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## North Coast Regional Water Quality Control Board

TO: File: Russian River; TMDL Development and Planning

FROM: Steve Butkus

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SUBJECT: SURVIVABILITY OF FECAL INDICATOR BACTERIA IN SURFACE WATERS

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Potential pathogen contamination has been identified in the surface waters of the lower and middle Russian River watershed leading to their placement on the federal Clean Water Act Section 303(d) list of impaired waters. Fecal indicator bacteria (FIB) were used to assess this impairment of the contact recreation (REC-1) and non-contact recreation (REC-2) designated beneficial uses and compliance with the Water Quality Control Plan for the North Coast Region (NCRQWCB 2011). Recreational beneficial use criteria have been developed for measurements of FIB concentrations to indicate a potential health risk from exposure to pathogens in surface waters. Most strains of FIB do not directly pose a health risk to swimmers (i.e., REC-1), but FIB often co-occur with human pathogens and are easier to measure than the actual pathogens that may pose a risk of illness.

Several different FIB have been used to assess the possibility of human pathogens in surface waters. *Escherichia coli* (*E. coli*), *Enterococcus*, and *Bacteroides* bacteria are not generally the cause of human illness, but they are being used to indicate the possible presence of sewage and pathogenic bacteria, viruses, and protozoans in surface waters (Butkus 2013). However, the presence of FIB may not accurately evaluate contamination from fecal sources since some of these bacteria have the potential to survive and replicate in surface waters outside of the animal host.

This memorandum reviews relevant scientific literature on the surface water survivability of the FIB that are used to assess impairment of recreation beneficial uses.

The following presents a summary of eight studies on the survivability of FIB:

### **1. Relative Decay of Bacteroidales Microbial Source Tracking Markers and Cultivated *Escherichia coli* in Freshwater Microcosms (Dick et al. 2010)**

This study compared the relative decay rates of three *Bacteroides* bacteria gene markers with *E. coli* bacteria concentrations in river water microcosms spiked with human wastewater. The *Bacteroides* bacteria gene markers measured were AllBac for general *Bacteroides* bacteria and HF183 and BacHum for human-host *Bacteroides* bacteria. Five sets of microcosms were incubated at 20°C under aerobic conditions and monitored over 11 days: (1) experimental control, (2) artificial sunlight, (3) sediment exposure, (4) reduced temperature (20°C), and (5) no autochthonous microbial predation (autoclaved spike). The concentrations of *E. coli* bacteria were measured with culture-based methods, whereas the numbers of *Bacteroides* bacteria gene markers were measured with qPCR assays.

The study found that:

- *Bacteroides* bacteria AllBac gene marker concentrations persisted longer than the human-host *Bacteroides* bacteria gene markers.
- Exposure to sunlight, sediment, and reduced microbial predation resulted in more rapid decay of the human-host *Bacteroides* bacteria gene markers relative to *E. coli* concentrations.
- No significant differences were observed in decay rates of three *Bacteroides* bacteria gene markers and *E. coli* bacteria at reduced temperature or under the experimental control conditions.
- The 99% decay rates for the experimental control condition were as follows:
  - E. coli* bacteria: 2.0 days
  - Bacteroides* bacteria gene marker AllBac: 3.3 days
  - Bacteroides* bacteria gene marker HF183: 2.2 days
  - Bacteroides* bacteria gene marker BacHum: 1.7 days

### **2. Relative Decay of Fecal Indicator Bacteria and Human-Associated Markers: A Microcosm Study Simulating Wastewater Input into Seawater and Freshwater (Jeanneau et al. 2012)**

This study compared the relative decay rates of *E. coli* and *Enterococcus* bacteria and two human-host *Bacteroides* bacteria gene markers: HF183 and BifAd. Freshwater microcosms spiked with human wastewater were incubated at 20°C in darkness under aerobic conditions. The concentration of *E. coli* and *Enterococcus* bacteria were

measured with culture-based methods, whereas the numbers of *Bacteroides* bacteria gene markers were measured with qPCR assays.

The study found that:

- In freshwater, the decay of the *Bacteroides* bacteria gene marker BifAd was significantly faster than the decay of *Enterococcus* bacteria concentrations.
- In freshwater, the decay of the *Bacteroides* bacteria gene marker HF183 was significantly faster than the decay of *E. coli* or *Enterococcus* bacteria concentrations.
- In freshwater, the decay of the *Bacteroides* bacteria gene marker BifAd was significantly faster than the decay of *E. coli* bacteria concentrations.
- The 90% decay rates for the freshwater microcosms were as follows:
  - E. coli* bacteria: 5.8 days
  - Enterococcus* bacteria: 3.1 days
  - Bacteroides* bacteria gene marker HF183: 1.7 days
  - Bacteroides* bacteria gene marker BifAd: 3.6 days

### **3. Relative inactivation of faecal indicator bacteria and sewage markers in freshwater and seawater microcosms (Ahmed 2014)**

This study compared the relative decay of *E. coli* and *Enterococcus* bacteria, and *Bacteroides* bacteria gene marker HF183. Freshwater and seawater microcosms spiked with human wastewater under ambient subtropical climatic conditions were monitored over 14 days. The microcosm water temperatures ranged from 14°C - 18°C. The concentration of *E. coli* and *Enterococcus* bacteria were measured with culture-based methods, whereas the numbers of *Bacteroides* bacteria gene markers were measured with qPCR assays.

The study found that:

- There was no significant difference between the decay of *E. coli* and *Enterococcus* bacteria and the *Bacteroides* bacteria gene marker HF183 marker in either freshwater and seawater microcosms.
- The 90% decay rates for the saltwater microcosms were as follows:
  - E. coli* bacteria: 1.7 days
  - Enterococcus* bacteria: 1.9 days
  - Bacteroides* bacteria gene marker HF183: 2.7 days
- The 90% decay rates for the freshwater microcosms were as follows:
  - E. coli* bacteria: 2.2 days
  - Enterococcus* bacteria: 1.9 days
  - Bacteroides* bacteria gene marker HF183: 3.5 days

#### **4. Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water (Kreader 1998)**

This study measured the decay of *Bacteroides distasonis* using PCR. The measurements were for detection of the DNA gene marker and not quantified. The gene marker used was not provided. Whole human feces were dispersed into water from the Ohio River and incubated with different temperatures and microbial predation microcosms.

The study found that *Bacteroides distasonis* was detected for:

- 14 days at 4°C
- 4 to 5 days at 14°C
- 1 to 2 days at 24°C
- 1 day at 30°C
- 14 days at 24°C without microbial predation, at least 12 days longer than with microbial predation.

#### **5. Factors influencing the persistence of fecal *Bacteroides* in stream water (Bell et al. 2009)**

This study compared decay rates of the total *Bacteroides* bacteria gene marker, AllBac, from equine fecal samples incubated in stream water microcosm experiments. Microcosm experiment were conducted to evaluate the effects of initial fecal waste concentration and temperature on decay rates. The measurements were for detection of the DNA gene marker and not quantified.

The study found:

- No significant difference in the decay rate of the AllBac *Bacteroides* bacteria gene marker based on initial fecal waste concentration.
- The time required for the *Bacteroides* bacteria gene marker concentrations to become significantly different from the experimental control microcosm ranged from 1 day incubation at 35°C to 11 days incubation at 5°C.

## **6. Persistence of host-associated Bacteroidales gene markers and their quantitative detection in an urban and agricultural mixed prairie watershed (Tambalo et al. 2012)**

This study evaluated the decay rates of *E. coli* bacteria concentration and several *Bacteroides* bacteria gene markers in freshwater stream water spiked with fecal waste and incubated in the field for 14 days with temperature ranging from 20°C to 25°C. Two experiments were conducted in July and August. *Bacteroides* bacteria gene markers evaluated were AllBac (total *Bacteroides*), BacH (Human), BacR (ruminant), and CowM2 (Bovine). The concentrations of *E. coli* bacteria were measured with culture-based methods, whereas the numbers of *Bacteroides* bacteria gene markers were measured with qPCR assays. The measurements were for detection of the DNA gene marker and not quantified.

The study found:

- The decay of the host-specific *Bacteroides* gene markers were significantly faster than the *E. coli* concentration and the total *Bacteroides* gene marker AllBac.
- The 99% decay rates for the freshwater microcosms were as follows:
  - E. coli* bacteria: 6-15 days
  - Bacteroides* bacteria gene marker AllBac: 8.3 – 13.4 days
  - Bacteroides* bacteria gene marker BacH: 1.5 – 7.7 days
  - Bacteroides* bacteria gene marker BacR: 1.4 – 5.6 days
  - Bacteroides* bacteria gene marker CowM2: 1-4 days

## **7. Differential decay of human faecal Bacteroides in marine and freshwater (Green et al. 2011)**

This study compared the decay rates of culturable *Enterococcus* bacteria concentrations, *Enterococcus* bacteria gene marker (Entero1) and several total and human-host *Bacteroides* bacteria gene markers (GBSteriF1, BuniF2, GenBac3, Hf183 Taq., HF 134/303R, HF 183/303R. and HumM2). Freshwater microcosms were spiked with human wastewater and exposed to either sunlight and dark experimental treatments and monitored for 21 days. The concentrations of *E. coli* bacteria were measured with culture-based methods, whereas the numbers of *Bacteroides* bacteria gene markers were measured with qPCR assays.

The study found:

- Culturable *Enterococcus* bacteria concentrations decayed faster than the *Bacteroides* bacteria and *Enterococcus* bacteria gene markers

### **8. Influence of wastewater disinfection on densities of culturable fecal indicator bacteria and genetic markers (Chern 2013 in press)**

This study investigated the effect of disinfection (chlorination or ultraviolet light) on effluents from secondary wastewater treatment in different seasons on the decay of *E. coli* bacteria, *Enterococcus* bacteria, and several *Bacteroides* bacteria concentrations. The concentrations of *E. coli* bacteria, *Enterococcus* bacteria, and several *Bacteroides* bacteria were measured with both culture-based methods, and bacteria gene markers using qPCR assays. The following bacteria gene markers were assayed: EC23S857 (*E. coli* bacteria), Entero1 (*Enterococcus* bacteria), GenBac2 (total *Bacteroides* bacteria), Hf183 and HumM2 (human-host *Bacteroides* bacteria).

The study found:

- Disinfection of secondary treatment effluents showed significantly reduced culturable *E. coli*, *Enterococcus*, and *Bacteroides* bacteria.
- Disinfection of secondary treatment effluents showed no significant reduction in bacteria gene markers for *E. coli*, *Enterococcus*, and *Bacteroides* bacteria.
- No significant differences were observed in the decay of culturable bacteria and genetic marker densities to type of disinfection (chlorination vs. UV) or season.

### **9. *Escherichia coli*, enterococci, and *Bacteroides thetaiotaomicron* qPCR signals through wastewater and septage treatment (Srinivasan et al 2011)**

This study evaluated the concentration *Escherichia coli*, enterococci, and *Bacteroides thetaiotaomicron* throughout a wastewater treatment facility and septage treatment facility. *E. coli* bacteria and *Enterococcus* bacteria were measured by both cultivation methods and qPCR assays.

The study found:

- Concentrations and decay rates were significantly correlated between *Bacteroides thetaiotaomicron* gene markers and *E. coli* bacteria and *Enterococcus* bacteria (as measured by both cultivation methods and qPCR assays) through the waste treatment process for both raw sewage and septage.

## Findings

Based on the review of the literature on FIB survivability summarized in this memorandum, Regional Water Board staff can make the following findings:

- The decay of host specific *Bacteroides* gene markers was most affected by higher temperatures, followed by microbial predation.
- During warm summer temperatures, *Bacteroides* gene markers may be found for only 1 to 2 days, whereas in during cooler winter months the markers could be detected for more than a week.
- Most of the studies reviewed found that during warm summer temperatures, *Bacteroides* gene markers decay more rapidly than *E. coli* bacteria and *Enterococcus* bacteria concentrations.
- Wastewater disinfection (either chlorination or ultraviolet light) significantly reduces culturable (i.e., living) *E. coli*, *Enterococcus*, and *Bacteroides* bacteria, but may not reduce the amount of the gene markers due ambient DNA that has not yet decayed (Chern 2013 in press)
- Genetic markers measured using qPCR may not be suitable for monitoring the efficacy of wastewater disinfection on the inactivation of bacterial pathogens due to the detection of non-living bacterial DNA (Chern 2013 in press).

## Citations

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