MSAR Special Study - Additional Responses to Comments on July 7, 2020 Draft

Riverside County Flood Control & Water Conservation District

- Comment - Draft memorandum stated: "Conduct a special study to evaluate releases of naturalized *Escherichia coli* (*E. coli*) from the Santa Ana River bottom." The Uncontrollable source study (June 2016) was designed to do this as well however the conclusions were disappointing with resuspension decided upon by process of elimination as no other sources were identified. The issues encountered in that study should be reflected upon during design of this special study.

  Response: We agree with this comment. Also for consideration during the final design of this special study design is a need to better understand how the Regional Board would consider the use of such data in the context of a TMDL revision. It may be appropriate to have discussion on that issue prior to implementing the study.

- Comment - Table Note 3 in Table 2 stated: "Note that this proposal does not include analyzing water samples for constituents representing environmental factors that could potentially influence bacteria populations, e.g., DOC or nutrients." As this is mentioned as a possible addition to the study can these costs be included as an optional task?

  Response: The additional costs would be for laboratory analyses and would depend on the constituents analyzed and the selected laboratory. Given that 12 water samples total would be analyzed, the additional cost would be $1200 to $2,400 for the Study assuming a range of $100 to $200/per sample. We can obtain specific costs if the Task Force decides it wants to move forward with the basic study as presented. At that time we would then discuss with the Task Force which constituents it wants to include and then obtain the most current costs from the selected laboratory.

Santa Ana Regional Water Quality Control Board

The sampling design appears insufficient for the presented data analysis/interpretation approaches and study objectives. Specific comments follow excerpts from the Tech Memo.

Excerpt 1: Technical Memorandum Page 5, 1st (original text in quotes):

"Analytical methods exist that can be employed to develop quantitative information about potential fecal bacterial sources within the non-MS4 segment of the Santa Ana River. Specifically:

- Approximate the load from recent fecal deposits as a whole by quantifying a universal Bacteroidetes concentration, which measures the total fecal bacteria load. If this concentration in water at the MISSION site is correlated to the general indicator *E. coli* concentration, then it can be concluded that fresh deposits play an important role in the downstream fecal indicator bacteria loading. Conversely, if there is no correlation between universal Bacteroidetes and *E. coli* in water samples from the MISSION site, then it can be inferred that the releases from naturalized *E. coli* colonies (generally void of fresh fecal matter and associated *Bacteroides*) are the most important source for downstream fecal bacteria loading."

Comments regarding above text (Responses in italics):

The methodologies as described seems inappropriate and insufficient to prove fresh fecal deposits vs. naturalized *E. coli* as the most important source for downstream fecal bacteria loading.

1) Total *Bacteroidetes* concentration may or may not approximate total fecal bacteria concentration. Which "Total *Bacteroidetes*" assay will be used? Some assays have been shown to capture a large indigenous aquatic microbial population.

   Numerous studies have demonstrated that Bacteroidales *species can survive for only a few hours in the environment; appropriately indicating recent fecal contamination (Balleste and Blanch 2010). Additionally, previous studies have successfully used both total* Bacteroides *(Bacspp marker) and the human* Bacteroides *genetic marker (HF183 marker) to identify recent sewage sources in surface waters (Sauer et al. 2011 and Cao et al. 2017). Specifically, ratios of the total* Bacteroides *marker that characterizes recent total fecal contamination were compared to ratios of human* Bacteroides *marker that differentiates human fecal sources from other sources. This approach has been used to distinguish sewage sources of contamination from non-human sources (Sauer et al. 2011).*

2) It is likely total *Bacteroidetes* would be measured by genetic methods (qPCR or digital PCR) and *E. coli* measured by culture-based method. With a small sample size (3 sites x 4 samples/site =12 samples) and unknown data distribution (plus possibility of censored data), it is unclear if it is feasible to conduct a statistically defensible correlation analysis.

   The Special Study is proposed to provide preliminary scoping level information and is not intended to make definitive conclusions about the source of fecal bacteria loads in the non-MS4 segment of the MSAR. Two sources are hypothesized: (1) releases of naturalized E. coli *from channel bottom sediments; and (2) freshly deposited feces from human/animal sources. If preliminary results suggest evidence of one of these sources is strong and there is a value to the Task Force as a whole to draw a scientifically defensible conclusion, then the Task Force may authorize design of a more robust study.*

3) Also, even if the selected total *Bacteroidetes* assay approximated total fecal bacteria concentration, *E. coli Bacteroidetes* ratios can differ greatly among different hosts (human and animals). It seems more effective to form hypotheses on potential fecal source hosts based on local watershed knowledge and evaluate the feasibility of this approach first.

   The occurrence of indicator organisms, including Bacteroides*, in surface waters can be a function of both spatial and temporal variability. Thus, identifying the sources of fecal contamination can be challenging because their concentrations can vary substantially. However, in a tiered approach, trends of* E.coli *and total Bacteroidetes can be compared to determine if* E.coli *concentrations are consistently low compared to total Bacteroidetes, suggesting recent fecal contamination that can then be followed up by the human* Bacteroides *marker that distinguishes human from other natural sources.*

4) Additionally, a lack of correlation between A and B, may not necessarily mean that C is the most important source for B.

   A key purpose of this study is to determine the importance of releases of naturalized colonies of E. coli from the channel bottom, e.g., through resuspension. Obtaining insight into this question will help refine our understanding of sources of bacteria in the watershed, i.e., is it a naturalized source or a fresh source of bacteria. Further understanding of this dichotomy will support decisions regarding where to allocate resources to mitigate sources of bacteria in the watershed.

Excerpt 2: Technical Memorandum Page 5, 2nd Bullet (original text in quotes):

- "Findings from the above Bacteroidetes and *E. coli* correlation analysis can be used to focus subsequent experimental sampling on either (1) potential sources of recent fecal deposits to the water or (2) factors that influence location, persistence, and population of naturalized *E. coli* colonies within channel bottom sediment and biofilms over the ~1.5-mile non-MS4 segment of the Santa Ana River. For the former, host specific *Bacteroides* markers will be used to apportion the Total Bacteroidetes concentration into known (e.g., human, dog, avian, pig, or horse) and unknown hosts. For the latter, the concentration of *E. coli* within the channel bottom will be determined at multiple sites by collecting sediment and biofilm samples. If desired, differences between sites (e.g., varying environmental conditions over different sampling events) could also be investigated to better understand the drivers for colony growth and *E. coli* release. Findings from such analyses could be used to inform future management actions, if any, that could be implemented in the watershed."

Comments regarding the above text (Responses in italics):

1) "subsequent experimental sampling" – is this referring to the sampling described in Table 2, or additional sampling/study to what is described in the Technical Memo? It seems the former. It is confusing as the study is not presented as a tiered approach, e.g. analyzing water first then sediment? Please be explicit.

   The subsequent experimental sampling would involve additional sample collection (not included in Table 2) to develop a sufficient data set to provide more robust scientific support to any preliminary findings. Such sample collection would be coordinated with Regional Board staff and watershed stakeholders to support the regulatory direction of the group.

2) "apportion the total Bacteroidetes…" – Source apportionment is very challenging and requires a lot of assumptions. How this would be done needs to be described. Which host-associated markers would be used for the different animals? Is the Avian marker also going to be Bacteroides? Are these marker results generated from a sample size of 12 amenable for the selected source apportionment method?

   Even in a well characterized watershed, source apportion can be challenging, therefore, a tiered approach has been recommended to determine if the observed trends in E.coli *concentrations are suggestive of recent fecal contamination, or a result of gradual releases from channel bottoms. If the* E.coli *trends suggest recent fecal contamination, then a more*

specific Bacteroides *genetic marker (i.e., HF183) can be used to identify human sources from non-human, natural sources. We have revised the referenced paragraph to provide additional clarity.*

3) "differences between sites …" – Sediments and biofilm microbial distribution can be extremely patchy (spatially within a site) and highly dependent upon many variables (particle size, organic content) which could vary greatly by site as well as by season. Combining this with the relative high variance in MPN measurements for *E. coli*, the study design of limited number of samples per site (4 samples per site) across a long temporal spam (a whole year) is insufficient.

   We agree that this is a key challenge to an assessment of the role of sediment releases to downstream E. coli *loads. We may continue to hypothesize this source through a process of elimination of other possible sources. If it is determined that a scientifically defensible quantification of this source is needed to support the regulatory approach being implemented by Task Force members, we will need to implement a far more robust study design than is presented in this scoping study.*

References

Ballesté, E. and A.R. Blanch. 2010. Persistence of *Bacteroides* Species Populations in a River as Measured by Molecular and Culture Techniques. Applied and Environmental Microbiology 76: 7608-7616.

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Sauer, E.P., J.L. VandeWalle, M.J Bootsma and S.L. McLellan. 2011. Detection of the Human Specific *Bacteroides* Genetic Marker Provides Evidence of Widespread Sewage Contamination of Stormwater in the Urban Environment. Water Research 45: 4081-4091.