TO: File: Russian River; TMDL Development and Planning

FROM: Steve Butkus

DATE: January 15, 2013

SUBJECT: VARIABILITY ASSESSMENT OF FECAL INDICATOR BACTERIA SAMPLING

The North Coast Regional Water Board staff are developing Russian River Total Maximum Daily Loads (TMDLs) for pathogen indicators to identify and control contamination impairing recreational water uses. Potential pathogen contamination has been identified in the lower and middle Russian River watershed leading to the placement of waters within these areas on the federal Clean Water Act Section 303(d) list of impaired waters. The contamination identified has been linked to impairment of the contact recreation (REC-1) and non-contact recreation (REC-2) designated beneficial uses. Health advisories for these waters have been published and posted by Sonoma County and the City of Santa Rosa authorities.

Regional Water Board staff conducted a source analysis study for the development of the Russian River Pathogen TMDL. The study was organized into individual tasks and sampling plans designed to collect information which will address the identified management questions (Fadness and Butkus 2011). Task 1 evaluated the temporal and spatial variability of fecal indicator bacteria (FIB) at high use public recreation beaches. Tasks 2 and 3 evaluated the influence of land use and beach recreational use on FIB concentrations. *Enterococcus*, *Escherichia coli (E. coli)* and total coliform bacteria concentrations were measured by the Regional Water Board Microbiology Laboratory using the IDEXX, Enterolert® and Colilert® microbial tests. *Enterococcus* bacteria concentrations were also measured by the Sonoma County Public Health Laboratory using the multiple tube fermentation test (Standard Method 9010). Results of the study were documented in a report by the NCRWQCB (2012).

The following assessments of FIB variability are presented in this memorandum:

- Censored Data Estimation
- Data Transformation
- Analytical Methods Variability
- Intra-Analytical Laboratory Variability
- Inter-Analytical Laboratory Variability
- Sample Temporal Variability
- Sample Spatial Variability

Censored Data Estimation

Collected samples were analyzed at a 10% dilution factor to measure the FIB concentrations in the range of 10 to 24,196 Most Probable Number (MPN)/100mL. Based on previous sampling, we expected that the FIB concentrations would be within the analytical method range for this dilution factor. However, the results showed that many of the samples collected were actually below the analytical reporting limit of 10 MPN/100mL after the 10% dilution factor was applied. Measurements analyzed below the reporting unit are called "censored" data (Helsel and Hirsch 2002). Estimates of summary statistics, which best represent the entire distribution of data, both below and above the reporting limit, are necessary to accurately analyze environmental conditions. Unbiased estimates of the censored data are needed to assess the spatial and temporal variation in measured FIB concentrations.

Numeric methods exist that allow the estimate of summary statistics from data sets that include censored values. One commonly used method is simple substitution. Simple substitution methods replace a single value such as one-half the reporting limit for each less-than value. Summary statistics are calculated using both these artificial numbers along with the values above the reporting limit. The simple substitution method is widely used, but has no theoretical basis because it assumes something is known (the value for nondetects) that really is not known. Studies have determined that simple substitution methods performed poorly in comparison to other procedures. Substitution of zero produced estimates of mean and median which were biased low, while substituting the reporting limit resulted in estimates above the true value (Helsel and Hirsch 2002).

Regression on order statistics (ROS) was applied to estimate censored data prior to use in statistical hypothesis tests. ROS is based on the modified probability plotting (Helsel 1990; Helsel and Cohn 1988). The method is also known as Helsel's Robust Method. The approach fits a regression line to log transformed observation values above the reporting limit against their standard score and Wiebull plotting positions. The regression line is used to estimate the values of each censored value. The data are then transformed back to the measurement unit. The fitted distribution is used only to extrapolate the measurement values below the analytical reporting limit. These extrapolated values are not considered estimates for specific samples, but are only used collectively to estimate summary

statistics. The method is considered robust since the estimates are made from observed data rather than a fitted distribution above the reporting limit. The method also avoids bias from data transformation since the summary statistics are calculated in original measurement units. The censoring percentage can be as high as 80% (Helsel, 2005).

Data Transformation

Some of the statistical tests applied to evaluate the compiled data require that the distribution meets certain criteria. These parametric statistical methods require that the data distributions meet four assumptions:

1. linearity of the relationship between dependent and independent variables
2. independence (i.e., no serial correlation)
3. homoscedasticity (constant variance)
4. normal data distribution

If any of these assumptions are not met, then the results of the parametric statistical test may be biased or misleading. FIB data often do not meet the assumption of a normal distribution and often exhibit a log-normal distribution. These log-normal distributions can be transformed to a normal distribution by using the logarithm of the measured FIB concentration. The log-transformed data become normally distributed to meet the assumption required for valid application of parametric statistical methods. Figures 1 – 6 show the effect of base-10 logarithmic transformation on the data distribution for the FIB concentrations. Figures 1, 3 and 5 compare the distribution of the distribution of the untransformed data to a normal distribution. Figures 2, 4, and 6 present the same comparison after logarithmic transformation. The visual comparison demonstrates that the log transformed FIB data distribution more closely approximate a normal distribution. The logarithmic transformation resulted in a data distribution that closely matches a normal distribution for each FIB.

Analytical Methods Variability

Multiple analytical laboratory procedures for pathogenic indicator bacteria in water samples have been approved as standard methods. Tests for coliform groups of bacteria have traditionally been conducted using the multiple-tube fermentation (MTF) procedure as a most probable number index (Standard Method 9010). IDEXX's Quanti-Tray Colilert® and Enterolert® procedures have been adopted as standard methods for monitoring recreational water quality by the U.S. Environmental Protection Agency (IDEXX, 2001; USEPA, 2003). The Colilert® and Enterolert® methods are relatively recent MPN procedures that produce results in 24 hours.

The IDEXX Quanti-Tray Colilert® and Enterolert® methods use an adaptation of the MTF procedure (Standard Method 9010) that provide results as a most probable number (Cochran 1950; Oblinger and Koburger 1975). The MPN does not provide an exact count of

FIB cells in a sample, but estimates the concentration based on the probability of incubation in replicated cultures across several serial dilution steps. The probability is based on the Poisson distribution for extreme values and has two assumptions: (1) The FIB organisms are distributed randomly throughout the sample, and (2) the sample will exhibit growth in the culture media whenever one or more of the FIB organisms are present (Greenwood and Yule 1971). With multiple dilutions and tubes, the statistical calculation of the MPN value becomes complex. Instead, the IDEXX methods apply an approximation that can be used for any combination of dilutions and tubes (Thomas 1942). The IDEXX Quanti-Tray®/2000 methods apply a table based on the number of large and small incubation cells showing a positive culture. Counting the number of positive tubes provides the MPN and the 95% confidence levels for the FIB concentration estimate.

The California Department of Public Health has issued guidance on listing freshwater beaches using Enterolert® analyses (CDHS 2006). Enterolert® analyses from freshwater samples can result in false positive measurements due to potential interference from algae (SYRCL, 2002). To assess the difference between the analytical methods, replicate samples were collected at each sample location and analyzed for *Enterococcus* bacteria concentration using both MTF (Standard Method 9010) and IDEXX's Enterolert® procedure.

The sampling locations and results of the *Enterococcus* bacteria concentration analyses are presented in Tables 1 and 2. The multiple tube fermentation tests resulted in high percentages of measurements below the analytical reporting limit. Samples collected at Healdsburg Memorial Beach and Monte Rio Beach resulted in 100% and 96% censored data, respectively, when measured with the multiple tube fermentation test. ROS cannot be used to estimate censored data at these high percentages. Samples collected from Santa Rosa Creek resulted in 75% censored data, just below the 80% threshold for acceptable application of the ROS method. Therefore, only the data collected from Santa Rosa Creek were used to assess the difference between the two analytical methods after applying the ROS method to estimate censored data.

Figure 7 shows a comparison of the distribution of *Enterococcus* bacteria concentrations measured by the different analyses and laboratories. The visual comparison shows that the *Enterococcus* bacteria concentration distributions are similar between the Enterolert® analyses by Regional Water Board Microbiology Laboratory and the MTF analyses of the Sonoma County Public Health Lab. The Enterolert® analyses conducted by Sonoma County Public Health Lab show much higher *Enterococcus* bacteria concentrations than the other two analyses.

The Mann-Whitney U statistical hypothesis test was applied to assess the difference between the distributions of FIB concentrations measured by the different analytical methods. The Mann-Whitney U Test is a non-parametric test for assessing whether two samples of observations come from the same distribution (Helsel and Hirsch 2002). The method is also known as the Wilcoxon Rank-Sum test. The test null hypothesis is that the

two samples are drawn from a single population. The test requires the two samples to be independent and the observations to be continuous measurements. The test is similar to performing an ordinary parametric two-sample t test, but is based on ranking the data set. This statistical test is a nonparametric (i.e., distribution-free) inferential statistical method. The test makes no assumption of the frequency distributions. Nonparametric methods are the most appropriate approach for assessing water quality data which can have widely varying frequency distributions.

The results of the hypothesis tests confirm the visual observation of the box plots distribution. The test showed there be to a large statistical difference between Enterolert® analyses conducted by Sonoma County Public Health Lab and the Enterolert® analyses by Regional Water Board Microbiology Laboratory ($U = 4.5$, $p < 0.0001$)[1] and the MTF analyses of the Sonoma County Public Health Lab ($U = 6$, $p < 0.0001$). The hypothesis tests also showed no statistical difference between the Enterolert® analyses by Regional Water Board Microbiology Laboratory and the MTF analyses of the Sonoma County Public Health Lab ($U = 359.5$, $p < 0.9362$).

Intra-Analytical Laboratory Variability

The IDEXX Quanti-Tray®/2000 methods provide the MPN and the 95% confidence levels for the FIB concentration estimates. Figures 8 – 10 present a comparison of the MPN value with the 95% confidence interval for the analytical method. The best fit lines shown in the figures were derived using non-linear regression. All three FIB showed an explained variance greater than 90%. For each indicator, the measurement variability increases with increasing FIB concentration. FIB measurements of samples analyzed at different dilutions are shown separately to demonstrate the effect of sample dilution on the variability of the FIB concentration MPN estimate. Most samples were analyzed at a 10% dilution factor to measure the FIB concentrations in the expected range of 10 to 24,196 MPN/100mL. However, based on the many censored results received during the routine sampling, some of the analyses were conducted at different dilution factors to avoid the reporting of censored results. Figure 10 best demonstrates the effect the sample dilution on the confidence of the FIB concentration estimates. The 10% dilution resulted in a larger confidence interval (i.e., less accuracy) than samples analyzed with no dilution. The effect is diminished at higher FIB concentrations.

Knowledge of analysis method variability is most important near the threshold for advising the public on the impairment of primary and secondary contact recreation (i.e., REC beneficial use). The U.S. Environmental Protection Agency (USEPA) has recently issued FIB concentration criteria for beach notification purposes (USEPA 2012). The criteria are for *Enterococcus* and *E. coli* bacteria concentrations analyzed with culture methods. The

[1] *U is the Mann-Whitney U test statistic, p is the probability that one population is different than another population.*

criteria identify a Beach Action Value (BAV) to be used with a single FIB sample to inform public health authorities whether a beach swimming advisory should be posted for public notification. BAV criteria are presented for the same estimated illness rate as the USEPA (1986) criteria (i.e., 36 illnesses per 1000 primary contact recreators). The USEPA advises that any single sample above the BAV could trigger a beach notification until another sample below the BAV is collected. The BAV criteria are presented in FIB concentration units of colony-forming units (cfu)/100mL, and not the MPN concentration units resulting from the IDEXX, Enterolert® and Colilert® microbial tests. The USEPA recommends using the membrane filtration technique to measure *Enterococcus* bacteria (USEPA 2002a) and *E. coli* bacteria (USEPA 2002b). Large differences are often observed between FIB concentrations when measured by MPN estimates versus cfu estimates (Gronewold and Wolpert 2008).

The USEPA (2012) advises that any single sample above the BAV could trigger a beach notification. However, this assumes that the single FIB sample is representative of the actual ambient FIB concentrations. The BAV criteria does not account for the variability of the IDEXX Quanti-Tray Colilert® and Enterolert® methods. For MPN estimates that are close to the BAV criteria, the comparison of a single FIB sample to the BAV criterion only provides about a 50% level of confidence that the actual ambient FIB concentration is below the BAV criterion. In other words, , the actual ambient FIB concentration has a nearly equal chance of being higher than the BAV criterion when assessed with a single FIB sample.

Because a greater level of confidence is desirable, regression analysis was applied to measured *Enterococcus* and *E. coli* data to determine the value of an MPN estimate of an FIB concentration needed to assure compliance with the BAV values at 95% confidence. Regressions were conducted between the MPN estimates with the 95% upper confidence levels. The samples analyzed with no dilution were used for determining the needed *Enterococcus* bacteria concentration since the criteria are within the range of samples collected. The 10% dilution analyses were used for the *E. coli* bacteria concentration without the ROS estimates of censored measurements since these data largely influence the regression equation (Figure 8).

Table 3 shows the values that the MPN estimate of FIB concentration needs to be in order to meet the BAV criteria at a 95% confidence. The analysis assumes that the BAV criteria provided as cfu is equivalent to the MPN estimated value. These MPN values account for the analytical method variability, assuring that the actual ambient FIB concentrations are below the BAV criteria. As shown in Table 3, the MPN estimate of *E. coli* bacteria would have to be at or below 139 MPN/100mL to provide a 95% confidence that the actual ambient concentration is below the 235 cfu/100mL BAV criterion. Similarly, the MPN estimate of *Enterococcus* bacteria would have to be at or below 49 MPN/100mL to provide a 95% confidence that the actual ambient concentration is below the 70 cfu/100mL BAV criterion.

Inter-Analytical Laboratory Variability

Laboratories following standard method procedures develop quality assurance information on the variability of the laboratory procedures for indicator bacteria measurements (i.e., Standard Method 9020B.4). Variability has been observed between split samples sent to different laboratories in other studies (Griffith et al. 2006). A small study conducted by the North Coast Regional Water Board at Monte Rio Beach has shown that there were differences between the results of concurrently collected samples analyzed by different laboratories. However the sample size was inadequate to allow a statistical comparison. To assess variability that may be reported between different laboratories, concurrently collected samples (i.e., collected within a 1-minute timeframe) were analyzed for FIB concentrations by both the Sonoma County Public Health Laboratory and the Regional Water Board Microbiology Laboratory. The samples were collected to assess any inter-laboratory variability.

Enterococcus, *E. coli* and total coliform bacteria concentrations were measured at both laboratories using the IDEXX Quanti-Tray Enterolert® and Colilert® microbial tests. Four samples were concurrently collected at each location and three samples were analyzed by the Regional Water Board Microbiology Laboratory and the fourth sample was analyzed by the Sonoma County Public Health Laboratory. A statistical approach using *Control Charts* was applied to assess if there was a significant difference in the results between laboratories. The statistical approach, also known as Process-Behavior Charts, is often used in manufacturing. The Control Chart is a statistical approach used to assess how a process changes over time. A Control Chart defines the upper and lower confidence limits based on historical data. The log-transformed FIB data were used for the application of the control chart method since it is a parametric method.

A control chart approach was used to compare the results from the fourth sample (analyzed at the Sonoma County Public Health Laboratory) to the three samples (analyzed at the Regional Water Board Microbiology Laboratory). The approach will test if the fourth sample result is significantly different the first three sample results. Censored values cannot be estimated for application in a Control Chart since the estimates cannot be considered for specific samples, but can only be used collectively in estimating summary statistics. Therefore, only samples with all four results above the reporting limit were used to assess inter-laboratory variability.

Table 4 presents the results of the Control Chart analysis. The upper and lower statistical control limits were established at two sample standard deviations to represent the 95% confidence interval. The results showed that *E. coli* bacteria concentrations measured by the Sonoma County Public Health Laboratory were significantly different than the results from the Regional Water Board Microbiology Laboratory in 31% of the analyses. *Enterococcus* bacteria concentrations measured by the Sonoma County Public Health Laboratory were significantly higher than the results from the Regional Water Board

Microbiology Laboratory in 43% of the analyses. Finally, total coliform bacteria concentrations measured by the Sonoma County Public Health Laboratory were significantly higher than the results from the Regional Water Board Microbiology Laboratory in 12% of the analyses.

Sample Temporal Variability

Samples collected at any location can vary temporally. One needs to know the variance over a short time (i.e., between samples collected at the same time and location) in order to compare between results from sampling events over time. The measured variation of replicate samples can be used to assess the serial variation between samples collected within the sampling time period. To assess this component of variability, three replicate samples were collected in rapid succession at each location sampled for Task 1. In addition, replicate samples were collected for some events for Tasks 2 and 3 of the study, and duplicate samples were collected for the Regional Water Board Microbiology Laboratory quality assurance requirements. All replication sample results were compiled to provide an estimate of temporal variability at a sample location.

Figures 11 – 13 present a comparison between the geometric mean of the replicate and the variability of the replicates described by the 95% confidence interval. All three FIB concentrations showed increasing variability with larger geometric means. In other words, the variability of a sample increases with higher FIB concentrations. The correlation between the replicate geometric mean and variability was assessed using the *Correlation Coefficient* (R). The correlation coefficient is a measure of the strength and direction of the linear relationship between two variables. The correlation between replicate geometric mean and variability was highest with total coliform bacteria (R = 70%), followed by *E. coli* bacteria (R = 43%) and *Enterococcus* bacteria (R = 28%).

In addition to analytical method variability, the knowledge of sampling variability is most important near threshold for advising the public on the impairment of primary and secondary contact recreation (i.e.,REC beneficial use). The USEPA (2012) advises that any single sample above the BAV could trigger a beach notification. However, this assumes that the single FIB sample is representative of the actual ambient FIB concentrations. The BAV criteria does not account for any sampling variability. For sample FIB values that are close to the BAV criteria, the comparison of a single FIB sample to the BAV criterion only provides about a 50% level of confidence that the actual ambient FIB concentration is below the BAV criterion. For sample FIB values that are close to the BAV criteria, the actual ambient FIB concentration has a nearly equal chance of being higher than the BAV criterion when assessed with a single FIB sample.

Regression analysis was applied to estimate the value a single FIB sample should be in order to assure compliance with the BAV values. Regressions were conducted between the geometric mean of replicate samples with several different upper confidence levels (i.e.,

80%, 90%, 95% and 99% confidence levels). The analysis assumes that the BAV criteria provided as cfu as equivalent to the MPN estimated value. Table 5 shows the values that a single FIB sample needs to be to meet the BAV criteria at different levels of confidence. These FIB values account for the sample variability to make certain that the actual ambient FIB concentrations are below the BAV criteria. To account for the sampling variability, the measurement of a single sample for *E. coli* bacteria measurement would have to be at or below 100 MPN/100mL to provide a 95% confidence that the actual ambient concentration is below the 235 cfu/100mL BAV criterion. Similarly, the measurement of a single sample for *Enterococcus* bacteria would have to be at or below 37 MPN/100mL to provide a 95% confidence that the actual ambient concentration is at or below the 70 cfu/100mL BAV criterion.

Sample Spatial Variability

Samples collected at any location can vary spatially. The variability of FIB concentrations across the general area of a sampling reach is needed in order to compare between different stream reaches. The measured variation of replicate samples can be applied to single sample FIB concentrations in order to make comparisons between single samples collected from other stream reaches. The sampling was conducted to provide an estimate of spatial variability at various stream reaches. To assess the variability of FIB concentrations at a stream reach, nine spatially distributed samples were collected at three different stream reaches in the watershed: Healdsburg Memorial Beach, Monte Rio Beach, and Santa Rosa Creek near Railroad Street. Triplicate samples were collected at each of nine locations at each stream reach: three longitudinal locations along the river channel (upstream ½ width of stream, at the center of the reach, downstream ½ width of stream) and at three locations along the lateral transect (off of far bank, mid-channel, off of near bank) (Table 1). *Enterococcus*, *E. coli* and total coliform bacteria concentrations were measured for each sample by the Regional Water Board Microbiology Laboratory using the IDEXX, Enterolert® and Colilert® microbial tests (Table 6).

The Kruskal-Wallis test statistical was used to assess if any sample showed a statistical difference between the nine samples collected at each stream reach. The Kruskal-Wallis test was also applied to assess if there was a significant difference longitudinally (i.e., upstream to downstream) or laterally (i.e., from bank-to-bank) at each site. The Kruskal-Wallis test is a one-way analysis of variance conducted using ranked data (Helsel and Hirsch 2002). It is an extension of the Mann–Whitney U test to three or more groups. This non-parametric method was used for testing if samples originate from the same distribution by assessing the equality of population medians among the groups. The parametric equivalent of the Kruskal-Wallis test is the one-way analysis of variance (ANOVA). When the Kruskal-Wallis test indicates significant results, then at least one of the samples is different from the other samples in the group. FIB concentrations were considered significantly different if the probability was lower than $\alpha = 0.05$.

Table 7 presents the results of applying the Kruskal-Wallis test to assess the difference between the FIB concentrations from the nine stream reach locations sampled. No significant difference between sampling locations within the reach were found for most FIB concentrations and stream reaches sampled. Only the total coliform bacteria concentrations at Healdsburg Memorial Beach showed a significant difference. The median FIB concentration at the downstream-right bank location was much lower than other locations within the reach. The stream flow exist the beach are at this location on the reach. The lower total coliform number may be due to an increase in stream velocity at this location as compared to other more quiescent areas of the beach.

Table 8 presents the results of applying the Kruskal-Wallis test to assess the difference between the FIB concentrations laterally (from bank-to-bank). No significant difference between sampling locations within the reach were found for most FIB concentrations and stream reaches sampled. Only the total coliform bacteria concentrations at Monte Rio Beach showed a significant difference. The median FIB concentrations along the left-bank locations were much higher than the center of the channel or the right-bank. The left-bank locations are where the public has direct access to the stream breach from the beach. The statistically higher total coliform concentrations along the beach shoreline may be due to increased human contact increasing the disturbance of the bottom sediment.

Table 9 presents the results of applying the Kruskal-Wallis test to assess the difference between the FIB concentrations longitudinally (from upstream-to-downstream). No significant difference between sampling locations within the reach were found for most FIB concentrations and stream reaches sampled. Only the total coliform bacteria concentrations at Healdsburg Memorial Beach showed a significant difference. The median FIB concentrations increase nearly 2-fold between the upstream and downstream sampling transects. The statistically higher total coliform concentrations along the channel thalweg (i.e. upstream to downstream) may be due to increased human contact from the public access to the beach.

Findings

The following findings were made from the assessments of FIB variability presented in this memorandum:

- Censored Data Estimation – Many of the FIB concentration measurements were reported below the analytical reporting limit. Estimates of these censored results were made to allow statistical comparisons between measured FIB concentrations.

- Data Transformation – The FIB concentrations showed a log-normal distribution. These data were transformed to normal space to conduct the parametric statistical methods used in this assessment.

- Analytical Methods Variability – A statistically significant difference was observed between Enterolert® analyses conducted by Sonoma County Public Health Lab and the Enterolert® analyses by Regional Water Board Microbiology Laboratory and the MTF analyses of the Sonoma County Public Health Lab. No statistically significant difference was observed between the Enterolert® analyses by Regional Water Board Microbiology Laboratory and the MTF analyses of the Sonoma County Public Health Lab.

- Intra-Analytical Laboratory Variability – The measurement variability increases with increasing FIB concentrations. The measurement variability also increases with sample dilutions before analysis. The effect of dilutions on the FIB concentration estimate is diminished at higher FIB concentrations. To account for the analytical variability of the IDEXX Quanti-Tray Colilert® and Enterolert® analytical methods, the MPN estimate of *E. coli* bacteria would have to be at or below 139 MPN/100mL to provide a 95% confidence that the actual ambient concentration is below the 235 cfu/100mL BAV criterion. Similarly, the MPN estimate of *Enterococcus* bacteria would have to be at or below 49 MPN/100mL to provide a 95% confidence that the actual ambient concentration is at or below the 70 cfu/100mL BAV criterion.

- Inter-Analytical Laboratory Variability - *E. coli* bacteria concentrations measured by the Sonoma County Public Health Laboratory were significantly higher than the results from the Regional Water Board Microbiology Laboratory in 31% of the analyses. *Enterococcus* bacteria concentrations measured by the Sonoma County Public Health Laboratory were significantly higher than the results from the Regional Water Board Microbiology Laboratory in 43% of the analyses. Total coliform bacteria concentrations measured by the Sonoma County Public Health Laboratory were significantly higher than the results from the Regional Water Board Microbiology Laboratory in 12% of the analyses. These differences were found between duplicate samples collected at the same time. It is recommended that split samples be analyzed by both laboratories to further evaluate the differences observed in the FIB concentration results.

- Sample Temporal Variability - The variability of a sample increases with higher FIB concentrations. The correlation between replicate geometric mean and variability was highest with total coliform bacteria (R = 70%), followed by *E. coli* bacteria (R = 43%) and *Enterococcus* bacteria (R = 28%). To account for the sampling variability, the measurement of a single sample for *E. coli* bacteria measurement would have to be at or below 100 MPN/100mL to provide a 95% confidence that the actual ambient concentration is below the 235 cfu/100mL BAV criterion. Similarly, the measurement of a single sample for *Enterococcus* bacteria would have to be at or below 37 MPN/100mL to provide a 95% confidence that the actual ambient concentration is at or below the 70 cfu/100mL BAV criterion. The sample variability includes the variability associated with the analytical method.

- Sample Temporal Variability - No significant difference between sampling locations within the reach were found for most FIB concentrations and stream reaches sampled. Only total coliform bacteria concentrations were found to be significantly different at a few locations: downstream at Healdsburg Memorial Beach and along the beach access bank at Monte Rio Beach. These differences in total coliform bacteria concentrations at may be due to increased human contact within the benthic sediments of the stream reach due to the public beach.

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TABLES

Table 1. Stream reach sampling locations.
(Stream bank orientation was defined by looking downstream).

Stream Reach	Location	Description
Santa Rosa Creek at Railroad St	SRRU	Right Bank, Upstream Transect
	SRCU	Channel Center, Upstream Transect
	SRLU	Left Bank, Upstream Transect
	SRRM	Right Bank, Center Transect
	SRCM	Channel Center, Center Transect
	SRLM	Left Bank, Center Transect
	SRRD	Right Bank, Downstream Transect
	SRCD	Channel Center, Downstream Transect
	SRLD	Left Bank, Downstream Transect
Healdsburg Memorial Beach	HMRU	Right Bank, Upstream Transect
	HMCU	Channel Center, Upstream Transect
	HMLU	Left Bank, Upstream Transect
	HMRM	Right Bank, Center Transect
	HMCM	Channel Center, Center Transect
	HMLM	Left Bank, Center Transect
	HMRD	Right Bank, Downstream Transect
	HMCD	Channel Center, Downstream Transect
	HMLD	Left Bank, Downstream Transect
Monte Rio Beach	MRRU	Right Bank, Upstream Transect
	MRCU	Channel Center, Upstream Transect
	MRLU	Left Bank, Upstream Transect
	MRRM	Right Bank, Center Transect
	MRCM	Channel Center, Center Transect
	MRLM	Left Bank, Center Transect
	MRRD	Right Bank, Downstream Transect
	MRCD	Channel Center, Downstream Transect
	MRLD	Left Bank, Downstream Transect

Table 2. Results of *Enterococcus* bacteria concentration using two analytical tests.

Stream Reach	Location	Enterolert® Test						Multiple Tube Fermentation Test		
		Sonoma Co. Public Health Lab			Region-1 Microbiology Laboratory			Sonoma Co. Public Health Lab		
		Replicate 1 (MPN/100mL)	Replicate 2 (MPN/100mL)	Replicate 3 (MPN/100mL)	Replicate 1 (MPN/100mL)	Replicate 2 (MPN/100mL)	Replicate 3 (MPN/100mL)	Replicate 1 (MPN/100mL)	Replicate 2 (MPN/100mL)	Replicate 3 (MPN/100mL)
Santa Rosa Creek at Railroad St	SRRU	97	216	121	10	10	<10	<20	20	20
	SRCU	97	63	171	<10	52	10	<20	80	<20
	SRLU	175	134	146	<10	52	41	20	<20	<20
	SRRM	41	86	122	20	20	10	<20	<20	<20
	SRCM	185	120	73	<10	<10	<10	<20	<20	<20
	SRLM	169	218	158	20	<10	10	<20	20	<20
	SRRD	52	95	62	<10	<10	<10	<20	20	40
	SRCD	52	84	85	<10	10	<10	<20	<20	<20
	SRLD	121	86	189	<10	10	<10	<20	<20	<20
Healdsburg Memorial Beach	HMRU	10	20	<10	<10	<10	<10	<20	<20	<20
	HMCU	20	30	10	<10	<10	<10	<20	<20	<20
	HMLU	20	41	10	<10	<10	<10	<20	<20	<20
	HMRM	<10	31	10	<10	10	<10	<20	<20	<20
	HMCM	<10	20	20	<10	<10	<10	<20	<20	<20
	HMLM	20	<10	<10	<10	<10	<10	<20	<20	<20
	HMRD	10	<10	10	<10	<10	<10	<20	<20	<20
	HMCD	10	<10	20	10	<10	<10	<20	<20	<20
	HMLD	<10	41	<10	10	10	10	<20	<20	<20
Monte Rio Beach	MRRU	<10	<10	10	<10	<10	<10	<20	<20	<20
	MRCU	<10	20	20	<10	<10	<10	<20	<20	<20
	MRLU	31	41	10	<10	<10	<10	20	<20	<20
	MRRM	<10	<10	<10	<10	<10	10	<20	<20	<20
	MRCM	20	<10	<10	<10	<10	<10	<20	<20	<20
	MRLM	20	20	20	<10	<10	<10	<20	20	<20
	MRRD	<10	<10	10	<10	<10	<10	<20	<20	<20
	MRCD	10	<10	<10	<10	<10	<10	<20	<20	<20
	MRLD	98	41	<10	<10	<10	10	<20	<20	<20

Table 3. Ninety-five Percent (95%) Confidence Level for a MPN estimate of FIB concentrations at the Beach Action Value (BAV) criteria

Threshold	***E. coli*** **(cfu/100ML)**	***Enterococcus*** **(cfu/100ML)**
Beach Action Value	235	70
95% Confidence Level	139	49

Table 4. Inter-Analytical Laboratory Results Comparison

FIB	Station	Date	Significant Difference between Lab Results
E. Coli	Alexander Valley Road	6/7/2011	No
	Camp Rose	6/7/2011	No
		6/14/2011	No
	Forestville Access Beach	6/7/2011	No
		6/14/2011	No
	Healdsburg Memorial Beach	6/7/2011	Yes
		6/14/2011	No
		6/21/2011	No
	Johnson's Beach	6/7/2011	No
		6/21/2011	Yes
	Steelhead Beach	6/7/2011	Yes
		6/14/2011	No
		6/21/2011	Yes
Enterococcus	Alexander Valley Road	6/7/2011	No
	Camp Rose	6/7/2011	No
	Forestville Access Beach	6/7/2011	Yes
	Healdsburg Memorial Beach	6/7/2011	Yes
	Johnson's Beach	6/7/2011	No
		6/21/2011	No
	Steelhead Beach	6/7/2011	Yes
Total Coliform	Alexander Valley Road	6/7/2011	No
	Camp Rose	6/7/2011	No
		6/14/2011	No
		6/21/2011	Yes
	Forestville Access Beach	6/7/2011	No
		6/14/2011	No
		6/21/2011	Yes
	Healdsburg Memorial Beach	6/7/2011	No
		6/14/2011	No
		6/21/2011	No
	Johnson's Beach	6/7/2011	No
		6/14/2011	No
		6/21/2011	No
	Steelhead Beach	6/7/2011	No
		6/14/2011	No
		6/21/2011	No

Table 5. Confidence Levels for a single FIB sample at the Beach Action Value (BAV) criteria

Threshold	***E. coli* (cfu/100ML)**	***Enterococcus* (cfu/100ML)**
Beach Action Value	235	70
80% Confidence Level	163	40
90% Confidence Level	131	38
95% Confidence Level	100	37
99% Confidence Level	47	37

Table 6. Measured FIB concentrations at each stream reach location

Stream Reach	Location	*E. coli*			*Enterococcus*			Total coliform		
		Replicate 1 (MPN/100mL)	Replicate 2 (MPN/100mL)	Replicate 3 (MPN/100mL)	Replicate 1 (MPN/100mL)	Replicate 2 (MPN/100mL)	Replicate 3 (MPN/100mL)	Replicate 1 (MPN/100mL)	Replicate 2 (MPN/100mL)	Replicate 3 (MPN/100mL)
Santa Rosa Creek at Railroad St	SRRU	309	301	185	10	10	<10	1,860	2,595	1,785
	SRCU	573	211	187	<10	52	10	3,873	1,552	1,850
	SRLU	337	211	336	<10	52	41	2,987	2,481	3,448
	SRRM	269	199	197	20	20	10	2,723	2,415	1,281
	SRCM	324	243	342	<10	<10	<10	2,098	2,098	1,935
	SRLM	355	187	199	20	<10	10	2,755	2,359	1,850
	SRRD	238	315	269	<10	<10	<10	673	3,076	2,382
	SRCD	256	288	211	<10	10	<10	2,755	3,076	2,310
	SRLD	187	231	218	<10	10	<10	2,046	1,935	1,553
Healdsburg Memorial Beach	HMRU	10	<10	<10	<10	<10	<10	1,607	1,211	1,439
	HMCU	10	10	<10	<10	<10	<10	1,455	1,785	1,291
	HMLU	<10	<10	10	<10	<10	<10	1,935	2,755	1,664
	HMRM	10	10	<10	<10	10	<10	1,396	1,658	1,211
	HMCM	<10	20	10	<10	<10	<10	2,382	2,046	1,658
	HMLM	<10	20	<10	<10	<10	<10	1,396	1,467	1,274
	HMRD	<10	<10	<10	<10	<10	<10	905	749	1,187
	HMCD	<10	<10	10	10	<10	<10	1,354	1,081	1,274
	HMLD	<10	<10	<10	10	10	10	1,483	884	860
Monte Rio Beach	MRRU	41	63	72	<10	<10	<10	1,467	1,178	1,334
	MRCU	20	20	10	<10	<10	<10	1,439	1,333	1,198
	MRLU	41	<10	10	<10	<10	<10	1,860	1,664	2,143
	MRRM	<10	<10	41	<10	<10	10	1,112	1,723	1,334
	MRCM	20	<10	30	<10	<10	<10	1,274	1,317	1,291
	MRLM	<10	10	10	<10	<10	<10	1,785	1,722	1,198
	MRRD	<10	10	41	<10	<10	<10	1,439	959	1,780
	MRCD	<10	10	30	<10	<10	<10	1,421	1,291	909
	MRLD	<10	10	20	<10	<10	10	1,515	1,597	1,664

Table 7. Assessment of the stream reach spatial variability of measured FIB concentrations

Stream Reach	Statistical Metrics	*E. coli*	*Enterococcus*	Total coliform
Santa Rosa Creek at Railroad St	Kruskal-Wallis Statistic	6.032	8.699	7.694
	Probability	0.644	0.368	0.464
	Significant Difference	No	No	No
Healdsburg Memorial Beach	Kruskal-Wallis Statistic	4.840	11.563	18.340
	Probability	0.775	0.172	0.190
	Significant Difference	No	No	**Yes**
Monte Rio Beach	Kruskal-Wallis Statistic	8.894	8.910	11.351
	Probability	0.351	0.350	0.183
	Significant Difference	No	No	No

Table 8. Assessment of the lateral (bank-to-bank) spatial variability of measured FIB concentrations

Stream Reach	Statsitical Metrics	*E. coli*	*Enterococcus*	Total coliform
Santa Rosa Creek at Railroad St	Kruskal-Wallis Statsitic	0.680	1.076	0.494
	Probability	0.712	0.584	0.781
	Significant Difference	No	No	No
Healdsburg Memorial Beach	Kruskal-Wallis Statsitic	1.223	2.598	2.839
	Probability	0.543	0.273	0.242
	Significant Difference	No	No	No
Monte Rio Beach	Kruskal-Wallis Statsitic	2.788	1.273	9.693
	Probability	0.248	0.529	0.008
	Significant Difference	No	No	**Yes**

Table 9. Assessment of the longitudinal (upstream-to-downstream) spatial variability of measured FIB concentrations

Stream Reach	Statsitical Metrics	*E. coli*	*Enterococcus*	Total coliform
Santa Rosa Creek at Railroad St	Kruskal-Wallis Statsitic	6.032	8.699	7.694
	Probability	0.644	0.368	0.464
	Significant Difference	No	No	No
Healdsburg Memorial Beach	Kruskal-Wallis Statsitic	4.840	11.563	18.340
	Probability	0.775	0.172	0.190
	Significant Difference	No	No	**Yes**
Monte Rio Beach	Kruskal-Wallis Statsitic	8.894	8.910	11.351
	Probability	0.351	0.350	0.183
	Significant Difference	No	No	No

FIGURES

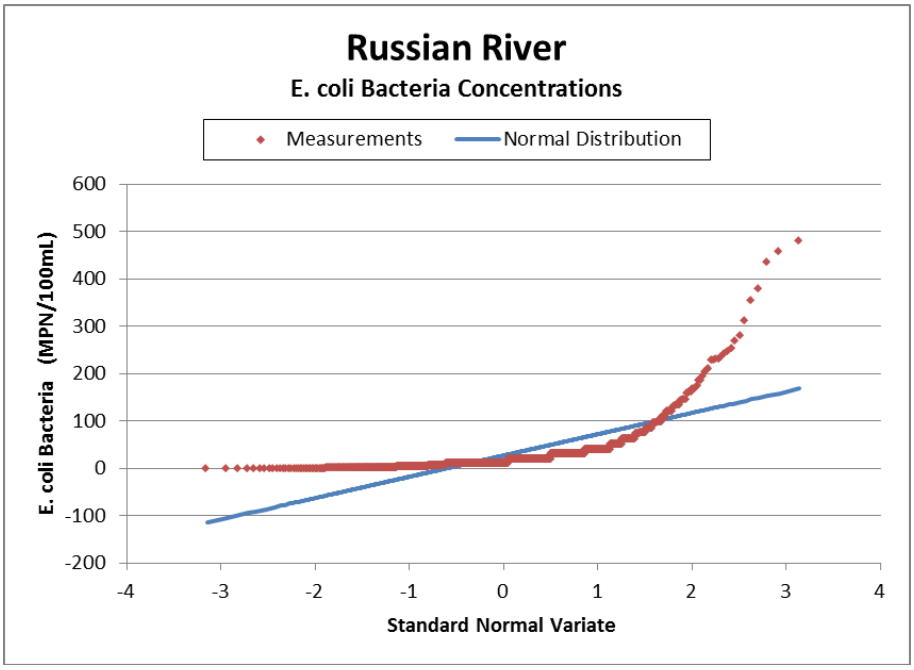

Figure 1. Comparison of *E. coli* Bacteria Measurements to a Normal Distribution

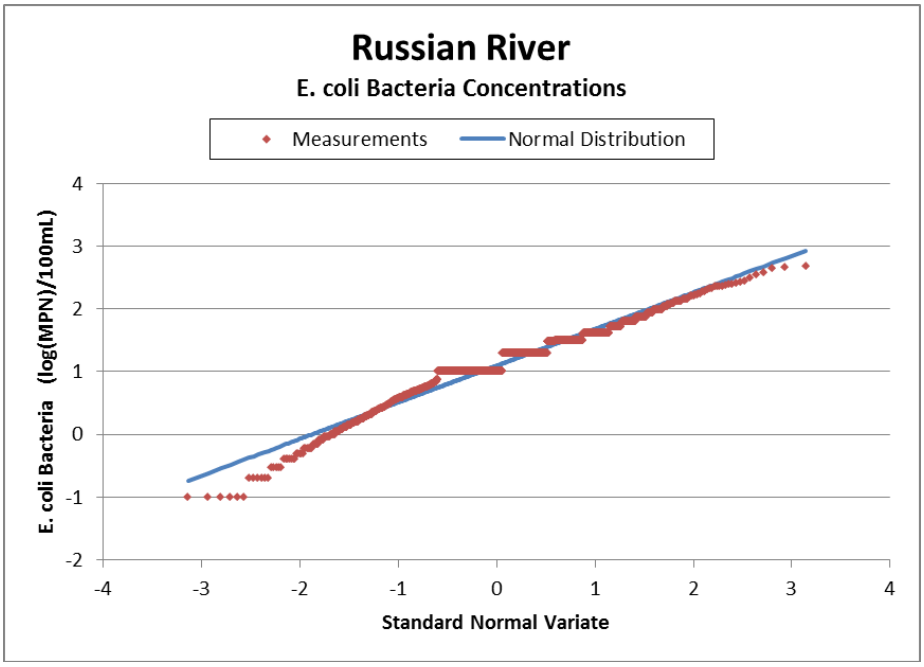

Figure 2. Comparison of logarithmically transformed *E. coli* Bacteria Measurements to a Normal Distribution

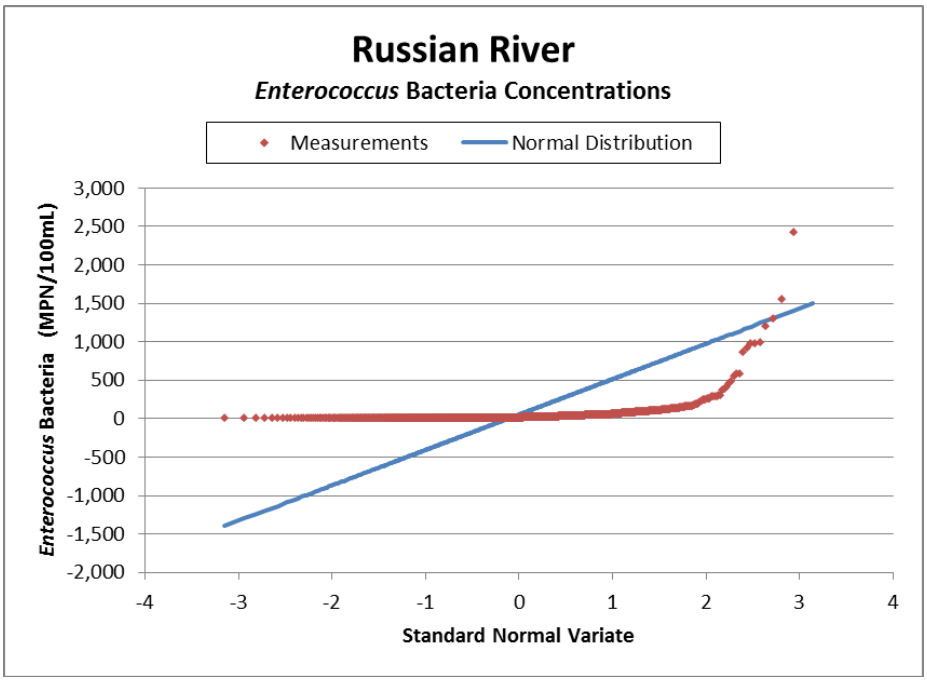

Figure 3. Comparison of *Enterococcus* Bacteria Measurements to a Normal Distribution

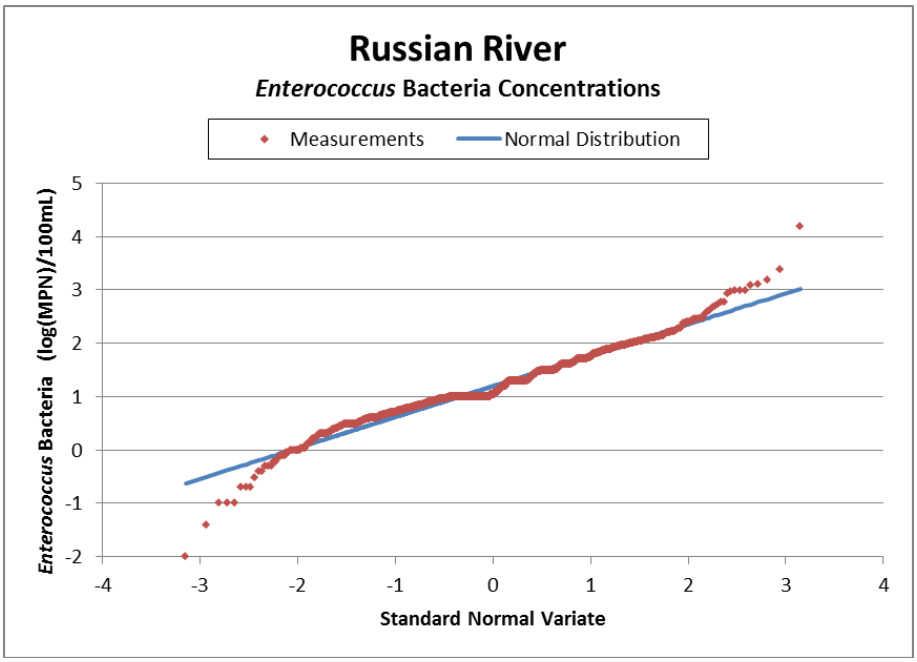

Figure 4. Comparison of logarithmically transformed *Enterococcus* Bacteria Measurements to a Normal Distribution

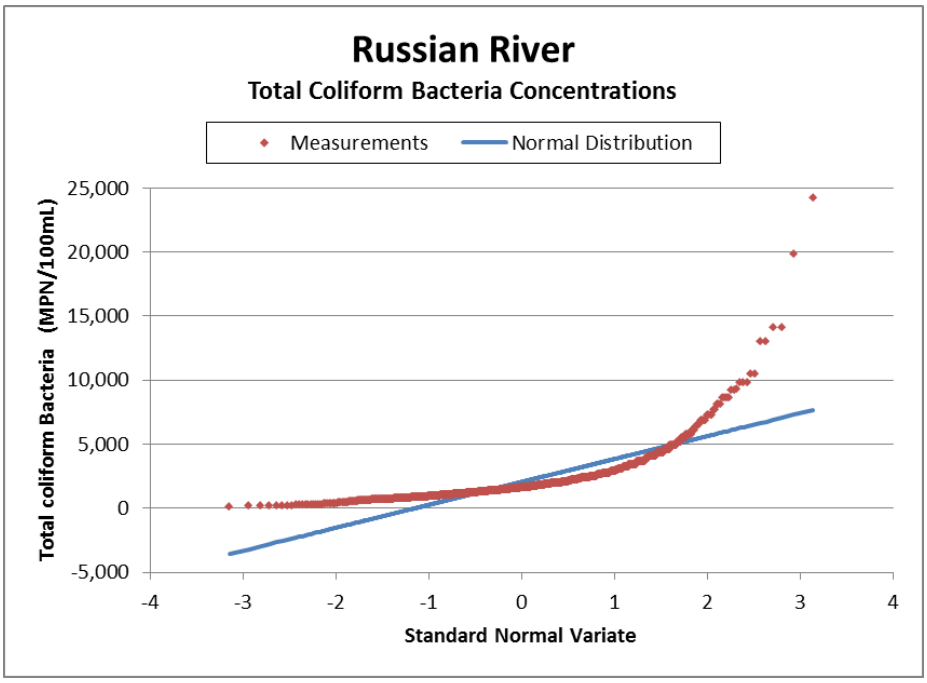

Figure 5. Comparison of Total Coliform Bacteria Measurements to a Normal Distribution

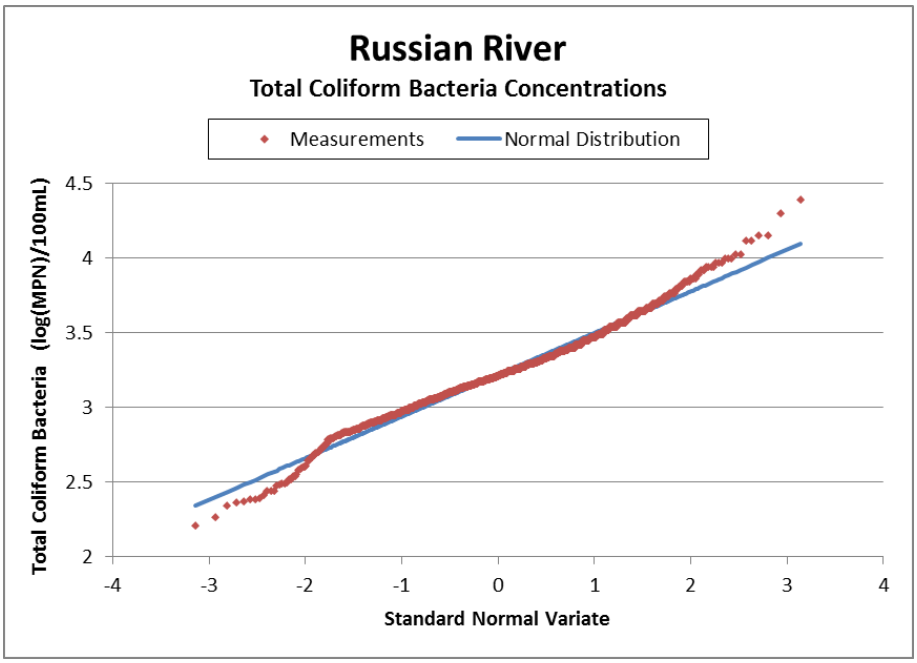

Figure 6. Comparison of logarithmically transformed Total Coliform Measurements to a Normal Distribution

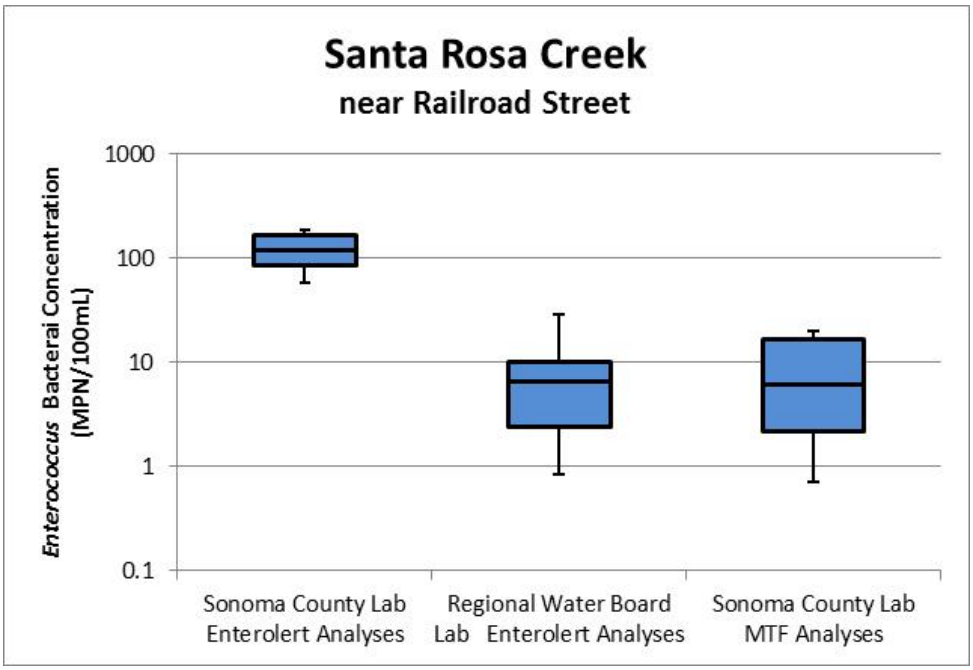

Figure 7. Distribution Comparison of the Analytical Measurements of *Enterococcus* Concentrations in Santa Rosa Creek near Railroad Creek on September 1, 2011.

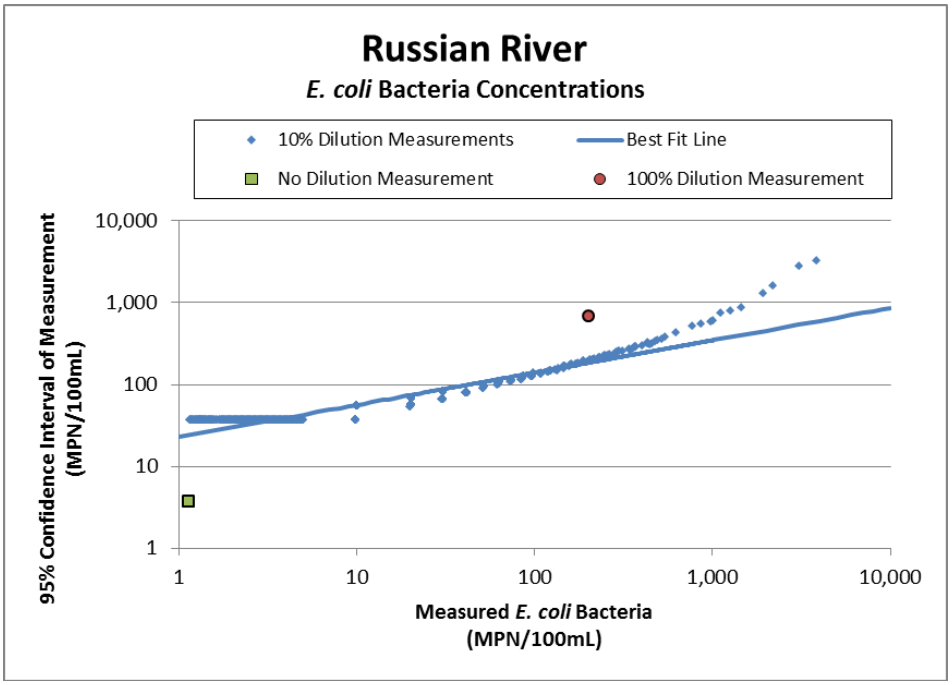

Figure 8. Comparison of *E. coli* Bacteria Measurements with the 95% Confidence Interval for the IDEXX Quanti-Tray Colilert® Analytical Method
(Explained variance = 94%).

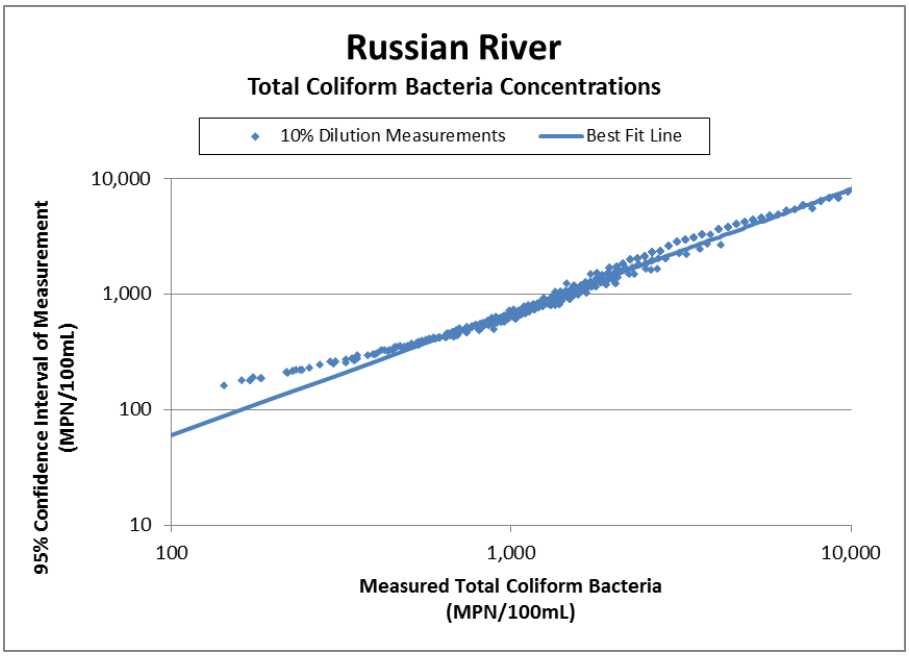

Figure 9. Comparison of Total Coliform Bacteria Measurements with the 95% Confidence Interval for the IDEXX Quanti-Tray Colilert® Analytical Method (Explained variance = 99%).

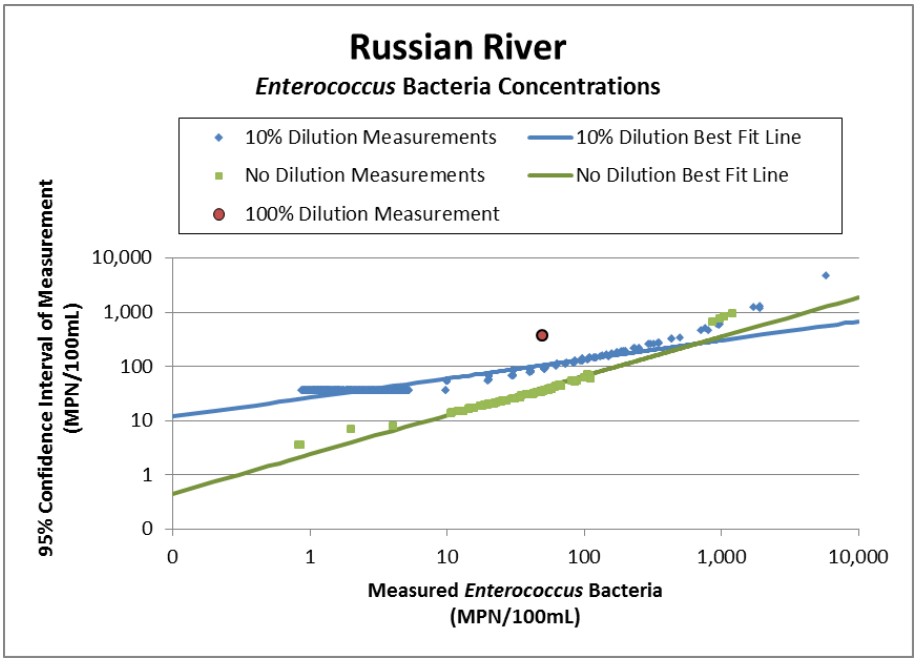

Figure 10. Comparison of *Enterococcus* Bacteria Measurements with the 95% Confidence Interval for the IDEXX Quanti-Tray Enterolert® Analytical Method (Explained variance: No Dilution = 97%; 10% Dilution = 92%).

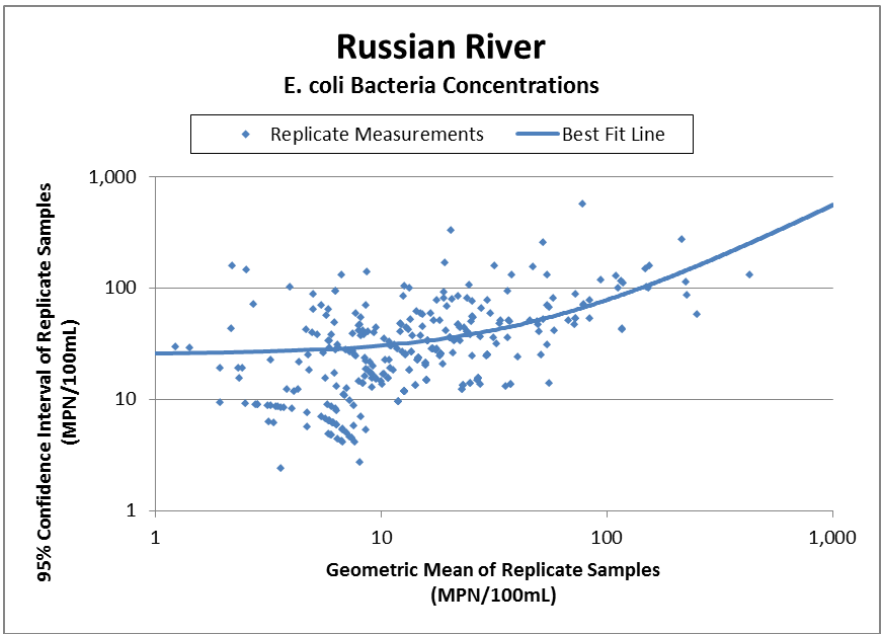

Figure 11. Comparison of the *E. coli* bacteria geometric mean of replicate samples to the variability of the samples. (Correlation coefficient = 43%)

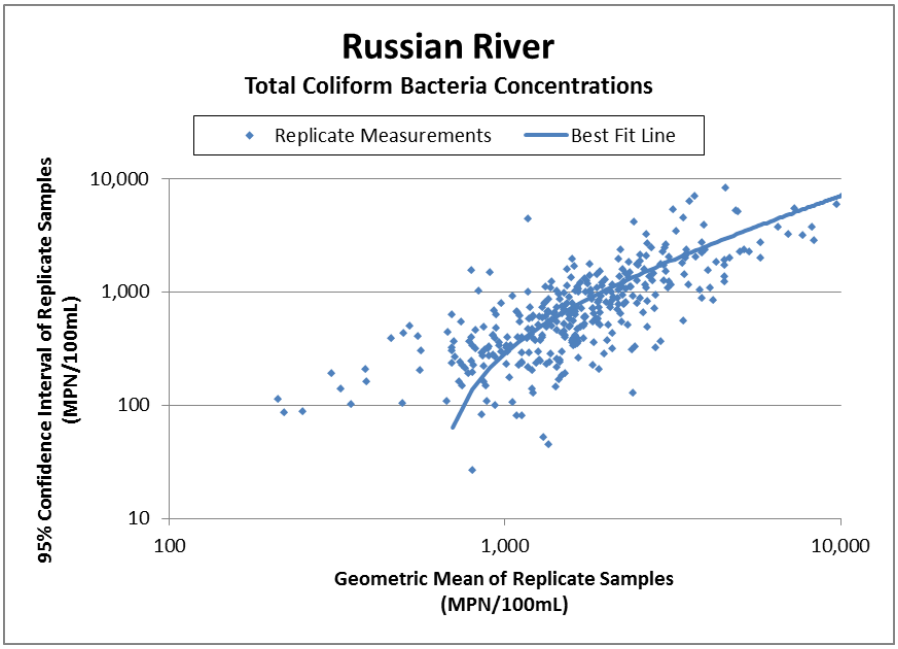

Figure 12. Comparison of the Total coliform bacteria geometric mean of replicate samples to the variability of the samples. (Correlation coefficient = 70%)

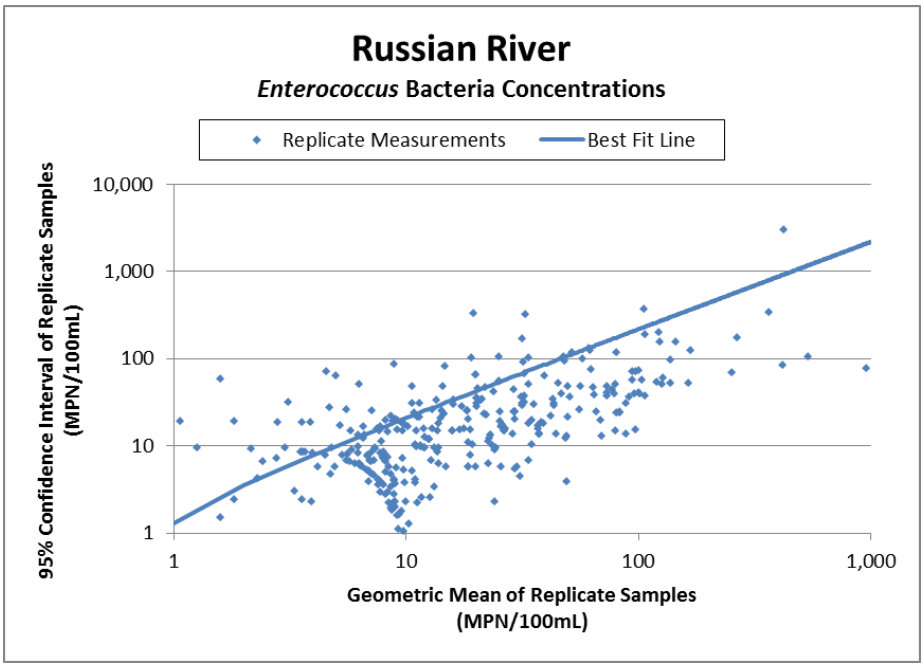

Figure 13. Comparison of the *Enterococcus* bacteria geometric mean of replicate samples to the variability of the samples. (Correlation coefficient = 28%)