TECHNICAL REPORT

2020
Reassessment of
Fecal Indicator Bacteria and Microbial Source Tracking Data
for the
Russian River Watershed Pathogen Total Maximum Daily Load

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LIST OF ABBREVIATIONS

cfu	colony forming units
CWA	Clean Water Act
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
FIB	Fecal Indicator Bacteria
GM	Geometric Mean
HSA	Hydrologic Subarea
HUC	Hydrologic Unit Code
ISWEBE	Inland Surface Waters, Enclosed Bays, and Estuaries
LBNL	Lawrence Berkeley National Laboratory
mL	milliliters
MST	Microbial Source Tracking
NCRWQCB	North Coast Regional Water Quality Control Board
NGI	National Epidemiological and Environmental Assessment of Recreational Water gastrointestinal illness rate
OWTS	Onsite Wastewater Treatment System
ppth	parts per thousand
QA/QC	Quality Assurance/Quality Control
REC-1	Water Contact Recreation
RNA	Ribonucleic Acid
rRNA	ribosomal Ribonucleic Acid
RWQC	Recreational Water Quality Criteria
SCWA	Sonoma County Water Agency
SETAC	Society of Environmental Toxicology and Chemistry
STV	Statistical Threshold Value
SWRCB	State Water Resources Control Board
TMDL	Total Maximum Daily Load
USGS	United States Geological Survey
WQO	Water Quality Objective
WQS	Water Quality Standard

Contents

1 INTRODUCTION

In August 2019, the North Coast Regional Water Quality Control Board (Regional Water Board) adopted Resolution R1-2019-0038, to amend the *Water Quality Control Plan for the North Coast Region* (Basin Plan) to include the Action Plan for the Russian River Watershed Pathogen TMDL (Action Plan). The Action Plan summarizes the findings of the Total Maximum Daily Load (TMDL) analyses conducted across the Russian River Watershed to address pathogen pollution and listings of the Russian River on the Clean Water Act Section 303(d) list of impaired waters [303(d) list]. These analyses and the results are reported in the *2019 Staff Report for the Action Plan for the Russian River Pathogen TMDL* (TMDL Staff Report). The Regional Water Board's adoption of the Action Plan must be approved by the State Water Resources Control Board (State Water Board) and the Office of Administrative Law before it is implemented as state regulation. It has not yet been brought before the State Water Board for approval, due to: 1) a delay associated with the requirement to make all documents posted to the web to be accessible (i.e. compliant with the Americans with Disabilities Act); and 2) staff redirection to support State Water Board staff with the State Water Board's October 2020 adoption of the 2018 Integrated Report and 303(d) listing of impaired waters.

In October 2020, the State Water Board adopted the 2018 Integrated Report, including an updated 303(d) list of impaired waters for three of the state's regions, including the North Coast Region. Section 303(d) of the Clean Water Act envisions an assessment of water quality impairment (e.g., exceedance of water quality standards) to precede the development of a TMDL and the implementation of an approved TMDL to be the mechanism for restoring water quality and attaining water quality standards, as required. Following adoption/approval of the TMDL, the 303(d) list is typically updated in the next listing cycle to reflect the findings of the TMDL. The timing of the State Water Board's adoption of the 2018 Integrated Report and updated 303(d) list interrupted this normal flow, by requiring consideration of Russian River pathogen listings after the Regional Water Board's adoption of the applicable TMDL and Action Plan, but before the State Water Board's consideration of the TMDL and Action Plan. Further, public comment on the draft 303(d) listing proposal for the Russian River released by the State Water Board in preparation for its October 2020 hearing, indicated concerns of some Russian River residents that would be best addressed before the State Water Board in the TMDL approval hearing, rather than the Integrated Report hearing. As such, the State Water Board chose to postpone consideration of the Russian River pathogen related 303(d) listing considerations until a time after it conducts a hearing on approval of the Regional Water Board's adoption of the TMDL and Action Plan. A future update of the 303(d) list relative to the Russian River can then be made, which is consistent with the adopted and approved Russian River TMDL.

1.1 Purpose of the 2020 Data Reassessment

The purpose of the 2020 Data Reassessment is to update the Russian River watershed geographic-based fecal indicator bacteria (FIB) and microbial source tracking (MST) data analyses in consideration of discussions between the State Water Board and Regional Water Board staffs and public comments received on the draft 2018 Integrated Report and proposed 303(d) list. As above, the State Water Board postponed consideration of its staff's Russian River 303(d) listing proposal until a time after it conducts a hearing on the Regional Water Board's adoption of the TMDL and Action Plan.

For the Regional Water Board, the geographic-based analysis of FIB and MST data, as described below, was primarily conducted for the narrow purpose of defining the Advanced Protection Management Program (APMP) boundary within which investigation of cess pools, failing Onsite Wastewater Treatment Systems (OWTS), and substandard OWTS would be prioritized. The APMP is a key component of the TMDL and Action Plan as the APMP definition and associated implementation actions are necessary to comply with the State Water Board's OWTS Policy. Following approval by the State Water Board of the Regional Water Board's adoption of the TMDL and Action Plan, including the APMP boundary, Regional Water Board staff will propose that a future update to 303(d) list for the Russian River watershed simply reference the findings of the TMDL as the basis for impairment listing.

1.2 Overview of TMDL Study Results for Reference

On a larger scale and as described in detail in the 2019 TMDL Staff Report, several TMDL studies were conducted to support development of the Russian River Pathogen TMDL and Action Plan. The key TMDL studies were landscape-scale studies that related elevated FIB results with identified categories of landuse, high use recreational beaches, and areas of high parcel density served by OWTS. The 2019 TMDL Staff Report also summarizes a special study of ambient water quality using the PhyloChip™ phylogenetic DNA microarray to estimate the percentages of bacteria in water samples that matched DNA profiles for reference fecal waste sources.

In summary, the TMDL studies identified evidence of fecal waste discharge throughout the whole watershed, particularly associated with:

- Wet weather runoff
- Developed areas, both sewered and non-sewered
- Shrubland (including rural residential areas)
- Agricultural lands (also including rural residential areas)
- High density parcel neighborhoods served by OWTS
- Recreational beaches during high volume use (e.g., holiday weekends)

In each of the landuse categories (developed areas, agricultural areas, and shrubland), both human and bovine fecal waste signatures were present. Specifically, the data from the PhyloChip™ [1] showed evidence of human-sourced fecal waste discharge in the Russian River mainstem with a significant human signal at Johnson's Beach and Monte Rio Beach during winter months when recreational use is low. Human signals were detected in some high parcel density catchments served by OWTS (e.g., Healdsburg, Forestville, and Monte Rio). Human signals were detected in tributaries to the Russian River in some sewered neighborhoods (e.g., Piner Creek and Copeland Creek); shrubland, which includes rural residential (e.g., Blucher Creek, Crane Creek, Gossage Creek); and agricultural lands, which also include rural residential (e.g., Abramson Creek and Woolsey Creek). The only tributary identified predominantly as forestland that showed a human signal was Mays Creek (or Mays Canyon).

On the basis of these results, the Action Plan establishes a waste discharge prohibition against the discharge of waste containing fecal waste material from humans or domestic animals to waters of the state within the Russian River Watershed. The prohibition applies to the whole watershed, because the TMDL studies indicated evidence of fecal waste discharge from numerous landuse categories that exist throughout the watershed. A list of 6 means of complying with the prohibition are identified in Section V.A of the Action Plan.

1.2.1 APMP Boundary Process

Relative to OWTS, compliance with the prohibition is achieved when an OWTS owner implements "adequate treatment and best management practices to prevent the discharge of fecal waste material from humans…from entering a water of the state either directly, or indirectly as a result of stormwater runoff."

For the purpose of establishing an Advanced Protection Management Program (APMP) boundary as required under the statewide OWTS Policy, the data collected for the TMDL studies were also evaluated on a geographic basis, though they were not originally collected in a manner intended to characterize water quality conditions geographically. But a geographic based assessment was useful as a means of narrowing down from the whole watershed, the areas of highest priority with respect to further OWTS investigation. The results of the geographic based assessment were used to delineate an APMP boundary within which identification of individual cess pools, failing OWTS and substandard OWTS would be prioritized. In addition, special provisions to correct identified cess pools, failing OWTS, and substandard OWTS apply within the APMP boundary.

FIB and MST data were assessed using the USGS hydrologic unit code (HUC) HUC-12 scale[2] as the most appropriate geographic scale given the distribution of the monitoring

[1] Dubinsky and Andersen (2014)

[2] The full extent of surface water drainage for the United States is mapped using a hierarchical system of hydrologic units at various scales. Each hydrologic unit is assigned a hydrologic unit code (HUC). The hydrologic units are organized by hierarchy which is indicated by the number associated with the HUC code (e.g. HUC-4, HUC-8, and HUC-12, where HUC-4 represents the subregion level, delineating large

locations. The clustering of monitoring locations made it inadvisable to assess data on a reach scale. Similarly, the number of data were insufficient to reasonably represent findings to whole hydrologic subareas, which though smaller than the scale of the watershed are still overly large to effectively prioritize further OWTS investigation.

1.2.2 APMP Boundary Water Quality Tests

As above, the findings of the TMDL indicated an association of FIB exceedances and evidence of human-sourced fecal waste with developed areas (sewered and unsewered), shrubland (including rural residential), and agricultural land (also including rural residential). Notably, areas with a high parcel density serviced by OWTS were statistically corelated with downstream FIB exceedances. Staff initially attempted to design an APMP boundary based on the density of parcels serviced by OWTS, in accordance with the findings of the TMDL. But, the number of OWTS that fell within such a boundary was too big to reasonably serve as a method of prioritization. As such, after two rounds of public review, staff proposed and the Regional Water Board adopted an APMP boundary encompassing all HUC-12 subwatersheds that met both of two water quality tests, identified as “Tier One” and “Tier Two” below.

1.2.2.1 Tier One Test

In the first test, FIB data were binned by HUC-12 subwatershed and evaluated to determine impairment[3] or pollution[4] status. Data were evaluated using all relevant thresholds, not restricted by the direction provided in the statewide objective specific to 303(d) listing under the evaluation criteria of the Listing Policy. HUC-12 subwatersheds were identified as impaired/polluted if:

1) The freshwater *E. coli* data collected in the HUC-12 subwatershed exceeded either the geomean or statistical threshold value (STV) contained in the statewide bacteria objective as calculated across the whole year, the winter period, or the summer period.

2) The saline enterococci data collected in the HUC-12 subwatershed exceeded either the geomean or statistical threshold value (STV) contained in the statewide

river basins, HUC-8 represents the subbasin level, delineating medium-sized river basins, and HUC-12 represents a more local subwatershed level that captures tributary systems).

[3] “Impairment” is a federal term describing waters that are water quality limited because they do not consistently meet water quality standards.

[4] “Pollution” is defined in the Porter Cologne Water Quality Control Act to mean “an alteration of the quality of the waters of the state by waste to a degree which unreasonably affects either of the following: (A) The waters for beneficial uses. (B) Facilities which serve these beneficial uses. “Pollution” may include “contamination.” “Contamination” means an impairment of the quality of the waters of the state by waste to a degree which creates a hazard to the public health through poisoning or through the spread of disease. “Contamination” includes any equivalent effect resulting from the disposal of waste, whether or not waters of the state are affected.

bacteria objective as calculated across the whole year, the winter period, or the summer period.

3) The freshwater enterococci data collected in the HUC-12 subwatershed exceeded either the geomean or STV contained in the national Recreational Water Quality Criteria (RWQC) as calculated across the whole year, the winter period, or the summer period AND there were Public Health Advisories based on concerns about pathogen exposure any time in the period of 2013-2018.

Public Health Advisory data were evaluated in association with enterococci data as confirmation of the real potential for beneficial use impact in the HUC-12 in question. This is because in waters rich in organic matter, enterococci measurements can sometimes result in false positives. *E.coli* and enterococci geomeans and STVs were recalculated as part of the 2020 Data Reassessment. The Public Health Advisory data was deemed accurate and required no reconsideration.

1.2.2.2 Tier Two Test

In the second test, microbial source data were binned for impaired/polluted HUC-12 subwatersheds and evaluated to determine the degree to which exceedances of FIB criteria coincided with evidence of human-sourced fecal waste. Both human-sourced PhyloChip™ and human-sourced *Bacteroides* (HuBac) data were used. For PhyloChip™ data results, a threshold of 10% gene sequence match with human-sourced bacteria was used to indicate at least moderate certainty that human fecal waste was present in the water column; this threshold is consistent with that used in the 2019 Staff Report. For HuBac, a threshold of 10,000 gene copies/100 mL was used to indicate strong evidence that human fecal waste was present in the water column; this threshold is consistent with that used in the 2019 Staff Report.[5] The purpose of the second test was to refine the number of impaired/polluted HUC-12 subwatersheds identified for inclusion within the APMP boundary to only those with evidence of human-sourced bacteria. This was to ensure that further investigation of cess pools, failing OWTS, and substandard OWTS would be prioritized in those areas with the highest potential of human fecal waste discharge and water quality impact.

1.2.2.3 Additional Considerations

It must be noted that the geographic-based analysis of FIB and MST data in the Russian River watershed is limited by the fact that FIB and MST data was collected to support TMDL studies, not characterize pathogen status across the watershed. As such, only a subset of the HUC-12 subwatersheds were sampled. Further, of those HUC-12 subwatersheds within which FIB and MST data were collected, only a subset had a sufficient number of data to allow for a geomean calculation. Despite this

[5] During the 303(d) listing process in 2020 under the State Board's leadership, staff discovered thresholds in Sauer et. al. (2011) of 1,000/100 mL as moderate evidence and 5,000/100 mL as strong evidence of a given fecal waste signal. This is considerably lower than the 10,000/100 mL used in the 2019 TMDL Staff Report to help define the APMP boundary.

limitation, the approach was deemed appropriate for the specific purpose of prioritizing a narrower area within which to assess the status of individual OWTS.

2 DATA REASSESSMENT PROCESS

As part of the 2020 Data Reassessment, Regional Water Board staff reviewed all the FIB and MST data collected in the Russian River watershed, conducted additional QA/QC procedures, and applied the statewide bacteria objectives, RWQC and applicable elements of Listing Policy consistently across all relevant data.

Additional QA/QC procedures included:

1) Update monitoring station naming conventions to ensure a consistent convention across all TMDL studies;[6]
2) Unify monitoring station codes for 6 locations that previously were identified with multiple codes,[7]
3) Update monitoring station geolocation data to ensure that all stations are accurately located within the proper Hydrologic Unit Code (HUC) HUC-12 subwatershed area;[8]
4) Correct 2 types of replicate errors;[9]
5) Ensure that censored data were treated consistently;[10]
6) Correct typographical errors; [11]

[6] See Appendix A for a list of reconciled monitoring station names.

[7] See Appendix B for a table with corrected station location codes.

[8] The location of two monitoring stations was corrected to place station a) 113UR3929 from the Lower Santa Rosa Creek subwatershed into the Porter Creek-Mark West Creek subwatershed and b) 114DDRC59 from Dutch Bill Creek-Russian River subwatershed into Porter Creek-Russian River subwatershed. See Appendix C for a map representing all monitoring stations.

[9] A) While assessing the FIB dataset staff discovered that although most replicate readings had been averaged some had not. These inconsistencies were found and corrected. Since replicates were collected in a random manner, averaging replicates ensures that there is only one reading per sampling date. B) While assessing the FIB dataset staff discovered that there were replicate data included for two sampling locations in the FIB dataset that had the same sampling location name (Unnamed Tributary at Sanford Road) and the same geographic coordinates, but slightly different sampling location codes. These two samples were considered as replicates and averaged and the average value used for the location for the sampling date. The sampling location code 114US1675 was chosen for this sampling location. This correction ensures that there is only one reading per sampling location per sampling date.

[10] Censored data refer to those data that have unknown values because their actual values are below the analytical reporting limit. While assessing the FIB dataset staff discovered that although most censored readings had been substituted with the corresponding reporting limit, some had not. Therefore, these inconsistencies were found and corrected. Specifically, during the 2020 Data Reassessment, staff identified readings listed as “<10 cfu/100 mL” and “<100 cfu/100 mL” in the FIB dataset. Staff replaced these readings with the corresponding reporting limit of 10 cfu/100 mL and 100 cfu/100 mL respectively.

[11] The enterococci reading for the sample collected on June 3, 2010 from Russian River at Healdsburg Veterans Memorial Beach (114RR2940), in the Brooks Creek HUC-12 subwatershed, was originally

Regional Water Board staff conducted the initial TMDL analysis of the FIB data (prior to the August 2019 adoption), using Microsoft Excel to determine exceedance of statewide bacteria objectives and the RWQC. For the 2020 Data Reassessment, Regional Board staff chose to use R, a programming language and software environment designed for statistical and data analysis (R Core Team, 2017). R is an open source software supported by the R Foundation for Statistical Computing and is free to download and use. Programming languages are preferable when assessing large datasets and provide the benefit of reproducibility, clarity, and ease of documentation.

Staff applied the statewide bacteria objectives and RWQC based on assessment guidelines represented by an FIB Assessment Flowchart (see Appendix D). In accordance with the statewide bacteria objectives, RWQC, and applicable provisions of the Listing Policy and as described in detail in (Appendix E), staff applied the following criteria consistently across all relevant datasets:

1) As with the initial analysis, for the 2020 Data Reassessment, staff grouped and assessed data by HUC-12 subwatershed. The data for each parameter were considered collectively for both the mainstem Russian River and tributary segment sampling locations within a given HUC-12 subwatershed.[12]
2) For the 2020 Data Reassessment, staff used the same minimum data requirements to calculate geometric mean (GM) and statistical threshold value (STV) as were used during the initial analysis. Specifically, a minimum of five (5) data points, per sampling location, was used to compute a single GM for an assessment year (year-round) and for winter/wet season samples. A minimum of three (3) data points, per sampling location, was used to compute a single GM for the summer/dry season. A minimum of one (1) data point, per calendar month, per sampling location, was used when determining the STV.

transcribed as “1010 cfu/100 mL”. Staff assumes this entry to be a typographical error since the three readings for this location immediately following June 3, 2010 (June 4, June 8, and June 15, 2010 respectively) are all listed as “10 cfu/100 mL”. Furthermore, the remaining two readings for this location from June 2010 are 31 cfu/100 mL (June 22, 2010) and 41 cfu/100 mL (June 29, 2010). Therefore, the dataset entry for enterococci for June 3, 2010 for Russian River at Healdsburg Veterans Memorial Beach (114RR2940) sampling location has been corrected to 10 cfu/100 mL. The correction of this typographical error prevents the reading for June 3, 2010 from erroneously being calculated to be above the allowed thresholds for enterococci as per the US EPA RWQC.

[12] The landuse based studies conducted as the basis of the TMDL associates landuses present in the Brooks Creek-Russian River, Porter Creek-Russian River, and Dutch Bill Creek-Russian River HUC-12 subwatersheds with evidence of fecal waste pollution. In addition, the fecal indicator bacteria, *Bacteroides*, Phylochip™, and public health advisory data collected within the boundaries of each of these HUC-12 subwatersheds exceed established thresholds, presenting another line of evidence of fecal waste pollution. Finally, when mainstem reaches are assessed separate from the associated tributaries, each of these HUC-12 subwatersheds present evidence of fecal waste pollution. For example, the mainstem reaches of Brooks Creek-Russian River HUC-12 have enterococci, HuBac, and beach posting results that exceed established thresholds. Porter Creek-Russian River HUC-12 mainstem reaches have HuBac and Phylochip™ data that exceed established thresholds. The mainstem reaches of Dutch Bill Creek-Russian River HUC-12 have Phylochip™ and beach posting data that exceed established thresholds.

3) For the 2020 Data Reassessment, staff interpreted the 30-day interval to be equivalent to a static calendar month. Staff selected this approach because calendar months are sufficient approximations of 30 days, as the average number of days in a month is 30. Using calendar months does not influence the calculations in a statistically significant manner, it reduces the introduction of arbitrary start dates, and it simplifies the calculation for different time periods with respect to wet or dry seasons
4) GMs and STVs were calculated for each sampling location, based on minimum sample requirements. The exceedances of the calculated GMs and STVs based on the appropriate WQOs or RWQCs were determined. Staff then grouped the exceedances of GM or STV sampling location results by HUC-12 subwatershed providing a total exceedance of GM or STV of the appropriate WQO or RWQC for each HUC-12 subwatershed included in the analysis. The total exceedances calculated, compared to the total number of GM or STV samples, for each HUC-12 subwatershed were then evaluated in light of the appropriate binomial tables in the Listing Policy to determine whether the regulatory standards were exceeded for that particular HUC-12 subwatershed.
5) Water samples were analyzed for human-source *Bacteroides* using HuBac and for bovine-source *Bacteroides* using BoBac. Hubac and BoBac data were grouped separately by HUC-12 subwatershed and combined to calculate a median value. A median value at or above 10,000 gene copies/100 mL was considered strong evidence of the presence of the associated fecal waste in the water column.
6) Water samples were analyzed for human-source, grazer-source, and bird-source bacteria using the PhyloChip™ phylogenetic DNA microarray, which calculates the percentage of DNA in a given water sample that matches a library of bacterial DNA, sorted by source. A value at or above 10% DNA match with either human-source, grazer-source, or bird-source reference bacteria was considered at least moderate evidence of the presence of the given fecal waste source.

There were no changes to the Public Health Advisory data as presented in the 2019 Staff Report. From the period of 2013 to 2018 there were 15 Public Health Advisories in the Oat Valley Creek-Russian River HUC-12 subwatershed, 4 in the Brooks Creek-Russian River HUC-12 subwatershed, 19 in the Dutch Bill Creek-Russian River HUC-12 subwatershed, and the Lower Santa Rosa Creek HUC-12 subwatershed is posted with a permanent Public Health Advisory.

3 DATA REASSESSMENT SUMMARY OF FINDINGS

The 2019 Staff Report, Chapter 4, presents *E. coli* (Table 4.2), enterococci (Table 4.3), *Bacteroides* (Tables 4.4 and 4.5), and PhyloChip™ (Tables 4.6, 4.7, 4.8) data in separate tables based on geomean, STV, maximum, and median findings, as appropriate, for each of the 42 HUC-12 subwatersheds. It also combines the findings for each of these parameters in a weight of evidence table (Table 4.12) wherein the HUC-12 subwatersheds that meet the Tier One Test as described above are highlighted.

The 2020 Data Reassessment produces a few different results than those reported in Chapter 4 of the 2019 Staff Report. Importantly, there are three differences between the list of HUC-12 subwatersheds meeting Tier One Test as represented in the 2019 Staff Report (and Action Plan) and as reassessed and reported here.

1) Lake Mendocino-East Fork Russian River HUC-12-- Though not identified in the 2019 Staff Report, E. coli data in the Lake Mendocino-East Fork Russian River HUC-12 subwatershed surpasses the exceedance frequency threshold from the binomial tables in the Listing Policy.
2) Sausal Creek-Russian River HUC-12-- Similarly, E. coli data in the Sausal Creek-Russian River HUC-12 subwatershed surpasses the exceedance frequency threshold from the binomial tables in the Listing Policy.
3) Oat Valley-Russian River HUC-12-- Though identified in the 2019 Staff Report as impaired/polluted based on freshwater enterococci data and public health advisories, a reassessment of the freshwater enterococci data in Oat Valley-Russian River HUC-12 subwatershed indicates that results do not surpass the exceedance frequency threshold from the binomial tables in the Listing Policy. Fewer exceedances of the geomean for enterococci were identified in the 2020 reassessment.
4) Brooks Creek-Russian River HUC-12— Reassessment of the E. coli data in the Brooks Creek-Russian River HUC-12 subwatershed surpasses the exceedance frequency threshold from the binomial tables in the Listing Policy, adding to the enterococci and public health advisory data by which this HUC-12 subwatershed was already identified as polluted/impaired.

There is also one difference between the list of HUC-12 subwatersheds meeting the Tier Two Test as reported in the 2019 Staff Report (and Action Plan) and as reassessed and reported here.

1) Porter Creek-Mark West Creek HUC-12-- Specifically, the Porter Creek-Mark West Creek HUC-12 subwatershed was identified in the 2019 Staff Report as impaired/polluted based on *E. coli* data, which remains the case following the 2020 Data Reassessment. There are no *Bacteroides* data with which to specifically assess the presence of human fecal waste, but there are PhyloChip™ data. The maximum percentage of human-sourced bacteria measured using PhyloChip™ was reported in the 2019 Staff Report as 5%. The 2020 Data Reassessment included an evaluation of the geolocation data for monitoring stations and determined that a station, which previously had been identified as belonging in the Lower Santa Rosa Creek HUC-12, actually belonged in the Porter Creek-Mark West Creek HUC-12 subwatershed. As a result, the PhyloChip™ data now shows 12% of the measured bacteria DNA as matching human-sourced bacteria in the reference library, thereby meeting the Tier Two Test.

The specific results of the 2020 Data Reassessment are presented in Appendices D, E, F, and H which includes tables that compare, parameter by parameter, the findings of the 2020 Data Reassessment to the findings as presented in the 2019 Staff Report. In summary, the 2020 Data Reassessment found the following:

- Freshwater E. coli – The 2020 Data Reassessment confirmed E. coli data in all of the HUC-12 subwatersheds reported in the 2019 Staff Report continue to surpass the exceedance frequency threshold from the binomial tables in the Listing Policy indicating impairment/pollution. In fact, the 2020 Data Reassessment resulted in a higher number of exceedances in most of the reported HUC-12 subwatersheds, except the Upper Laguna de Santa Rosa13 and Porter Creek-Russian River14 HUC-12 subwatersheds, which never-the-less, continue to surpass the exceedance frequency threshold from the binomial tables from the Listing Policy indicating impairment/pollution. In addition, the 2020 Data Reassessment found that the geomean as calculated for summer months in the Lake Mendocino-East Fork Russian River HUC-12 subwatershed15 surpass the exceedance frequency threshold from the binomial tables in the Listing Policy indicating impairment/pollution, which was not reported in the 2019 Staff Report.
- Saline Enterococci - The 2020 Data Reassessment confirmed exceedance of the statewide Enterococci objective in the Willow Creek HUC-12 subwatershed that surpasses the exceedance frequency threshold in the binomial tables from the Listing Policy indicating impairment/pollution. In fact, the 2020 Data Reassessment resulted in a higher number of exceedances than reported in 2019 for the summer period.
- Freshwater Enterococci – The 2020 Data Reassessment confirmed exceedance of the RWQC in all of the HUC-12 subwatersheds reported in the 2019 Staff Report as surpassing the binomial tables from the Listing Policy indicating impairment/pollution, except the Oat Valley-Russian River HUC-12 subwatershed.16
- Human Bacteroides-- The HuBac results exceeding a threshold of 10,000 gene copies/100 mL as represented in the 2019 Staff Report are consistent with the 2020 Data Reassessment, except for Porter Creek-Russian River HUC-12. In Porter Creek-Russian River HUC-12, a value of 48,200 gene copies/100 mL was reported in the 2019 Staff Report, which is updated to a value of 48,600 gene copies/100 mL as a result of the 2020 reassessment.

[13] Fourteen exceedances out of 21 calculations of the STV as reported in the 2019 Staff Report versus 13 exceedances out of 20 calculations of the STV as calculated in the 2020 Data Reassessment.

[14] Six exceedances out of 6 calculations of the winter geomean as reported in the 2019 Staff Report versus 0 exceedances out of 141 calculations of the geomean as calculated in the 2020 Data Reassessment.

[15] Four exceedances out of 4 calculations of the summer geomean as calculated in the 2020 Data Reassessment.

[16] Seven exceedances out of 17 calculations of the geomean as reported in the 2019 Staff Report versus 2 exceedances out of 7 calculations of the geomean as calculated in the 2020 Data Reassessment.

- Human PhyloChipTM-- The PhyloChipTM results as represented in the 2019 Staff Report are also consistent with the 2020 Data Reassessment, except for the results reported for Porter Creek-Mark West Creek wherein a value of 5% gene sequence match with human-source reference bacteria DNA is actually a 12% gene sequence match.

4 CONCLUSIONS

The 2020 Data Reassessment required that all *E. coli*, enterococci, *Bacteroides*, and PhyloChip™ data collected in the Russian River watershed as part of several TMDL studies be compiled and additional QA/QC procedures conducted in preparation for geographic-based analyses.[17] As a general matter, the 2020 Data Reassessment confirms the impairment/pollution findings as reported in the 2019 Staff Report and as used as the basis for the Action Plan. There are four exceptions. Based on the Tier 1 Test, Lake Mendocino-East Fork Russian River HUC-12 and Sausal Creek-Russian River HUC-12 are newly determined to exceed established thresholds. Also based on the Tier 1 Test, Oat Valley-Russian River HUC-12 is no longer determined to exceed established thresholds. Based on the Tier 2 Test, Porter Creek-Mark West Creek HUC-12 indicates the presence of human-source fecal waste in the water column.

[17] An R code routine was written to automatically calculate geomeans for E. coli and enterococci data across each of the HUC-12 subwatersheds and across 3 time periods (i.e., all year, winter period, and summer period) using a minimum of 5 sample points for the all year and winter period categories and a minimum of 3 sample points for the summer period. Similarly, an R code routine was written to calculate the STV for E. coli and enterococci data across each of the HUC-12 subwatersheds using a minimum of 1 sample and a static 30-day period based on a calendar month. An R code routine was also written to calculate on a HUC-12 basis the median data results for each of the 2 Bacteroides data types (i.e., HuBac and BoBac). Finally, an R code routine was written to calculate the maximum and median DNA matches for each of 3 PhyloChipTM category types (i.e., human-source, grazer-source, and bird-source reference bacteria

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6 GLOSSARY

Binomial Distribution: A binomial distribution is a mathematical distribution that describes the probabilities associated with the possible number of times particular outcomes will occur in a series of observations (i.e., samples). Each observation may have only one of two possible results (e.g., standard exceeded or standard not exceeded) (SWRCB, 2015).

Calendar year: Twelve consecutive calendar months (SWRCB, 2019).

Calendar month: A period of time from a day of one month to the day before the corresponding day of the next month if the corresponding day exists, or if not to the last day of the next month (e.g., from January 1 to January 31, from June 15 to July 14, or from January 31 to February 28) (SWRCB, 2019).

Data Point: Data acquired from a laboratory that measures concentrations of indicator bacteria or other water quality parameters. Each sampling station has one data point per sampling date.

- ***Sample***: A sample is either a single weekly geometric mean from a given subwatershed or, if assessing based on Statistical Threshold Value calculated for the subwatershed over the calendar month for which there are lab sample data.

Exceedance: A sample above a statewide water quality objective threshold as per Part 3 of the Inland Surface Waters, Enclosed Bays and Estuaries Plan (ISWEBE Plan), above a federal water quality criterion threshold as per the United States Environmental Protection Agency Recreational Water Quality Criteria (USEPA RWQC), or other established threshold.

***Fecal Indicator Bacteria* (*FIB*)**: The statewide REC-1 WQO and US EPA RWQC, require the use of fecal indicator bacteria to assess the microbiological quality of water and to detect evidence of fecal pollution. FIB live in the intestines of warm-blooded animals (including humans) and enter water bodies through fecal matter. These bacteria are often harmless to human beings, but are used as indicators of, or proxies for, harmful pathogens, that may also exist in the intestines of animals along with FIB. This type of indicator method is used since it is impossible to measure all potentially harmful pathogens that may exist in the waterbody being investigated. The use of FIB in this manner is very common, scientifically accepted, and approved by the US EPA and the California EPA. FIB testing is performed in government-accredited labs using culture-based methods.

Geometric mean (GM): A type of mean or average that indicates the central tendency or typical value of a set of numbers by using the product of their values (as opposed to the arithmetic mean which uses their sum). The geometric mean is defined as the n^{th} root of the product of n numbers (SWRCB, 2019).

Line of Evidence: A line of evidence refers to a single piece of evidence used when performing scientific assessments. While performing scientific inquiry, several different lines of evidence are used together to employ a weight-of-evidence approach and reach a scientifically defensible conclusion. (SETAC, 2018; EFSA, 2017). The weight of evidence assessment comprises three basic steps: (1) assembling the evidence into lines of evidence of similar type, (2) weighing the evidence and (3) integrating the evidence (EFSA, 2017).

***Microbial Source Tracking* (*MST*)**: Microbial source tracking is a tool used by scientists, and approved for use by the California EPA, the United States EPA and by the United States Geological Survey (USGS), to identify the source of fecal contamination. The basis of this tool is that the microorganisms present in the feces of different types of animals (including humans) have unique characteristics that allow us to determine what specific animal the fecal matter being tested came from. In other words, the physiological differences in various hosts (animals whose feces is being analyzed) lead to differences in specific characteristics of microorganisms that are present in their intestines. Microbial source tracking is a culture-independent method performed in government-accredited labs.

- ***PhyloChip™***: The PhyloChip is a low-cost Affymetrix GeneChip microarray, developed at Lawrence Berkeley National Laboratory (LBNL), designed to detect and quantify abundance of bacterial and archaeal taxa using signature probes targeting all known 16S rRNA gene sequences (Schatz et al, 2010). 16S rRNA gene sequencing is used to identify bacteria. PhyloChip™ technology can be used for microbial source tracking to determine the source of fecal pollution. Specifically, whether the fecal matter came from humans, grazers, shorebirds or other animal hosts.

Null Hypothesis: A null hypothesis is a statement used in statistical testing that has been put forward either because it is believed to be true or because it is to be used as a basis for argument, but has not been proved (SWRCB, 2015).

Period: A single time frame for which pathogen data are assessed. A period can be one Assessment Year, one winter season, or one dry season, defined below.

- ***Assessment Year (year-round)***: November 1 of Calendar Year 1 through October 31 of Calendar Year 2. *e.g.* November 1, 2014 through October 31, 2015.

- ***Winter or wet season***: November 1 through March 31 of the Assessment Year. *e.g.* November 1, 2014 through March 31, 2015.

- ***Summer or dry season***: April 1 through October 31 of the Assessment Year. *e.g.* April 1, 2015 through October 31, 2015.

- ***Rolling window***: A time frame for deriving the GM. The applied GM is calculated in a window that rolls one week at time. The GM is calculated in a window that rolls one week at time. See the figure below.

Window 1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6		
Window 2		Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	
Window 3			Week 3	Week 4	Week 5	Week 6	Week 7	Week 8

When calculating a GM, the window, for this assessment, starts on a Sunday and ends on a Saturday. In cases where a period's first window does not start on a Sunday and/or the last window does not end on a Saturday, the dates are "padded," but any data from those dates are excluded. If the final window's Saturday is outside the period, then that is not calculated. More information on rolling windows used for geometric mean calculations can be found in Appendices H and I.

Statewide Water Quality Objectives (WQO) for Bacteria: Part 3 of the Inland Surface Waters, Enclosed Bays and Estuaries Plan (ISWEBE Plan) - the Bacteria Provisions and Variance Policy which establishes water quality objectives for reasonable protection of people that recreate within all surface waters, enclosed bays, and estuaries of the state that have the water contact recreation beneficial use (REC-1) (SWRCB, 2019). The ISWEBE Plan lists two Bacteria WQO applicable to waters with the REC-1 beneficial use, depending on the salinity level, as discussed below (SWRCB, 2019).

- ***Escherichia coli (E. coli)***: The bacteria water quality objective for all waters where the salinity is equal to or less than 1 part per thousand (ppth) 95 percent or more of the time during the CALENDAR YEAR is: a six-week rolling GEOMETRIC MEAN of *Escherichia coli* (*E. coli*) not to exceed 100 colony forming units (cfu) per 100 milliliters (mL), calculated weekly, and a STATISTICAL THRESHOLD VALUE (STV) of 320 cfu/100 mL not to be exceeded by more than 10 percent of the samples collected in a CALENDAR MONTH, calculated in a static manner (SWRCB, 2019).

- **Enterococci:** The bacteria water quality objective for all waters where the salinity is greater than 1 ppth more than 5 percent of the time during the CALENDAR YEAR is: a six-week rolling GEOMETRIC MEAN of enterococci not to exceed 30 cfu/100 mL, calculated weekly, with a STV of 110 cfu/100 mL not to be exceeded by more than 10 percent of the samples collected in a CALENDAR MONTH, calculated in a static manner (SWRCB, 2019).

REC-1 Bacteria Water Quality Objectives (SWRCB, 2019)

Applicable Waters	Objective Elements	Estimated Illness Rate (NGI): 32 per 1,000 water contact recreators	Estimated Illness Rate (NGI): 32 per 1,000 water contact recreators
	Indicator	Magnitude GM (cfu/100 mL)	Magnitude STV (cfu/100 mL)
All waters where salinity is equal to or less than 1 ppth 95 percent or more of the time	*E. coli*	100	320
All waters where the salinity is greater than 1 ppth more than 5 percent of the time	Enterococci	30	110

The waterbody GM shall not be greater than the applicable GM magnitude in any six-week interval, calculated weekly. The applicable STV shall not be exceeded by more than 10 percent of the samples collected in a CALENDAR MONTH, calculated in a static manner

NGI = National Epidemiological and Environmental Assessment of Recreational Water gastrointestinal illness rate

GM = geometric mean

STV = statistical threshold value

cfu = colony forming units

mL = milliliters

ppth = parts per thousand

Statistical Significance: Statistical significance occurs when it can be demonstrated that the probability of obtaining a difference by chance only is relatively low (SWRCB, 2015)

Statistical Threshold Value (STV): The STV for the bacteria water quality objectives is a set value that approximates the 90th percentile of the water quality distribution of a bacterial population (SWRCB, 2019).

Water Contact Recreation (REC-1): Uses of water for recreational activities involving body contact with water, where ingestion of water is reasonably possible. These uses include, but are not limited to, swimming, wading, water-skiing, skin and scuba diving, surfing, white-water activities, fishing, or use of natural hot springs (NCRWQCB, 2018).

- ***Primary Contact Recreation***: The primary contact recreation classification protects people from illness due to activities involving the potential for ingestion of, or immersion in, water. Primary contact recreation usually includes swimming, water-skiing, skin-diving, surfing, and other activities likely to result in immersion (USEPA, 2012).

United States Environmental Protection Agency Recreational Water Quality Criteria (RWQC): The US EPA RWQC provides the EPA's recommended CWA §304(a) RWQC for states consisting of the magnitude, duration, and frequency of excursions for enterococci and *E. coli* as measured by culture-based methods (USEPA, 2012). EPA provides two sets of recommended criteria, each of which correspond to two different illness rates. The designated use of primary contact recreation would be protected if either set of criteria recommendations are adopted into state water quality standards (WQS) and approved by EPA (USEPA, 2012). An estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators was used during the initial analysis and for the 2020 reassessment. Table 1 (Recommended 2012 RWQC) (USEPA, 2012) is displayed below

Recommended 2012 RWQC (USEPA, 2012)

Criteria Elements	**Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators**	**Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators**		**Estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators**	**Estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators**
	Magnitude	**Magnitude**		**Magnitude**	**Magnitude**
Indicator	GM (cfu/100 mL)	STV (cfu/100 mL)	OR	GM (cfu/100 mL)	GM (cfu/100 mL)
Enterococci – marine and fresh	35	130		30	110
OR					
E. coli - fresh	126	410		100	320

Duration and Frequency: The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a ten percent excursion frequency of the selected STV magnitude in the same 30-day interval

[a] EPA recommends using EPA Method 1600 (U.S. EPA, 2002a) to measure culturable enterococci, or another equivalent method that measures culturable enterococci and using EPA Method 1603 (U.S. EPA, 2002b) to measure culturable *E. coli*, or any other equivalent method that measures culturable *E. coli*.

7 APPENDICES

APPENDIX A – MONITORING STATION NAMES

Initial Microbial Source Tracking (MST) Analysis Sampling Locations with Alternative Names and their corresponding FIB Analysis Sampling Location Names

	Initial Analysis	**2020 Reassessment**	**2020 Reassessment**
HUC-12 subwatershed	**Alternative Sampling Location Name (MST Analysis)**	**Sampling Location Name (FIB Analysis)**	**Sampling Location Name (MST Analysis)**
Cummiskey Creek-Russian River	Commisky Station Road	Russian River at Highway 101	Russian River at Highway 101
West Slough-Dry Creek	Healdsburg LD-LR2	Unnamed Tributary at West Dry Creek Road	Unnamed Tributary at West Dry Creek Road
West Slough-Dry Creek	Foss Creek	Foss Creek at Matheson Street	Foss Creek at Matheson Street
West Slough-Dry Creek	Lambert Creek	Unnamed Tributary at Lambert Bridge Road	Unnamed Tributary at Lambert Bridge Road
Mill Creek	Palmer Creek	Palmer Creek at Palmer Creek Road	Palmer Creek at Palmer Creek Road
Oat Valley Creek-Russian River	Cloverdale River Park	Russian River at Cloverdale River Park	Russian River at Cloverdale River Park
Sausal Creek-Russian River	Healdsburg LD-LR1	Unnamed Tributary at Fredson Road	Unnamed Tributary at Fredson Road
Sausal Creek-Russian River	Healdsburg LD-LR3	Unnamed Tributary at Alexander Valley Road	Unnamed Tributary at Alexander Valley Road
Sausal Creek-Russian River	Alex Valley Campground	Russian River at Alexander Valley Road	Russian River at Alexander Valley Road
Sausal Creek-Russian River	Geyserville Hwy Bridge	Russian River at Highway 128	Russian River at Highway 128
Brooks Creek-Russian River	Healdsburg AOC	Unnamed Tributary at Redwood Drive	Unnamed Tributary at Redwood Drive

HUC-12 subwatershed	Initial Analysis	2020 Reassessment	2020 Reassessment
	Alternative Sampling Location Name (MST Analysis)	Sampling Location Name (FIB Analysis)	Sampling Location Name (MST Analysis)
Brooks Creek-Russian River	Healdsburg HD-HR	Unnamed Tributary at Fitch Mountain Road	Unnamed Tributary at Fitch Mountain Road
Brooks Creek-Russian River	Memorial Beach	Russian River at Healdsburg Veterans Memorial Beach	Russian River at Healdsburg Veterans Memorial Beach
Brooks Creek-Russian River	Camp Rose	Russian River at Camp Rose	Russian River at Camp Rose
Upper Santa Rosa Creek	Santa Rosa Creek at Los Alamos Road	Santa Rosa at Los Alamos Road	Santa Rosa at Los Alamos Road
Lower Santa Rosa Creek	Piner Creek	Piner Creek at Fulton Road	Piner Creek at Fulton Road
Lower Santa Rosa Creek	Santa Rosa Creek at Railroad Street	Santa Rosa at Railroad Street	Santa Rosa at Railroad Street
Lower Santa Rosa Creek	Abramson Creek	Abramson Creek at Willowside Road Levy	Abramson Creek at Willowside Road Levy
Upper Laguna de Santa Rosa	Blucher Creek	Blucher Creek at Lone Pine Road	Blucher Creek at Lone Pine Road
Upper Laguna de Santa Rosa	Copeland Creek	Copeland Creek at Commerce Drive	Copeland Creek at Commerce Drive
Upper Laguna de Santa Rosa	Crane Creek	Crane Creek at Snyder Lane	Crane Creek at Snyder Lane
Upper Laguna de Santa Rosa	Gossage Creek	Gossage Creek at Gilmore Avenue	Gossage Creek at Gilmore Avenue
Upper Laguna de Santa Rosa	Turner Creek	Unnamed Tributary at Turner and Daywalt Road	Unnamed Tributary at Turner and Daywalt Road

HUC-12 subwatershed	Initial Analysis Alternative Sampling Location Name (MST Analysis)	2020 Reassessment Sampling Location Name (FIB Analysis)	2020 Reassessment Sampling Location Name (MST Analysis)
Lower Laguna de Santa Rosa	Laguna de Santa Rosa	Laguna de Santa Rosa at Sebastopol Community Center	Laguna de Santa Rosa at Sebastopol Community Center
Lower Laguna de Santa Rosa	Irwin Creek	Unnamed Tributary at Sanford Road	Unnamed Tributary at Sanford Road
Porter Creek-Mark West Creek	Woolsey Creek	Unnamed Tributary at River Road	Unnamed Tributary at River Road
Porter Creek-Mark West Creek	Van Buren Creek	Van Buren Creek at St. Helena Road	Van Buren Creek at St. Helena Road
Porter Creek-Russian River	Forestville HD-LR1	Unnamed Tributary at Trenton Road	Unnamed Tributary at Trenton Road
Porter Creek-Russian River	Forestville HD-LR2	Unnamed Tributary at Del Rio Court	Unnamed Tributary at Del Rio Court
Porter Creek-Russian River	Steelhead Beach	Russian River at Steelhead Beach	Russian River at Steelhead Beach
Porter Creek-Russian River	Limerick Creek	Unnamed Tributary at Old Redwood Highway	Unnamed Tributary at Old Redwood Highway
Green Valley Creek	Green Valley Creek	Green Valley Creek at Martinelli Road	Green Valley Creek at Martinelli Road
Dutch Bill Creek-Russian River	Monte Rio HD-HR	Unnamed Tributary at River Road near Duncan Road	Unnamed Tributary at River Road near Duncan Road
Dutch Bill Creek-Russian River	Monte Rio HD-LR	Unnamed Tributary at Main Street	Unnamed Tributary at Main Street
Dutch Bill Creek-Russian River	Monte Rio AOC	Unnamed Tributary at Foothill Drive	Unnamed Tributary at Foothill Drive
Dutch Bill Creek-Russian River	Monte Rio Beach	Russian River at Monte Rio Beach	Russian River at Monte Rio Beach
Dutch Bill Creek-Russian River	Forestville HD-HR	Unnamed Tributary at River Drive	Unnamed Tributary at River Drive

HUC-12 subwatershed	Initial Analysis Alternative Sampling Location Name (MST Analysis)	2020 Reassessment Sampling Location Name (FIB Analysis)	2020 Reassessment Sampling Location Name (MST Analysis)
Dutch Bill Creek-Russian River	Northwood HD-LR	Unnamed Tributary at Old Monte Rio Road	Unnamed Tributary at Old Monte Rio Road
Dutch Bill Creek-Russian River	Rio Nido LD-HR	Unnamed Tributary at River Road near Rio Nido	Unnamed Tributary at River Road near Rio Nido
Dutch Bill Creek-Russian River	Camp Meeker AOC	Unnamed Tributary at Market Street	Unnamed Tributary at Market Street
Dutch Bill Creek-Russian River	Dutch Bill Creek	Dutch Bill Creek at Fir Road	Dutch Bill Creek at Fir Road
Dutch Bill Creek-Russian River	Johnson’s Beach	Russian River at Johnson’s Beach	Russian River at Johnson’s Beach
Dutch Bill Creek-Russian River	Forestville Access Beach	Russian River at Forestville Access Beach	Russian River at Forestville Access Beach
Dutch Bill Creek-Russian River	Mays Creek	Mays Creek at Neeley Road	Mays Creek at Neeley Road
Willow Creek-Russian River	Cassini LD-HR	Unnamed Tributary at Moscow Road	Unnamed Tributary at Moscow Road
Willow Creek-Russian River	Jenner Boat Ramp	Russian River at Jenner Boat Ramp	Russian River at Jenner Boat Ramp

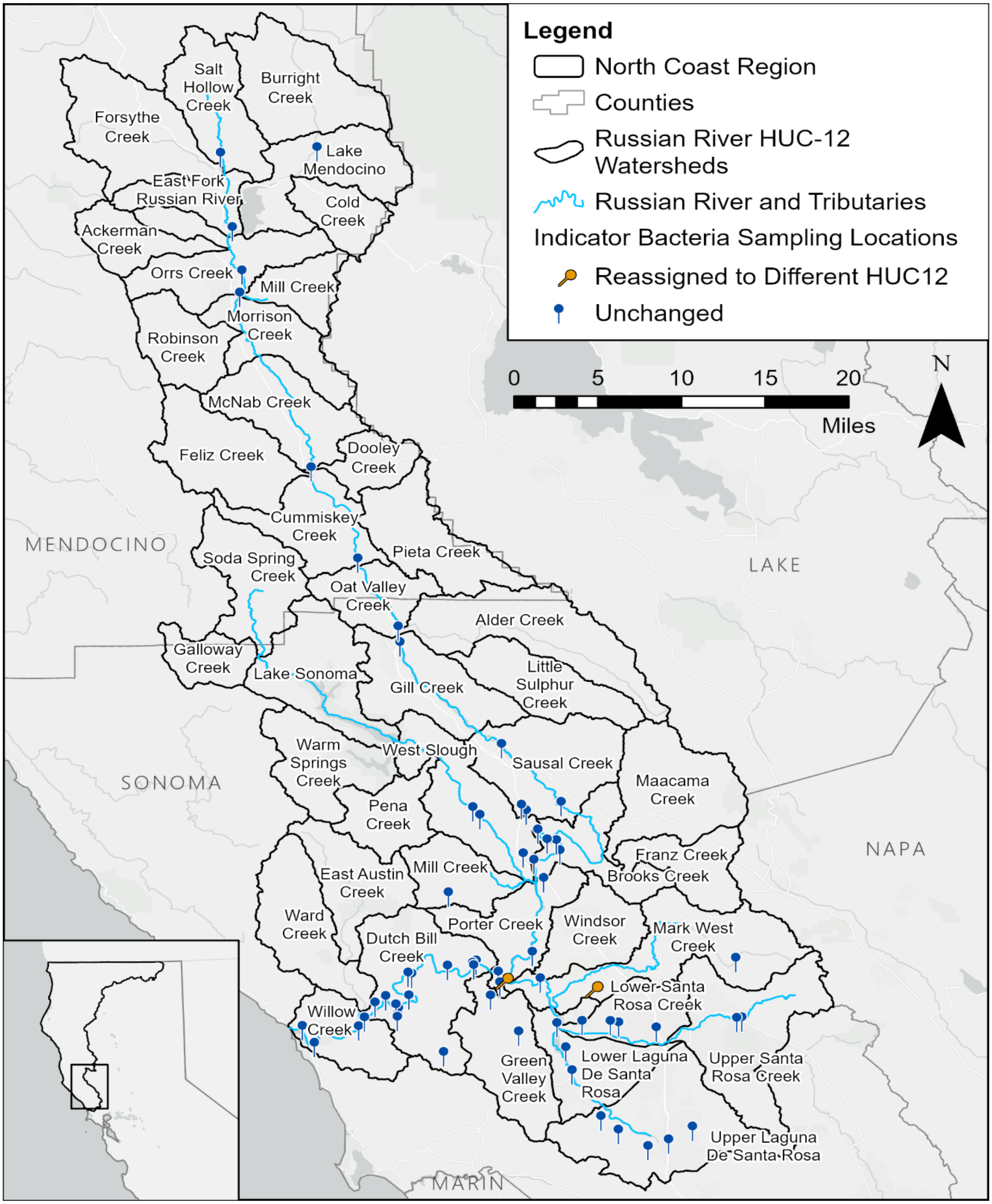
APPENDIX B – MONITORING STATION CODES

Corrected Sampling Location Codes

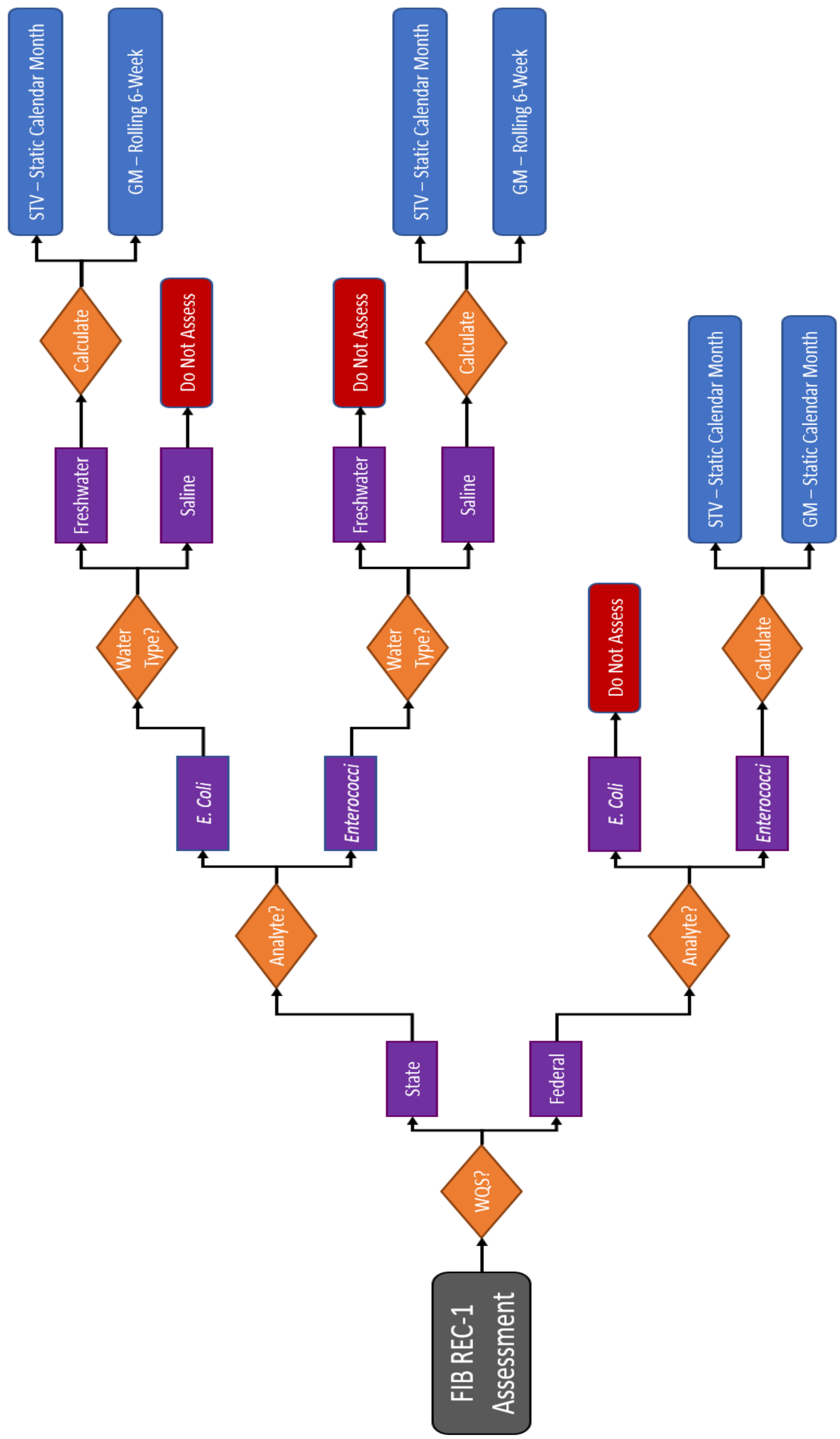
Incorrect Sampling Location Code	**Correct Sampling Location Code**	**Sample Location Name**
114RR6966	114RR6968	Russian River at River Road near Hopland
114RR6967	114RR6968	Russian River at River Road near Hopland
114RR6969	114RR6968	Russian River at River Road near Hopland
114EF6318	114EF6320	Russian River, East Fork at East Road
114EF6319	114EF6320	Russian River, East Fork at East Road
114RR8263	114RR8265	Russian River at Talmage Road
114RR8264	114RR8265	Russian River at Talmage Road
114RR8400	114RR8402	Russian River at Vichy Springs Road
114RR8401	114RR8402	Russian River at Vichy Springs Road
114RR8707	114RR8709	Russian River at Mendocino Drive
114RR8708	114RR8709	Russian River at Mendocino Drive
114RR9179	114RR9181	Russian River at East School Way
114RR9180	114RR9181	Russian River at East School Way

APPENDIX C – MAP OF MONITORING STATIONS

Fecal Indicator Bacteria Sampling Locations



APPENDIX D – FIB ASSESSMENT FLOW CHART



APPENDIX E – ASSESSMENT FUNDAMENTALS

Assessment Fundamentals

The fundamental business rules applied during data assessment and the state and federal water quality thresholds against which the data are compared are described below. The business rules applied during the 2020 reassessment are identical to those applied during the initial analysis.

Assessment Periods

As with the initial analysis, during the 2020 reassessment, staff applied three (3) assessment periods when evaluating FIB data for exceedances of regulatory standards:

- summer/dry season (April 1 through October 31 of an assessment year),
- winter/wet season (November 1 through March 31 of an assessment year), and
- year or year-round (November 1 of Calendar Year 1 through October 31 of Calendar Year 2).

The Listing Policy provides specific guidance on which binomial table to use depending on the assessment period being considered (SWRCB, 2015). For an assessment year (year-round) and for winter/wet season assessments, a ten (10) percent exceedance frequency as described in Table 3.2 (The Minimum Number Of Measured Exceedances Needed To Place A Water Segment On The Section 303(D) List For Conventional Or Other Pollutants) of the Listing Policy binomial tables is used to reject the null hypothesis (SWRCB, 2015). For assessment of bacterial measurements from inland waters collected April 1 through October 31, a four (4) percent exceedance percentage shall be used if (1) bacterial measurements are indicative of human fecal matter, and (2) there is substantial human contact in the water body (SWRCB, 2015). For these samples, the Listing Policy states that the binomial table, Table AB 411-Coastal Beaches List (Pursuant To AB 411: The Minimum Number Of Measured Exceedances Of Bacterial Standards For Coastal Beaches Needed To Place A Water Segment On The Section 303(D) List When Water Quality Monitoring Was Conducted April 1 Through October 31) should be used to reject the null hypothesis (SWRCB, 2015). As presented in the FIB datasets (Appendices A and B) and described in sections 3.3 and 3.4 of this Technical Memorandum, these criteria are met for Russian River data collected April 1 through October 31. Therefore, staff applied the four (4) percent exceedance percentage to assess summer/dry season data.

Assessment Sample Size Threshold

For the 2020 reassessment, staff used the same minimum data requirements to calculate geometric mean (GM) and statistical threshold value (STV) as were used during the initial analysis. Specifically, a minimum of five (5) data points, per sampling location, was used to compute a single GM for an assessment year (year-round) and for winter/wet season samples. A minimum of three (3) data points, per sampling location, was used to compute a single GM for the summer/dry season. A minimum of one (1) data point, per calendar month, per sampling location, was used when determining the STV.

Exceedances

An exceedance is defined as a calculated GM or STV sample value, for a particular sampling location, that is above the maximum allowable GM or STV value listed in the regulatory standards. For the initial analysis, and the 2020 reassessment, staff utilized the Listing Policy's binomial tables to identify exceedances of both the REC-1 WQO and the US EPA RWQC. The Listing Policy uses a binomial distribution and applies a statistical test known as the null hypothesis to assess any statically significant difference in the data that could be caused by sampling or laboratory error.

Staff determined exceedances of the regulatory standards by HUC-12 subwatershed using the following procedure. GMs and STVs were calculated for each sampling location, based on minimum sample requirements. The exceedances of the calculated GMs and STVs based on the appropriate WQOs or RWQCs were determined. Staff then grouped the exceedances of GM or STV sampling location results by HUC-12 subwatershed providing a total exceedance of GM or STV of the appropriate WQO or RWQC for each HUC-12 subwatershed included in the analysis. The total exceedances calculated, compared to the total number of GM or STV samples, for each HUC-12 subwatershed were then evaluated in light of the appropriate binomial tables (as described in section 2.5 below) in the Listing Policy to determine whether the regulatory standards were exceeded for that particular HUC-12 subwatershed.

Objectives and Criteria Applied to Fecal Indicator Bacteria (FIB)

Statewide REC-1 Water Quality Objectives

Part 3 of the Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California (ISWEBE Plan) establishes water quality objectives (WQO) for reasonable protection of people that recreate within all surface waters, enclosed bays, and estuaries of the state with the contact recreation beneficial use (REC-1). As with the initial analysis, staff considered data during the 2020 reassessment in light of the ISWEBE REC-1 objectives for *E. coli* in freshwaters, and enterococci where waters are saline, to determine if values exceeded the statewide criteria.

Table 1 (REC-1 Bacteria Water Quality Objectives) of Part 3 of the ISWEBE Plan defines the REC-1 WQO as either a six-week rolling GM or a monthly STV (SWRCB, 2019). The REC-1 WQO for freshwaters (all waters where the salinity is equal to or less than 1 part per thousand (ppth) 95 percent or more of the time during the calendar year) is a six-week rolling GM of *E. coli* not to exceed 100 colony forming units (cfu) per 100 milliliters (mL), calculated weekly, and an STV of 320 cfu/100 mL not to be exceeded by more than 10 percent of the samples collected in a calendar month, calculated in a static manner (SWRCB, 2019).

The statewide REC-1 WQO for saline waters (all waters where the salinity is greater than 1 ppth more than 5 percent of the time during the calendar year) is a six-week rolling GM of enterococci not to exceed 30 cfu/100 mL, calculated weekly, with a STV of 110 cfu/100 mL not to be exceeded by more than 10 percent of the samples collected in a calendar month, calculated in a static manner (SWRCB, 2019).

While all other HUC-12 subwatersheds included in Russian River contain water segments classified solely as freshwater, the Willow Creek-Russian River HUC-12 subwatershed contains both saline and freshwater water segments. Therefore, in accordance with the ISWEBE Plan , and similar to the initial analysis, when assessing the Willow Creek-Russian River HUC-12 subwatershed, only those segments classified as freshwater were included in the statewide REC-1 *E. coli* objectives assessment, and only those segments classified as saline were included in the statewide REC-1 enterococci objectives assessment (SWRCB, 2019). Saline water segments in the Willow-Creek-Russian River HUC-12 subwatershed are – Russian River at Jenner Boat Ramp (114RR0066), Russian River at Bridgehaven (Bridgehaven_SCWA), and Russian River at Duncan Mills (Duncan_Mills_SCWA). There is only one freshwater segment in the Willow Creek-Russian River HUC-12 subwatershed – Unnamed Tributary at Moscow Road (114C07MRC). A flowchart describing the FIB assessment process that staff followed during initial analysis as well as the 2020 reassessment has been provided in Appendix H.

US EPA Recreational Water Quality Criteria

As with the initial analysis, in addition to the statewide REC-1 WQO, staff also reassessed all enterococci data within the Russian River Watershed to identify any exceedance of the 2012 US EPA Recreational Water Quality Criteria (US EPA RWQC). Table 1 (Recommended 2012 RWQC) of the US EPA RWQC, sets the enterococci criteria for the protection of human health in marine and freshwater designated for use for swimming, bathing, surfing, or similar water contact activities (primary contact recreation) (USEPA, 2012). The 2020 reassessment applied the US EPA RWQC for an estimated illness rate of 32 per 1000 primary contact recreators - a GM of 30 cfu/100 mL and an STV of 110 cfu/100 mL for enterococci in marine and freshwaters (USEPA, 2012). This was the same estimated illness rate used during the initial analysis.

The EPA recommends that the waterbody GM not be greater than the selected GM magnitude (30 cfu/100 mL) in any 30-day interval and that there should not be greater than a ten percent excursion frequency of the selected STV magnitude (STV of 110 cfu/100 mL) in the same 30-day interval (USEPA, 2012). The EPA allows for the calculations to be performed in a static or rolling manner (USEPA, 2012).
For the 2020 reassessment, staff interpreted the 30-day interval to be equivalent to a static calendar month. Staff selected this approach because calendar months are sufficient approximations of 30 days, as the average number of days in a month is 30. Using calendar months does not influence the calculations in a statistically significant manner, it reduces the introduction of arbitrary start dates, and it simplifies the calculation for different time periods with respect to wet or dry seasons. A flowchart describing the FIB assessment process has been provided in Appendix H. During the initial analysis staff used a static 30-day month interval

which resulted in the exclusion of data associated with the last five (5) or six (6) days of the year in order to get an even number of 360 days per year.

Water Bodies Included in the Analysis

The full extent of surface water drainage for the United States is mapped using a hierarchical system of hydrologic units at various scales. Each hydrologic unit is assigned a hydrologic unit code (HUC). The hydrologic units are organized by hierarchy which is indicated by the number associated with the HUC code (e.g. HUC-4, HUC-8, and HUC-12, where HUC-4 represents the subregion level, delineating large river basins, HUC-8 represents the subbasin level, delineating medium-sized river basins, and HUC-12 represents a more local subwatershed level that captures tributary systems). As with the initial analysis, for the 2020 reassessment, staff grouped and assessed data by HUC-12 subwatershed. The data for each parameter were considered collectively for both the mainstem Russian River and tributary segment sampling locations within a given HUC-12 subwatershed.

APPENDIX F – COMPARISON OF *E. COLI* DATA

HUC-12 Subwatershed Exceeding Statewide REC-1 WQO for E. coli (Initial Analysis) and HUC-12 Subwatershed Exceeding Statewide REC-1 WQO (2020 Data Reassessment)

HUC-12 subwatershed[a]	Initial Analysis	Initial Analysis	2020 Reassessment	2020 Reassessment
	Geometric Mean Exceedance / Sample Count[b]	Statistical Threshold Value Exceedance / Sample Count[b]	Geometric Mean Exceedance / Sample Count[b]	Statistical Threshold Value Exceedance / Sample Count[b]
Lake Mendocino-East Fork Russian River[c]	0/0 - Year-Round	1/1 - Year-Round	0/0 - Year-Round 0/0 - Winter 4/4 - Summer	1/1 - Year-Round 0/0 - Winter 1/1 - Summer
West Slough-Dry Creek	0/0 - Year-Round	5/8 - Year-Round	2/2 - Year-Round 2/2 - Winter 0/0 - Summer	15/20 - Year-Round 12/15 - Winter 3/5 - Summer
Sausal Creek-Russian River[c]	0/91 - Year-Round	5/38 - Year-Round	0/91 - Year-Round 0/0 - Winter 0/105 - Summer	7/42 - Year-Round 5/10-Winter 2/32 - Summer
Brooks Creek-Russian River[c]	0/284 - Year-Round	6/110 - Year-Round	0/267- Year-Round 0/0 - Winter 0/341 - Summer	8/108 - Year-Round 6/10-Winter 2/98 - Winter

[a] All exceedances above the statewide REC-1 WQO for E. coli are marked in bold.

[b] All exceedances are year-round assessments unless specified.

[c] HUC-12 subwatersheds with exceedances above the statewide REC-1 WQO for E. coli that were identified during the 2020 reassessment but not during the initial analysis

HUC-12 subwatershed[a]	Initial Analysis Geometric Mean Exceedance / Sample Count[b]	Initial Analysis Statistical Threshold Value Exceedance / Sample Count[b]	2020 Reassessment Geometric Mean Exceedance / Sample Count[b]	2020 Reassessment Statistical Threshold Value Exceedance / Sample Count[b]
Upper Santa Rosa Creek	0/0 - Year-Round	8/49 - Year-Round	22/26 - Year-Round 0/0 - Winter 30/34-Summer	15/61 - Year-Round 4/20 - Winter 11/41-Summer
Lower Santa Rosa Creek	55/57 - Year-Round	50/93 - Year-Round	55/57- Year-Round 2/2 - Winter 89/91-Summer	55/95 - Year-Round 22/31-Winter 33/64-Summer
Upper Laguna de Santa Rosa	0/0 - Year-Round	14/21 - Year-Round	0/0 - Year-Round 0/0 - Winter 0/0 - Summer	13/20 - Year-Round 11/15-Winter 2/5 - Summer
Lower Laguna de Santa Rosa	7/17 - Year-Round	7/10 - Year-Round	11/21 - Year-Round 4/4 - Winter 10/21-Summer	15/22 - Year-Round 11/13-Winter 4/9-Summer
Porter Creek-Mark West Creek	4/6 - Summer	1/7 - Year-Round	4/6 - Year-Round 0/0 - Winter 5/10-Summer	2/11- Year-Round 2/6 - Winter 0/5 - Summer

HUC-12 subwatershed[a]	Initial Analysis Geometric Mean Exceedance / Sample Count[b]	Initial Analysis Statistical Threshold Value Exceedance / Sample Count[b]	2020 Reassessment Geometric Mean Exceedance / Sample Count[b]	2020 Reassessment Statistical Threshold Value Exceedance / Sample Count[b]
Porter Creek-Russian River	6/6 - Winter	7/59 - Year-Round	0/141 - Year-Round 0/0 - Winter 0/182 - Summer	10/64 - Year-Round 8/10-Winter 2/54 - Summer
Green Valley Creek	8/19 - Year-Round	10/17 - Year-Round	8/19 - Year-Round 2/2 - Winter 10/21-Summer	11/19 - Year-Round 8/11-Winter 3/8-Summer
Dutch Bill Creek-Russian River	1/369 - Year-Round	8/18 - Winter	1/369 - Year-Round 0/0 - Winter 6/568 - Summer	17/162 - Year-Round 11/24 - Winter 6/138 - Summer

[a]All exceedances above the statewide REC-1 WQO for *E. coli* are marked in **bold**.

[b]All exceedances are year-round assessments unless specified

[c]HUC-12 subwatersheds with exceedances above the statewide REC-1 WQO for *E. coli* that were identified during the 2020 reassessment but not during the initial analysis

APPENDIX G – COMPARISON OF ENTEROCOCCI DATA

HUC-12 Subwatersheds Exceeding Statewide US EPA RWQC for enterococci (Initial Analysis) and HUC-12 Subwatershed Exceeding Statewide REC-1 WQO (2020 Data Reassessment)

	Initial Analysis	**Initial Analysis**	**2020 Reassessment**	**2020 Reassessment**
HUC-12 subwatershed[d]	**Geometric Mean** **Exceedance / Sample Count[e]**	**Statistical Threshold Value** **Exceedance / Sample Count[b]**	**Geometric Mean** **Exceedance / Sample Count[b]**	**Statistical Threshold Value** **Exceedance / Sample Count[b]**
Cummiskey Creek-Russian River	14/27 - Year-Round	6/27 - Year-Round	3/7 – Year-Round 0/0 - Winter 7/17-Summer	5/27 – Year-Round 1 / 2 - Winter 4/25-Summer
West Slough-Dry Creek	17/18 - Year-Round	15/18 - Year-Round	0/0 – Year-Round 0/0 - Winter 0/0 - Summer	15/18 – Year-Round 13/14-Winter 2/4 - Summer
Oat Valley Creek-Russian River[c]	7/17 - Year-Round	2/17 - Year-Round	2/7 – Year-Round 0/0 - Winter 2/10 - Summer	2/17 – Year-Round 1 / 2 - Winter 1/15 - Summer
Sausal Creek-Russian River	18/42 - Year-Round	14/42 - Year-Round	5/14 – Year-Round 0/0 - Winter 6/21-Summer	14/42 – Year-Round 7/10-Winter 7/32-Summer

[d] All exceedances above the statewide REC-1 WQO for E. coli are marked in bold.

[e] All exceedances are year-round assessments unless specified.

Brooks Creek-Russian River	19/87 - Year-Round	10/87 - Year-Round	5/27 - Year Round 0/0 - Winter 5/45-Summer	9/81 - Year-Round 6/10-Winter 3/71 - Summer
Upper Santa Rosa Creek	10/11 - Year-Round	9/11 - Year-Round	3/3 - Year-Round 0/0 - Winter 6/6-Summer	10/13 - Year-Round 3 / 4 - Winter 7/9-Summer
Lower Santa Rosa Creek	37/42 - Year-Round	29/42 - Year-Round	2/5 - Year-Round 0/0 - Winter 9/12-Summer	34/49 - Year-Round 10/16-Winter 24/33-Summer
Upper Laguna de Santa Rosa	19/20 - Year-Round	16/20 - Year-Round	0/0 – Year-Round 0/0 - Winter 0/0 - Summer	16/21 - Year-Round 12/16-Winter 4/5-Summer
Lower Laguna de Santa Rosa	14/17 - Year-Round	13/17 - Year-Round	2/2 - Year-Round 0/0 - Winter 4/4-Summer	16/22 - Year-Round 11/13-Winter 5/9-Summer
Porter Creek-Mark West Creek	5/7 - Year-Round	3/7 - Year-Round	0/0 - Year-Round 0/0 - Winter 2/2 - Summer	5/11 - Year-Round 3/6 - Winter 2/5 - Summer
Porter Creek-Russian River	14/50 - Year-Round	9/50 - Year-Round	2/14 - Year-Round 0/0 - Winter 2/2 - Summer	12/53 - Year-Round 8/10 - Winter 4/43 - Summer
Green Valley Creek	13/18 - Year-Round	14/18 - Year-Round	1/2 – Year-Round 0/0 - Winter 3 / 4 - Summer	13/18 – Year-Round 8/10-Winter 5/8-Summer

Dutch Bill Creek-Russian River	42/137 - Year-Round	36/137 - Year-Round	6/43 - Year Round 0/0 - Winter 7/66 - Summer	31/124 - Year Round 17/23-Winter 14/101-Summer
Willow Creek-Russian River[d]	5/66 - Year-Round	7/32 - Year-Round	0/2 - Year-Round 0/0 - Winter 3/12-Summer	7/25 - Year Round 1/3 - Winter 6/22-Summer
Willow Creek-Russian River[e]	5/66 - Year-Round	7/32 - Year-Round	5/42 - Year-Round 0/0 - Winter 10/56-Summer	7/24 - Year Round 1 / 2 - Winter 6/22-Summer

[a] All exceedances above the US EPA RWQC or statewide REC-1 WQO for enterococci are marked in **bold**.

[b] All exceedances are year-round assessments unless specified

[c] HUC-12 subwatersheds with exceedances above the US EPA RWQC for enterococci that were identified during the initial analysis but not during the 2020 reassessment

[d] Willow Creek-Russian River HUC-12 exceedances of the US EPA RWQC for enterococci

[e] Willow Creek Russian River HUC-12 exceedances of the statewide REC-1 WQO for enterococci

APPENDIX H – COMPARISON OF BACTEROIDES DATA

HUC-12 subwatershed with strong evidence of a human fecal waste source of pollution identified during the initial analysis and during the 2020 Data Reassessment

HUC-12s with strong evidence of human fecal source*	Initial Analysis	Initial Analysis	2020 Reassessment	2020 Reassessment
	Median Human-specific *Bacteroides* (gene copies/100 mL)	Number of *Bacteroides* measurements	Median Human-specific *Bacteroides* (gene copies/100 mL)	Number of *Bacteroides* measurements
Orrs Creek-Russian River	10,548	6	10,548	6
Lower Santa Rosa Creek	32,909	2	32,909	2
Porter Creek-Russian River	48,200	7	48,600	10
Green Valley Creek	17,016	2	17,016	2

APPENDIX I – COMPARISON OF PHYLOCHIP DATA

Comparison of Initial Analysis and 2020 Reassessment of Phylochip data showing a moderate (≥ 10%) or strong (≥ 20%) human fecal waste match

HUC-12 subwatershed	Max of Gene Sequence Percent Match Initial Analysis	Median of Gene Sequence Percent Match Initial Analysis	Number of Gene Sequence Measurements Initial Analysis	Max of Gene Sequence Percent Match 2020 Reassessment	Median of Gene Sequence Percent Match2020 Reassessment	Number of Gene Sequence Measurements 2020 Reassessment
West Slough-Dry Creek	16	5	5	16	5	5
Brooks Creek-Russian River	10	2	8	10	2	8
Lower Santa Rosa Creek	32	6	8	32	6	6
Upper Laguna De Santa Rosa	24	10	10	24	10	10
Porter Creek-Mark West Creek	5	3	2	12	4	4
Porter Creek-Russian River	54	7	7	54	6	9
Dutch Bill Creek-Russian River	89	2	36	89	2	35
Willow Creek-Russian River	16	2	3	16	2	3

Attachment 1 : FIB_MST_Raw_Data.xlsx (Fecal Indicator Bacteria and Microbial Source Tracking Datasets used during the 2020 reassessment)

Attachment 2: FIB_MST_Results.xlsx (Results of the 2020 Reassessment)