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Decay of sewage-sourced microbial source tracking markers and fecal indicator bacteria in marine waters

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ABSTRACT

The decay of sewage-sourced enterococci, *Escherichia coli*, three human-associated microbial source tracking (MST) markers, *Salmonella*, *Campylobacter*, and norovirus GII was measured in situ in coastal, marine waters. Experiments examined the effects of sunlight intensity and season on decay. Seawater was seeded with untreated sewage, placed into permeable dialysis bags, and deployed in the coastal ocean near the water surface, and at 18 cm, and 99 cm depths, to vary solar intensity, during winter and summer seasons. Microbial decay was modeled using a log-linear or shoulder log-linear decay model. Pathogen levels were too low in sewage to obtain kinetic parameters. Human-associated MST markers all decayed with approximately the same rate constant (k ~ 1.5 d^{-1}) in all experimental treatments, suggesting markers could be detectable up to ~6 days after a raw sewage spill. *E. coli* and enterococci (culturable and molecular marker) k significantly varied with season and depth; enterococci decayed faster at shallow depths and during the summer, while *E. coli* decayed faster at shallow depths and during the winter. Rate constants for MST markers and culturable FIB diverged except at the deepest depth in the water column potentially complicating the use of MST marker concentrations to allocate sources of FIB contamination.

1. Introduction

Culturable fecal indicator bacteria (FIB) *Escherichia coli* and enterococci have been used for decades to evaluate the microbial quality of recreational water. FIB are commensal bacteria present in high numbers within intestinal tracts of animals. However, because FIB are shed by many types of animals (Field and Samadpour, 2007) and can even be found in environmental reservoirs like sand and wrack (Byappanahalli et al., 2012), FIB presence in water does not provide information as to the source of the fecal contamination (Field and Samadpour, 2007). Identifying the source of fecal contamination is important because the health risk associated with microbial pollution in recreational waters depends on the source of pollution (Soller et al., 2010). Moreover, identifying the source of fecal contamination can allow for polluted waterbodies to be remediated.

Microbial source tracking (MST) is the process of identifying the FIB sources. MST can incorporate fecal source-associated MST markers (hereafter "MST markers") to differentiate animal hosts contributing FIB to waters. MST markers are genetic sequences unique to bacteria or viruses from specific animal hosts, many of which are genetic sequences from the bacterial order *Bacteroidales* (Boehm et al., 2013). By determining major fecal pollution sources, water resource managers and public health officials can evaluate the health risks posed to recreational water users and target major pollution sources for remediation (Harwood et al., 2014).

MST markers are being applied world-wide (e.g., Heaney et al., 2015; Odagiri et al., 2015) to glean insight into sources of fecal contamination. However, there is no clear guidance for interpreting MST marker concentrations, and this has led to somewhat subjective interpretation of measured concentrations. Recent work has aimed to inform the interpretation of MST marker concentrations. For example, Wang et al. (2013) proposed that MST marker concentrations can be used to allocate sources of *E. coli* and enterococci using a simple "ratio model" if the MST marker and FIB decay characteristics in ambient water are similar. Boehm et al. (2015) used quantitative microbial risk assessment to identify a risk-based threshold for human MST markers to guide the interpretation of measured concentrations; however, the authors suggest that

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further information on the relative decay of human MST markers and pathogens is needed to refine the threshold estimate. Both of these studies highlight the need for better understanding of the relative decay of MST markers, pathogens, and FIB in environmental waters in order to better guide the application of MST markers for fecal source identification.

In the present study, we measured the concurrent decay of three human-associated MST markers, FIB, and several enteric pathogens in situ in marine waters subject to natural sunlight cycles. Raw sewage was added to marine water and placed in dialysis bags in the coastal ocean. The dialysis bags were then floated at various depths in the water column to receive different doses of solar radiation. The dialysis bags allow water and solutes to exchange between the experiment and the coastal ocean, but retain microbial targets and thus represent in open system experimental design (Korajkic et al., 2014). Whereas five previous studies (Ahmed et al., 2014; Bae and Wuertz, 2009; Green et al., 2011; Jeanneau et al., 2012; Walters et al., 2009) have investigated the decay of human-associated MST markers in seawater, four (Ahmed et al., 2014; Green et al., 2011; Jeanneau et al., 2012; Walters et al., 2009) of them used a closed, batch microcosm design, which physically separates fecal-seeded seawater from the ocean and can lead to experimental artifacts. A recent meta-analysis of MST marker and FIB decay studies suggests closed, batch studies may be of limited value to understanding MST marker decay in the environment (Brooks and Field, 2016). None of the previous studies quantitatively investigated the effect of solar intensity on MST marker decay nor did they investigate the effect of season and the accompanying changes in water temperature. Here we carefully designed our experiments to investigate whether a quantitative relationship exists between sunlight intensity and target decay, as has been previously well documented for FIB. We also investigated the effect of season on decay by conducting our experiments during both winter and summer. The open experimental design that allowed investigation into quantitative sunlight effects and seasonal effects on MST marker decay in seawater fills important knowledge gaps as summarized by Brooks and Field (2016).

We aim to answer three applied research questions through our study design: (1) How long do human-associated MST markers persist in marine waters? If their signal is extremely long-lived, it may limit their usefulness as they may indicate very old contamination. (2) Are the decay rates of culturable enterococci (cENT), qPCR-measured total enterococci (tENT), and culturable *E. coli* (EC) the same as those of the human-associated MST markers? If so, then it suggests that FIB source allocation using these MST markers and a simple ratio model (Wang et al., 2013) may be feasible. Our focus is on these three FIB as they are currently regulated through USEPA Ambient Water Quality Criteria (USEPA, 2012b). (3) Are the decay rates of human-associated MST markers the same as the decay rates of bacterial and viral enteric pathogens? If so, then it will simplify the use of a risk-based framework for interpreting concentrations of MST markers (Boehm et al., 2015).

2. Methods

2.1. Study design

Field microcosm experiments were conducted at Pillar Point Harbor (37.502467° N, 122.4838829° W) in Half Moon Bay, CA, USA during September 2014 (summer experiments) and February 2015 (winter experiments). To simulate sewage pollution in marine waters, experiments were conducted in 1 L volume dialysis bags (120 mm flat width, ~30 cm long) with a 6–8 kDa molecular weight cutoff (~1 nm pore size, Spectra/Por Standard RC Tubing, Spectrum Laboratories Inc., Rancho Dominguez, CA) containing 95% by volume ocean water from the deployment site mixed with 5% by volume raw sewage influent from the a local wastewater treatment plant (see supporting information (SI)). 5% sewage was chosen for the experimental set-up using our best professional judgment to provide sufficient initial starting concentrations of MST markers and FIB, while also representing a realistic vol/vol fraction of sewage expected after a raw sewage spill in the coastal ocean. The bag thickness was ~6 cm when filled. The pore size of the dialysis bags allowed passage of nutrients and water, but prevented passage particles and molecules larger than 6–8 kDa. The spectral absorbance of the dialysis bag material was measured using a spectrophotometer and is reported elsewhere (Maraccini et al., 2016).

Dialysis bags were deployed by securing them to floating rigs constructed of polyvinylchloride pipes attached to air-filled plastic cylinders that acted as buoys (Fig. 1). The bags were oriented in the horizontal direction to allow maximal sunlight exposure. We evaluated the impact of sunlight irradiation by placing separate rigs at different depths: 5 cm and 99 cm below surface during the summer deployment and 5 cm, 18 cm, and 99 cm depths during the winter deployment (measurements represent distance from water surface to center of the bag).

Summer and winter experiments were conducted for 10 and 7 days, respectively. Each day of the experiments, two dialysis bags representing biological replicates were destructively sampled from each experimental treatment at approximately 8:00 h. Three bags were collected from the deepest rig (99 cm) on day 5 during the summer deployment and from all three depths on day 6 during the winter. Control bags containing only ocean water were placed at each depth and were collected on days 5 and 10 for the summer deployment and day 7 for the winter. Additional control bags containing only molecular grade water were placed at 5 and 99 cm for both deployments and collected on the same days as the other control bags to evaluate the ability of the dialysis bags to prevent genetic materials and microorganisms from entering the bag while deployed in the ocean or during sample collection in the field and processing in the lab.

The salinity, temperature, and dissolved oxygen of the ambient ocean water were measured upon sample collection using a using a handheld probes and a deployed thermistor (see SI). A 1 L sample of the ambient ocean water was collected daily in a sterile 1 L bottle at the time of sampling. Water from the dialysis bags was aseptically poured into a sterile bottle, stored in the dark at 4 °C, and processed within 4 h of collection at Stanford University. Water from the dialysis bags and ambient ocean water were processed each day for the physical and molecular measurements listed below. The raw sewage used to create the 5% sewage-ocean water mixture was also processed.

2.2. Physical measurements

Turbidity and chlorophyll *a* concentrations of the ambient ocean water were measured within 12 h of sampling. Nonpurgeable organic carbon (NPOC) and nutrient concentrations of both the bag water and ambient ocean water were also measured. See SM for details.

The daily average UVB transmitted to the middle of the deployed dialysis bags within the water column is reported by Maraccini et al. (2016) and was calculated using information on incident UVB at the water surface, dialysis bag depth and absorbance, and water absorbance.

2.3. Culture-based FIB

Culturable enterococci (cENT) and *E. coli* (EC) were enumerated using Enterolert® and Colilert-18®, respectively (IDEXX, Westbrook,

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