California Regional Water Quality Control Board
North Coast Region

Russian River Pathogen TMDL

Upper Russian River
Fecal Indicator Bacteria
Monitoring Report

November 2013

INTRODUCTION

The North Coast Regional Water Board staff are developing the Russian River Total Maximum Daily Loads (TMDLs) for pathogen indicators to identify and control contamination. Potential pathogen contamination has been identified in three areas of the lower and middle Russian River watershed (Hydrologic Units 114.10 and 114.20). Identification of the contamination led 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 have been published and/or posted by Sonoma County and City of Santa Rosa authorities.

North Coast Regional Water Board staff and the Sonoma County Water Agency have measured fecal indicator bacteria (FIB) concentrations throughout the lower and middle Russian River watershed, downstream of Hopland (NCRWQCB 2012). These data are being used to support the development of the watershed-wide Russian River Pathogen Indicator TMDL. Water samples have been collected for measurement of FIB concentrations since 2001 at various locations from the mainstem Russian River. Until 2013, water samples had not been collected in the upper Russian River watershed (i.e., upstream of Hopland).

During August of 2013, Regional Water Board staff collected water samples from the upper Russian River watershed to compare FIB concentrations there with concentrations measured in the lower and middle watershed. A Sampling Plan (NCRWQCB 2013) and a Quality Assurance Project Plan (Butkus 2012) were developed that detailed the water sample collection and analysis of the *E. coli*, *Enterococcus*, and *Bacteroides* bacteria concentrations from water samples collected in the upper Russian River watershed.

MONITORING QUESTION

The measurement of FIB concentrations in the upper Russian River was designed to answer the following management question:

- Are similar fecal indicator bacteria concentrations observed in the mainstem of the Russian River?

WATER SAMPLING

Water samples were collected for analysis of *E. coli*, *Enterococcus*, All *Bacteroides*, and human-host *Bacteroides* bacteria concentrations. Water samples were collected at five (5) locations in the upper Russian River mainstem and one (1) from the East Fork Russian River (Table 1). Water samples were collected on August 14, 21, and 28, 2013. Triplicate samples were collected once during each sampling event to assess variability of replicate samples.

ASSESSMENT METHODS

The measured FIB concentrations were used to assess whether FIB concentrations measured in the upper Russian River were significantly different than FIB concentration measurements in the middle and lower Russian River.

Visual comparisons and statistical hypothesis tests were made between different groupings of the measured FIB concentrations and other metrics. Distributions of the measured FIB concentrations are compared visually using box and whisker plots. The boxes represent the interquartile range of the distribution around the median and the whiskers represent the 10th and 90th percentiles. Measurement results that were reported as below the analytical detection limit are shown in the figures as ½ the detection limit.

A nonparametric (i.e., distribution-free) inferential statistical method was used to assess differences between FIB concentrations in the upper, middle, and lower Russian River. The nonparametric hypothesis test makes no assumption about the frequency distributions of the measured data. Nonparametric methods are the most appropriate approach for assessing water quality data, which can have widely varying frequency distributions (Helsel and Hirsch 2002).

The Mann-Whitney U test is a non-parametric hypothesis test for assessing whether two samples of observations come from the same distribution (Helsel and Hirsch 2002). The test null hypothesis is that the two samples are drawn from a single population. 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 inferential statistical method that makes no assumption about the frequency distributions.

The Mann-Whitney U statistical test was applied to assess the difference between the measurements of FIB concentrations in the upper, middle, and lower Russian River. The hypothesis tests were considered statistically significantly different if the resulting probability of rejecting the null hypothesis (H_o) was equal or lower than $\alpha = 0.05$.

RESULTS

The FIB concentration measurements are presented in the following series of tables and figures. In several figures*, E. coli* and *Enterococcus* bacteria concentrations are visually compared to the U.S. Environmental Protection Agency (USEPA 2012) guidelines for posting swimming advisories at beaches (i.e., Beach Action Values). Based on a presumptive risk of 36 illnesses per 1000 recreators, the swimming advisory guidelines presented for *E. coli* and *Enterococcus* bacteria concentrations are 235 and 70 cfu/100mL, respectively.

The USEPA (2012) criteria are expressed as colony-forming units per sample volume (cfu/100mL) based on membrane filtration methods (USEPA 2002a; USEPA 2002b). Many laboratories, including the Regional Water Board Microbiology Laboratory, use a different analysis method to measure *E. coli* (and *Enterococcus*) bacteria concentrations (IDEXX 2001). Two of these methods, (Colilert® and Enterolert® Quanti-Tray/2000) have been shown to produce equivalent results as the membrane filtration methods (Budnick et al. 1996; Yakub et al. 2002) and have been approved by the USEPA for analysis and sampling under the Clean Water Act.

The following results are presented:

- FIB concentrations (i.e., *E. coli*, *Enterococcus*, All *Bacteroides*, and human-host *Bacteroides* bacteria) are presented in Table 2 (pages 7 – 8) and in Figures 1 – 5 (pages 11 - 13).

- Results for tests of statistically significant differences between FIB concentrations collected in the upper Russian River and the lower-middle Russian River watersheds are presented for mainstem and tributary locations in Tables 3 – 4 (page 9) and Figures 6 – 15 (pages 14-18).

FINDINGS

Based on the assessments of FIB concentrations presented in this report, Regional Water Board staff can make the following findings:

- All *E. coli* and *Enterococcus* bacteria concentrations measurements in the East Fork Russian River (in Potter Valley) exceeded the USEPA (2012) Beach Action Values used to advise the possible risk of illness for swimming recreators.

- Several *Enterococcus* bacteria concentrations measurements in the mainstem Russian River at East School Way (Redwood Valley) and at Lake Mendocino Drive (northern Ukiah) also exceeded the USEPA (2012) Beach Action Values.

- High concentrations of All *Bacteroides* bacteria were observed in the East Fork Russian River (in Potter Valley), but with a relatively low percentage (0.5%) from human-host *Bacteroides* bacteria.

- Higher relative concentrations of human-host *Bacteroides* bacteria were observed in the middle and southern Ukiah locations as compared to locations in the northern Ukiah locations.

- *E coli* bacteria measurements collected in the upper Russian River mainstem locations were significantly higher than measurements collected in the middle and lower locations, downstream of Hopland, during August 2013.

- All *Bacteroides* bacteria measurements collected in the upper Russian River mainstem locations were significantly lower than measurements collected in the middle and lower locations, downstream of Hopland, during August 2013.

- No significant difference was observed in FIB concentrations from measurements in Russian River tributaries in the upper and lower-middle watershed locations. The box-and-whisker plots visually show large differences that were not statistically significant. The hypothesis test had a low power to detect a significant difference due to the small sample size (i.e., only 3 samples were collected on the East Fork to represent upper Russian River watershed tributary locations).

CITATIONS

Budnick, G.E., Howard, R.T., and D.R. Mayo. 1996. Evaluation of Enterolert for enumeration of enterococci in recreational waters. Applied and Environmental Microbiology 62(10): 3881–3884.

Butkus, S. 2012a. Russian River Human Impact Study Quality Assurance Project Plan. North Coast Regional Water Quality Control Board, Santa Rosa, CA.

Helsel, D.R. and R.M. Hirsch. 2002. Statistical Methods in Water Resources, Techniques of Water Resources Investigations, Book 4, Chapter A3, 510 p. U.S. Geological Survey, Washington DC. Available at http://water.usgs.gov/pubs/twri/twri4a3/

IDEXX. 2001. Colilert® and Enterolert® Test Pack Procedures IDEXX Laboratories, Inc., Westbrook, Maine. (http://www.idexx.com/view/xhtml/en_us/water/water-microbiology.jsf).

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USEPA 2002a. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). Available at: http://www.epa.gov/microbes/1600sp02.pdf

USEPA 2002b. Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified Membrane-Thermotolerant Escherichia coli Agar (Modified mTEC). Available at: http://www.epa.gov/microbes/1603sp02.pdf

USEPA 2012. Recreational Water Quality Criteria. Publication No. EPA 820-F-12-058. U.S. Environmental Protection Agency, Washington, DC.

Yakub,G.P., Castric, D.A., Stadterman-Knauer, K.L., , Tobin, M.J., Blazina, M., Heineman, T.N., Yee, G.Y. and L. Frazier. 2002. Evaluation of Colilert and Enterolert Defined Substrate Methodology for Wastewater Applications. Water Environment Research Vol. 74, No. 2 (Mar. - Apr., 2002), pp. 131-135.

TABLES

Table 1. Sampling Locations

Location	Reach Represents	Latitude	Longitude
East Fork Russian River at East Road	Potter Valley	39.270379	-123.100581
Russian River at East School Way	Redwood Valley	39.264964	-123.208231
Russian River at Lake Mendocino Drive	Northern Ukiah	39.195557	-123.194882
Russian River at Vichy Springs Road	Middle Ukiah	39.155140	-123.184108
Russian River at Talmage Road	Southern Ukiah	39.134388	-123.186425
Russian River at River Road	Hopland	38.971371	-123.106683

Table 2. FIB Concentration Measurements
Red Bold font indicates the measurement exceeds the USEPA (2012) Beach Action Value

Location	Sample Collection Date	*E. coli* Bacteria (MPN/100mL)	*Enterococcus* Bacteria (MPN/100mL)
East Fork Russian River at East Road	8/14/2013	**339***	**376***
	8/21/2013	**426**	**216**
	8/28/2013	**880**	**364**
Russian River at East School Way	8/14/2013	31	52
	8/21/2013	36*	**71***
	8/28/2013	41	**72**
Russian River at Lake Mendocino Drive	8/14/2013	20	52
	8/21/2013	52	10
	8/28/2013	52*	**588***
Russian River at Vichy Springs Road	8/14/2013	31	<10
	8/21/2013	31	10
	8/28/2013	10	<10
Russian River at Talmage Road	8/14/2013	63	10
	8/21/2013	52	10
	8/28/2013	41	10
Russian River at River Road	8/14/2013	41	31
	8/21/2013	96	<10
	8/28/2013	41	20

***** Median value of duplicate samples

Table 2. FIB Concentration Measurements *continued*

Location	**Sample Collection Date**	**All *Bacteroides* Bacteria (16SrRNA genes/100mL)**	**Human-host *Bacteroides* Bacteria (16SrRNA genes/100mL)**	**Human-host *Bacteroides* Bacteria (Percent of All *Bacteroides* Bacteria)**
East Fork Russian River at East Road	8/14/2013	990,483	2,978	0.3%
	8/21/2013	1,070,960	5,949	0.6%
	8/28/2013	1,658,520	8,733	0.5%
Russian River at East School Way	8/14/2013	14,916	86	0.6%
	8/21/2013	19,695	979	5.0%
	8/28/2013	57,229	3,030	5.3%
Russian River at Lake Mendocino Drive	8/14/2013	187,963	915	0.5%
	8/21/2013	297,026	3,275	1.1%
	8/28/2013	964,962	5,982	0.6%
Russian River at Vichy Springs Road	8/14/2013	87,763	5,778	6.6%
	8/21/2013	112,772	11,803	10.5%
	8/28/2013	325,244	33,863	10.4%
Russian River at Talmage Road	8/14/2013	75,116	4,473	6.0%
	8/21/2013	93,045	9,293	10.0%
	8/28/2013	320,863	27,860	8.7%
Russian River at River Road	8/14/2013	45,464	1,898	4.2%
	8/21/2013	53,661	1,742	3.2%
	8/28/2013	107,003	7,644	7.1%

Table 3. Mann-Whitney U Hypothesis Test Results of FIB Concentrations Measured at in the Russian River
Bold Blue font indicates a statistically significant difference in FIB concentrations between the upper watershed and the middle and lower watershed concentrations in the mainstem Russian River.

Fecal Indicator Bacteria	**Mann-Whitney *U* Test Statistic**	**Probability**
E. coli	17.5	**<0.001**
Enterococcus	175	0.757
All Bacteroides	148	**0.001**
Human-host Bacteroides	107	0.204
Percent of Human-host Bacteroides	61	0.264

Table 4. Mann-Whitney U Hypothesis Test Results of FIB Concentrations Measured at in tributaries to the Russian River
Bold Blue font indicates a statistically significant difference in FIB concentrations between the East Fork of the Russian River (upper watershed) and tributaries in the lower watershed.

Fecal Indicator Bacteria	**Mann-Whitney *U* Test Statistic**	**Probability**
E. coli	7	0.066
Enterococcus	11	0.173
All Bacteroides	2	0.101
Human-host Bacteroides	4	0.297
Percent of Human-host Bacteroides	13	0.101

FIGURES

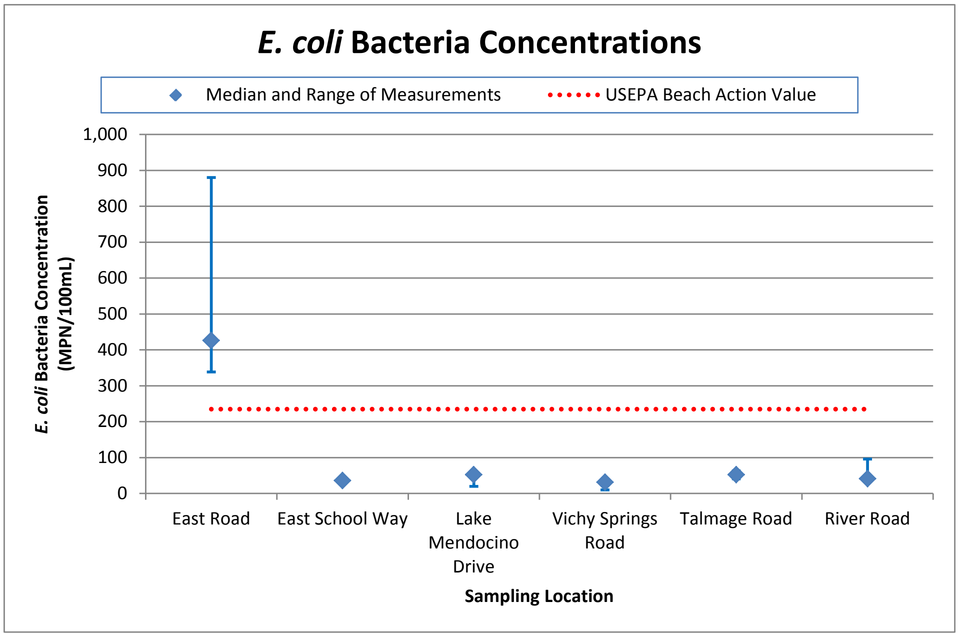

Figure 1. *E. coli* bacteria concentrations measured in the Upper Russian River watershed during August 2013.

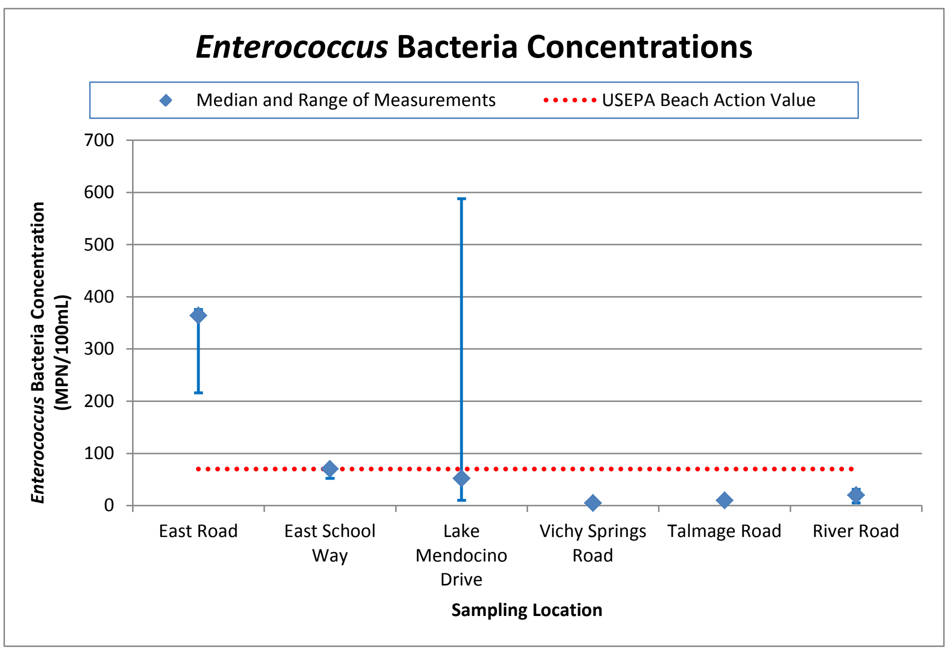

Figure 2. *Enterococcus* bacteria concentrations measured in the Upper Russian River watershed during August 2013.

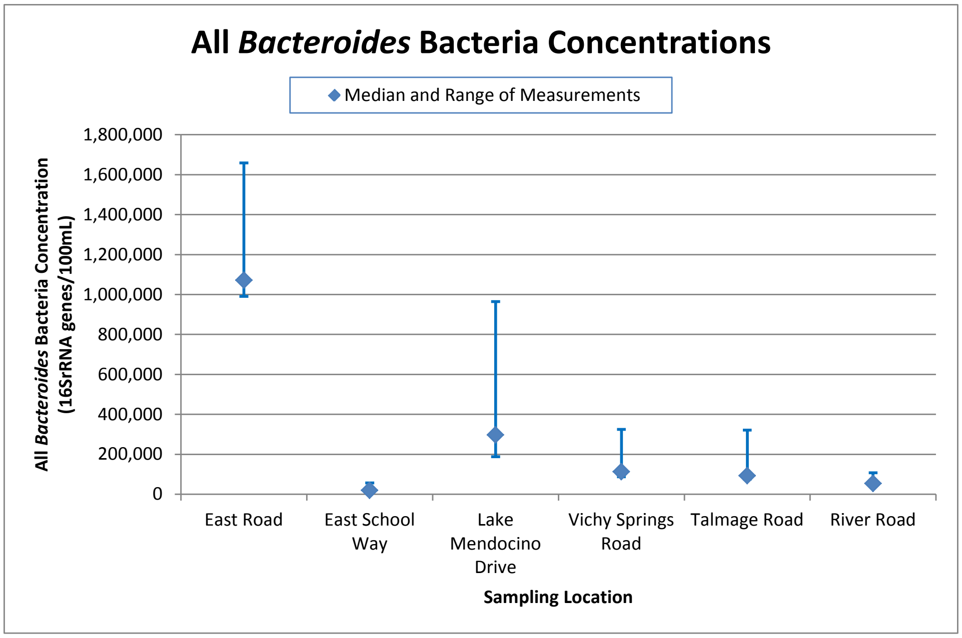

Figure 3. All *Bacteroides* bacteria concentrations measured in the Upper Russian River watershed during August 2013.

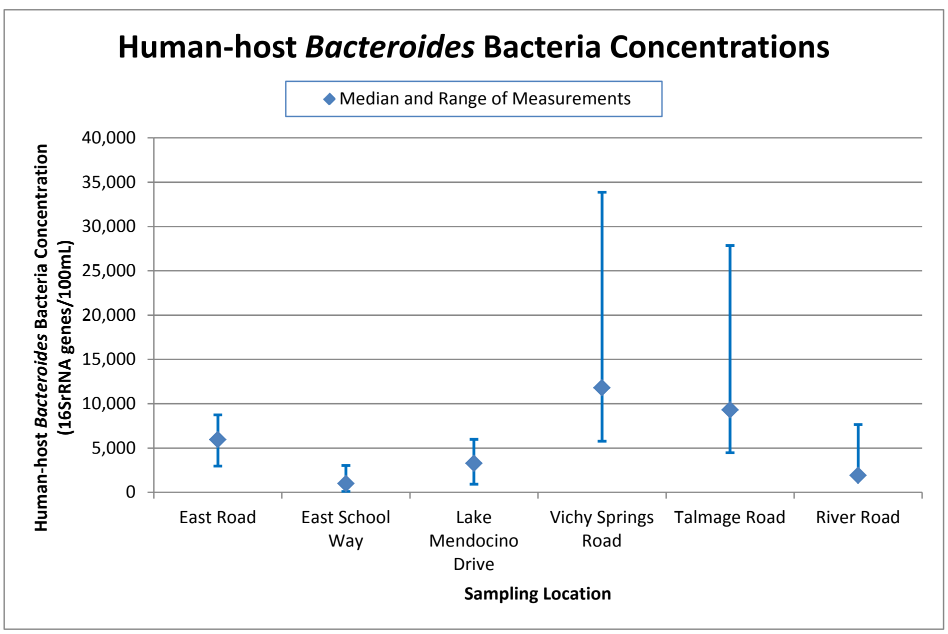

Figure 4. Human-host *Bacteroides* bacteria concentrations measured in the Upper Russian River watershed during August 2013.

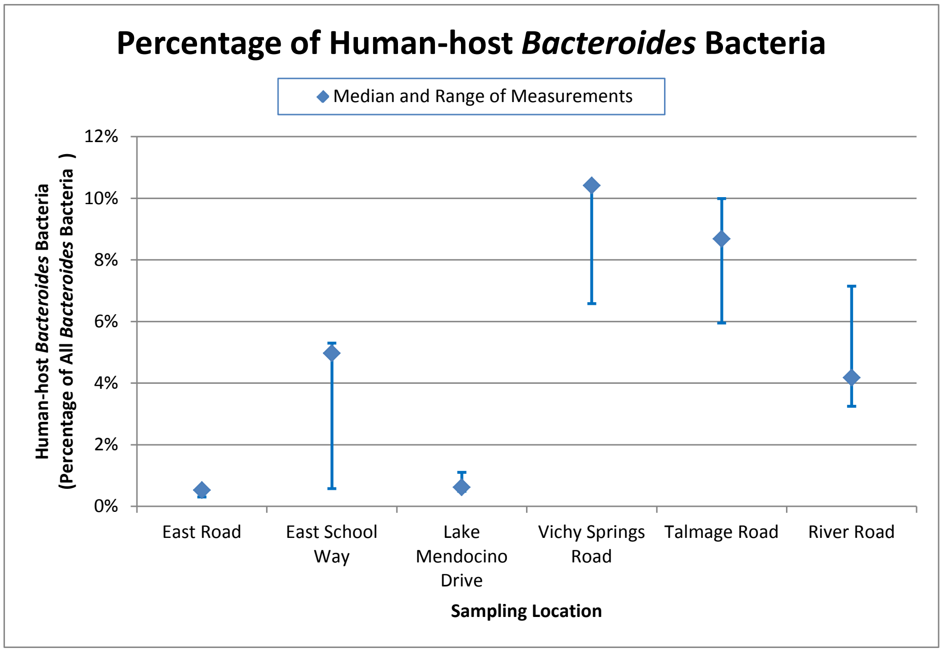

Figure 5. Percent of Human-host *Bacteroides* bacteria measured in the Upper Russian River watershed during August 2013.

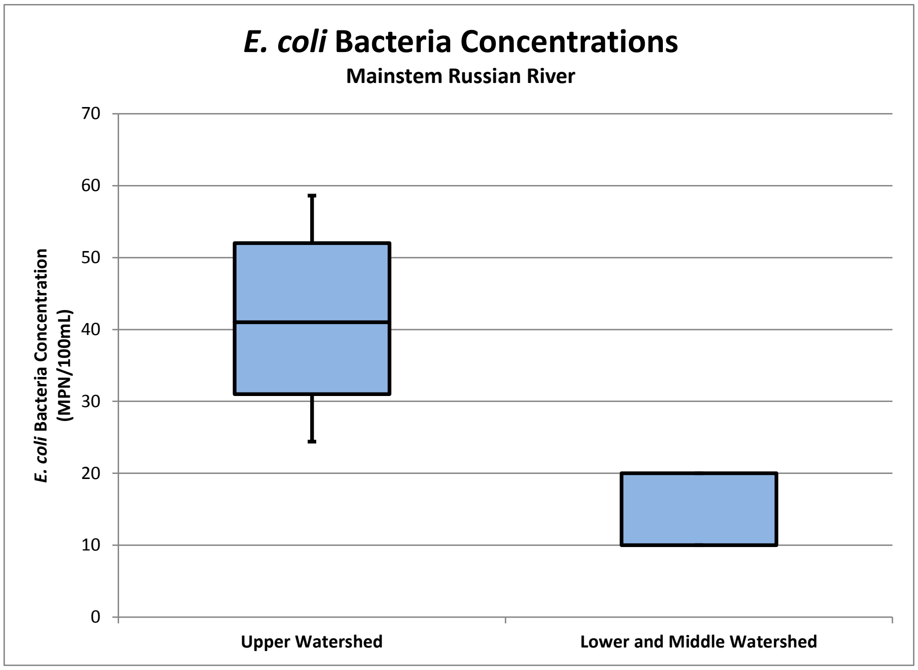

Figure 6. Comparison of *E. coli* Bacteria concentrations measured in the upper and the lower-middle watershed locations in the mainstem Russian River.

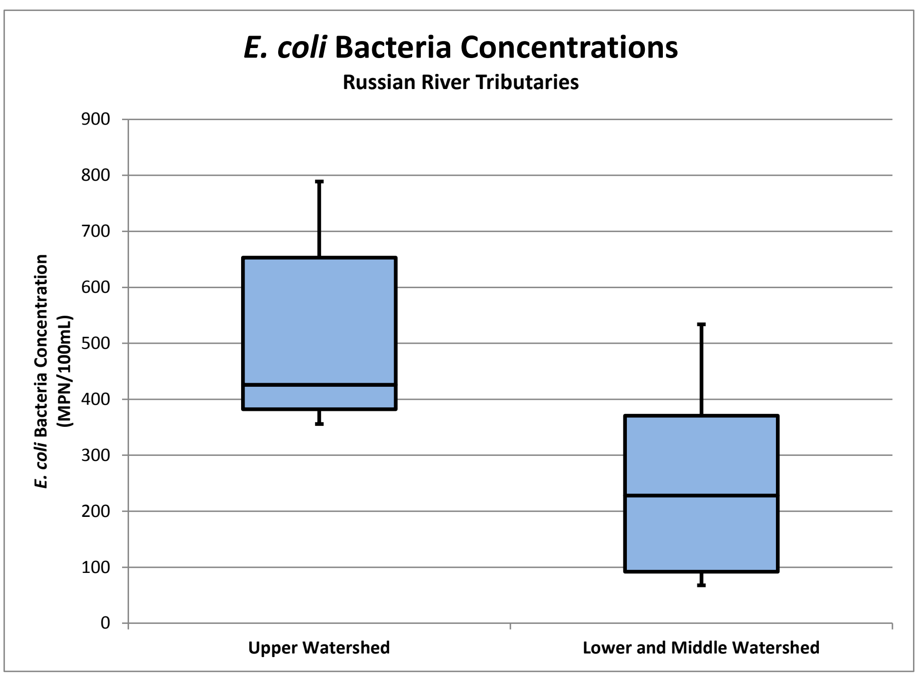

Figure 7. Comparison of *E. coli* Bacteria concentrations measured in the upper and the lower-middle watershed locations in tributaries to the Russian River.

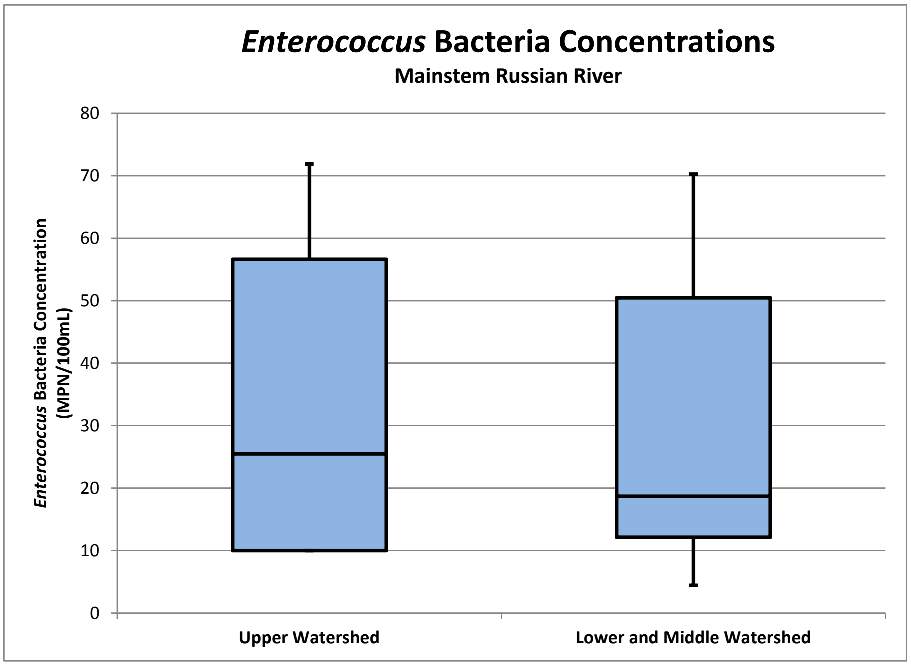

Figure 8. Comparison of *Enterococcus* Bacteria concentrations measured in the upper and the lower-middle watershed locations in the mainstem Russian River.

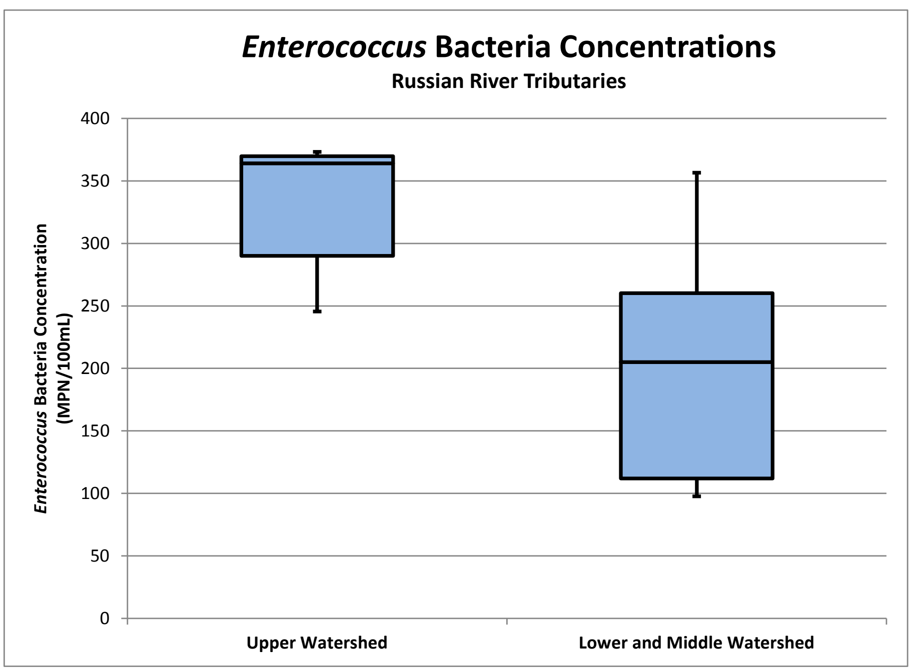

Figure 9. Comparison of *Enterococcus* Bacteria concentrations measured in the upper and the lower-middle watershed locations in tributaries to the Russian River.

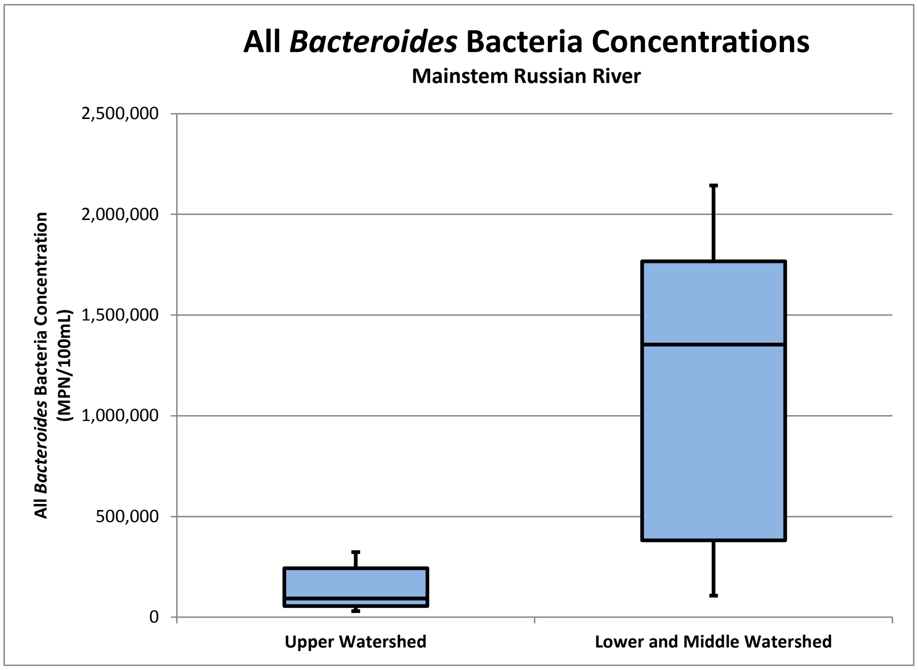

Figure 10. Comparison of All *Bacteroides* Bacteria concentrations measured in the upper and the lower-middle watershed locations in the mainstem Russian River.

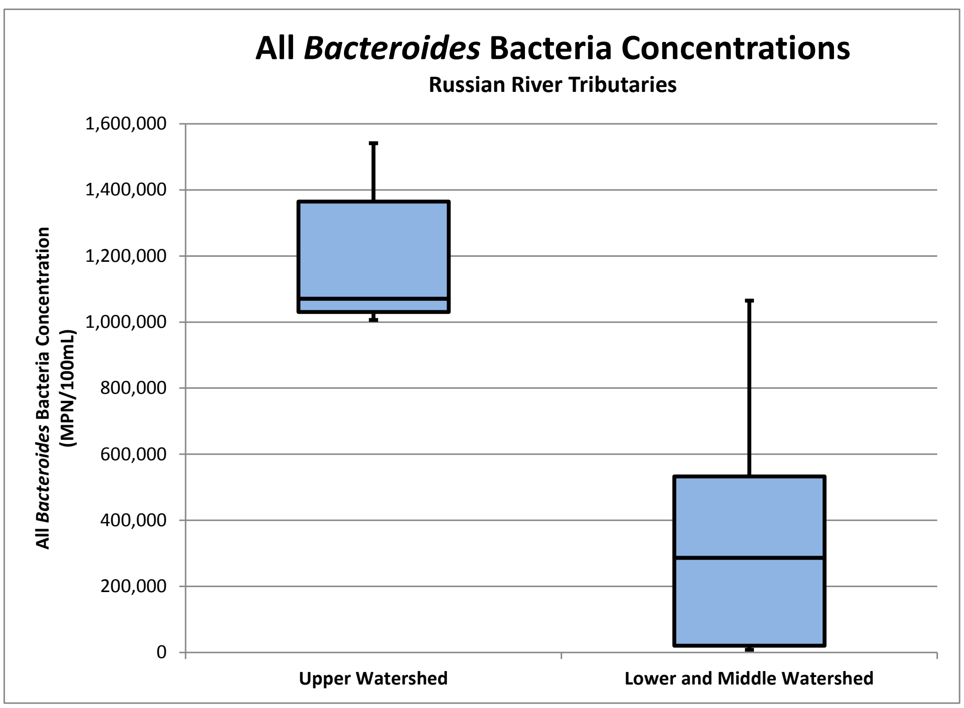

Figure 11. Comparison of All *Bacteroides* Bacteria concentrations measured in the upper and the lower-middle watershed locations in tributaries to the Russian River.

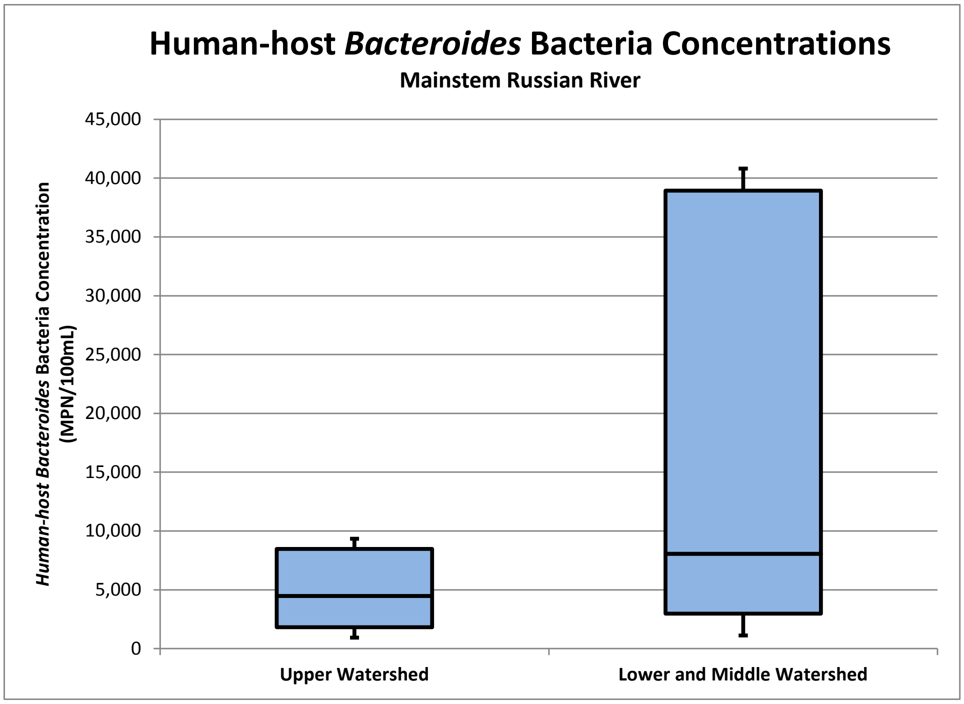

Figure 12. Comparison of Human-host *Bacteroides* Bacteria concentrations measured in the upper and the lower-middle watershed locations in the mainstem Russian River.

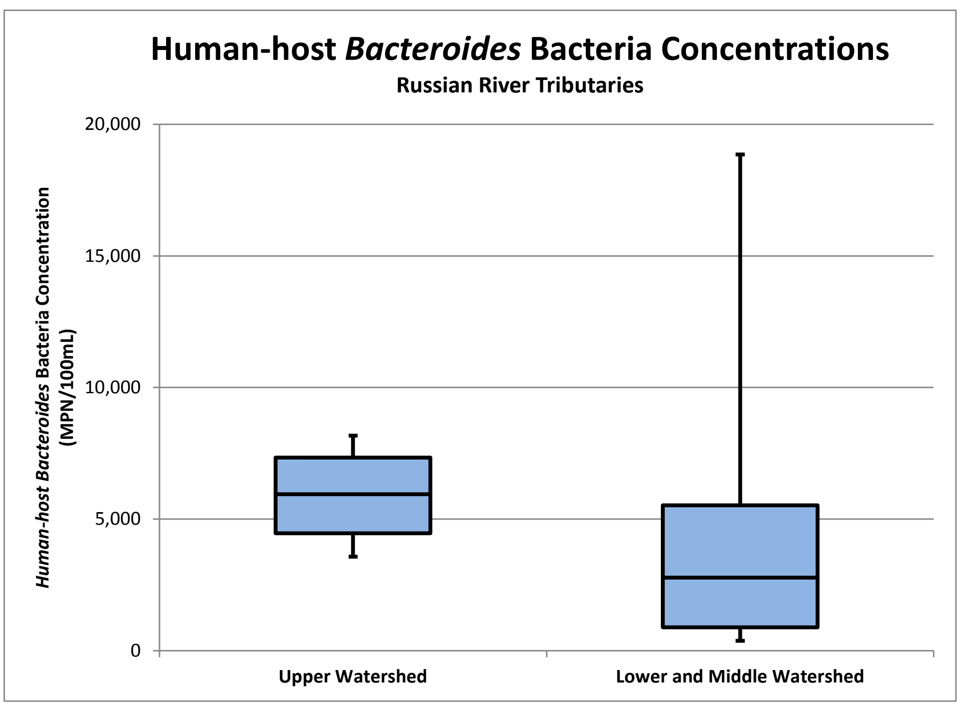

Figure 13. Comparison of Human-host *Bacteroides* Bacteria concentrations measured in the upper and the lower-middle watershed locations in tributaries to the Russian River.

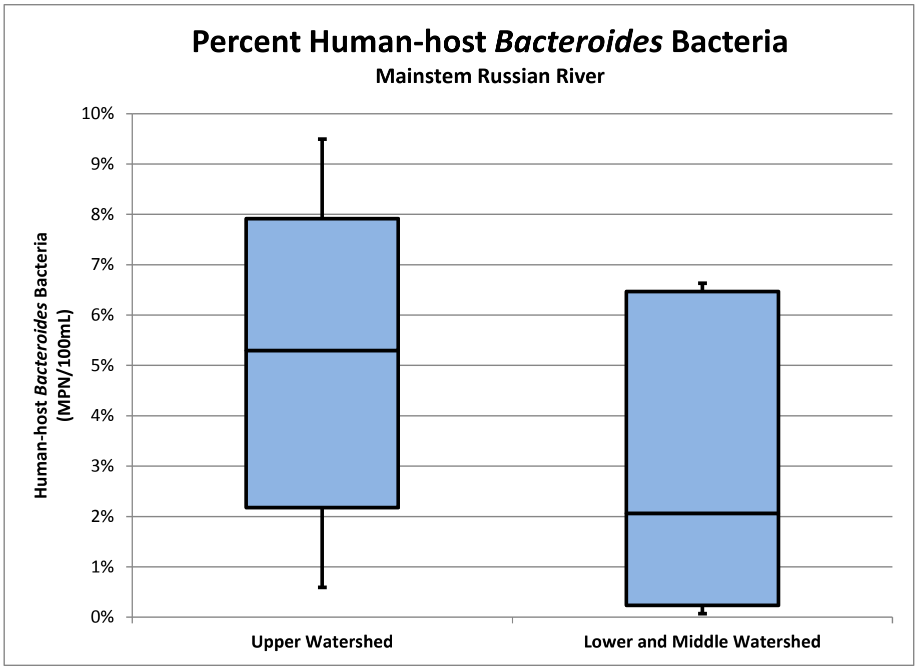

Figure 14. Comparison of Human-host *Bacteroides* Bacteria concentrations measured in the upper and the lower-middle watershed locations in the mainstem Russian River.

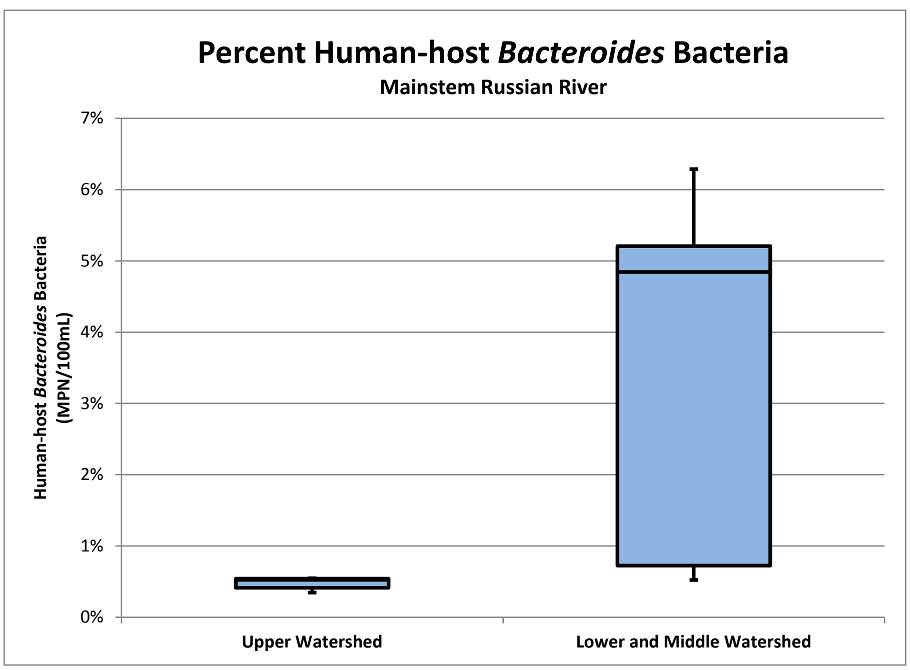

Figure 15. Comparison of Human-host *Bacteroides* Bacteria concentrations measured in the upper and the lower-middle watershed locations in tributaries to the Russian River.