Effect of freshwater sediment characteristics on the persistence of fecal indicator bacteria and genetic markers within a Southern California Watershed

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Abstract

In this study, the aging of culturable FIB and DNA representing genetic markers for *Enterococcus* spp. (ENT1A), general *Bacteroides* (GB3), and human-associated *Bacteroides* (HF183) in freshwater sediments was evaluated. Freshwater sediment was collected from four different sites within the upper and lower reach of the Topanga Creek Watershed and two additional comparator sites within the Santa Monica Bay, for a total of six sites. Untreated (ambient) and oven-dried (reduced microbiota) sediment was inoculated with 5% sewage and artificial freshwater. Microcosms were held for a 21-day period and sampled on day 0, 1, 3, 5, 7, 12, and 21. There were substantial differences in decay among the sediments tested, and decay rates were related to sediment characteristics. In the ambient sediments, smaller particle size and higher levels of organic matter and nutrients (nitrogen and phosphorus) were associated with increased persistence of the GB3 marker and culturable *Escherichia coli* (cEC) and enterococci (cENT)). The HF183 marker exhibited decay rates of -0.50 to -0.96 day^{-1} , which was 2-5 times faster in certain ambient sediments than decay of culturable FIB and the ENT1A and GB3 markers. The ENT1A and GB3 markers decayed at rates of between -0.07 to -0.28 and -0.10 to -0.44 day^{-1} , and cEC and cENT decayed at rates of between -0.22 and -0.81 and -0.03 and -0.40 day^{-1} , respectively. In the oven-dried sediments, increased persistence of all indicators and potential for limited growth of culturable FIB and the GB3 and ENT1A markers was observed. A simplified two-box model using the HF183 marker and cENT decay rates generated from the microcosm experiments was applied to two reaches within the Topanga Canyon watershed in order to provide context for the variability in decay rates observed. The model predicted lower ambient concentrations of enterococci in sediment in the upper (90 MPN g^{-1}) versus lower Topanga watershed (530 MPN g^{-1}) and low ambient levels of the HF183 marker (below the

LLOQ) in sediments in both lower and upper watersheds. It is important to consider the variability in the persistence of genetic markers and FIB when evaluating indicators of fecal contamination in sediments, even within one watershed.

1. Introduction

Decay of bacteria and fecal markers in sediments is particularly understudied even though sediments, soils, and beach sands have been implicated as potential reservoirs in a variety of climates and environments (Alm et al., 2014; Craig et al., 2004; Garzio-Hadzick et al., 2010; Haller et al., 2009; Lee et al., 2006). Sediments can promote persistence of fecal organisms both by providing protection from ultraviolet radiation, temperature fluctuations, and predation by microorganisms in the overlying water column (Davies et al., 1995; Korajkic et al., 2013; Wanjugi and Harwood, 2013; Wheeler Alm et al., 2003) and by supplying critical nutrients (Craig et al., 2004; Labelle et al., 1980; Mika et al., 2009; Wanjugi et al. 2016). Under favorable sediment conditions, fecal indicator bacteria (FIB) exhibit extended survival and even regrowth, which can lead to false positive indications of recent fecal pollution in water quality monitoring when organisms colonize sediment and levels no longer correspond with the presence of fecal pollution and pathogens (Byappanahalli and Fujioka, 1998; Byappanahalli et al., 2012; Davies and Bavor, 2000; Garzio-Hadzick et al., 2010; Lee et al., 2006).

FIB are used as proxies for the myriad of pathogens present in fecal matter, and their presence has been linked to adverse health effects (Cabelli et al., 1982; Kay et al., 1994; USEPA, 2010). However, FIB do not provide information regarding the source of fecal contamination (Field and Samadpour, 2007). Quantification of source-associated DNA markers using quantitative polymerase chain reaction (qPCR) methods have greatly advanced microbial source tracking (MST) efforts. Molecular MST methods enable successful source tracking by allowing

for same-day water quality monitoring results and information regarding the source of the fecal contamination (Boehm et al., 2013; Griffith et al., 2013, 2009; Noble et al., 2006). Although there has been limited research evaluating the decay rates of source tracking markers along with culturable FIB in water (e.g. Bae and Wuertz, 2015, Mattioli et al. 2017), marker decay has not yet been fully investigated in sediments. FIB are typically measured by culture-based technique, whereas DNA-based markers measure DNA from both culturable, viable but not culturable (VNBC), and dead cells. Further, decay of fecal microorganisms is dependent on both the bacterium itself and on physical (e.g. organic matter content, temperature, salinity) (Haller et al., 2009; Shelton et al., 2014; Thelaus et al., 2009) and biotic factors (predation and competition) (Kinnaman et al., 2012; Korajkic et al., 2013; Wanjugi and Harwood, 2013). Ideally, indicators should provide a warning that fecal contamination by human-derived sewage has recently occurred, and their presence should correlate with the presence of human pathogens.

Studies so far evaluating genetic marker decay in sediments are limited and have focused on evaluating the effect of moisture content on decay of genetic markers in freshwater sand (Eichmiller et al. 2014) and in marine beach sand (Yamahara et al. 2012), decay in freshwater sediment under aerobic and anaerobic conditions (Kim and Wuertz, 2015), decay of the LA35 poultry litter marker in fresh and marine sediments and waters (Nayak et al. 2015), and decay of a single genetic marker: *Catelliboccus marimmalium* in wetted and unwetted beach sand (Brown and Boehm, 2015). With the exception of the Nayak et al. (2015) study, these efforts consider decay in one sediment type only; the relative aging of genetic markers and FIB may vary among sites due to differences in particle size, nutrient concentrations, and ambient microbial communities which has not yet been addressed.

In this study, laboratory microcosm experiments were used to investigate differences in the relative aging of FIB and genetic markers in six different freshwater sediments. In addition, the effect of ambient microbiota on decay was evaluated by oven-drying four of the six sediments and comparing decay rates between the ambient and oven-dried sediments. The main objectives were to: 1) examine the variability in genetic marker and FIB decay rates with respect to varied freshwater sediment characteristics, 2) investigate the role of the ambient microbiota on persistence of genetic markers and FIB in freshwater sediments, and 3) assess the variability in sediment decay rates within one watershed (Topanga Creek Watershed). In addition, a simplified two-box model was applied, using HF183 marker and cENT decay rates generated from the microcosm experiments, to better contextualize the variability in decay rates observed within the Topanga Canyon watershed.

2. Methods

2.1 Sediment Microcosm Experiments

2.1.1 Sediment collection and characterization

Freshwater sediments were collected from six sites: a total of four sites were selected within the Topanga Canyon Watershed (S1-S4), two within the upper reach of Topanga Canyon and two within the lower reach of the Topanga Canyon; and, for comparison, two additional sites: Ballona Creek freshwater marsh (C1) and Medea Creek in Malibu Canyon (C2). In a previous study, chronically high levels of fecal contamination entering the upper Topanga Creek watershed were observed (Riedel et al., 2015). Inputs include runoff from the town of Topanga and Topanga Canyon Boulevard, which parallels the creek. The upper reach (4500-4650m) is pool-dominated and larger substrate, such as cobbles, boulder, and bedrock are more frequent, while the lower reach of Topanga Canyon is dominated by riffles and runs (25 and 75%) and a

higher percentage of smaller substrates, such as fines and gravel (Dagit et al., 2014). Site coordinates, map of sites selected, and site descriptions can be found in the Supplemental Information (SI).

Approximately the top 5 cm sediment was collected from at least three locations within a 5 m x 5 m transect at each site using sterile sediment cores. Sediments were held at 4 °C in the dark for approximately 48 hours before microcosm experiments. Particle size, organic matter content, and nutrient, and chlorophyll *a* levels were measured in each sediment type; for detailed methods see the SI.

2.1.2 Microcosm setup

Microcosms were constructed in 2L Pyrex beakers with a 2:1 sediment: water ratio by volume using homogenized sediment and artificial fresh water ((AFW) distilled water with 0.3 mM MgCl₂, 0.6 mM CaCl₂, and 1.4 mM NaHCO₃). Two types of sediment treatments were conducted: ambient (untreated) and reduced microbiota (oven-dried).

Sediment collected from selected sites (S2, S3, S4, and C2) was oven-dried at 180°C for 48 hours to reduce the impact of the ambient microbial community and predators (Korajkic et al., 2013; Wanjugi and Harwood, 2013). Reduction of the ambient microbial community was assured by running an Enterolert and Coli-ert IDEXX on sediment resuspension and by streaking the sediment resuspension onto Tryptic Soy Agar; bacterial levels after oven-drying were below the LOD on all media. Oven-dried sediment moisture content was adjusted with AFW to match moisture content of ambient sediment prior to seeding of sewage inoculum.

Sewage influent was collected from the Orange County Sanitation District in Fountain Valley, CA and held for 24 hours prior to sediment inoculation. On Day 0 of the microcosm experiments, ambient and oven-dried sediment from each site was mixed with sewage (5%

primary influent) for two hours in separate 26 L Sterilite containers. After two hours, 400 mL seeded sediment was distributed into each of three replicate, 2 L Pyrex beakers. 200 mL AFW was then added to each beaker. A preliminary microcosm experiment showed no difference in cENT decay in freshwater sediment collected from Site S2 when ambient site water or filter-sterilized site water was used instead of AFW (see SI for details, Figure S2).

Microcosm experiments were conducted in a Precision Environmental Chamber set at 20° C on a 12-hour light/dark cycle. Each beaker had an airstone to ensure mixed and oxygenated conditions (Whisper 100 Aquarium Air Pump, Tetra, Blacksburg, VA). Sediment was inspected visually to ensure that it was not suspended by air movement.

2.1.3 Microcosm Sampling

Prior to sampling the sediment at each time point, water was carefully pipetted off into sterile 1L Nalgene bottles, so as not to disturb the sediment, and stored on ice for processing. Sediment was then sampled using a sterile 15 mL falcon tube core. Three cores were taken from approximately the top 10 cm of each beaker, composited, and stored on ice until processed. Preliminary testing showed no difference in cENT decay when the top 5 cm was sampled versus a 5-15 cm core (see SI for details, Figure S2). Each beaker was then replenished with 200 mL new AFW, simulating semi-continuous flow conditions. Sediment from each of the three replicate beakers was processed for culturable FIB (cEC and cENT) and genetic markers for human (HF183), enterococci (ENT1A), and general *Bacteroides* (GB3) on days 0, 1, 3, 5, 7, 12, and 21. Water from one of the three replicate beakers was processed on days 1, 3, 5, 7 and 12, to account for resuspension to the water column occurring between day 1 and day 12.

2.2 Marker Processing, DNA extraction, and qPCR Analyses

Water samples were processed by filtering sample through a 47 mm, 0.4 μ m pore size, HTP polycarbonate filter (EMD Millipore, Billerica, MA) in triplicate. Each filter was placed in an individual 2 ml polypropylene screw cap tube, containing 0.3 g, 212 – 300 μ m (50 – 70 U.S. sieve) acid washed glass beads (Sigma-Aldrich, St. Louis, MO) and stored at -80°C until DNA extraction. DNA extraction was conducted with the DNA-EZ ST1 Extraction Kit (GeneRite, North Brunswick NJ) following the manufacturer’s protocol. Eluted DNA samples were stored at -20°C until analysis.

0.25 g sediment was placed into an individual 2 mL polypropylene screw cap tube with 750 μ Ls of Bead Solution (MoBio Inc., Carlsbad, CA) and 1 g of garnet beads (MoBio Inc., Carlsbad, CA). Sediment samples were extracted according to manufacturer’s protocol with the MoBio Power Soil DNA Extraction Kit (MoBio Inc., Carlsbad, CA) with minor modifications: DNA was eluted into 50 μ L of elution buffer, and material in the tubes was homogenized by bead beating for 60 seconds (BeadBeater, Biospec, Bartlesville, OK).

QPCR assays were performed according to previously published protocols (see SI for details, Table S3). Reaction mixtures consisted of 1X Taqman Environmental Master Mix (Applied Biosystems, Foster City, CA), forward and reverse primers, probes, bovine serum albumin, and 2 μ L of template DNA. Triplicate samples were run on a StepOnePlus™ real-time PCR system (Applied Biosystems, Carlsbad, CA). Samples and calibration standards were run in triplicate. A five-point standard calibration curve was run alongside samples on each 96-well plate. Standard curves had efficiencies between 89 - 100% and $R^2 > 0.99$ (see SI, Table S4). Quantification thresholds (Ct) were converted into units of gene copies using a pooled master standard calibration model. Negative controls (no template controls on 96-well plate) and

filtration and extraction blanks were included to ensure contamination of samples did not occur during either the filtration or extraction processes or during PCR.

2.3 FIB Enumeration

For each treatment, a 10 g subsample was placed in a sterile 100 mL polypropylene container with 100 mL PBS, hand shaken for two minutes, and allowed to settle for 30 seconds (Boehm et al, 2009). Sample wash was decanted and processed for FIB.

cEC and cENT were measured with Colilert-18TM and EnterolertTM (IDEXX, Westbrook ME) reagents and protocols to determine the most probable number (MPN) of cells per 100 mL or g sample. Samples were analyzed at a 1:10 dilution as recommended by the manufacturer, or a 1:100 dilution on an as needed basis. The limit of detection (LOD) for these assays was 10 MPN/100 mL and any sample below the limit of detection was assigned a value of 5 MPN/100 mL or g for analysis.

2.4 Statistical Analysis

Decay rate constants were calculated with a first-order decay equation: $C=C_0e^{-kt}$, where $C(t)$ is the concentration at time point (t), C_0 is the initial concentration, and k is the first order decay constant. Natural log normalized concentration ($\ln C/C_0$) was regressed over time. Decay was analyzed between day 0 and day 12 due to increased frequency of sampling points during this duration and lower rate of decline observed beyond day 12 for certain indicators, unless levels fell below the limit of detection before day 12, in which case decay was analyzed between day 0 and the last day of detectable concentration of FIB or marker. Concentrations measured on day 21 were used to evaluate if concentrations significantly changed over the longer time period. Biphasic and shoulder log-linear models were tested but generally did not improve fit of the model, and application of the first-order decay equation allowed for a more direct comparison

among the different decay rate constants. Multiple linear regression (MLR) analyses were used to compare significant differences between slope (decay rate constant) for the different indicators and sediment types tested. Additional MLR models were run to evaluate impact of sediment characteristics on decay rate constant in the six ambient sediments tested; sediment characteristics were included as predictor variables in individual MLRs testing effect of % fines, total nitrogen (TN), phosphate, % organic matter, and the microbiota treatment on decay rate constants. Student's t-tests were run comparing FIB and genetic marker levels on day 0 and day 21 to determine if there was a significant reduction in marker and FIB concentration over the course of the experiments. All statistical analyses were conducted in Stata 12.0 (Stata Corp, College Station, Texas).

2.5 Topanga Canyon Watershed ambient sediment and water sampling

Ambient sediment samples were collected from four sites along Topanga Creek on seven different days between July 2014 and March 2015. Samples were only collecting during dry weather with at least 10 days since active rainfall. Sediment samples were collected into 50 mL falcon tubes using sterile soil cores and transported on ice. Water samples were collected within the Topanga upper watershed sites from 2012-2014 as part of a three-year microbial source tracking study. Detailed results and sampling methods are described in an earlier study (Riedel et al., 2015). For detailed site descriptions and locations see the SI, Table S2.

2.6 Simplified Two-Box Model

A simplified stream model was applied to two stream reaches in the Topanga watershed in order to highlight sediment-related processes (see SI for model schematic, Figure S2). Two creek reaches were modeled: 1) the upper watershed reach, which represented a 1000 m stretch between 6500 m and 4800 m, where significant reductions in FIB levels were observed during

field sampling and which represented an impacted stream segment, and 2) a 1000 m stretch within the lower Topanga watershed (3600 m to 1700 m). Both reaches are located between the town of Topanga and Topanga State Beach, a heavily populated recreational site and have the potential to mediate urban inputs from the town of Topanga.

The model applied in this study considered the scenario where there is a constant input from an upstream site of 300 MPN 100 mL⁻¹ cENT and 400 copies 100 mL⁻¹ HF183 marker. Initial concentrations in the sediment and water column were set to zero.

The elements modeled include time-variant cENT and HF183 marker sediment and water levels. Stream dimensions (depth of the water column and sediment) and average flow (u) were based on average measurements taken in Topanga Creek from 2012 to 2014 (see SI, Table S5). The concentration in the water column was governed by settling of particle-attached microbes and is described in Eqn.1.

$$\text{Eqn.1 } \frac{dC_w}{dt} = \frac{u}{L}(C_{in}-C_w) - \left(\frac{Vs_f C_w}{D_w}\right)$$

In Eqn (1), the terms on the right side of the equation describe an input of FIB and HF183 marker (C_{in}) and removal by settling and lateral flow.

The time-variant concentration in sediment was calculated as a function of deposition and decay only. The concentration in the sediment is described by Eqn 2.

$$\text{Eqn 2. } \frac{dC_{sed}}{dt} = \frac{C_w V_s f}{D_s} - k_{sed} C_{sed}$$

In Eqn (2), the terms on the right side of the equation describe deposition of particle-associated cells and subsequent decay in the sediment. Resuspension from the sediment was not included in the model. Sediment decay rate constants for the two reaches were calculated using data from the microcosm experiments described above. Decay rate constants were averaged from sites S3 and S4 for the upper watershed reach simulation for cENT and for HF183. For decay within the

lower watershed, decay rate constants were applied based on results for Site S2. For details regarding input parameters and variables included in the model see the SI (Table S5).

3. Results

3.1 Sediment characteristics

Sediment collected from Ballona Creek FW marsh (C1), had the smallest particle size (50.0 % fines), highest organic matter content (4.6 %) and highest levels of nutrients of the sites tested. Sediment collected from C2 (Medea Creek, Malibu) and S2 (Topanga Creek lower watershed) had intermediate levels of organic matter (1.4, 1.2 %) and percent fines (9.7, 10.6 %), respectively. Sites S1, S3, and S4 were mostly comprised of sand (93%), and had low levels of organic matter and nutrients, respectively (Table 1). Total nitrogen (TN) and carbon levels were measured in sediment after the addition of the 5% sewage inoculum. Overall, TN and C levels were increased with the sewage addition- on average by 56% and 63%, respectively. Differences observed between sites (described above) were maintained at these elevated levels; total nitrogen and carbon levels measured before and after the addition of the 5% sewage inoculum were significantly correlated: TN- $r=0.96$, $p=0.002$; TC- $r=0.94$, $p=0.006$.

3.2 Decay of FIB and Source Markers

First order decay rate constants in both ambient and oven dried sediments are presented in Table 2 and decay dynamics are presented in Figures 1 and 2. Two additional MLR models for the ambient ($R^2=0.70$, $P=0.00$) and oven-dried sediments ($R^2=0.61$, $P=0.00$) were run in order to evaluate variability in decay rate constant among the different sediments and assays tested. Fixed independent variables sediment and assay type and interaction terms for time, site, and assay were included in the two MLR models to allow for change in concentration over time to vary as a function of both sediment type and assay. Decay rate constants were compared pair-wise by

applying post-hoc simple contrasts in Stata 12.0 (Stata Corp, College Station, Texas) on differences in slopes. Differences in decay rate constants between the different indicators and sediment-types are presented in Table 3: significant differences in decay at $p < 0.05$ are indicated by non-overlapping letters (e.g. decay rate constant assigned GH represents significantly slower than decay rate constant assigned ABC).

3.2.1 HF183

Over the course of the 21-day microcosm, the HF183 marker fell below the LLOQ (lower limit of quantification) in all ambient sediments by day 7. In the oven-dried (OD) sediments, the HF183 marker fell below the LLOQ by day 7 for sites S3-OD and S4-OD (Topanga upper watershed sites) and was quantifiable until day 21 in oven-dried sediments from sites C2-OD (Ballona Creek) and S2-OD (Topanga Lower Watershed). Although there was a trend for increased persistence of the HF183 marker in sediment from sites C1 and S2, decay rate constants were not significantly different across the six sites tested. Decay of the HF183 marker was slower in the oven-dried sediments for all four sites tested.

3.2.2 GB3

The GB3 marker was quantifiable until at least day 21 in all ambient sediments. There was a trend for slower decay of the GB3 marker in sediments with higher levels of fines, nutrients, and organic matter content (Sites C1, C2, and S2). In the oven-dried sediment microcosms, GB3 marker levels increased over time until day 3, indicating initial growth of cells followed by stabilization of the GB3 marker at elevated levels. Levels of the GB3 marker measured on day 21 versus day 0 were not significantly different ($p < 0.05$) in any of the four oven-dried sediments, indicating extended persistence of the marker in the reduced microbiota treatment.

3.2.3 ENT1A

The ENT1A marker remained stable over the course of the 21-day sampling period in all six ambient sediments tested. Excluding Site S3 (Topanga Upper Watershed), there were no significant differences between ENT1A marker levels measured on day 21 and day 0. In the oven-dried sediments, levels of the ENT1A marker initially increased in all four sediments and levels were higher on day 21 than on day 0 for sediments collected from Site S2-OD (lower Topanga watershed) ($p=0.06$) and site C2-OD (Medea Creek) ($p<0.05$), indicating cell growth.

3.2.4 cEC and cENT

In ambient sediments from Site C1 (Ballona FW Marsh) and S2 (Topanga Creek Lower Watershed), increased persistence of cEC and cENT was observed compared to all other sites and both cEC and cENT were detectable until day 21. In these sediments, cENT exhibited low R^2 values (Table 2), indicating persistence and a poor fit of the first-order decay model. cEC and cENT levels fell below the LOD by day 21 for sites S1, S3, and S4 and these sites exhibited significantly faster decay rates. In the oven-dried sediment microcosms, cEC levels measured on day 21 were not significantly different from levels measured on day 0 for all four sediments tested. Sites S2-OD and C2-OD had significantly higher concentrations of cENT at day 21 than at day 0, indicating cell growth ($p<0.05$). cENT levels declined after day 3 in Site S3-OD and S4-OD sediment, and levels fell below the LOD by day 21 in S3-OD microcosms (Figure 2).

3.3 Sediment characteristics and decay rates

Individual MLR models were run for each indicator; normalized concentration ($\ln C/C_0$) was regressed over time, and sediment characteristic (TN, P, % fines, % organic matter, reduced microbiota) were included as predictor variables. MLR results, coefficient (β) of the interaction term testing impact of each sediment characteristic on decay rate constant and R^2 of each MLR model, are presented in Table 4. Increased levels of nutrients (N and P), percent organic matter,

and percent fines were associated with increased persistence of culturable FIB and the GB3 marker (Table 4). Decay rate constants was also found to vary as a function of the microbiota treatment: culturable FIB and the GB3 and ENT1A markers exhibit decreased persistence in the presence of ambient microbiota in freshwater sediments.

3.4 Genetic Markers and FIB in the water column

Time series results of normalized FIB and MST marker concentrations in the water column are included in SI: Figure S4 and S5. Water column was always sampled first and carefully pipetted off, so as to not resuspend sediments. In ambient sediments, the HF183 marker was detected in the water column only on day one for all six sites. The ENT1A marker was quantified at levels above the LLOQ in all sites until day 12. The GB3 marker was quantified at levels above the LLOQ in all sites except Site S2 (Topanga Lower Watershed) until day 21. cEC was measured in the water column until day 5 in Site S4 microcosms, until day 7 in Site C1, C2, S1, and S2 microcosms, and at least until 12 in Site C3 microcosms. cENT fell below detectable levels by day 12 for all sites except Site S2 (Topanga lower watershed). In the oven-dried microcosms, cEC, cENT, and the GB3 and ENT1A markers were all quantified at detectable levels until at least day 12. The HF183 marker was not detected in the water column in any of the oven-dried microcosms beyond day 5.

3.5 Differential sediment decay within the Topanga Canyon Watershed- Simplified Two-Box Model Results

With a consistent input simulated, the sediment cENT concentrations led to a modeled prediction of cENT stabilization at at 90 MPN/g in the upper watershed reach, which was similar to average ambient levels measured at the two upper watershed sites during the field survey (73 MPN/g at 6500 m and 37 MPN/g at 4800 m), respectively. In the lower watershed, increased

persistence of cENT within the sediment led to cENT being predicted to stabilize at 530 MPN/g, which is approximately five times higher than cENT levels in the upper watershed (Figure 3). These levels were consistent with cENT levels measured during the field survey in the lower watershed (450 MPN/g- see Supplemental Information, Figure S6). Water column concentrations were predicted to stabilize at 240 MPN/100 mL in the simulated upper watershed stream stretch and at 250 MPN/100 mL in the simulated lower stream stretch, respectively.

The HF183 marker was predicted to stabilize at levels below the LLOQ in the sediment in both the lower and upper watershed stretches (70 copies/g and 95 copies/g, respectively). Water column concentrations were predicted to stabilize at 320 copies/100 mL in the simulated upper watershed stream stretch and at 335 copies/100 mL in the simulated lower stream stretch, respectively (Figure 3).

4. Discussion

4.1 Decay in ambient sediments

There were substantial differences in decay among the six freshwater sediments tested, and decay rate constants were related to sediment characteristics in most cases. Nutrient (N and P) levels and to a lesser extent percent fines and organic matter levels were associated with increased persistence of culturable FIB and the GB3 marker. To our knowledge, this is the first study evaluating the impact of freshwater sediment characteristics on the relative aging of genetic markers. These findings are consistent with other studies evaluating impact of freshwater sediment characteristics on persistence of culturable FIB survival. Lee et al. (2006) attributed increased concentrations of *E. coli* in beach sands with presence of natural organic matter. Garzio and Hadzick (2009) observed an increase in sediment *E. coli* concentrations in stream

sediments with increasing silt content. Results from Haller et al. (2009) showed long-term persistence of FIB in freshwater sediments, up to 50 days, and found that small particle size and higher levels of organic matter and nutrients enhanced survival of FIB.

In contrast to the other indicators tested, the ENT1A marker exhibited increased persistence in all ambient sediment types tested, and decay rate constants was not predicted by sediment characteristics. The genetic marker for enterococci, ENT1A, decayed more slowly than did culturable *Enterococcus* spp. This finding was in accord with other studies evaluating decay of the ENT1A marker in freshwater (Eichmiller et al. 2014; Kim and Wuertz et al. 2015) and marine beach sand (Yamahara et al. 2012). The ENT1A marker decayed at rates comparable to cEC and cENT at sites C1 and S2. These sites had comparably higher levels of nutrients and percent fines and represent sediments that may promote persistence of FIB. The long-term persistence of the ENT1A marker in sediments may make it a more appropriate marker in sediments with characteristics that serve to promote persistence of FIB and pathogens. The ENT1A marker may be less useful in source tracking applications, where an idea of recent inputs is necessary to identify potential sources of fecal contaminants.

In sandy sediments with low nutrients (sites S1, S3, and S4), both the GB3 and the HF183 markers exhibited decay rate constants similar to those measured for cENT and cEC. These trends are in accord with other studies looking at decay in sandy sediments. Eichmiller et al. (2014) showed that HF183 and FIB decay rate constants were comparable in freshwater sand with low total organic carbon content (0.05%) and a low percentage of fines. Kim and Wuertz (2015) observed *Bacteroidales* decay rate constants that were comparable to cENT and cEC decay rate constants in core sediments with low organic carbon and nitrogen content. In sediments with higher organic matter content, nutrients, and percent fines (Sites C1 and S2),

GB3 marker decay was most similar to decay of cEC and cENT. The GB3 marker may be useful as a complementary and rapid indicator of general fecal contamination in freshwater sediments. In contrast, in sediments from Sites C1 and S2, there was a decoupling of HF183 marker and FIB decay rates. The HF183 marker targets a subset of strains within the *Bacteroides* genus, an obligate anaerobic bacteria (Dick et al., 2005; Green et al., 2011), which could lead to the decreased persistence of the HF183 marker in sediments that have characteristics associated with enhanced survival of other indicators (Craig et al. 2004; Lee et al. 2006; Haller et al. 2009). The HF183 marker decayed significantly faster than cENT and cEC at sites C1 and S2 and was not detected beyond day seven in any of the six sediments tested. The HF183 marker may provide less conservative estimates than traditional FIB in freshwater sediments. In this study, microcosms were continuously oxygenated, which may have had implications on persistence of the HF183 marker. The HF183 marker may be more likely to accumulate and exhibit increased persistence under anaerobic conditions. Kim and Wuertz (2015) observed extended survival and persistence of *Bacteroides* cells and DNA in freshwater sands under anaerobic conditions, and Nayak et al. (2015) observed an increase over time of *Bacteroidales* cells in fresh and marine sediments inoculated with poultry fecal material.

4.2 Decay in oven-dried sediments

Interactions with indigenous microbiota have been shown to be an important factor controlling culturable FIB in water and sediment (Korajkic et al. 2013; Wanjugi et al. 2013) and to a lesser extent in controlling genetic marker concentrations in the water column (Dick et al. 2010; Okabe et al. 2007). In the oven-dried sediment microcosms, long-term persistence and limited growth of culturable FIB and the GB3 and ENT1A markers was observed, signaling the importance of the natural community on controlling fecal inputs. GB3 and cEC levels increased

over the 21-day time series in all four oven-dried sediments, and initial growth and increased persistence of cENT and the ENT1A marker was observed. Our results support previous findings where growth of FIB in sediment has been observed in inoculated sediment without active predators present or with predators inhibited (Byappanahalli and Fujioka, 1998; Davies et al., 1995; Haller et al. 2009). Other studies examining *Bacteroides* marker aging have observed positive decay rates (i.e. growth or an increase in cell or cell-equivalent counts) under certain environmental conditions including in freshwater sand at 28% moisture for the general *Bacteroides* AllBac marker (Eichmiller et al. 2014) and in anaerobic freshwater sediment microcosms within the first two days of inoculation (Kim and Wuertz, 2015). HF183 marker decay was slowed to a lesser extent by the reduced microbiota treatment, and the HF183 marker showed no signs of potential for re-growth in any of the four sediments tested.

4.3 Concentration of FIB and genetic markers in overlying water column

Artificial freshwater was used in the microcosm experiments; therefore, FIB and markers found in the water column were derived from the spiked sediments. Water column indicator concentrations paralleled sediment decay patterns. In the ambient sediment microcosms, concentrations of all indicators consistently declined over time with the HF183 marker only being detected on day one in the water column for all six sediments tested. In the oven-dried sediments, increased detection of all indicators in the water column was observed. All indicators were measurable until day 21 with the exception of the HF183 marker, which was only detectable in the water column until a maximum of day 5.

4.4 Box Model Results: Role of sediments within the Topanga Canyon Watershed

Differences in decay rate constants of indicators were observed even within one watershed. Differences in decay due to changing sediment characteristics led to scenarios where

cENT was predicted to accumulate in sediment in the lower Topanga Watershed reach at high levels (530 MPN g⁻¹) and stabilize at lower levels (90 MPN g⁻¹) in the upper Topanga watershed reach. These results paralleled field data. Previous studies have observed similar substantial differences in *E. coli* streambed concentrations within short distances (Badgley et al., 2011; Bai and Lung, 2005).

In both the upper and lower watershed reaches, deposition of particle-associated cells led to a reduction in levels of cENT and the HF183 marker in the water column. Deposition of sediment-associated bacteria is likely one mechanism contributing to the reduction of fecal indicators seen in the upper watershed reach. Observed reductions may also be a result of dilution from natural seeps and decay in the water column (not addressed in this study). Decreased vegetative cover was reported in the upper watershed (Dagit et al., 2014), increasing the potential for UV inactivation of FIB in the water column, which has been shown to be an important factor influencing the survival of enteric bacteria (Boehm et al., 2009; Maraccini et al., 2015; Sinton et al., 2002).

This study illustrates that freshwater sediment characteristics have the potential to impact persistence of indicators, having important ramifications for water quality. Particle-associated bacteria can be deposited and accumulate in sediments, leading to creation of reservoirs of fecal bacteria and pathogens (Boehm et al., 2014; Byappanahalli et al., 2012; Davies et al., 1995; Garzio-Hadzick et al., 2010; Hipsey et al., 2006). Natural turbulence can lead to resuspension events that be an important source of microbes to the water column (Pandey et al., 2012; Steets and Holden, 2003; Walters et al., 2014) and can lead to an increased risk of human infection (Haller et al., 2009; Halliday and Gast, 2011; Heaney et al. 2012). Box-model results suggest that cENT will accumulate in the lower Topanga watershed sediments, while the sediments tested in

this study are not likely to act as a reservoir of the HF183 human marker: the faster decay rate of the HF183 marker seen in all six sediments tested resulted in predicted HF183 marker levels stabilizing at levels below the LLOQ in sediment in both the Topanga upper and lower watershed reaches.

More work is needed to correlate aging of indicators to that of pathogenic bacteria in sediments from different sites. The simulation run in this study is limited in that several parameters were assumed, namely particle settling velocity and fraction of particle-attached cells, which has been shown to vary temporally and by organism (Schillinger and Gannon, 1985; Krometis et al. 2007; Characklis et al. 2005).

5. Conclusions

- Sediments can play an important role in water quality that can vary even within one watershed and with regard to different indicators, suggesting the importance of considering what suite of indicators is most appropriate on an individual site basis and including sediments and sediment-bacterial interactions in watershed models.
- FIB and genetic marker decay rate constants were similar in sediments with low levels of organic matter and fines. In ambient sediments with high levels of organic matter and fines, there was a decoupling of HF183 marker and culturable FIB decay rates with increased persistence of cENT and cEC.
- The HF183 marker exhibited statistically similar decay rate constants in all six freshwater sediments tested in this study, and is not likely to accumulate. The HF183 marker fell below the LLOQ within seven days in all six sediments, confirming reliability of the HF183 for recent inputs to freshwater sediment.

- The ENT1A marker showed increased persistence in all sediments, relative to the other indicators, and decay was not dependent on sediment type. Detection of the ENT1A marker may not represent recent inputs in the freshwater sediments tested in this study.
- Biotic interactions are an important factor in controlling decay of culturable FIB and genetic markers (GB3 and ENT1A) in freshwater sediments.

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685 **Tables**

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687 **Table 1.** Characteristics of the six freshwater sediments used in microcosm study.

Site	Chla	Particle Size			CHN Analysis				Nutrients					Moisture	Organic
	mg/g	%			Weight %				umol/g					Content	Matter
		Clay	Sand	Silt	C		TN		PO ₄ ³⁻	TN	NH ₃	NO ₃ ⁻	NO ₂ ⁻	%	%
					Amb	+Sew	Amb	+Sew							
C1	3.76	27.49	50.05	22.46	1.098	0.758	0.124	0.112	1.10	1.35	0.12	1.10	0.12	44.68	4.60
C2	8.31	9.31	89.50	1.19	0.165	0.310	0.016	0.043	0.17	0.20	0.02	0.17	0.02	16.35	1.20
S1	0.62	2.85	97.15	0.00	0.020	0.034	0.007	0.008	0.02	0.03	0.01	0.02	0.01	14.28	0.39
S2	1.22	7.50	90.20	2.30	0.505	0.493	0.047	0.052	0.51	0.60	0.05	0.51	0.05	16.28	1.40
S3	0.00	5.51	93.19	1.30	0.125	0.120	0.015	0.017	0.13	0.16	0.02	0.13	0.02	14.30	0.80
S4	1.19	5.50	93.20	1.30	0.079	0.284	0.013	0.031	0.08	0.11	0.01	0.08	0.01	13.85	0.66

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689 *Chla= chlorophyll *a*, C= carbon, , TN= total nitrogen, Amb= ambient sediments (not inoculated), +Sew= Sewage inoculated sediments

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Table 2. Decay rate constants of FIB and specific markers in sediment microcosms inoculated with primary WWTP influent given. Initial concentrations (C_0) of FIB and specific markers measured within the microcosms are given in either copies (specific markers) or cells (FIB). R^2 are given for the relationship between time and natural log normalized concentration.

Treatment	Ambient Sediment				Oven-Dried Sediment			
	Co copies or cells	k	SE	R^2	Co copies or cells	k	SE	R^2
Assay/Site	(g^{-1})	(day^{-1})			(g^{-1})	(day^{-1})		
cENT								
C1	7.5E+02	-0.03	0.08	0.01				
C2	6.6E+02	-0.35	0.14	0.40	5.9E+02	0.32	0.08	0.51
S1	5.1E+02	-0.40	0.06	0.79				
S2	1.6E+02	-0.07	0.08	0.06	4.8E+02	0.25	0.12	0.24
S3	3.1E+02	-0.39	0.05	0.81	2.4E+02	-0.18	0.07	0.28
S4	5.4E+02	-0.37	0.08	0.65	2.3E+02	0.10	0.10	0.06
cEC								
C1	7.9E+03	-0.22	0.04	0.67				
C2	3.9E+04	-0.52	0.06	0.89	5.8E+04	0.25	0.13	0.20
S1	2.4E+04	-0.38	0.11	0.43				
S2	6.9E+03	-0.16	0.09	0.21	1.5E+04	0.52	0.14	0.51
S3	7.2E+03	-0.81	0.21	0.56	1.8E+04	-0.09	0.14	0.10
S4	9.7E+03	-0.80	0.14	0.84	2.2E+04	0.28	0.11	0.30
HF183								
C1	1.3E+04	-0.50	0.07	0.83				
C2	8.0E+03	-0.96	0.10	0.93	6.6E+03	-0.20	0.03	0.70
S1	7.1E+03	-0.86	0.16	0.81				
S2	7.8E+03	-0.52	0.08	0.82	6.6E+03	-0.24	0.08	0.38
S3	4.7E+03	-0.73	0.17	0.73	5.7E+03	-0.49	0.08	0.79
S4	5.1E+03	-0.62	0.22	0.54	6.2E+03	-0.49	0.08	0.80
ENT1A								
C1	1.0E+05	-0.18	0.03	0.72				
C2	1.3E+05	-0.05	0.02	0.26	1.1E+04	0.85	0.13	0.81
S1	3.8E+04	-0.28	0.07	0.51				
S2	3.4E+04	-0.14	0.05	0.27	6.3E+03	0.26	0.08	0.37
S3	3.8E+04	-0.07	0.03	0.22	4.3E+03	-0.04	0.04	0.1
S4	3.7E+04	-0.07	0.03	0.23	7.5E+03	0.12	0.05	0.22
GB3								
C1	7.5E+06	-0.22	0.07	0.38				
C2	2.5E+06	-0.18	0.03	0.68	6.1E+05	0.34	0.11	0.36
S1	8.1E+05	-0.44	0.10	0.56				
S2	2.6E+06	-0.10	0.05	0.22	4.8E+05	0.5	0.15	0.44
S3	2.1E+06	-0.34	0.07	0.61	1.3E+06	0	0.08	0
S4	6.8E+05	-0.33	0.04	0.84	7.5E+05	0.14	0.09	0.14

Table 3. MLR pairwise comparison of decay rate constants of FIB and markers for different indicators in the six ambient sediments and the four oven-dried sediments tested were conducted. Significant differences in decay rate constant at $P < 0.05$ are indicated by non-overlapping letters. Red letters indicate groups with lower decay rate constant (any group with letter H) and bolded letters indicate groups with higher decay rate constant (any group with letter A).

Treatment	Site	Assay				
		cENT	cEC	HF183	ENT1A	GB3
Ambient	C1	H	CDEFG	ABC	CDEFGH	CDEFG
	C2	BCDEF	ABC	A	H	CDEFGH
	S1	ABCD	BCD	AB	CDEF	ABC
	S2	GH	DEFGH	ABC	EFGH	FGH
	S3	ABCD	A	ABC	FGH	BCDEF
	S4	ABCDE	A	ABCDEFG	EFGH	BCDEF
Oven-Dried	S2	DEF	GH	AB	DEF	FGH
	S3	A	ABC	A	ABC	ABCD
	S4	BCDE	EFG	A	BCDE	CDE
	C2	EFG	DEF	A	H	EFG

Table 4. Slope of the interaction term testing illustrating if decay rate constant of each assay is affected by % fines, total nitrogen (TN), phosphate, % organic matter, or microbiota treatment included below. R² values of each MLR provided in parentheses.

Sediment Characteristic	Assay				
	cEC	cENT	HF183	GB3	ENT1A
% Fines	0.003 (0.72)	0.004 (0.55)**	0.002 (0.43)	0.002 (0.61)*	0.000 (0.39)
PO₄³⁻	0.15 (0.76)**	0.18 (0.66)**	0.07 (0.42)	0.14 (0.67)**	0.00 (0.39)
TN	0.12 (0.76)**	0.15 (0.66)**	0.06 (0.43)	0.11 (0.66)**	0.00 (0.39)
% OM	0.03 (0.73)	0.04 (0.59)**	0.02 (0.43)	0.03 (0.64)*	0.00 (0.39)
Microbiota +	-0.43 (0.74)**	-0.12 (0.41)**	-0.01 (0.47)	-0.24 (0.66)**	-0.12 (0.49)**

* Significant at P<0.1 Level

** Significant at P<0.05 Level

Figures

Figure 1. Time series of normalized FIB and MST marker concentrations ($\ln C/Co$) in the six freshwater ambient sediments tested.

Figure 2. Time series of normalized FIB and MST marker concentrations ($\ln C/C_0$) in the four freshwater oven-dried sediments tested (reduced microbiota treatment).

Figure 3. Upper and lower watershed reach simulation results estimating cENT and HF183 marker levels in upper and lower watershed reaches with a constant input of 300 MPN/100 mL enterococci and 400 copies/100 mL HF183 marker.