Review of Draft Staff Scientific Report for the Action Plan for the Russian River Watershed Pathogen Indicator Bacteria Total Maximum Daily Load – Jan 16, 2015

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General Comments

The Staff Report reviewed was clearly articulated, yet appear caught between two slightly different goals; being to meet water quality standards based on bacteriological indicators (via setting of bacterial discharge limits to meet these bacteriological indicators and associated management actions) versus protection of public health. The first is the regulatory requirement of the TMDL, the second is the intent of the regulation, but at times lost in the translation with regulatory bacteriological criteria, some of which are problematic and not related to health risk. This discrimination and concern over bacteriological measures and actual pathogen risks may arise due to the heavy urbanization of parts of the Russian R. watershed where human enteric viruses could behave differently to the bacteriological indicators (for example, human virus ratios to pathogen indicators would expect to be higher from sites impacted by homeless people, given higher likelihood of infections than the general population, so per gram of excreta very high concentrations to that in municipal sewage or sewage-impacted stormwater); and soil-filtered groundwaters, surface-impacted by sewage that may remove most bacteriological indicators but still contain infectious human enteric viruses. Conversely, bacteriological measures in regions predominantly impacted by non-cattle-derived manures are likely to be over-protective of actual human pathogen risks when pathogen indicator bacteria are used. As the report describes, fecal coliform bacteria are particularly problematic, due to their environmental growth unrelated to health risk – as noted in the report, the U.S. EPA removed recommending these indicators back in 1986[1].

Nonetheless, given that 303(d) listing is based on set pathogen indicator bacteria, the latter consideration of actual health risk is only added here not as a criticism of the report, but rather of the nature of the TMDL regulation. Hence, I have attempted to only add constructive comment to improve public health protection. Overall the authors should be congratulated on a detailed and through report that addresses the 16 hydrologic areas of the Russian R TMDL Project and is probably one of the first to include state-of-the-art molecular fecal indicators to complement their interpretations.

In general, relative human health risks from fecally-contaminated water is highest from human excreta/sewage/septic seepage, then possibly similar from cattle feces, but less from other domesticated animals and waterfowl (e.g. [2]). Hence, it was particularly useful to read of human and cattle fecal source estimates by sub-

watershed. Recreator health risks are evident, except when human/cattle impact maybe low (as identified in Table 1.5, Figure 1.4). However, as enterococci results are not present, the relative level of potential impact is unclear – given the lack of dose-response relationship between *E. coli* in water and gastrointestinal (GI) illness in recreators[1]. For example, for the same level of culturable fecal indicator bacteria used you can not resolve between high to minimal risk[3]. The presence of shellfish potentially eaten raw or poorly cooked across the watershed (as identified in Table 2.1) further exaggerates the difference in actual human viral risks versus bacterial indicators that may behave very differently[4-7].

It is therefore obvious that the water quality objectives based on fecal coliform concentrations within the box in Section 2.1.2 are very out of date with respect to risk – but again not a criticism of the report, but rather what was apparently set as report targets (noting reflection of this by the authors on p29, Section 2.1.2.2). The inclusion of additional indicators (*E. coli* and *Bacteroides* microbial source trackers [MST]), but noting these are also not necessarily very useful for shellfish risk assessments (i.e. unclear what the absence of MST human markers means given greater virus persistence over bacteria). This reviewer is unaware of the science that supports fecal coliform numeric objectives (43 or 49 cfu/ 100 mL) for shellfish described in Section 2.1.2.3. In Section 2.1.2 the authors refer to the new (2012) EPA recreational water criteria[8], which does not recommend fecal coliforms, but enterococci, and to a lesser degree due to a poorer epidemiologic relationship, *E. coli*. So a general overall criticism is why were enterococci not used, but the obvious answer seems to be due to lack of State regulations, not science. Noting that human- and cattle-targeted *Bacteroides* markers were used to good effect, and indeed are more useful than enterococci results alone.

Another potential concern at times arise from the interpretation of 'Natural Background' in Chapter 2. The lack of significant human disturbance seem very appropriate, but it was unclear how a sound sanitary inspection, such as suggested by EPA in their new criteria toolbox approach (see: http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/index.cfm) was used? It is clear by reading Chapter 3, however with the detection of human and cattle markers at most sites, that significant impact occurs across the watershed. Also, the initial description on the use of *Bacteroides* bacteria is possibly misleading, as 1) there is apparently no recognition of the non-enteric members of *Bacteroides* (e.g. plant matter degraders [9, 10]), and 2) one has to assume that human+cattle fecal markers are implied, as not stated in Chapter 2.

Therefore Recommend (1): that the initial Chapter 2 tables state which MST markers were targeted (one could infer that total *Bacteroides* or specific markers are being referred to?). A general point here is that it is important to confirm animal fecal sources by multiple MST markers, not general *Bacteroides* marker presence (e.g. [11]). EPA used the general *Bacteroides* marker to increase sensitivity for known sewage-contaminated beaches in its epidemiology studies (see specific comments on use of *Bacteroides* bacteria below). It was excellent to read that a more thorough

Bacteroides study is planned for 2016, and as no single MST marker is 100% specific, use of multiple markers will be better integrated in the future report.

Specific Comments

***Bacteroides* bacteria**: As raised above, Chapter 2 is unclear in relation to which MST markers are being referred to. Further, on page 28-29, it infers that the laboratory actually counted viable *Bacteroides* bacteria, which is possible, but strict anaerobes only provide a culturable count from recent fecal contamination. Generally, *Bacteroides* cells rapidly lose their ability to grow when exposed to dissolved oxygen[12]. Hence what EPA and most researchers undertake is an estimation of genomic DNA presence (which is clearer latter on in the report too), being reflective of *Bacteroides* bacteria presence (dead and alive). As there is no single relationship between targeted gene target copies per cell, varying by a factor of at least 1-10 (depending on the gene copy number that varies by species and cell growth cycle) it is not recommended to report as cell equivalents (so good to see the reported values are expressed in gene copies not cell equivalents).
The statement on page 31 ("Bacteroides bacteria are not found in ambient surface waters without sources of mammalian waste."), and similarly on the last paragraph of p72 (Chapter 4, "*E. coli* and *Bacteroides* bacteria are found in the intestinal tracts of warm-blooded animals. Because they are not warm-blooded animals, salmon and other fish do not contribute these bacteria to streams."), however, is clearly not true, unless referring to HF183 and HumM2 markers for human-host *Bacteroides* analyses and CowM2 and Rum2Bac markers for bovine-host analyses (cited on page 32, Section 2.2.1.2). However, even HF183 has been identified from fish[13].
So while it is recognized that human-direct or animal-direct *Bacteroides* MST markers were used (e.g. Table 2.4), it is unclear from the tables which actual markers were used, which does make a big difference [14-16].

Therefore Recommend (2): Please identify the actual markers and protocols used associated with tables and figures. Also note that the reporting limit is likely to vary depending on the specific matrix used, so performance for each sample or at least type of sample also needs to be specified to interpret the 30 or 60 gene copies per 100 mL limits reported on pages 32 & 33 (Tables 2.4 & 2.5). Nonetheless, the logic reported in Section 2.2.1.1 for the 30/60 genes per 100mL reporting limit seems reasonable.
In Chapter 3, use of the 60 human-targeted MST and 30 bovine-targeted MST marker genes per 100 mL seems appropriate to support impaired water status, given traditional fecal indicators are inadequate alone to make a call relative to potential human health risk. Also, as fecal sources may change (mobilize) differently during rain events it was also good to see rain-event resolution in the data (e.g. Fig 3.1). However, as illustrated in Tables 3.1 & 3.2, with < 3 samples taken for a number of sites it is unclear what the real range of MST marker was.

Therefore Recommend (3): Given the concern for interpreting the < 100%

exceedence (e.g. Tables 3.3 & 3.4), further samples should be included before making the exceedence call or else leave undetermined. A minimum number of samples of 5-10 is probably more appropriate for these sites without 100% exceedence.

However, what is unclear is the contribution from human recreators and homeless groups along the river? Excellent to see data presented in Section 4.4.2 on the potential contributions from recreational use of the river system at some sites, which showed clear increased human-target MST markers downstream of recreators. Is there similar data available for homeless 'settlements', given the high estimates of people unsheltered (some 7,500)? Both recreators and homeless represent a difficulty in addressing the TMDL if the fraction from each is not documented.

In Chapter 4, page 69, the comment 'However, it is not clear whether the sources of detected *E. coli* bacteria are of human origin and therefore pose a more significant threat to public health or whether their presence is a result of contamination by birds and other wildlife that frequent the storage ponds.' identifies a limitation of the MST markers. In that as sewage was the original fecal source and that UV or chlorination disinfection was used, the human-directed MST markers would likely still be detected, even if pathogen risk had been controlled by the disinfection step. However, it would still have been useful to have included bird vs human MST data to indicate the level of waterfowl fecal contribution.

Fecal coliforms and enterococci bacteria: page 29 Section 2.1.2.2 correctly discusses *Klebsiella* spp. as one of a number of fecal coliforms that may have no relationship to fecal matter, but just one of many genera! Further, while *E. coli* is more specific to fecal matter, it too is not exclusively fecal nor just to warm-blood animals (as claimed on page 35, Section 2.2.2 and elsewhere), in that cold-blooded animals and insects harbor it too (e.g. [17-19]) and under certain conditions *E. coli* bloom in surface waters [20, 21].
Section 2.2.3 on fecal coliform bacteria and possible shellfish risk is not based on science, but follows policy-directed values (43 & 49 MPN per 100 mL) so while a regulatory target is discussed, this whole section is problematic when trying to interpret likely human risk – it is a 'book of fiction' to this reviewer. Like coliforms, enterococci are also well known to be symbionts of various insects, so they too can come from cold-blooded animals and accumulate in soils/sediments, particularly in heavily vegetation environments[22]. Filter feeders will also accumulate coliforms and enterococci sourced from fecal and non-fecal environments, but sadly depurate them much more effectively than the real risk, human enteric viruses. This has been known for decades but regulations have not changed [23-27].
What is clear, however, is that when sewage is known to contaminate recreational waters (human enteric viruses maybe present and considered to cause the majority of recreational water users' GI illness[28]), but there does not seem to be a dose-response relationship between *E. coli* concentration and gastrointestinal illness (just a threshold, which aligns with the GM < 100 cfu/100 mL cited in Table 2.7); whereas

there is a dose-response relationship with enterococci [8, 29] – hence EPA's preference for enterococci and this reviewers if the presence of human sewage or cattle manure are confirmed by sanitary survey and/or use of specific *Bacteroides* markers or equivalent via microarray analysis.
The fewer sites exceeding the set *E. coli* criteria (using a 30-day period), compared to those with positive human and/or bovine MST marker presence (and at high levels) is a concern but consistent with the lack of a dose-response relationship between risk and *E. coli* concentrations. All the more reason not to rely on *E. coli* data alone.
As discussed above, EPA maintained *E. coli* in the 2012 recreational water criteria, but not because it was better than enterococci, rather, because of historical precedent and stakeholder interest in maintaining an *E. coli* criterion. So it is not correct to express (p 104) "U.S. EPA recommends *E. coli* bacteria criteria as the best indicator of health risk from water contact in freshwater." – read their criteria report[8], but it does recommend enterococci as the best, due to the dose-response nature in sewage-impacted waterbodies.

Therefore Recommend (4): Reword Section 6.2 so it does not suggest *E. coli* is the best indicator for freshwater. That was a political/regulator interpretation to continue with the previous approach, but is not backed by science in the US or Europe[30].

In reference to possible impacts from fish hatcheries (pp72-73), it is probably incorrectly to assume that environmental *E. coli* are not excreted by fish. The paper cited [31] clearly shows that sediment-borne *E. coli* were present in fish intestines, and others have shown the maintenance of *E. coli* through growth in sediments[32]. Also, the term 'vector' is actually correct (meaning from infection within a host), but I understand the authors actually meant to say 'transport vehicle', assuming no replication of *E. coli* within fish?

Potential pathogens as per phylochip: this section is great to see, giving a very different perspective on assessing fecal contamination, and seems to generally support the MST results. There is however, some potential mis-representation in the results, such as given in Table 3.7. For example *Klebsiella pneumoniae* maybe a human pathogen when isolated from people, but in the environment, much less likely to be. For example it is common in pulp industry wastewaters [33] and even grows in drinking water biofilms[34]. Streptococcus spp. are also common from various sources, so it makes no sense to identify this genus or an unnamed species as a pathogen. Also, *Vibrio cholerae* is a common species in streams and has no human health impact unless it is carrying specific toxin genes, normally not found in developed regions of the world, e.g. [35].

Therefore Recommend (5): Remove the misleading percent of samples with pathogens detected in Table 3.7. What is much more of value is the estimation for specific faecal sources given in Tables 4.1 to 4.3. Furthermore, that human-targeted markers are present in all locations, all land use categories (least from forested) and

higher during rain event periods (Section 4.2.1). Noting that once over some 15-20% human impacted water, then human viruses may be the dominant pathogens of concern[3] and very different risk estimates result if the fecal indicator is assayed by culture versus qPCR[36], which highlights the importance of using molecular tools to clarify potential risks (as in Figs 4.6 & 4.7, and 4.8 & 4.9) versus compliance to a culture-based standard (as given in Figs 4.4 & 4.5). [Minor typo on Y-axis title for Fig 4.8, correct spelling of '*Bacteroides*']

***Cryptosporidium* and *Giardia* detections**: In Section 3.4.2 while it is correct that *C. hominis and C. parvum* are species that are very likely to cause human infections, other genotypes of *Cryptosporidium*, which are many, are generally not considered of human health concern[37]. Further, the EPA method (1623) used to assay the presence of these two genera do not resolve between genotypes. However, given the presence of human and cattle fecal matter it is a reasonable assumption to make that at least some of the recovered oo/cysts were of human-infectious genotypes[38]. Normally *Giardia* cysts are orders of magnitude higher than oocysts of *Cryptosporidium* in fresh feces and sewage – so why are the *Giardia* results not reported? Also, it is important to report method recoveries to interpret environmental monitoring data, please provide these recoveries. In summary, raw waters should have < 10 oo/cysts per 100-L to not be of concern in source drinking waters (assuming a 3-log reduction by treatment to meet EPA target level of 0.075 oocysts per 1000-L in ESWTR), but seems only (660/48) ~10-L size samples were collected, but how many from any one site? Please provide by site the number of samples and the recoveries for each so the data can be adequately interpreted – would appear that the drinking water safety statement cannot be justified unless more data is collected, and there is likely a direct risk to recreations?
The high likelihood of calves within the watershed (Table 4.14) and eventual retention ponds losses to the river (Page 95) pushes up the likelihood of human-infectious oo/cysts making their ways into the Russian R. system[39-41]. Also, it is notable that some of the bovine MST markers do not identify calf fecal pollution[15]. Hence, to reiterate, there is a need to utilize a number of markers and clarify their target groups vs risk issues.

Recycled Water Holding Ponds, SSOs & Exfiltration: Section 4.3.1.2 described the general use of wastewater storage ponds, and their potential for fecal indicator bacterial recontamination via wildlife. No data is present on rain-induced overflows of these ponds, does it not occur? There is unclear discussion on pages 68-9 of holding pond waters reaching the river system? However, there is clearly rain-induced sewage contamination into stormwater drains or directly into the river system that is the main concern, not these holding ponds. Has treatment of stormwater discharges or containment in treatment ponds and reuse been considered as a way of controlling what appears to be the major health risk wastewater within the watershed, other than the lack of compliance to 'treated' wastewater discharges (e.g. Table 4.4)?

Therefore Recommend (6): It is important to try and document the amount of

exfiltration from sewers in the watershed, as that is probably one of the largest single source to manage, but costly. Based on data provided, it appears that sanitary sewer overflows (SSOs) could occur over the 1,151 miles of gravity sewers (Table 4.5), so why are these not mapped, in addition to the fact that all sewers leak (exfiltration) [42]; noting if well below the groundwater table, will suffer infiltration (Section 4.3.1.4). Therefore, the reported (Table 4.6) SSO discharges and miles of gravity sewers infers at least 30 gals/mile of gravity sewer per year – which may well be a large underestimation? As stated above, bird or wildlife coliform contributions to ponds probably comes with minimal risk compared to human sewage/seepage into the river system.

The reported lack of epidemiological studies directed to specific wet weather stormwater impacted sites is inappropriate and appears deliberately misleading (end of Section 4.3.3.1). Given the clear identification of human sewage in stormwater, these discharges can be considered a likely major human pathogen contributing source.

Therefore Recommend (7): It is the view of this reviewer that EPA or others do not need to undertake what end up being very expensive epi studies to yet again show that sewage-contaminated recreational waters increase human health GI illness rates over EPA thresholds of concern (8/1000 HCGI or 32 NGI/1000)[8].

Biosolids applied to land: There appears to be no data on actual assessment of rain-induced biosolids pathogens/indicators nor mapping as to where this may occur? The comment about >10% slop is relevant, but proximity to riparian zones and high intensity rain periods would be higher risk scenarios if possible? Why are these not discussed?

Private wastewater discharges > 1,500 GPD & Mobile home parks, Onsite systems: The 19 private system discharges are clearly a potential major concern, particularly with septic tank/leachfield or spray irrigation. The concern comes from there being no apparent discussion on maintenance or survey of these systems for actual performance or contribution to watershed fecal pollution? Similar concern for the mobile home parks and campgrounds, and the large number of onsite systems, given the high likelihood of at least partial failures and surfacing of poorly treated sewage from onsite systems. As demonstrated in Sydney's drinking water catchment in Australia, it was lack of maintenance rather than set-back distances from waterways that dictated pathogen risks from onsite systems, which may still presents a risk when most members of a community are connected to a sewer[43]. The Sydney results seem relevant to the Russian R. watershed based on data presented in Section 4.4.1.

Livestock waste, manure holding ponds & land application of manure: Aspects described in Section 4.4.6 seems to exclude discussion of human-infectious *Giardia*, which from adult cattle is probably of key concern to human recreator health[44], probably much more likely than any obscure cattle viral zoonoses discussed. Of viral

zoonoses however, the more likely is hepatitis E serogroup C from hogs[45, 46] and this virus will also accumulate in shellfish[47]. Also, given the highest density of animals being chickens, at least for Sonoma County, the principle pathogen group missed is *Campylobacter jejuni*[48].
It seems bizarre to describe (Section 4.4.7) pond liner seepage rates in millionths of a cm/s without any indication of follow-up performance testing or life-time expectation for such performance? Having said that, the key problem with all ponds is the high rain event-driven overflow that is all but inevitable within the lifetime of most ponds – and indicated to be within the 20-y peak stream flow rate in the report. Why no follow-up barrier protection discussion?
Lastly, given land application of manures, what specific data was collected to ascertain problematic regions of this activity – I seem to have missed this? Overall, Figs 5.1 & 5.2 indicate significant human and bovine fecal pollution increases during rain events, but no resolution between manure application areas vs others?

Therefore Recommend (7): Suggest correct pathogen issues to the livestock waste and related manure sections.

TMDL Loadings: In Section 7.2, p110, there is a misunderstanding over the use of *Bacteroides* and the epidemiologic study conditions used to derive the new EPA criteria. The study sites with human sewage impact included wastewater discharges from UV or chlorinated secondary effluent, and qPCR Enterococcus spp. and total *Bacteroides* were the best indexes for GI health outcome. This is presumably due to enteric viruses and other pathogens being somewhat resistant to the standard disinfection processes used, hence, why drop information from disinfected wastewaters when applying the *Bacteroides* WLAs? Also, the fundamental science in assaying infectious enteric viruses has improved greatly since the time the total coliform 2.2. MPN / 100 mL etc. were derived and described in Title 22, and reflects a need to move onto more relevant pathogen performance measures for wastewaters in the State of California. Noting that there have been updates related to recycled water, however, the 12-log virus reductions for recharge of groundwater is probably going too far[49], but at least helps identify the inconsistency of a 2.2 Total Coliform target versus the real need to have much greater enteric virus removal.

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