

## Memorandum

TO: Charles Reed, NCRWQCB

CC: Gerald Bowes, SWRCB

FR: Patricia A. Holden, UCSB

DATE: 2-22-15

RE: Peer Review of the Scientific Basis of the

*Draft Staff Report for the Action Plan for the Russian River Watershed Pathogen Indicator Bacteria Total Maximum Daily Load*

This memo provides the requested peer review of the “Draft Staff Report for the Action Plan for the Russian River Watershed Pathogen Indicator Bacteria Total Maximum Daily Load”, herein referred to as the Staff Report. The primary charge to peer reviewers is to assess the data and analytical methodologies used to develop the Staff Report, which recommends load reductions and numeric targets that are necessary to attain bacterial water quality standards. For each finding or conclusion of the Staff Report, the reviewers are to determine whether each is “based on sound scientific knowledge, methods, and practices”. This review is based on the revised request for External Peer Review, dated January 15, 2015.

### **1. Nature of the water quality problem**

The scientific basis is sound for establishing the conclusion that “the Bacteria Water Quality Objective is not being fully supported in the subject watershed”. This peer review assessment is based upon the indicator bacterial results as reported in the Staff Report and supporting documents.

### **2. Desired Target Conditions**

The validity of using *Bacteroides* DNA-based markers to define natural background and to use the same markers as pathogen indicator organisms hinges on strictly defining the analysis methodology and adhering to that, since there are several potential markers and variations in approaches that would affect the strict setting of numerical limits on gene copy numbers. If there is a standard reference that can be consistently used, this will legitimize setting numeric targets for one marker, but it is crucial that the specific marker and methods used to quantify it be specified and adhered to. Otherwise, the numeric targets, the basis for setting those, and the later monitoring data could be based on incongruent methodologies resulting in incomparable data. It should be noted that the closest equivalent to a “Standard Methods (for the Examination of Water and Wastewater)” in DNA-based analysis in this context is Griffith et al., (2013). However, if there are standardized methods used for setting, and monitoring, natural background, then the proposal as stated is valid and justified.

The *Bacteroides* DNA-based markers of fecal sources have weakly understood relationships to fecal indicator bacteria (FIB), and FIB concentrations have been related to risk of illness to swimmers or fishers. In the absence of epidemiological justification, source-specific DNA-based markers should not be used in isolation. However, it is scientifically valid and justified to use, in tandem with

fecal indicator bacterial data, *Bacteroides*-based host specific markers to infer fecal sources and thus to infer potential for contaminated waters to impinge on human health.

As stated above, the validity of decision-making around the detection of host-specific *Bacteroides* markers of concern hinges on specifying the method of analysis and applying this consistently. Otherwise, it is valid, as long as the methods, including minimum sample numbers, are specified and scientifically justified.

It is scientifically valid and justified to use *E. coli* concentrations in place of fecal coliform in this context.

As stated above, the validity of the numeric targets for *Bacteroides* markers hinges on the methodologies used to set the targets and the methods used to assess attainment. These must be standardized and accepted, e.g. as per Griffith et al. (2013). The numbers of samples and frequency should be specified, or reviewed and approved.

### **3. Source Analysis**

An overall assessment is that there were studies performed that appear to be valid that provide background data in support of potential sources, particularly of human origin. It is unclear the exact methodologies that were used, but it is unlikely that the methods were the same as in Griffith et al. (2013). However, the BLRPs include determining sources, and thus such efforts would likely be performed using current, and consistent (across the watershed), approaches. The range of potential sources (Table 1) appears to be comprehensive and reasonably derived from studies to identify potential sources.

### **4. Seasonal Variation and Critical Conditions**

Fecal indicator bacterial concentrations are known to vary in individual streams during periods of high rainfall and runoff; DNA-based markers also vary. The seasonality of the co-variances of these indicators is not well understood. “Wet and dry” periods occur throughout the year in this watershed. Therefore, it is scientifically valid to apply the same loading criteria throughout the year.

### **5. Linkage Analysis**

The basis for linking *E. coli* concentrations to attaining beneficial uses as described is valid.

### **6. TMDL, Loading Capacity, and Allocations**

There is scientific validity to base TMDLs on *E. coli* concentrations; for *Bacteroides*, valid methods used to establish the TMDLs should be specified, as should be the methods used for monitoring target attainment into the future.

### **7. Margin of Safety**

The basis for selecting the MOS appears to be scientifically valid.

## **8. Implementation Plan**

The implementation plan would likely result in progress towards attaining water quality standards and supporting recreational beneficial uses. There is a concern, however, that the time allowed for source identification is not sufficiently long and thus it will be difficult to produce scientifically based BLRPs (e.g. in 1 year). Also, it is unclear how source identification will be funded. Finally, related to remarks made previously in this peer review, it is important to select scientifically valid methods for the DNA-based approaches, and to standardize across those methods, in time and in space.

### **Other Issues**

There are two sets of remarks included in this section. The first set (bulleted list) is general and may reiterate, expand upon, or complement, the answers to specific issues above. The other remarks more specifically relate the Staff Report, i.e. suggesting where some improvements could be made, if staff resources allow.

General points include:

- The pathogen TMDL does not regard CECs or other water pollutants. A more holistic approach to watershed management is advocated. Where there are bacterial markers of human or bovine waste, there may also be compounds of emerging concern (CECs, e.g. pharmaceuticals, or personal care product additives) and nutrients. Where there are no markers of human or bovine waste, there still may be these compounds that have negative implications for ecological health. These do not occur in isolation from pathogens if they are from the same source.
- Pathogen indicators are used to infer the presence of pathogens; the presence of pathogens is used to infer the risk to human health from water contact or shellfish consumption. Where possible, it is best to increase the direct relevance to human, or ecological, health of information used in water quality management.
- Standardizing the methods used in identifying sources for the BLRPs and the monitoring efforts would enhance the possibility that work performed basin wide is internally comparable, but it would also provide a body of data for the State, and could model to other regions. Standardization in sampling, analysis, and data analysis methods is strongly recommended, and should in fact be required.
- The funding for implementation should be identified. It is a concern that the environmental improvement objectives can be met without causing undue burden to the Counties and other jurisdictions or stakeholders. How source identification studies are to be funded is unknown (for individual BLRPs), as is how monitoring is to be funded, particularly for DNA-based markers of human or bovine waste.
- Where BLR strategies are ineffective, watershed management methods should be investigated that would promote better water quality, for example (related to the soil attenuation outcomes described in Chapter 2) increasing percolation

through soils, increasing natural sunlight incidence so that solar radiation can generate free radicals to attenuate fecal pathogens, and similar.

- DNA-based marker persistence, or decay, in the environment relative to fecal indicator bacterial decay is uncertain. An internal report to this Staff Report has reviewed and summarized a good body of scientific literature, but published studies have been performed over varying conditions and there are few such studies. It is very difficult to generalize relationships between DNA-based marker decay relative to fecal indicator bacterial decay. Often, it is found that there is low correspondence between concentrations of these two types of indicators.
- The scientific literature does not support quantitative interchange of *Bacteroides* with fecal coliform. Even when a specific host appears to be the source of fecal pollution based on host specific markers, fecal coliform levels rarely correspond to *Bacteroides* levels in affected waters.
- Available DNA-based markers vary in their specificity and sensitivity, as described in the Report. The targets that are described, their basis, and protocols for measuring, should be selected and should be consistent. A source is Griffith et al. (2013).

#### Detailed comments about the Staff Report

Overall, this is a very readable and accessible Report. Below are some recommendations or comments that are intended as helpful.

It would be very helpful if maps with sampling locations were provided, in order to relate sampling data reported in tables or charts back to the physical setting. For example, Table 2.1 and Figure 1.2 are challenging to relate to one another. The sub watersheds labeled on Figure 1.2 are not all listed on Table 2.1 and vice versa. It would certainly be helpful if these were congruent.

In some places, the Staff Report refers to “domestic animals” instead of “livestock”. If the source were really cows, then it would be better to define the animal host of concern as such. Otherwise, “domestic animals” could include pets (dogs, mainly), which can be significant sources but don’t appear to be a focus here.

Section 2.1.2, P27: The box “Bacteria Water Quality Objective” relates “natural background levels” to first “coliform”, which should be clarified as either “fecal coliform” or “total coliform”. The 2<sup>nd</sup> and 3<sup>rd</sup> uses specify “fecal coliform”.

Section 2.1.2, P27: last paragraph has a typographical error: “pathogen” should be plural “pathogens”.

Section 2.1.2.1, P27, p28: It is unclear what “significant human disturbance” means in this context. The phrase “zero human waste” in the use on P28 implies that it is acceptable to discharge human waste into water bodies. The distinction between

“treated” and otherwise altered, versus untreated, would seem useful here. Surely the intent is not to suggest that it is acceptable to allow known discharge low amounts of raw sewage or human feces into natural waters.

Section 2.2.1, P23: It should be noted that not all of the assays available are “quantitative”. For example, the horse assay that is listed in Griffith et al., 2013, yields presence / absence, not gene copy numbers. While Griffith et al. 2013 mentions that a quantitative marker for horse is available, it was not validated in that study.

Section 2.2.1.1, P33: It would be useful to provide more detail about the scientific basis, or to cite literature. How is it known that above 10% of the reporting limit is above “natural background”? How long are markers able to persist in disinfected waters? What is the evidence that markers in disinfected waters are, or are not, able to indicate persistent pathogens in disinfected waters?

P34, same section: Ashbolt et al. (2010) specify the HF183 marker, and this is based on personal communication internally at the U.S. EPA. The text in this section should be specific: if it is HF183, then this needs to be stated. Not all human markers have the same detection limits and not all are similarly specific to human waste. Further, Wade et al. (2010) did not test HF183, but rather the general *Bacteroidales* marker. These are not interchangeable. In sum, the reporting limit is specific to the marker and likely the qPCR conditions. Reading on to Section 2.1.1.2, the Report could address this if the numeric limits are set based on detection limits for the selected markers from Griffith et al. (2013), using the approaches specified in the latter reference.

Chapter 3, P39: in point “1.”, The numeric targets should be related specifically to a specific host-specific marker (e.g. HF183) and using specified qPCR analysis methods. While one can assume that fecal coliform will be measured according to “Standard Methods for the Examination of Water and Wastewater”, and that doing so is implied when one states that fecal coliform are being measured (for example), this is not yet the case for qPCR methods for host specific markers. A recommendation is that Griffith et al. (2013) be specified throughout, to standardize around a specific qPCR approach.

Figures 3.1 and 3.2: The legend appears to be mislabeled. The red is “Target Exceeded”, not “Target Attained”. This change would make the labeling consistent with the data in Table 3.1.

Tables 3.1 and 3.2: Here and in prior sections of the text, the authors are encouraged to change the nomenclature from “genes / 100 mL” to “gene copies/ 100 mL”. It would be helpful if the Tables had a footnote with the citation to the original source of these data, since whether the samples were acquired during dry or wet weather would be useful to know, and other timing or conditions of sampling.

The numeric targets for *Bacteroides* gene markers do not specify the number of samples (Table 2.5). Does this mean that if only one sample is acquired, the numeric target applies within a calendar year?

Table 3.5, P47: It would be very useful if the single sample *E. coli* data corresponding to the *Bacteroides* data (Tables 3.3 and 3.4) were reported, preferably alongside the latter. Otherwise, this report gives the impression that the main diagnosis of non-attainment is made on the basis of a few select host-specific qPCR results.

Section 6.1, P103: The need to be specific about *Bacteroides* numerical criteria, i.e. tethered to a specific qPCR marker and method, is reiterated here, as commented upon for Chapter 2 sections.

Chapter 7, Sections 7.1 and 7.1.1, P 108: The number of samples for *Bacteroides* monitoring is not specified.

Table 7.3: These percent reductions hinge on whether the methods for DNA-based analysis were exactly the same in the monitoring studies. That is, the exact same laboratory protocols, and primers for the DNA-based marker, would need to be used. The selection of “60 gene copies / 100 mL” hinges on a specific DNA-based protocol having been used exactly for the data that resulted in monitoring and the monitoring moving forward.

Table 8.1: The time schedule for submitting plans for Load Allocations is short, particularly for the “homeless” sources, which were not identified in the Staff Report. The time frame is of concern as it may place a large financial burden on the Counties, particularly if they need to perform studies to determine sources, and need to identify funding for doing so.

Section 8.2.2, P119-120: The possible compliance actions refer to disinfection of wastewater pathogens. This is the goal, but compliance is measured by indicator organisms, not pathogens.

BLRP, P134: The time frame of 1 year for BLRPs to identify sources of bacteria is short. This doesn’t allow for determining the influence of seasonality on patterns. A tiered approach is advocated by the Clean Beaches Initiative in the SWRCB, which is described in Griffith et al., 2013.