TO: File: Russian River; TMDL Development and Planning

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SUBJECT: EVALUATION OF FECAL INDICATOR BACTERIA TYPES

The North Coast Regional Water Board staff are developing Russian River Total Maximum Daily Loads (TMDLs) for pathogen indicators to identify and control contamination impairing recreational water uses. Potential pathogen contamination has been identified in the lower and middle Russian River watershed leading to the placement of waters within these areas on the federal Clean Water Act Section 303(d) list of impaired waters. The contamination identified has been linked to impairment of the contact recreation (REC-1) and non-contact recreation (REC-2) designated beneficial uses. Health advisories for these waters have been published and posted by Sonoma County and the City of Santa Rosa authorities.

The Regional Water Board and others have collected water samples for analysis of fecal indicator bacteria concentrations to assess support of recreation beneficial use (REC-1) and compliance with the Water Quality Control Plan for the North Coast Region (Basin Plan). Recreational beneficial use criteria have been developed for measurements of bacteria concentrations to indicate a potential health risk from exposure to pathogens in surface waters. Most strains of fecal indicator bacteria do not directly pose a health risk to swimmers (i.e., primary contact recreators), but fecal indicator bacteria often co-occur with human pathogens and are easier to measure than the actual pathogens that may pose a risk of illness.

This memorandum reviews some of the types of fecal indicator bacteria found in surface waters and identifies appropriate indicators.

Types of Pathogens

Pathogens most commonly identified and associated with waterborne diseases can be grouped into the three general categories: bacteria, protozoans, and viruses (USEPA 2001).

Bacteria

Bacteria are unicellular organisms that lack an organized nucleus and contain no chlorophyll. Bacteria contain a single strand of DNA and typically reproduce by binary fission, during which a single cell divides to form two new cells. Wastes from warm-blooded animals are a source for many types of pathogenic (disease-causing) bacteria found in surface waters, including the coliform group and *Streptococcus*, *Lactobacillus*, *Staphylococcus*, and *Clostridia*. Some bacteria are pathogenic and some are not. Total coliform, fecal coliform, *E. coli*, *Enterococcus*, and *Bacteroides* bacteria are not generally the cause of human illness, but they have been or are being used to indicate the possible presence of sewage and pathogenic bacteria, viruses, and protozoans that also live in human and animal digestive systems.

Protozoans

Protozoans are unicellular organisms that reproduce by fission and occur primarily in the aquatic environment. Pathogenic protozoans constitute almost 30 percent (or 10,000) of the 35,000 known species of protozoans. Pathogenic protozoans exist in the environment as cysts that hatch, grow, and multiply after ingestion, manifesting as the associated illness. Encystation of protozoans facilitates their survival, protecting them from harsh conditions such as high temperature and salinity. Two protozoans of major concern as waterborne pathogens are *Giardia lamblia* and *Cryptosporidium*. The *Giardia* organism inhabits the digestive tract of a wide variety of domestic and wild animal species, as well as humans. *Giardia* can cause giardiasis (i.e., gastroenteritis in humans), infects approximately 200 million people worldwide and is one of the most prevalent waterborne diseases in the United States. *Cryptosporidium* can cause cryptosporidiosis, with symptoms of acute or persistent diarrhea that can last for a few weeks. Individuals with cryptosporidiosis are still infective for weeks after the symptoms have vanished.

Viruses

Viruses are a group of infectious agents that require a host in which to live. Viruses are composed of highly organized sequences of nucleic acids, either DNA or RNA, depending on the virus. All viruses have a protein covering that encloses the nucleic acid. Some viruses have a lipoprotein envelope over the protein covering. The protein or lipoprotein covering determines to what surface the virus will adhere. The most significant virus group affecting water quality and human health originates in the gastrointestinal tract of infected individuals. These enteric viruses are excreted in feces and include hepatitis A, rotaviruses, Norwalk-type viruses, adenoviruses, enteroviruses, and reoviruses.

Types of Fecal Indicator Bacteria

The most common fecal bacteria indicators used to assess the human health risk from recreation beneficial uses are total coliform, fecal coliform, *E. coli*, and *Enterococcus* bacteria. With the exception of *E. coli* bacteria, these indicators are composed of specific groups of bacteria species that share common characteristics. *E. coli* bacteria are a single species within the fecal coliform bacteria group.

Total Coliform Bacteria

Total coliforms are a group of bacteria that are widespread in nature. All members of the total coliform group can occur in human feces, but some can also be present in animal manure, soil, submerged wood, and other places outside the human body. Thus, the usefulness of total coliforms as an indicator of fecal contamination depends on the extent to which the bacteria species found are fecal and human in origin. Because total coliforms can come from non-fecal sources, they are no longer recommended as an indicator for assessing the support of recreation beneficial use. However, total coliform is still recommended for use in assessing support of shellfish consumption based on criteria adopted in 1925. These criteria were based on investigations made by the Public Health Service which assessed the occurrence of typhoid fever or other enteric diseases attributed to shellfish harvesting and have been used since adoption (NSSP 2009).

Fecal Coliform Bacteria

Fecal coliform bacteria are a subset of total coliform bacteria but are more fecal-specific in origin. Fecal coliform bacteria concentration criteria were initially recommended by USEPA (1976) for assessing support of recreational use. However, even this bacteria group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* bacteria are commonly associated with soils and the surfaces of plants, so that areas with allochthonous organic debris may show high levels of fecal coliform bacteria that do not have a fecal-specific bacteria source.

Additionally, studies show that fecal coliform bacteria concentrations are relatively useless for evaluating illnesses caused by water contact recreation. Subsequent to the publication of the USEPA (1976) recommended criteria for fecal coliform bacteria concentration, several key epidemiological studies were conducted to evaluate the criteria for effectiveness at protecting public health from water contact recreation (Cabelli et al. 1982; Cabelli et al. 1983; Dufour 1983; Favero 1985; Seyfried et al. 1985a, Seyfreid et al. 1985b) The studies concluded that the USEPA (1976) recommended fecal coliform bacteria criteria had no scientific basis. As a result of the new information derived from epidemiological studies, the USEPA (1986) changed the criteria recommendation to use the fecal bacteria indicators of *E. coli* and *Enterococcus* bacteria, instead of fecal coliform bacteria.

Escherichia coli (E. coli) Bacteria

E. coli is a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals. USEPA (2012) compiled numerous epidemiological studies and concluded that *E. coli* bacteria are the best indicator of human health risk from water contact in recreational freshwaters. USEPA (2012) published recommended criteria in the U.S. Federal Register for protection of contact recreation for *E. coli* bacteria.

Enterococcus Bacteria

Enterococcus bacteria are a subgroup within the fecal streptococcus bacteria group. *Enterococcus* bacteria are distinguished by their ability to survive in salt water, and therefore more closely mimic many pathogens than the other indicators in marine environments. USEPA (2012) recommends enterococcus bacteria concentration as the best indicator of human health risk in salt water for recreation.

USEPA (2012) states that *Enterococcus* bacteria concentrations may also be used as an indicator of human health risk in fresh water. Similar to *E. coli* bacteria, the *Enterococcus* bacteria criteria were published in the U.S. Federal Register for protection of water contact recreation. However, concerns have been identified for application of the *Enterococcus* bacteria concentration criteria (USEPA 2012) as an indicator of fecal contamination in freshwater.

First, there is concern about applying the *Enterococcus* bacteria concentration criteria in freshwater as some *Enterococcus* bacteria can come from non-fecal sources. The criteria are based on epidemiological studies that found association between illness and *Enterococcus* bacteria concentrations in surface waters with known sources of human fecal waste, specifically *Enterococcus faecalis* and *Enterococcus faecium*. Most research has found that the bacteria species *Enterococcus faecalis* is found mostly in humans, dogs, and chickens, and may or may not come from other warm-blooded animals (Wheeler et al. 2002). *Enterococcus faecium* is commonly found in production animals (Fisher and Philips 2003). *Enterococcus hirae* is frequently found to originate from domestic animals (Devriese et al. 2002). However, sources of *Enterococcus* bacteria in many surface waters may also be from non-fecal, natural sources. For example, *Enterococcus mundtii* and *Enterococcus casseliflavus* are associated with plant sources (Ferguson et al. 2005; Ferguson et al. 2011). Furthermore, additional epidemiological studies were conducted in waters impacted by urban runoff sources but no domestic wastewater sources found low illness rates after exposure (USEPA, 2010).

Second, using *Enterococcus* bacteria concentrations to assess whether there is potential for sewage and human pathogens assumes that the bacteria do not persist or regrow in the environment. Studies have shown that these bacteria persist in benthic sediment and can regrow when re-suspended into the water column. Hartel et al. (2005) found that *Enterococcus* bacteria survived desiccation and regrew in rewetted sediment. Sediment

collected in the riparian habitat and from naturally occurring drain surface biofilms in fresh water urban streams were found to be significant reservoirs of *Enterococcus* bacteria (Roberts 2012). Anderson et al. (1997) found that a large portion of *Enterococcus* bacteria load in urban and rural waterways came from non-human sources, including large loads from senescing algae. Urban runoff samples have been found to contain relatively higher proportions of *Enterococcus mundtii* and *Enterococcus casseliflavus* suggesting runoff sources are associated with plant species (Ferguson et al. 2013). Bacterial growth of *Enterococcus casseliflavus* on drain surfaces have been found to serve as a chronic low-level source of *Enterococcus* bacteria measurements collected in urban runoff (Ferguson et al. 2013). These studies indicate that elevated *Enterococcus* bacteria concentrations in water samples might be due to instream conditions that lead to regrowth and not due to contributions from fecal matter.

Finally, there are numerous reports concerning the high rates of false positive results from measurements in freshwater samples using the IDEXX Enterolert® method. Several factors can cause interference with the test methods resulting in the over-quantification of *Enterococcus* bacteria concentrations, including suspended sediment in the water (Hartel et al. 2006). Other bacteria types (*Vibrio*, *Shewanella*, *Bacteroides* and *Clostridium*) have also been found to be enumerated as *Enterococcus* bacteria with the method (Sercu et al. 2010). Analytical tests for *Enterococcus* bacteria concentrations measure all species of the genus *Enterococcus* (i.e., cultural incubation methods, like the IDEXX Enterolert® or membrane filter methods). The composition of *Enterococcus* species show much more diversity than fecal wastes from human sources (Ahmed et al. 2005).

<u>*Bacteroides* Bacteria</u>

Bacteroides bacteria are another group of fecal indicator organisms that are used to measure fecal contamination. *Bacteroides* bacteria is the genus name of the bacteria from the phylum Bacteroidetes and order Bacteroidales. *Bacteroides* bacteria contribute a significant fraction of the fecal bacteria species in animal feces. *Bacteroides* bacteria are anaerobic and make up a substantial portion of gastrointestinal flora of mammals (Wexler 2007). *Bacteroides* bacteria are not found in ambient surface waters without mammalian sources.

Due to their anaerobic-nature, *Bacteroides* bacteria have a low potential for survival and regrowth in the environment. *Bacteroides* bacteria show survival of only 1-day at the higher water temperatures typically observed in the Russian River during the summer period (Kreader 1998; Bell et al. 2009). Therefore, analysis of *Bacteroides* bacteria concentrations are often used to detect recent fecal contamination of surface waters.

Quantitative real-time polymerase chain reaction (qPCR) methods have been used to measure the levels of Bacteroides by amplifying specific DNA sequences. *Bacteroides* bacteria concentrations can be measured immediately since these methods are conducted without culturing the bacteria. In addition, the use of a host-specific genetic marker (16S rRNA) can also quantify the percentage of the *Bacteroides* bacteria population that

originates from specific animal-hosts (i.e., human, bovine, etc.) (Molina 2007). However, since qPCR methods can measure the DNA from dead *Bacteroides* bacteria, wastewater discharges that have been disinfected with chlorine could have measureable levels of dead bacteria until degradation of the DNA occurs (Bell et al. 2009). Numeric criteria for *Bacteroides* bacteria are not available as epidemiological studies have not yet be conducted to link concentrations to illness rates.

Microbiome Community

Analytical measurement technology has advanced to a point where entire bacterial communities are quantified instead of just specific fecal indicator bacteria groups or species. High-throughput DNA sequence analysis can potentially identify all sources of microbial contaminants in a single test by measuring the total diversity of fecal microbial communities. The PhyloChip™ (Second Genome, San Bruno CA) is a phylogenetic DNA microarray that has 16S rRNA gene probes that can quantify 59,316 different bacterial taxa in a single water sample. Analyzing the comprehensive suite of bacteria in a sample can help identify the major sources of fecal contamination in surface waters (Hazen et al. 2010).

Cluster analysis revealed strong differences in community composition among fecal wastes from human, birds, pinnipeds, and livestock. Differences in the diversity among fecal sources reveal hundreds of unique taxa that are specific to human, bird, and livestock feces (Dubinsky et al. 2012). Actinobacteria, Bacilli, and many Gammaproteobacteria taxa discriminated birds from mammalian sources. Families within the Clostridia and Bacteroidetes taxa discriminated between humans, livestock, and pinniped animal sources. Comprehensive interrogation of microbial communities for these diverse identifier taxa has great potential to improve the reliability of source detection in the environment. Phylogenetic microarrays are an effective tool for rapidly measuring the full assortment of microbial taxa that discriminate sources of fecal contamination. However, the technology is costly. Numeric criteria for the microbome community are not available as epidemiological studies have not yet be conducted to link concentrations to illness rates.

Findings

- Fecal indicator bacteria are used to indicate the possible presence of sewage and pathogenic bacteria, viruses, and protozoans.

- *E. coli* bacteria and *Bacteroides* bacteria are appropriate indicators of fecal contamination in fresh water and human health risk from water contact recreation.

- Total coliforms and fecal coliform are no longer recommended as indicators for assessing the support of water contact recreation because they can come from non-fecal sources.

- *Enterococcus* bacteria are not appropriate indicators of sewage and pathogens in fresh water because they can come from non-fecal sources, can regrow in the stream environment, and because there is a likelihood of false positives results in fresh water using current analytical methods.

- Quantification of the microbiome community through DNA sequence analysis such as the PhyloChip has great potential to detect specific sources of fecal waste in water.

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