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Spatial and hydrologic variation of *Bacteroidales*, adenovirus and enterovirus in a semi-arid, wastewater effluent-impacted watershed

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ABSTRACT

Bacteroidales and viruses were contemporaneously measured during dry and wet weather conditions at a watershed-scale in a semi-arid watershed impacted by a mixture of agricultural runoff, municipal wastewater effluent and municipal runoff. The results highlight the presence of municipal wastewater effluent as a confounding factor for microbial source tracking (MST) studies, and thus data were segregated into groups based on whether they were impacted by wastewater effluent. In semi-arid environments such as the Calleguas Creek watershed, located in southern California, the relative contribution of municipal wastewater effluent is dependent on hydrology as storm events lead to conditions where agricultural and municipal stormwater dominate receiving waters (rather than municipal wastewater, which is the case during dry weather). As such, the approach to data segregation was dependent on hydrology/storm conditions. Storm events led to significant increases in ruminant- and dog-associated *Bacteroidales* concentrations, indicating that overland transport connects strong non-human fecal sources with surface waters. Because the dataset had a large number of non-detect samples, data handling included the Kaplan–Meir estimator and data were presented graphically in a manner that reflects the potential effect of detection limits. In surface water samples with virus detections, *Escherichia coli* concentrations were often below (in compliance with) the recreational water quality criteria. In fact, sites downstream of direct inputs of municipal wastewater effluent exhibited the lowest concentrations of *E. coli*, but the highest concentrations of human-associated *Bacteroidales* and highest detection rates of human viruses. The toolkit,

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comprised of the four *Bacteroidales* assays and human virus assays used, can be successfully applied to inform watershed managers seeking to comply with recreational water quality criteria. However, care should be taken when analyzing data to account for the effect of non-detect samples, sources with differing microbial viability, and diverging hydrologic conditions.

1. Introduction

Over 12,000 waterbodies in the United States are categorized as impaired by fecal indicator bacteria (FIB) discharges, and have been subject to total maximum daily loads (TMDLs), which describe the water quality improvement strategy to address FIB sources in the watershed (USEPA, 2009). Compliance with recreational water quality (REC) criteria in developed watersheds, both in the U.S. and elsewhere, represents a significant challenge to responsible agencies, as a myriad of non-point bacteria sources contribute to impairment. Some watersheds that are only subject to natural bacteria sources (e.g., birds) have been found to exceed REC criteria (Tiefenthaler et al., 2008), and some waterbodies have been subject to extensive remediation efforts yet exceedances of criteria persist (POLA, 2006). During storm events in urbanized watersheds, which may represent >99% of the annual bacteria discharge (Reeves et al., 2004), loading rates can be extraordinarily high – several times greater than the equivalent daily fecal loading from the entire human population within the watershed (Surbeck et al., 2006). The United States Environmental Protection Agency (USEPA) recently conducted extensive research including epidemiological studies and adopted revised federal REC criteria (Wade et al., 2006; USEPA, 2012). The revised criteria underscore the importance of the type of fecal source when evaluating potential REC health risks. Health risks associated with recreating in waters impacted by non-human sources can be orders of magnitude less than those with human sources (Colford et al., 2007; Soller et al., 2010).

Given the immense challenges involved with complying with REC criteria, and the importance of fecal source type to the level of risks, watershed managers often desire data regarding the fecal sources that are driving levels of FIB. Collectively referred to as microbial source tracking (MST), a plethora of methods have been developed to characterize the contribution of fecal discharges from different host populations to surface waters and are applied throughout the world (Field and Samadpour, 2007; Santo Domingo et al., 2007; Boehm et al., 2013). The most widely-applied and tested of these approaches targets host-associated 16S rRNA genes of the *Bacteroidales*, and assays based on quantitative PCR (qPCR) can be used to estimate genomic concentrations (Kildare et al., 2007; Shanks et al., 2008, 2009). Multiple comparison studies have tested and confirmed that, while not 100% sensitive or specific, many *Bacteroidales* markers are sufficiently sensitive and specific for detecting host-associated contamination (Boehm et al., 2013; Layton et al., 2013; Raith et al., 2013; Schriewer et al., 2013), are repeatable/reproducible (Ebentier et al., 2013), and the stable populations required for marker-based MST are present around the globe (Reischer et al., 2013).

Statistical and modeling approaches have been evaluated for using ratios of host-associated to universal *Bacteroidales* markers to quantify the contribution of human versus non-human sources on levels of FIB in watersheds (Stoeckel and Harwood, 2007; Wang et al., 2010, 2013; Russell et al., 2013). Applications of these ratios, which should account for differences in fate and transport characteristics along with the fact that MST assays are imperfect, are emerging as a tool for quantitative MST. Ratios and concentrations are interpreted differently; all host-associated concentrations represent the potential impact of that host population on downstream waters, while host-associated:universal ratios highlight the effect of that host population on the total *Bacteroidales* loading at the monitored site. Suppose that a runoff site has very high levels of the human marker BacHum (when compared to other sites) but a very low ratio of BacHum:BacUni. In this case, the site might pose an elevated risk to recreational users who come into contact with a waterbody impacted by human fecal sources, but on the other hand an agency that is responsible for remediating that site should also target potential non-human sources.

To support REC risk assessment, MST assays can be coupled with pathogen assays, particularly those for human viruses (McBride et al., 2013; Harwood et al., 2014). Virus assays with qPCR have been shown to be highly specific for mixed human fecal sources (Harwood et al., 2013), though they are often absent in individual fecal samples (Noble et al., 2003). Enterovirus, a single-stranded RNA virus, has been readily detected with qPCR during several studies of the coastal ocean and coastal watersheds in the western U.S. (Fuhrman et al., 2005; Noble et al., 2006; Viau et al., 2011). Adenovirus, a double-stranded DNA virus, is often detected in these same environments (Choi and Jiang, 2005; Sassoubre et al., 2012), and has been reported to have prolonged survival time and increased resistance to UV treatments (Nwachuku et al., 2005). Prior to this study, no known studies have contemporaneously measured *Bacteroidales* and viruses over the long-term at watershed-scale in waterbodies impacted by a mixture of agricultural runoff, municipal wastewater and municipal stormwater.

The objectives of this study were to (i) evaluate the abundance of four validated fecal *Bacteroidales* genetic markers (universal [BacUni], human- [BacHum], dog- [BacCan], and ruminant-associated [BacCow]) in treated and untreated municipal wastewater, (ii) compare quantitative data on host-associated fecal source identifiers based on *Bacteroidales* and human enteroviruses and adenoviruses with FIB measurements in surface waters, and (iii) utilize the spatial and