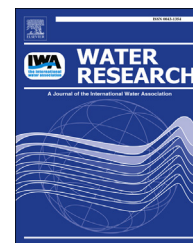


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# Long-term monitoring of molecular markers can distinguish different seasonal patterns of fecal indicating bacteria sources

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## ABSTRACT

Elevated levels of fecal indicator bacteria (FIB) have been observed at Topanga Beach, CA, USA. To identify the FIB sources, a microbial source tracking study using a dog-, a gull- and two human-associated molecular markers was conducted at 10 sites over 21 months. Historical data suggest that episodic discharge from the lagoon at the mouth of Topanga Creek is the main source of bacteria to the beach. A decline in creek FIB/markers downstream from upper watershed development and a sharp increase in FIB/markers at the lagoon sites suggest sources are local to the lagoon. At the lagoon and beach, human markers are detected sporadically, dog marker peaks in abundance mid-winter, and gull marker is chronically elevated. Varied seasonal patterns of FIB and source markers were identified showing the importance of applying a suite of markers over long-term spatial and temporal sampling to identify a complex combination of sources of contamination.

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## 1. Introduction

Fecal indicator bacteria (FIB) levels in coastal water and sand can correlate with the risk of visitors contracting an illness at beaches (Cabelli et al., 1982; Haile et al., 1999; Heaney et al., 2012; Kay et al., 1994; Wade et al., 2003) and are frequently used to monitor water quality in coastal waters. Often, sources of FIB at a beach are of unknown origin, making it difficult to take corrective action. FIB can originate from many possible sources such as urban runoff (Walters et al., 2011), agricultural runoff (Tiefenthaler et al., 2011), contaminated groundwater (Russell et al., 2013), beach sand and/or wrack (Halliday and Gast, 2011; Imamura et al., 2011), leaking sewage or septage systems (Sercu et al., 2009), and coastal wildlife (Layton et al., 2010). Library independent molecular methods (Boehm et al., 2013; Harwood et al., 2014) can help identify specific sources of fecal pollution through the use of host-associated sequence targeting that allows for identification of the original host of fecal pollution (for CA examples see Ervin et al., 2014; Russell et al., 2013). Some host-specific methods relevant to coastal watersheds include human-associated (Haugland et al., 2010), gull-associated (Sinigalliano et al., 2013), and dog-associated qPCR assays (Shibata et al., 2010).

Microbial source tracking (MST) studies (Hagedorn et al., 2011) are used to identify fecal sources and are often implemented using a tiered approach (Boehm et al., 2003; Griffith et al., 2013; Noble et al., 2006) where locations impaired for FIB are determined and then further analyzed for host-associated markers to distinguish sources contributing to elevated concentrations of FIB. However, some studies have found scenarios where FIB and markers do not correlate (Drozd et al., 2013; Flood et al., 2011; Mika et al., 2014) suggesting that overreliance on tiered sampling could miss important contamination sources. Further studies investigating this lack of correlation and long-term marker trends are warranted.

Topanga Beach is located between Santa Monica and Malibu CA, USA, at the terminus of the Topanga Creek watershed (Fig. 1). This watershed is the third largest watershed in the Santa Monica Mountains and the majority of the area is undeveloped public open space (Dagit and Webb, 2002; GeoPentech, 2006). The Topanga Creek watershed is home to reproducing populations of sensitive and endangered aquatic species including steelhead trout (CalTrout, 2006; Dagit and Krug, 2011; Dagit and Reagan, 2006; Dagit et al., 2007, 2005, 2004a, 2003; Tobias, 2006), western pond turtles (Dagit, 2004, 2003; Dagit et al., 2006), California newts, California tree frogs, western toads, Pacific tree frogs, and two-striped garter snakes. Within the watershed and adjacent to Topanga Beach, an estimated 4000 households exclusively use septic systems for waste treatment. Topanga Beach was ranked as one of the most polluted beaches in the state of California based on FIB levels from 2005 to 2006 (Barrett and Grimmer, 2006) and also from 2010 to 2012 (Greisbach et al., 2012, 2011). These rankings come despite numerous management efforts in the lower watershed to improve water quality including upgrades of public restroom facilities and removal of residences. The complexity of the development pattern in the Topanga watershed and the range of possible fecal inputs

have made it difficult to assess the causes of elevated FIB at Topanga Beach.

This study investigated sources of FIB to the Topanga watershed and the applicability of using MST technology over longer time scales. We tested the following hypotheses: 1) lagoon discharge negatively impacts coastal water quality, 2) upper watershed sources of FIB (human and non-human inputs such as dog) from developed areas are conveyed to the beach via the creek, 3) lower watershed and/or lagoon sources of FIB are correlated with elevated levels at Topanga Beach, and 4) coastal sources of FIB are conveyed to Topanga Beach via ocean currents. To address Hypothesis 1, six years of historical FIB levels were analyzed for the impact of lagoon discharge on coastal FIB levels. Hypotheses 2 to 4 were addressed by measuring FIB levels and culture-independent molecular markers associated with human, dog, and gull feces over a 21 month long MST study across ten sites strategically spaced throughout the watershed.

## 2. Materials and methods

### 2.1. Field site

#### 2.1.1. Topanga Creek watershed

Topanga Beach receives over 750,000 annual visitors (Given et al., 2006). Due to the Mediterranean climate, this region experiences a dry season (April–October) referred to in this study as the “recreation season” and a wet season (November–March) referred to in this study as the “winter season.” Typical rainfall averages 51 cm/year (Dagit and Webb, 2002) but was highly varied during this study (80 cm in 2011, 25 cm in 2012, Los Angeles County Department of Public Works Rain Gauge #318). The Topanga Creek watershed (approximately 47 km<sup>2</sup>) is 70% undeveloped with the majority of the development in the upper watershed, minimal development in the middle watershed, and business development in the lower watershed (Fig. 1). The watershed includes a creek and dynamic lagoon system that breaches and berms throughout the year, contributing variable flow to coastal waters. The lagoon system has been reduced 93% by coastal development from a historical size of approximately 0.12 km<sup>2</sup> (Dagit and Webb, 2002).

FIB sources to the beach may be conveyed from the upper watershed by the creek or may originate in the lower watershed. Potential sources of fecal contamination in the upper watershed include residential septic systems, transient populations residing in the watershed, pets, horse farms, and wildlife. The lower watershed near the beach is a complex mixture of development and hydrologic features including a lagoon system impacted by a major highway crossing over portions of it, a former residential neighborhood, a historic non-operational motel, multiple business establishments using septic systems, a state-of-the-art beach public restroom septic facility, and frequent habitation by transient persons. It is also possible that FIB are transported by a predominately down-coast marine current from the city of Malibu, which has a high density of coastal residential septic systems.

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