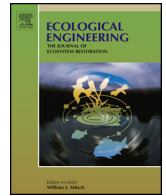




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# Towards sustainable protection of public health: The role of an urban wetland as a frontline safeguard of pathogen and antibiotic resistance spread

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### ABSTRACT

Wetlands are known for providing important ecosystem services, such as reduction of eutrophication. However, less research has focused on public health service of wetlands: their ability to protect microbial quality of water for downstream while they attract wildlife populations. For urban surface water, transmission of antibiotic resistance is also an important concern. Under anticipated climate change scenarios, more extreme precipitation patterns are emerging. Thus, runoff and flooding from increased precipitation will impair surface water quality more significantly. The objectives of this study were to 1) evaluate the dynamics of bacteria of public health concern in urban wetlands, including: *Escherichia coli*, *Campylobacter*, Shiga toxin-producing *Escherichia coli* (STEC), *Arcobacter*, and two antibiotic resistance genes (sulfonamide, *sul1* and tetracycline, *tetQ*); and 2) identify the major fecal contamination sources using microbial source tracking (MST). Water samples were collected across the urban wetlands in central Ohio from June 2013 to June 2014 and tested with a culture-based (*E. coli*) and quantitative PCR (the rest) methods. Since Canada geese were the most observed wildlife at the study sites and they are known to carry an array of infectious disease agents, their fecal samples were also collected. From the water samples, *E. coli* (fecal indicator) had the highest occurrence in 99.2% of the total samples, followed by *sul1* (98.3%), *Arcobacter* (94.9%), STEC (74.6%), *tetQ* (58.5%), and *Campylobacter* (1.7%). Overall reduction in *E. coli* was 22.3% across the wetlands, but variation was observed depending on the season. Microbial source tracking suggested a high prevalence of avian- (44.9%) and ruminant- (38.1%) originated fecal contaminations (e.g., Canada geese and deer). From the geese fecal samples, STEC (93.2%) and *Arcobacter* (4.5%) were detected, which could potentially compromise wetlands' functions in reductions in bacteria of public health concern and antibiotic resistance. The results suggest that careful wildlife management together with long-term monitoring of microorganisms of concern are needed for making urban wetlands as a sustainable frontline safeguard of pathogen and antibiotic resistance spread in surface water.

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## 1. Introduction

Wetlands can provide extensive ecosystem services, such as habitat for wildlife, biodiversity enhancement, flood prevention, water purification, and recreational and aesthetic values. Well-functioning wetlands provide beneficial ecosystem service and

improve human health status by providing clean water (Horwitz et al., 2012). For more than half a century, various types of wetlands have been constructed all over the world to mimic natural wetlands for water quality improvement (Vymazal, 2011). Research regarding wetland's functions on reductions in microorganisms of public health concerns has been documented (Thurston et al., 2001; Molleda et al., 2008; Hathaway et al., 2009). In a constructed wetland receiving municipal and livestock wastewater in Spain, removal rates of microorganisms were 99.9% for *Escherichia coli* and total coliforms, 100% for *Clostridium*, *Giardia* cysts, and *Cryptosporidium* oocysts (Molleda et al., 2008). In Arizona, a constructed

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wetland receiving secondary sewage effluent showed significant reductions in total coliforms, fecal coliforms, coliphage, *Giardia* cysts, and *Cryptosporidium* oocysts (Thurston et al., 2001).

Nonpoint source of microbial contaminations also contributes to water quality impairment, including stormwater runoffs (Rowny and Stewart, 2012). A model demonstrated a three-fold increase in *E. coli* concentration when precipitation events occurred within 24 h prior to the measurement in southwest Sweden (Tornevi et al., 2014). After heavy precipitation events, 10 to 1000-fold increases in fecal indicator bacteria were also found in Oslo, Norway (Tryland et al., 2014). Antecedent precipitation was one of the significant predictors of *E. coli* in suburban watersheds in Oregon, USA (Chen and Chang, 2014). Wetlands have been used as best management practices (BMPs) to treat microbial contaminants in stormwater. A 64.5% average reduction of *E. coli* numbers from influent to effluent samples was found in two stormwater BMP wetlands in North Carolina (Hathaway et al., 2009). In another constructed stormwater wetland in the North Carolina coastal plain, the *E. coli* reduction was 59% from inlet to outlet samples (Humphrey et al., 2014). However, compared to point sources (e.g. wastewater), wetlands treating stormwater demonstrated higher variability of performance (International Stormwater BMP Database, 2010).

In addition to pathogens, antibiotic resistance (AR) poses a significant threat on human health (CDC, 2015). Infection with resistant pathogens has been shown to increase the mortality and morbidity of diseases and costs of health care (Williams, 2002). AR has been detected in a wide range of environments (Cantas et al., 2013). In urban water bodies, sewage, outfalls from wastewater treatment plants, wastewater lagoons, hospital effluents, and ponds have been reported for AR bacteria and genes (Hamelin et al., 2007; Marion et al., 2015; Rodriguez-Mozaz et al., 2015). Abatement of AR genes has been observed as water moves through wetlands (Nölvak et al., 2013; Chen et al., 2015). In a constructed wetland receiving rural domestic wastewaters in China, significant reductions in AR genes (such as tetracycline and sulfonamide resistance genes, including *sul1*) were observed with the removal rates ranging from 83% to 100% (Chen et al., 2015). Horizontal subsurface flow wetland mesocosms treating municipal wastewater in Estonia demonstrated high frequencies of reduction in tetracycline and sulfonamide resistance genes (including *sul1*), although the removal rates varied throughout the study period (Nölvak et al., 2013).

In urban wetlands, wildlife are attracted to these habitats, which raises a concern since wildlife are known for harboring zoonotic pathogens, such as avian influenza and enteric pathogens including *Campylobacter*, *Salmonella*, and toxin-producing *E. coli* (Abulreesh et al., 2007). For example, various pathogenic *E. coli* serotypes have been isolated from Canada geese feces, including enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), and enteroagglomerative (EAEC) *E. coli* in urban areas (Kullas et al., 2002). In Great Lakes recreational region, Shiga toxin-producing *E. coli* has been identified from Canada geese feces (Hsu et al., 2016). In addition to avian species, enteropathogenic and Shiga-toxin producing *E. coli* have been isolated from shedding from mammals (Ishii et al., 2007; Jay et al., 2007; Laidler et al., 2013). In fact, an *E. coli* O157:H7 outbreak has been linked to strawberry consumption contaminated by deer feces, leading to deadly hemolytic uremic syndrome in Oregon, USA (Laidler et al., 2013).

For assessing microbial water quality, fecal indicator bacteria (FIB), such as total coliform, fecal coliform, *E. coli*, and enterococcus have been widely used (National Nonpoint Source Monitoring Program, 2013). In general, presence of FIB demonstrates a linkage to fecal contaminations and suggests potential presence of pathogens (National Nonpoint Source Monitoring Program, 2013). However, presence and levels of FIB do not necessarily indicate the presence/levels of specific pathogens and cannot tell the specific sources of fecal contamination. Given the fact that each pathogen

leads to different disease outcome when infected (Hunter, 1997; Vandenberg et al., 2004; Brooks et al., 2005), assessment of specific pathogen is suggested when investigating public health risk. For identifying the contamination sources, microbial source tracking (MST) determines the major sources of fecal contaminations, such as human and different types of animals (Lee and Lee, 2010; Lee et al., 2013; Ervin et al., 2014; Wilkes et al., 2014). Molecular markers in certain bacteria (e.g. *Bacteroidales*) associated with fecal materials from specific animal sources have been identified and applied to MST (Mieszkin et al., 2009; Haugland et al., 2010; Green et al., 2012). In conjunction with quantitative polymerase chain reaction (qPCR), direct detection and quantification of the molecular markers for pathogens and MST could be achieved in short amount of time.

Urban wetlands for this study are located in central Ohio (Olentangy River Wetland Research Park [ORWRP]) and have been established for research and education purposes. A significant amount of literature has been documented regarding vegetation and soil development, carbon and nitrogen dynamics, and water quality changes including physiochemical parameters, turbidity, nitrogen and phosphorus (Mitsch et al., 1998, 2012, 2014a,b). Reductions in nutrients (total phosphorus and nitrate-nitrogen) were observed in multi-year studies (Mitsch et al., 2014b). One previous study reported an overall reduction of 17% in *E. coli* from inflow to outflow at the ORWRP (Young, 2009). However, studies about the dynamics in bacterial pathogens and their potential sources, and antibiotic resistance were not conducted in the context of One Health, which recognizes the interconnection among the health of human, animal, and the environment (CDC, 2016). A healthy wetland provides beneficial ecosystem service in reducing pathogens and antibiotic resistance genes and thus better protects human health. This study covers two areas of One Health: whether an urban wetland performs an adequate ecosystem service to protect public health (environmental health), and whether there is a potential disease spread between animals and humans via surface water (animal–human health). As a consequence, both could affect public health in this urban area.

In order to investigate the potential roles of wetlands to protect urban water quality, the objectives of this study were to evaluate the dynamics and potential sources of bacteria of public health concern in urban wetlands, including fecal indicator bacteria, waterborne pathogens, and antibiotic resistance.

## 2. Materials and methods

### 2.1. Sample collections and data acquisition

This study was conducted at the Olentangy River Wetland Research Park (ORWRP) in Columbus, Ohio, USA (40.0204, –83.0186) (Fig. 1). The park includes two 1-ha experimental wetland basins. Water from the Olentangy River is continuously pumped into the wetlands, with rates adjusted based on river discharges. One liter of grab water samples were collected in duplicate using 1 L sterilized polypropylene bottles at the inflow, midpoint, and outflow of both wetland basins every other week at approximately 11:00 am from June 23, 2013 to June 21, 2014. Water samples were placed immediately in a cooler and holding time was within 6 h before being processed. Canada geese (*Branta canadensis*) feces were also surveyed because this area is often frequented by large numbers of geese. Fecal samples were identified and collected where geese were present or were recently observed on boardwalks at both wetlands at the same days when water samples were collected. Fecal samples were stored at –20 °C for further analysis. Water quality data, including water temperature, pH, dissolved oxygen, water conductivity, and reduction–oxidation

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