Water Research 136 (2018) 137–149

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Quantification of pathogens and markers of fecal contamination during storm events along popular surfing beaches in San Diego, California

Joshua A. Steele [a,*], A. Denene Blackwood [b], John F. Griffith [a], Rachel T. Noble [b], Kenneth C. Schiff [a]

[a] Southern California Coastal Water Research Project, 3535 Harbor Blvd. Ste 110, Costa Mesa, CA 92626, USA
[b] UNC Institute of Marine Science, 3431 Arendell Street, Morehead City, NC 28557, USA

ARTICLE INFO

Article history:
Received 27 January 2017
Received in revised form
19 January 2018
Accepted 24 January 2018

Keywords:
Human norovirus
Human adenovirus
Campylobacter
Microbial source tracking (MST) marker
Digital droplet PCR
Recreational water quality

ABSTRACT

Along southern California beaches, the concentrations of fecal indicator bacteria (FIB) used to quantify the potential presence of fecal contamination in coastal recreational waters have been previously documented to be higher during wet weather conditions (typically winter or spring) than those observed during summer dry weather conditions. FIB are used for management of recreational waters because measurement of the bacterial and viral pathogens that are the potential causes of illness in beachgoers exposed to stormwater can be expensive, time-consuming, and technically difficult. Here, we use droplet digital Polymerase Chain Reaction (digital PCR) and digital reverse transcriptase PCR (digital RT-PCR) assays for direct quantification of pathogenic viruses, pathogenic bacteria, and source-specific markers of fecal contamination in the stormwater discharges. We applied these assays across multiple storm events from two different watersheds that discharge to popular surfing beaches in San Diego, CA. Stormwater discharges had higher FIB concentrations as compared to proximal beaches, often by ten-fold or more during wet weather. Multiple lines of evidence indicated that the stormwater discharges contained human fecal contamination, despite the presence of separate storm sewer and sanitary sewer systems in both watersheds. Human fecal source markers (up to 100% of samples, 20-12440 HF183 copies per 100 ml) and human norovirus (up to 96% of samples, 25-495 NoV copies per 100 ml) were routinely detected in stormwater discharge samples. Potential bacterial pathogens were also detected and quantified: *Campylobacter spp.* (up to 100% of samples, 16-504 gene copies per 100 ml) and *Salmonella* (up to 25% of samples, 6-86 gene copies per 100 ml). Other viral human pathogens were also measured, but occurred at generally lower concentrations: adenovirus (detected in up to 22% of samples, 14-41 AdV copies per 100 ml); no enterovirus was detected in any stormwater discharge sample. Higher concentrations of avian source markers were noted in the stormwater discharge located immediately downstream of a large bird sanctuary along with increased *Campylobacter* concentrations and notably different *Campylobacter* species composition than the watershed that had no bird sanctuary. This study is one of the few to directly measure an array of important bacterial and viral pathogens in stormwater discharges to recreational beaches, and provides context for stormwater-based management of beaches during high risk wet-weather periods. Furthermore, the combination of culture-based and digital PCR-derived data is demonstrated to be valuable for assessing hydrographic relationships, considering delivery mechanisms, and providing foundational exposure information for risk assessment.

1. Introduction

Coastal southern California, with its Mediterranean climate patterns receives >95% of its precipitation during the winter season, with 70% of the precipitation occurring between January and March (NRC Report, 1990; Ackerman and Weisberg, 2003). Urban

* Corresponding author.
E-mail address: joshuas@sccwrp.org (J.A. Steele).

https://doi.org/10.1016/j.watres.2018.01.056
0043-1354/

stormwater runoff in southern California is known to contain high concentrations of fecal indicator bacteria (FIB) such as total and fecal coliforms and *Enterococcus*, which has been shown to have median concentrations ranging from 100-100,000 MPN per 100 ml (Griffith et al., 2010; Schiff and Kinney. 2001; Gannon and Busse, 1989; Brownell et al., 2007; Tiefenthaler et al., 2011; Parker et al., 2010). The result is an observed increase in FIB concentrations at marine bathing beaches from median Enterococcus concentrations of 10–100 MPN per 100 ml during dry weather and 10-10,000 MPN per 100 ml following storm events (Ackerman and Weisberg, 2003; Noble et al., 2003).

Concerns about exposure to pathogens and subsequent adverse human health effects (Given et al., 2006) have culminated in routine advisories against body contact with recreational waters from coastal Public Health Departments for up to 72 h following rain events (Thoe et al., 2014). Yet, in southern California, surfers regularly enter the ocean following rainstorms despite the well-advertised warnings of illness from public health officials because that is often when the best surf conditions occur. Even though stormwater is known as a major conduit of fecal contamination in southern California, relatively few previous studies exist that fully quantified bacterial and viral pathogens associated with fecal contamination (e.g. Jiang et al., 2001). Of those that have been published, little or no hydrographic information was available to provide context.

Monitoring FIB at marine beaches is useful for assessing public health risk because FIB concentrations have been previously determined to be related to illness rates of swimmers (e.g. Haile et al.,1999, Wade et al. 2010). FIB are also relatively easy and inexpensive to measure compared to actual pathogens. However, total coliforms, fecal coliforms, *E. coli*, and *Enterococcus spp.* are rarely the causative agents of illness. Instead, FIB co-occur with a wide range of pathogens found in human feces that may cause illness, including viruses, pathogenic bacteria, and/or protists (Prüss, 1998; Fong and Lipp, 2005); however, the relationships between presence of FIB and actual human pathogens in environmental waters are unpredictable (e.g. Bosch. 1998; Griffin et al., 2003; Fong and Lipp, 2005; Wu et al., 2011; McQuaig et al., 2012; Corsi et al., 2014). This is especially important in areas where storm drainage systems are separate from sanitary sewer systems, such as southern California, that present no *a priori* expectation to find human pathogens in stormwater runoff.

Several studies have been conducted to assess the impact of human noroviruses (NoV) and other human viral pathogens in sewage and on coastal waters in the context of sewage discharge and other potential anthropogenic sources (Eftim et al., 2017; Hassard et al., 2017). However, to our knowledge, there are very few reported calculations of human viral pathogen load from storm events, and likely none for stormwater discharge proximal to a high use recreational location. Therefore, identifying the sources of fecal contamination, and full quantification of pathogens are important steps toward understanding the risk to surfers using receiving waters for recreation following storm events (Soller et al., 2010, 2017).

Stormwater discharges (also called freshwater outlets in some previous documents) in southern California have previously been found to contain human fecal contamination confirmed through the presence of human viral pathogens such as enterovirus, NoV, and adenovirus (AdV) throughout the region (e.g. Noble et al., 2006; Noble and Fuhrman, 2001; Jiang et al., 2001). Several of the existing published studies measured the pathogen concentrations in stormwater samples from a range of locations, but techniques available at the time limited the ability to precisely quantify the pathogens (e.g. Jiang et al., 2001). New technological applications of droplet digital polymerase chain reaction (digital PCR) have enhanced the ability to measure microbial source tracking (MST) markers of host organisms and viral and bacterial pathogens present in stormwater runoff while being robust to inhibitory substances (Cao et al., 2015a; Coudray-Meunier et al., 2015).

The main objective of this study was to investigate viral and bacterial pathogen dynamics in the stormwater of two urban watersheds that discharge to high-use swimming/surfing beaches in southern California. To accomplish this, we used state-of-the-science quantitative analyses to determine concentrations of important viral and bacterial pathogens, along with MST markers selected for their high specificity and sensitivity (Boehm et al., 2013) and FIB quantification during multiple storms that occurred during winter 2014 and 2015. The microbial contaminant information was paired with available hydrographic information in order to assess emergent hydrographic relationships that drive illness risk to surfers during wet weather conditions. This exercise was conducted in tight coordination with a concurrent epidemiology study (Arnold et al., 2017) and QMRA modeling effort (Soller et al., 2017). We specifically selected these two distinct watersheds that discharge to popular surfing beaches in San Diego, CA to compare our findings in the context of land use, watershed size, and discharge impacts. Surfers at the beaches at the end of these watersheds reported a combined 4088 surfing days in wet and dry weather during the study (Arnold et al., 2017). A final objective of the study was to utilize principal component analysis and other relational statistical approaches to assess relationships across MST markers and pathogen types, yielding potentially valuable information for subsequent mitigation efforts.

2. Materials and Methods

2.1. Study design and water sample collection

The basic study design had two elements: ocean receiving waters and stormwater discharges. The ocean receiving water element focused exclusively on cultured FIB measurements, but was sampled at multiple sites at differing distances from the stormwater discharge point daily from January–March 2014 and December-March 2015, with the exception of Dec. 24, 25, and 31, 2014 and January 1, 2015. In this way, we captured the spatial and temporal influence of the stormwater discharges on the beach receiving water environment.

The stormwater discharge element focused on multiple microbial targets, but was limited to a single sampling location at the end of each watershed just before discharging to the beach, and exclusively during wet weather. Wet weather was defined to be consistent with the County of San Diego Public Health Department rain advisory; the day of rain $\geq$2.54 mm ($\geq$0.1 inch), plus 72 h (3 days). The additional measurements (in addition to the same FIB measured in the ocean) included host specific MST markers (human, avian, canine), viral pathogens (NoV genogroups I and II, enterovirus, AdV), and bacterial pathogens (*Campylobacter, Salmonella*).

2.1.1. Beaches

Daily ocean water samples were collected from January 15, 2014 to March 5, 2014 and from December 2, 2014 to March 31, 2015 at a total of six sites from two California beaches: Tourmaline Surfing Park (N = 2) and Ocean Beach in San Diego, CA (N = 4) (Fig. 1). Beach sampling details are described in the Supplementary Materials and Methods.

2.1.2. Watersheds

Tourmaline Creek is a small highly urbanized watershed (Fig. 1, S1). The watershed is approximately 3.3 km^2 and 86% developed