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Quantification of human-associated fecal indicators reveal sewage from urban watersheds as a source of pollution to Lake Michigan

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ABSTRACT

Sewage contamination of urban waterways from sewer overflows and failing infrastructure is a major environmental and public health concern. Fecal coliforms (FC) are commonly employed as fecal indicator bacteria, but do not distinguish between human and non-human sources of fecal contamination. Human *Bacteroides* and human *Lachnospiraceae*, two genetic markers for human-associated indicator bacteria, were used to identify sewage signals in two urban rivers and the estuary that drains to Lake Michigan. Grab samples were collected from the rivers throughout 2012 and 2013 and hourly samples were collected in the estuary across the hydrograph during summer 2013. Human *Bacteroides* and human *Lachnospiraceae* were highly correlated with each other in river samples (Pearson's r = 0.86), with average concentrations at most sites elevated during wet weather. These human indicators were found during baseflow, indicating that sewage contamination is chronic in these waterways. FC are used for determining total maximum daily loads (TMDLs) in management plans; however, FC concentrations alone failed to prioritize river reaches with potential health risks. While 84% of samples with >1000 CFU/100 ml FC had sewage contamination, 52% of samples with moderate (200–1000 CFU/100 ml) and 46% of samples with low (<200 CFU/100 ml) FC levels also had evidence of human sewage. Load calculations in the in the Milwaukee estuary revealed storm-driven sewage contamination varied greatly among events and was highest during an event with a short duration of intense rain. This work demonstrates urban areas have unrecognized sewage inputs that may not be adequately prioritized for remediation by the TMDL process. Further analysis using these approaches could determine relationships between land use, storm characteristics, and other factors that drive sewage contamination in urban waterways.

1. Introduction

Fecal pollution is an ongoing concern in urban waterways, especially those used for recreational purposes such as boating, fishing, kayaking, or canoeing. Recreational water contaminated with pathogenic bacteria, protozoa, and viruses, is a serious threat to human health (Arnone and Walling, 2007). Stormwater contaminated by sanitary sewage from leaking sewer lines or cross-connections can be a source of pathogens in urban areas (Sauer et al., 2011; Sercu et al., 2011, 2009) and has been associated with risks to human health (Gaffield et al., 2003; Haile et al., 1999). Additionally, combined sewer overflows (CSOs) and sanitary sewer overflows (SSOs) are sources of pathogens in urban waters (USEPA, 2004) and have the potential to affect recreational and drinking water sources (Marsalek and Rochfort, 2004). A significant association has been found between extreme rain events and gastrointestinal illness, which suggests that precipitation facilitates the delivery of waterborne pathogens from a variety of urban sources (Curriero et al., 2001; Drayna et al., 2010).

Fecal coliforms (FC), *Escherichia coli*, and enterococci have historically been used to monitor rivers and recreational beaches for fecal pollution (USEPA, 2012, 1976). Because these fecal indicator bacteria (FIB) are present in the gastrointestinal tract of humans and most warm-blooded animals, and are easily grown and quantified in the laboratory, they have been used as indicators of risk to human health in recreational waters for decades (Dufour and Schaub, 2007; McLellan et al., 2013). However, because of these same characteristics, their presence in almost all warm-blooded

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Nomenclature

CFU	Colony forming units
CN	Copy number
CSO	Combined sewer overflow
FIB	Fecal indicator bacteria
HB	Human *Bacteroides*
Lachno2	Human *Lachnospiraceae*
MMSD	Milwaukee Metropolitan Sewerage District
qPCR	Quantitative polymerase chain reaction
SSO	Sanitary sewer overflow
TMDL	Total maximum daily load
USGS	U.S. Geological Survey

animals does not distinguish the source of fecal pollution as human or non-human (McLellan and Eren, 2014) and often fail to specifically indicate the occurrence of harmful pathogens (Field and Samadpour, 2007; National Research Council, 2004). Identifying sources that are most likely to contribute pathogens is important to prioritize management strategies for mitigating fecal pollution. Several studies have had success employing molecular techniques using quantitative polymerase chain reaction (qPCR) to detect and quantify alternative indicators that are associated with human fecal pollution in urban waterways (Ahmed et al., 2010; Converse et al., 2011; Newton et al., 2013; Nshimyimana et al., 2014; Sercu et al., 2011). Specifically, qPCR assays developed for detecting genetic markers for human-associated *Bacteroides* (HB) and *Lachnospiraceae* (Lachno2) have exhibited a close correlation in sewage influent and in freshwater harbor samples, which suggests that using them in tandem, rather than individually, could provide a more accurate picture of human sewage contamination, particularly in urban waterways where many diffuse sources of fecal pollution have not been tested for cross-reactivity with new human indicators (Fisher et al., 2015b; Newton et al., 2011).

Many coastal Great Lakes cities experience fecal pollution, which threatens recreational water quality in nearby rivers and beaches. The metropolitan area of Milwaukee, Wisconsin is typical of Great Lakes coastal urban areas where the Milwaukee, Menomonee, and Kinnickinnic Rivers drain into Lake Michigan via the Milwaukee estuary. The densest urban areas of downtown Milwaukee have combined sewers, allowing the runoff from impervious surfaces to be treated by a water reclamation facility. Even in the absence of a CSO, chronic human sewage pollution has been identified in the Milwaukee estuary (Newton et al., 2013, 2011). Stormwater may be a major source of sewage pollution in Milwaukee's urban rivers (Sauer et al., 2011), potentially due to leaking sanitary sewage lines and sewer misconnections.

In this study, the degree of human sewage contamination in two urbanized watersheds and the Milwaukee estuary was evaluated. The goals of this study were to characterize human fecal contamination at assessment points that are used in total maximum daily load (TMDL) calculations, and demonstrate the value of using alternative fecal indicators to assess human health risks more accurately. Water samples were collected one to two times per month and during a variety of weather conditions, from June 2012 through August 2013. To gain a watershed scale assessment of sewage loads from the urban area, automated sampling was conducted across the hydrograph in the Milwaukee estuary for one to four days, under a variety of weather conditions. These measurements allowed for the examination of both quantitative loads entering Lake Michigan and the dynamics of genetic markers for human-associated indicators during storm events. This study demonstrates that human indicators can be especially useful in the TMDL implementation process for identifying and prioritizing river reaches based on human health risks caused by the presence of sewage contamination. Assuming that there is a higher likelihood that pathogens are present when sewage contamination is present, human-associated indicators have the potential to identify a human health risk that may not be revealed by using traditional FIB alone. Additionally, continuous sampling provides estimates of sewage loadings from urban areas and is useful for determining storm-driven sewage patterns.

2. Materials and methods

2.1. Study area and sampling methods

The Milwaukee estuary is the confluence of three major rivers that drain to Lake Michigan. The Kinnickinnic River (KK) is the smallest and most urban river with more than half of its drainage area covered by impervious surfaces. The Menomonee (MN) River is a smaller drainage area with mainly urban land uses, but is influenced by some agricultural land uses and natural space. The Milwaukee River (MKE) has the largest drainage area and has mainly agricultural land uses, but becomes highly urbanized near the mouth of the river. The characteristics of these watersheds are provided in Table 1. Land use and impervious surface percentages were determined for each watershed using National Land Cover Database 2011 data (Jin et al., 2013; Xian et al., 2011).

The primary focus of this study was the urbanized KK and MN Rivers. Four sampling sites were on the KK River, five sites were on the MN River (including one reference site upstream of urban land use), and one site was included below the confluence of the MN River and the MKE River, near the Milwaukee estuary. Sampling locations correspond to TMDL assessment points in Milwaukee's waterways (SEWRPC, 2013; SWWT, 2010a, 2010b). Sampling locations were selected for our study to represent the reaches of each river most likely to contain chronic fecal contamination. In the KK River, listed from downstream to upstream, the sampling locations were KK RI-13, KK RI-35, KK RI-34, and KK RI-33. In the urban area of the MN River, listed from downstream to upstream, the sampling locations were MN RI-20, MN RI-09, MN RI-32, and MN RI-22. One site in the MN River was selected as a rural, upstream comparison site (MN RI-36). Because the MKE River was not a focus in this study, only one sampling location was selected near the Milwaukee estuary (MKE RI-15), below the confluence of the MKE and MN Rivers.

Over the course of the study, a total of 202 grab samples from the ten sites were collected. Grab samples were collected at these locations by the Milwaukee Metropolitan Sewerage District (MMSD) in 2012 and 2013 as a part of their long-term monitoring program. Samples were collected approximately once per month during a variety of weather conditions from June 2012 through August 2013. Samples were collected in 1-L Nalgene bottles at the water surface in the center of the river channel and immediately placed on ice.

An automated Teledyne ISCO 3700 full size portable sequential sampler was used to collect hourly composite samples immediately downstream from the confluence of the MKE, MN, and KK Rivers in the Milwaukee estuary through event hydrographs for selected runoff events from May through September in 2013. The sampler was housed within the U.S. Geological Survey (USGS) monitoring station at Jones Island water reclamation facility in Milwaukee, Wisconsin. The sampler was programmed to collect 250 ml every 15 min for up to 24 h into 1-L polypropylene bottles, meaning each 1-L bottle represented a composite of four collections over 1 h. One-