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# Determination of DNA-based Fecal Marker Aging Characteristics for Use in Quantitative Microbial Source Tracking

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## EXECUTIVE REPORT

*Escherichia coli* and enterococci are fecal indicator bacteria (FIB) used to assess surface water quality around the world. In California's built environment, their presence in bathing water is associated with human illness when the bacteria arise from sources such as faulty sewer infrastructure, wastewater and urban runoff. However, FIB can originate from many other fecal and non-fecal sources, including animal feces, beach wrack, sands, and submerged vegetation, with little or reduced risk of human illness. Identifying FIB sources through microbial source tracking (MST) is therefore essential for two important management scenarios: remediating contaminated surface waters and determining potential health risks to swimmers. MST approaches have greatly advanced with the advent of fecal source-associated assays (i.e., qPCR quantification of DNA-based markers) able to identify the presence of an increasing number of animal sources of FIB, but implementation of MST in the two essential management scenarios remains difficult. This difficulty is due to serious limitations in the ability to accurately interpret DNA-based marker information. One major limitation is that knowledge of marker prevalence and performance has been obtained mostly from "fresh" fecal material in the laboratory, and it is not clear how aging of fecal material in the environment affects marker performance and data interpretation. During "aging," various environmental processes (e.g., inactivation, predation, adsorption to particles) may change the relative abundance of the different constituent microorganisms found in feces from that of the "fresh" sample. The goals of this study were to answer two main questions surrounding DNA-based MST markers: 1. What are the relative decay rates of DNA-based markers, FIB (*E. coli* and enterococci), and representative pathogens, as well as the whole microbial community, from sewage, cow feces, and bird feces, under environmental conditions relevant to the California coast? 2. Given the assessed patterns of DNA-based marker aging in fecal contamination, juxtaposed against FIB aging patterns, how can water quality managers best use MST data to diagnose the presence and amount of contamination from specific fecal hosts for a sampled site (i.e., beach or site along an MST sampling transect)? To answer these questions, this project examined relative degradation of FIB, DNA-based markers, and pathogens through both in-situ field experiments as well as ex-situ laboratory experiments. The in-situ field aging studies were conducted at three sites representing the typical surface water types in California: a freshwater site, a brackish water coastal lagoon site, and a marine, nearshore site. The design utilized diffusive dialysis bags that allow equilibrium of environmental conditions inside and outside the bags, thus allowing the decay of fecal microbes (inside the bags) to be monitored under realistic field conditions. The effects of environmental factors such as season and sunlight were assessed through replicating the study at each site during winter, summer with no shading, and summer with shading. Sewage, cow feces, and gull feces served as microbial seeds for these experiments. The laboratory experiments expanded what could be

assessed in the field by individually examining the effect of the water matrix and sediment on relative degradation of sewage fecal organisms. In the water matrix effect investigation, sewage aging in 12 waters (i.e., 3 from each of the 4 California regions represented by the project PIs) was investigated via an outdoor mesocosm study. In the sediment effect investigation, sewage aging in the presence of each of three types of sediment (i.e., freshwater, brackish and marine water sediment from the three sites of the in-situ field aging study) was investigated via a separate outdoor microcosm study. In all experiments, representative FIB, MST markers and pathogens, as well as the entire microbial communities, were measured for each fecal source.
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Overall, there was no universal trend regarding degradation of MST markers relative to FIB and pathogens observed in this project. Whether the three target groups experienced differential degradation, and to what extent, depended on the combination of environmental conditions (i.e., field site, season, sunlight) and fecal source, whether the MST markers were *Bacteroidales* or *Catellicoccus*. Nevertheless, while the *Bacteroidales* MST markers did not always show higher decay rates than pathogens and cultivable FIB, these MST markers rarely showed decay rates lower than the other two groups. The gram-positive *Catellicoccus* gull MST marker appeared to be a more conservative marker than the gram-negative *Bacteroidales* human, cow and dog MST markers, showing decay rates more similar to those of pathogens and *Enterococcus* DNA markers. While non-specific, the *Enterococcus* DNA markers generally appeared to be a conservative marker for indicating fecal contamination and potential human health risk. In practice, our results demonstrate that commonality regarding relative degradation may not be expected across sites, although similar decay between certain targets might be assumed under certain conditions. This was likely because different abiotic and biotic factors were the dominant factors affecting decay at different field sites. A holistic model integrating these various factors, if achievable, might be most useful for beach managers in predicting degradation behaviors at their local sites. A preliminary model integrating sunlight intensity, color and depth of environmental water has been developed, representing one step forward towards such a goal. To assist managers interpreting results, two quantitative MST models were developed: 1) A ratio model for potential fecal source allocation 2) A human fecal score for assessing the extent of human fecal contamination at a site Theoretically, the proportion of fecal contamination in a water sample attributable to a single source can be determined using a ratio method. However, the accuracy of this model relies on all markers decaying at a similar rate. As there is currently no simple method available for estimating the fecal contamination age, source allocation models must either be restricted to use on fresh fecal contamination scenarios or used with analytes that decay at a similar rate in the environment. Based on the results of our field studies, there are only certain rare conditions when decay rates are close enough to make this model viable. The ratio source allocation model therefore may only be used in environmental conditions where decay constants of FIB and MST markers are not expected to be different. Quantitatively characterizing a site regarding its extent of human fecal contamination for site remediation prioritization and effectiveness evaluation generally requires integrating human marker results across multiple samples from a site. Previously, this was mostly done via the best professional judgement (BPJ) available within the premise of each individual project. Recent research has demonstrated tremendous variability/uncertainty/inconsistency in the BPJ approaches for MST data interpretation, and it was concluded that a mathematically defined standardized algorithm was needed. Thus, a human fecal score (HFS) was developed to provide such an assessment based on HF183 qPCR measurements. The application of HFS is based on the collective results from daily morning water samples from a beach site. Model sensitivity analysis was conducted to demonstrate how HFS can be used to optimize study design parameters such as sample size (i.e., number of days sampled at the beach site) and qPCR replication (i.e., number of qPCR replicates analyzed per sample) HFS. Decision charts on sample size and PCR replication were developed for the management application of site prioritization (Appendix H). However, it is important to consider how HF183 marker degradation may affect application of HFS. A wide range of decay rates (k=<1 day-1 to 6 day-1, in C=C0e-kt) were observed for the HF183 marker in this project: marine waters (k=1-2), brackish waters

(k=1-6), freshwater (k=1-5), water matrix laboratory 3 study (k=<1 – 5), sediment (k=<1-2). These rates translate to <0.5 to <3 log10 reduction per day. Assuming a starting concentration of HF183 from fresh sewage input to be 6 log10 copy per 100ml (5% sewage measured in this project averaged 6.7 log10 copies of HF183 per 100ml), the HF183 signal would disappear in two days under the fastest decay rates observed in this project. If the sewage fecal source is introduced locally at the beach with a daily sampling scheme, the HFS would be integrating both the fresh and aged HF183 signals under most decay conditions. Nevertheless, with a less frequent sampling scheme (e.g., only once every two days), it would be possible to miss the HF183 signals under environmental conditions enabling the highest decay rates. It is therefore important for managers to consider the extent of potential decay on the spectrum of rates observed in this project, and adjust sampling design accordingly (more details in Appendix H). This project is the first ever to determine decay constants for such a large number of MST markers, FIB, and pathogens under environmental conditions. Although the Ratio Model may not be useful under most conditions, valuable information about how microbes behave in a range of environmental conditions was gleaned from this study. Even outside the context of a model, this information will help beach managers and researchers as they take on the arduous task of microbial source identification. Further, the Human Fecal Score Model will allow managers to prioritize beaches for remediation or Quantitative Microbial Risk Assessment studies.

**Full text:**
**http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/978_DNA_FecalMarkerAgingQuantMicrobialSourceTracking.pdf**