TO: File: Russian River; TMDL Development and Planning

FROM: Steve Butkus

DATE: June 2, 2014

SUBJECT: EVIDENCE OF WATER CONTACT RECREATION IMPAIRMENT IN THE RUSSIAN RIVER WATERSHED

The North Coast Regional Water Board staff are developing Russian River Total Maximum Daily Loads (TMDLs) for pathogen indicator bacteria to identify and control contamination impairing recreational water uses. Potential pathogen contamination has been identified in the Russian River Watershed leading to conclusion that the natural background levels of bacteriological quality are not being achieved in the mainstem Russian River and its tributaries, in violation of the Basin Plan's narrative Bacteria Water Quality Objective. The contamination identified has been linked to impairment of the water contact recreation (REC-1) and non-contact water recreation (REC-2) designated beneficial uses. *Escherichia coli (E. coli)* and *Bacteroides* bacteria concentrations were measured at numerous locations in the Russian River Watershed between 2001 and 2013 by the North Coast Regional Water Board and the Sonoma County Water Agency (NCRWQCB 2012, 2013a, 2013b).

The purpose of this memorandum is to summarize the evidence for non-attainment of the Bacteria Water Quality Objective collected as part of the Russian River Pathogen Indicator Bacteria TMDL process for use in interpreting the Bacteria Water Quality Objective in the 2012 303(d) and 305(b) Water Quality Assessment Integrated Report.

Assessment of *E. coli* Bacteria Concentrations

E. coli bacteria are appropriate indicators of human health risk during water contact in recreational freshwaters for the Russian River Pathogen Indicator Bacteria TMDL. *E. coli* bacteria are specific to fecal material from humans and other warm blooded animals, and they are linked with illness rates in epidemiological studies. The *E. coli* bacteria concentration measurements were compared to the *E. coli* bacteria numeric evaluation threshold. Table 1 presents the numeric evaluation thresholds for E. coli bacteria concentration for the Russian River Pathogen Indicator Bacteria TMDL based on samples collected over a 30-day period. The thresholds are based on the U.S. EPA (2012) criteria that correspond to a rate of 36 illnesses per 1,000 water contact recreators.

Table 1. *E. coli* Bacteria Numeric Targets

Pathogen Indicator Bacteria	Estimated Illness Rate 36 per 1,000 recreators	
	Geometric Mean (cfu/100mL)	Statistical Threshold Value (cfu/100mL)
E. coli	126	410

Note: Colony forming units (cfu) = most probable number (MPN)

Water samples were analyzed for *E. coli* bacteria concentrations using the IDEXX Colilert method. IDEXX's Colilert® procedures have been adopted as standard methods for monitoring recreational water quality by the United States Environmental Protection Agency (IDDEX, 2001; USEPA, 2003). Water samples were either undiluted or serially diluted 1:10, resulting in a minimum reporting limit of 1 or 10 MPN/100mL and a maximum reporting limit of 2,419 or 24,196 MPN/100mL. Measurements beyond the analytical reporting limits (i.e., censored data) were substituted with the reporting limit value.

The REC-1 beneficial use impairment was assessed using the numeric evaluation threshold and Table 3.2 of the *Water Quality Control Policy for California's Clean Water Act Section 303(d) List* (CSWRCB 2004), which is also known as the Listing Policy. The Listing policy applies a binomial distribution for listing decisions that minimizes decision error based on sample size and number of samples exceeding the criteria. In order to meet the statistical assumptions required for the application of the binomial distribution, *E. coli* bacteria concentrations were assessed using discrete 30-day averaging periods (Butkus 2013). Discrete 30-day periods were defined based on the Julian calendar date of each year (i.e., 30-day period 1 for Julian days 1-30; 30-day period 2 for Julian days 31-60, etc.).

The results of the assessment for *E. coli* bacteria concentrations are presented in Table 2. The table also indicates if the number of exceedances of the numeric threshold were high enough for the stream to be considered impaired per the Listing Policy. The results verify that there is evidence of REC-1 impairment due to high *E. coli* bacteria concentrations in five (5) tributaries of the Russian River Watershed.

Table 2. Impaired Russian River Tributaries from *E. coli* bacteria by Location

Location	Number of 30-day Periods Sampled	Number of Periods that Exceed Numeric Evaluation Threshold*	Considered Impaired per §303(d) Listing Policy
Foss Creek *at Matheson Street*	7	6	**Yes**
Green Valley Creek *at Martinelli Road and River Road*	11	7	**Yes**
Laguna de Santa Rosa *at Sebastopol Community Park*	11	6	**Yes**
Matanzas Creek *at Doyle Park and Bethards Drive*	8	7	**Yes**
Santa Rosa Creek *at Wildwood Drive, Highway 12, upstream of Rincon Creek, at Alderbrook Drive, and at Railroad Street*	61	50	**Yes**

* Number of 30-day periods that exceed either the geometric mean criterion (126 cfu/100mL) or the statistical threshold value (410 cfu/100mL).

Assessment of *Bacteroides* Bacteria Concentrations

Bacteroides bacteria are another group of pathogen indicator organisms that are used to measure fecal contamination. *Bacteroides* bacteria is the genus name of the bacteria from the phylum Bacteroidetes and order Bacteroidales. Bacteroides bacteria contribute a significant fraction of the fecal bacteria species in animal feces. *Bacteroides* bacteria are anaerobic (i.e., they do not live or grow in the presence of oxygen) and make up a substantial portion of the gastrointestinal flora of mammals. *Bacteroides* bacteria are not found in ambient surface waters without sources of mammalian waste.

Due to their anaerobic-nature, *Bacteroides* bacteria have a low potential for survival and regrowth in the environment. In addition, water temperature has been shown to affect the persistence of *Bacteroides* bacteria in surface water (Kreader 1998; Bell et al. 2009). For water temperatures typically observed in the Russian River during the summer period, *Bacteroides* bacteria would survive only one day. Because of their short life span, *Bacteroides* bacteria concentrations are often used to indicate recent fecal contamination of surface waters.

Quantitative real-time polymerase chain reaction (qPCR) methods were used to measure the concentration of *Bacteroides* bacteria by amplifying specific DNA sequences. In addition, the use of a host-specific genetic marker (16S rRNA) can also quantify the percentage of the *Bacteroides* bacteria population that originates from specific animal-hosts (i.e., human and bovine) (Molina 2007).

Numeric criteria for *Bacteroides* bacteria are not available as epidemiological studies have not yet been conducted to link concentrations to illness rates. However, U.S. EPA (2012) supports the development of *Bacteroides* bacteria criteria where site-specific information exists. In this assessment, the measurement of quantifiable concentrations of human-host or bovine-host *Bacteroides* bacteria was used to assess compliance with the narrative Water Quality Objective of the *Water Quality Control Plan for the North Coast Region* (NCRWQCB 2011), which is also known as the Basin Plan. The narrative Water Quality Objective states:

> *"The bacteriological quality of waters of the North Coast Region shall not be degraded beyond natural background levels."*

Regional Water Board staff collected water samples for measurement of human-host and bovine-host *Bacteroides* bacteria at numerous mainstem and tributary locations in the Russian River Watershed from 2011 to 2013 (NCRWQCB 2012; NCRWQCB 2013a; NCRWQCB 2013b). Data were compared to the *Bacteroides* bacteria numeric evaluation threshold of no quantitative reported concentration. The quantitative reporting limits for human-host and bovine-host *Bacteroides* bacteria concentrations used in this assessment were 60 and 30 16SrRNA genes per 100mL, respectively. The median concentrations measured at each of these locations are shown in Tables 3 through 6. In addition, the number of samples measured below the quantitative reporting limits is identified at each location.

The assessment of the *Bacteroides* bacteria data shows that human-caused bacteria concentrations in the Russian River Watershed are widespread. All locations sampled in the mainstem Russian River and most tributary locations resulted in measureable levels of both human-host and bovine-host *Bacteroides* bacteria concentrations. Of the 179 samples collected for human-host *Bacteroides* bacteria, only five percent were measured below the numeric evaluation threshold. Of the 83 samples collected for bovine-host *Bacteroides* bacteria, only five percent were measured below the numeric evaluation threshold.

Based on these results, it can be concluded that natural background levels of bacteriological quality are not being achieved in the mainstem Russian River and its tributaries, in violation of the Basin Plan's narrative Bacteria Water Quality Objective.

Table 3. Human-Host *Bacteroides* Bacteria Concentrations Measured in the Russian River

Location	**Median Human-host Bacteroides (genes/100mL)**	**Number of Measurements**	**Number of Measurements less than the Numeric Evaluation Threshold (60 genes per 100mL)**
East Fork at East Road, Potter Valley	5,949	3	0
East School Way, Redwood Valley	979	3	0
Lake Mendocino Drive, Ukiah	3,275	3	0
Vichy Springs Road, Ukiah	11,803	3	0
Talmadge Road, Ukiah	9,293	3	0
River Road, Hopland	1,898	3	0
Commisky Station Road, Cloverdale	2,731	2	0
River Park, Cloverdale	1,087	2	0
Hwy 128 Bridge, Geyserville	13,501	2	0
Jimtown Bridge, Healdsburg	37,052	2	0
Camp Rose Beach, Healdsburg	31,055	2	0
Veteran's Memorial Beach, Healdsburg	14,921	8	0
Steelhead Beach, Forestville	48,485	2	0
River Access Beach, Forestville	57,554	2	0
Johnson's Beach, Guerneville	1,677	10	0
Monte Rio Beach, Monte Rio	8,898	16	0
Public Boat Ramp, Jenner	4,837	2	0

Table 4. Human-Host *Bacteroides* Bacteria Concentrations Measured in Russian River Tributaries

Stream	Location	Median Human-host Bacteroides (genes/100mL)	Number of Measurements	Number of Measurements less than the Numeric Evaluation Threshold (60 genes per 100mL)
Abramson Creek	Willowside Road Path, Santa Rosa	273,401	4	0
Blucher Creek	Lone Pine Road, Cotati	18,022	2	0
Copeland Creek	Commerce Blvd, Rohnert Park	19,928	2	0
Crane Creek	Snyder Lane, Rohnert Park	26,703	2	0
Dutch Bill Creek	Main Street, Monte Rio	416	2	1
Foss Creek	Matheson Street, Healdsburg	37,346	2	0
Gossage Creek	Stony Glen Lane, Cotati	29,902	2	0
Green Valley Creek	Martinelli Road, Forestville	17,016	2	0
Laguna de Santa Rosa	Community Center, Sebastopol	7,469	2	0
Mays Creek	Neeley Road, Guerneville	1,325	2	0
Palmer Creek	Palmer Creek Road, Healdsburg	2,781	2	1
Piner Creek	Fulton Road, Santa Rosa	12,394	2	0
Santa Rosa Creek	Hwy 12 Bridge, Santa Rosa	2,727	2	0
Santa Rosa Creek	Railroad Street, Santa Rosa	32,909	2	0
Van Buren Creek	Erland Road, Santa Rosa	2,089	2	1

Table 4 *continued*. Human-Host *Bacteroides* Bacteria Concentrations Measured in Russian River Tributaries

Stream	Location	Median Human-host Bacteroides (genes/100mL)	Number of Measurements	Number of Measurements less than the Numeric Evaluation Threshold(60 genes per 100mL)
Unnamed Creek	Lambert Bridge Road, Healdsburg	5,257	2	0
Unnamed Creek	Fitch Mountain Road, Healdsburg	238	6	1
Unnamed Creek	Fredson Road, Healdsburg	8,580	5	0
Unnamed Creek	West Dry Creek Road, Healdsburg	4,040	5	0
Unnamed Creek	Alexander Valley Road, Healdsburg	2,031	5	1
Unnamed Creek	Redwood Drive, Healdsburg	2,310	5	0
Unnamed Creek	Limerick Road, Healdsburg	20,000	4	0
Unnamed Creek	Summerhome Park Road, Forestville	7,975	4	0
Unnamed Creek	Trenton Road, Forestville	48,200	5	0
Unnamed Creek	Del Rio Court, Forestville	3,460	3	0
Unnamed Creek	River Road, Rio Nido	3,600	3	1
Unnamed Creek	Foothill Dive, Monte Rio	371,000	1	0
Unnamed Creek	Duncan Road, Monte Rio	353	3	1
Unnamed Creek	Old Monte Rio Road, Monte Rio	25,100	4	0
Unnamed Creek	Main Street, Monte Rio	1,392	5	1
Unnamed Creek	Moscow Road, Duncans Mills	<60	1	1
Unnamed Creek	Lakeside Ave, Camp Meeker	9,090	4	0
Unnamed Creek	Sanford Road, Sebastopol	1,576	4	0
Unnamed Creek	Daywalt Road, Cotati	37,632	2	0
Unnamed Creek	River Road, Fulton	2,759	4	0

Table 5. Summary of Bovine-Host *Bacteroides* Bacteria Concentrations Measured in the Russian River

Location, Nearest City or Town	**Median Bovine-host Bacteroides (genes/100mL)**	**Number of Measurements**	**Number of Measurements less than the Numeric Evaluation Threshold (30 genes per 100mL)**
Commisky Station Road, Cloverdale	5,413	2	0
River Park, Cloverdale	710	2	0
Hwy 128 Bridge, Geyserville	236	2	0
Jimtown Bridge, Healdsburg	116	2	0
Camp Rose Beach, Healdsburg	286	2	0
Veteran's Memorial Beach, Healdsburg	381	2	0
Steelhead Beach, Forestville	23,684	2	0
River Access Beach, Forestville	14,710	2	0
Johnson’s Beach, Guerneville	85	7	0
Monte Rio Beach, Monte Rio	762	10	0
Public Boat Ramp, Jenner	2,682	2	0

Table 6. Summary of Bovine-Host *Bacteroides* Bacteria Concentrations Measured in Russian River Tributaries

Stream	Location, Nearest City or Town	Median Bovine-host Bacteroides (genes/100mL)	Number of Measurements	Number of Measurements less than the Numeric Evaluation Threshold (30 genes per 100mL)
Abramson Creek	Willowside Road Path, Santa Rosa	425,164	4	0
Blucher Creek	Lone Pine Road, Cotati	177,248	2	0
Copeland Creek	Commerce Blvd, Rohnert Park	51,685	2	0
Crane Creek	Snyder Lane, Rohnert Park	23,602	2	0
Dutch Bill Creek	Main Street, Monte Rio	15	2	2
Foss Creek	Matheson Street, Healdsburg	8,668	2	1
Gossage Creek	Stony Glen Lane, Cotati	76,895	2	0
Green Valley Creek	Martinelli Road, Forestville	72	2	0
Laguna de Santa Rosa	Community Center, Sebastopol	514	2	1
Mays Creek	Neeley Road, Guerneville	608	2	0
Palmer Creek	Palmer Creek Road, Healdsburg	106	2	1
Piner Creek	Fulton Road, Santa Rosa	3,274	2	0
Santa Rosa Creek	Hwy 12 Bridge, Santa Rosa	181	2	0
Santa Rosa Creek	Railroad Street, Santa Rosa	7,765	2	0
Van Buren Creek	Erland Road, Santa Rosa	2,265	2	1
Unnamed Creek	Sanford Road, Sebastopol	482	4	0
Unnamed Creek	Lambert Bridge Road, Healdsburg	453	2	1
Unnamed Creek	Limerick Road, Healdsburg	1,966	4	0
Unnamed Creek	Daywalt Road, Cotati	867,503	2	1
Unnamed Creek	River Road, Fulton	768	4	0

Assessment of the Presence of Potential Pathogenic Bacteria

Technology has advanced to a point where the species in an entire bacterial community can be identified instead of just a single pathogen indicator bacteria groups or species. DNA sequence analysis can identify possible fecal sources by measuring the total diversity of the microbial communities in a water sample (Dubinsky et al. 2012). The PhyloChip™ (Second Genome, San Bruno CA) is a phylogenetic DNA microarray that uses 16S rRNA gene probes to identify nearly 60,000 different bacteria taxa in a single water sample. Analyzing all known bacteria taxa identified the presence of potential human pathogens found in the surface waters of the Russian River Watershed.

Over one-hundred water samples were collected and processed using the PhyloChip™ microarray resulting in detection of over 10,000 different bacteria taxa in the Russian River watershed between 2011 and 2013 (Dubinsky and Andersen 2014). These samples were collected concurrently with pathogen indicator bacteria samples collected by the North Coast Regional Water Board (NCRWQCB 2012, 2013a, 2013b). .

Table 7 shows a list of ten (10) potential human pathogen taxa that were detected at various locations in the Russian River Watershed. Each of these pathogens is discussed below. Detection of pathogen related genes do not necessarily indicate that pathogenic strains are present, but that the bacteria community may or may not include the virulent strain. Detection of pathogen related genes do not necessarily indicate that pathogenic strains are present, but rather that closely related taxa are present that may or may not include the virulent strain. Additional analyses that specifically target pathogenic strains would be necessary to confirm their occurrence.

However, these measurements confirm that surface waters throughout the Russian River Watershed are potentially impaired with pathogenic bacteria.

Table 7. Summary of Human Pathogens Measured in Russian River Watershed

Pathogenic Bacteria	Number of Locations Measured		Percent of Samples with Detected Bacteria
	Mainstem Russian River	Tributaries	
Klebsiella pneumoniae	10	23	42%
Proteus mirabili	1	10	11%
Salmonella enterica	1	9	10%
Serratia marcescens	3	27	41%
Shigella flexneri	0	15	16%
Staphylococcus epidermidis	3	13	22%
Staphylococcus haemolyticus	2	0	2%
Streptococcus sp.	0	8	8%
Vibrio cholerae	0	1	1%
Yersinia sp.	4	7	15%

Findings

Based on the assessment of bacteria concentrations measured in the Russian River Watershed and presented in this memorandum, Regional Water Board staff can make the following findings:

- REC-1 beneficial uses are impaired due to high *E. coli* bacteria concentrations in five (5) tributaries of the Russian River Watershed:

- Both human-host and bovine-host *Bacteroides* bacteria concentrations exceed the numeric evaluation threshold (i.e., quantitative reporting limits) throughout the Russian River Watershed. Based on these results, it can be concluded that natural background levels of bacteriological quality are not being achieved in violation of the Basin Plan narrative bacteria Water Quality Objective.

- Measurements confirm the presence of potential pathogenic bacteria in surface waters throughout the Russian River Watershed.

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