From:	Bart Deamer
To:	Reed, Charles@Waterboards
Cc:	St.John, Matt@Waterboards; Mangelsdorf, Alydda@Waterboards; Butkus, Steve@Waterboards; Lee, Shin-Roei@Waterboards; Beryt, Elizabeth@Waterboards
Subject:	9-24-2015 2031291 BART DEAMER (2), NORTHWOOD
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Attachments:	Byappanahalli et al., Enterococcus in environment.pdf Weigand, Ashbolt et al., Genome Sequencing Enterococcus.pdf

Dear Charles-

Thank you for hosting the meeting at your offices last week and the public workshop this Tuesday in Monte Rio.

I would like to follow up on two new items that were announced at those meetings relating to the new draft TMDL's use of enterococci as indicator bacteria to measure health risk for swimmers in the lower Russian River mainstem. Health risk to swimmers is, of course, the foundation of the entire current TMDL process. The use of enterococci is crucial to the real-world impact of the proposed TMDL because, as I understand it, the other standard (E. coli) is already met everywhere on the mainstem. Enterococci are therefore positioned as the operative drivers of the far-reaching and expensive measures the TMDL appears to impose on residents along the lower Russian River.

The two new announcement items were:

1. There is no scientific peer review of the newly-proposed enterococcus standard pending and the Board does not intend to submit it for scientific peer review.

2. The staff believes that scientific peer review of the enterococcus standard is unnecessary because the peer review comments on its January draft TMDL, which proposed E. coli and Bacteroides standards, but not enterococcus, adequately reviewed the use of enterococcus as well, and recommended its use.

With respect, I hope that the Board will acknowledge the current scientific uncertainty surrounding the use of enterococci as pathogen indicators in heavily-forested, wildlife-rich, diffuse-source areas like the lower Russian River mainstem, and will take this uncertainty into account in implementing the TMDL.

Current scientific uncertainty of enterococci in natural areas like the lower Russian River

As you know, the epidemiological studies that are the basis for the EPA's 2012 enterococcus standard were conducted at point-source sites of human enterococcus: lake beaches near the outfalls of sewage treatment plants. Subsequent scientific studies have shown that, while enterococcus may be a reliable (indeed superior) indicator when a human fecal point source predominates the enterococci population, it is subject to a number of significant problems when applied to waters with diffuse sources and heavy vegetation like the lower Russian River. Rather than attempt to summarize all these problems, I am attaching two recent studies:

1. Byappanahalli, Nevers, Korajkic, Staley and Harwood, **Enterococci in the Environment** (Microbiology and Molecular Biology Reviews, December 2012). This article describes in detail the various reservoirs and habitats of non-fecal enterococci in natural settings that compromise their use as indicator bacteria in these settings. The general problem is summarized in the first paragraph as follows:

> The enterococci are most frequently used as fecal indicator bacteria (FIB), or general indicators of fecal contamination, but they are also used as surrogates for pathogens and/or health effects in risk assessment and other modeling applications [citations]. **Research spanning more than 3 decades, however, has shown that these bacteria are widely distributed in a variety of environmental habitats, even when there is little or no input from human and/or animal fecal sources** [emphasis added]. These extraenteric [i.e., non-fecal] habitats include soil and sediments, beach sand, aquatic and terrestrial vegetation, and ambient waters (rivers, streams and creeks); they may also be considered heterothermic habitats, in which temperatures are variable, in contrast to the gastrointestinal tract of warm-blooded animals, where the temperature is constant.

I commend the entire 21-page article for the full details in case the staff hasn't already had the opportunity to review it. After enumerating the many sources, habitats and reservoirs of enterococci in natural areas, the article reviews the current difficulty of distinguishing fecal-source enterococci from environmental-source enterococci, and summarizes current research on a variety of possible methods for doing this.

2. Weigand, Ashbolt, Konstantinidis and Santo Domingo, **Genome Sequencing Reveals the Environmental Origin of Enterococci and Potential Biomarkers for Water Quality Monitoring** (Environmental Science and Technology February 2014). In this article, the authors discuss the same issue, citing the article mentioned above. They summarize the problem in the first paragraph as follows:

> When detected in the environment, enterococci are interpreted as indicators of fecal pollution, and exposure to moderate cell densities **in sewage-impacted waters** correlates with increased risk of gastroenteritis [footnote citations; emphasis added]. As a result, current recreational water quality criteria issued by the U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO) focus on the densities of fecal indicator bacteria such as *Enterococcus* spp. [footnote citations]. **However, there is growing evidence to suggest that enterococci are present and may persist in a wide variety of environmental habitats, often in the absence of fecal contamination** (reviewed in [the above article]) [emphasis added]. Recent reports suggesting a primarily autochthonous [i.e., non-fecal] source for enterococci populations in marine beach sands and detritus, as well as in freshwater habitats, **highlight the potential for such populations to confound water quality monitoring, questioning the value of *Enterococcus* spp. as fecal indicators** [footnote citations; emphasis added]. Yet, it remains unknown how environmentally adapted strains relate to enteric enterococci targeted by current fecal indicator monitoring strategies. **Therefore, characterization of**

> **enterococci from such extra-enteric** [i.e., non-fecal] **habitats is needed to assess their genomic distinctiveness and potential for confounding the interpretation of microbial water quality assessments** [emphasis added].
>
> The article goes on to describe the current difficulty of distinguishing fecal-source enterococci from environmental-source enterococci, and reports an innovative method for using enterococcus genome sequencing to do this.

These articles and the many other articles cited in them show a strong need for scientific peer review and study before enterococci in heavily-forested, wildlife-rich, diffuse-source areas like the lower Russian River mainstem can be used to justify imposing extensive and burdensome new requirements on individual OWTSs.

Given the practical, real-world importance of the newly-proposed enterococcus standard in the Russian River TMDL and the difficulties of using enterococci as indicator bacteria in nature-heavy areas like the Russian River, why is the new enterococcus standard not being submitted to scientific peer review?

Peer review comments on January draft TMDL

At the meeting and the public workshop, the staff stated that the comments on the earlier-proposed E. coli and Bacteroides standards actually constituted sufficient scientific peer review of an enterococcus standard as well, and endorsed the use of enterococcus in the Russian River. Of the two reviewers, Professor Holden did not mention enterococcus in her comments, so I assume that Professor Ashbolt's comments were intended.

If I read them correctly, however, Professor Ashbolt's comments are consistent with the two studies above, one of which he co-authored. In his comments on the January draft TMDL, he points out that "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** [footnote citation; emphasis added]." The virtue of enterococci as an indicator bacteria applies "if the presences of **human sewage or cattle manure** are confirmed by sanitary survey and/or use of specific *Bacteroides* markers or equivalent via microarray analysis" due to "the dose-response nature in **sewage-impacted waterbodies**" [emphasis added]. Professor Ashbolt finally notes as to the EPA enterococcus standard, "The study sites **with human sewage impact** included wastewater discharges from **UV and chlorinated secondary effluent** [i.e., from sewage plants] and qPCR Enterococcus spp. and total *Bacteroides* were the best indexes for GI health outcome [emphasis added]." Professor Ashbolt's comments relate to sites, typically point-source sites, where fecal-source enterococci from human sewage are known to predominate, and expressly recognize that enterococci analysis is different in diffuse-source, heavily vegetated environments like the Russian River.

Further, Professor Ashbolt's passing comments about enterococcus in his review of an earlier draft TMDL that proposed E. coli and Bacteroides, not enterococcus, were clearly not intended to contradict the conclusions he and his co-authors clearly stated in their year-earlier article about

enterococcus in natural settings: that environmental-source enterococci are common in natural areas and can confound the use of enterococci as indicator bacteria, absent some sourcing method. I hope that the Board does not view Professor Ashbolt's incidental comments as a justification for ignoring the conclusions that he and his co-authors clearly and directly expressed in their article last year.

Current lack of knowledge of sources of Russian River enterococci

As I understand it, the Board hasn't made any analysis of its enterococcus samples to determine whether they come from fecal or environmental sources. In fact, if I understand it correctly, there is presently no feasible, scientifically-validated method available to do this, although research is underway in several laboratories to find a method. I note that extrapolating enterococci sources from sourced Bacteroides DNA currently appears to be infeasible. See Boehm and Sassoubre, **Enterococci as Indicators of Environmental Fecal Contamination** (2014): "Unfortunately, it may be difficult, if not impossible, to allocate enterococci sources using *Bacteroidales* genetic markers, due to the differential fate and transport of bacterial DNA and culturable enterococci in the environment [citations]. Ongoing work is assessing the feasibility of this approach and investigating the possibility of source tracking with enterococcal genetic markers."

Implementation of the TMDL

The bottom line is that the current science indicates that reliable sourcing of enterococci bacteria is a necessary precondition to using them to indicate health risk in heavily-forested, wildlife-rich, diffuse-source environments like the lower Russian River. Research is underway to identify feasible, scientifically-validated methods to do this, but they are not yet available. I therefore urge the Board to implement the TMDL in a manner consistent with the current uncertainty. Pending resolution of the current ambiguity of the enterococci in the mainstem, initial implementation should be focused on areas of the basin that are clearly impacted by fecal sources; these are all in tributaries of the mainstem, not in the mainstem itself. In this initial phase, the mainstem should of course be continuously monitored and analytical methods for sourcing enterococci developed. Once methods for sourcing enterococci have been developed and new sampling has been conducted, appropriate, science-based measures can be implemented to respond to any conditions found that are in fact unsafe for swimmers. <u>However, wide-spread, expensive, burdensome requirements should not be imposed based on mainstem enterococci until the current uncertain state of enterococcus knowledge can be and is resolved.</u>

Respectfully,
Bart Deamer
(Northwood resident)