudy (>4" vis), Murky (<4" vis)	WATERCOLOR:		Colorless, Green, Yellow, Bro	
OBSERVED FLOW:	NA, Dry Waterbody Bed, No Observed Flow, Isolated Pool, 0.1 - 1cfs, 1 - 5 cfs, 5 - 20 cfs, 20 - 50 cfs, 50 - 200 cfs, >200cfs				
Comments:					

Sample Information			
EVENT TYPE:	WaterTox_Chem, WaterChem, WaterTox	SAMPLE TYPE:	Grab / Integrated
OCCUPATION METHOD:	Walk-in, Bridge, Boat, Other _____	STARTING BANK:	LB / RB/ NA
SAMPLINGEQUIPMENT:	Indiv bottle by hand, By pole, Teflon tubing, Kemmer, Pole & Beaker, Other _____		
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water	HYDROMODLOC(to sample):	US / DS / NA
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert, Other _____		

GPS/DGPS

Target:

Actual:

GPS Model:

List of Samples Collected/Analyses

	DepthCollec (m)	E. Coli	E. Coli (dup)	E. Coli (blank)	Enterococcus	Enterococcus (dup)	Enterococcus (blank)	Bacteroidales
Sub/Surface	0.1	X			X			X

Comments:

Probe Measurements (Field Measurements)

	DepthCollec (m)	Air Temp (°C)	Water Temp (°C)	pH	Specific Conductivity (uS/cm)
Sub/Surface	0.1	0.1	0.1	0.1	0.1
Instrument:	NA				
Calib. Date:	NA				