INVESTIGATING THE DISTRIBUTION OF *LEGIONELLA PNEUMOPHILA* IN URBAN AND SUBURBAN HUDSON RIVER WATERSHEDS

A Final Report of the Tibor T. Polgar Fellowship Program

Azlan Maqbool
Polgar Fellow
School of Earth and Environmental Sciences
CUNY Queens College
Flushing, NY 11367

Project Advisors:

Gregory O'Mullan
School of Earth and Environmental Sciences
Queens College, City University of New York
Flushing, NY 11367

M. Elias Dueker
Department of Environmental and Urban Studies
Bard College
Annandale-on-Hudson, NY 12504

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ABSTRACT

The presence of *Legionella pneumophila* was assessed using a cultivation-based approach in New York City waterways, a freshwater portion of the lower Hudson River Estuary near Kingston NY, and in urban and suburban street water. *Legionella pneumophila* was detected in 51% of brackish New York City Estuary samples, most with concentrations near minimum detection (10 organisms/ mL). In contrast, the bacterium was detected in 22% of suburban freshwater Hudson River Estuary samples. Levels of the bacterium were found to be higher during wet weather compared to dry weather in the highly dense urban setting, but not in the less dense suburban/rural settings. *Legionella pneumophila* was also detected in 95% of New York City street water samples and in 88% of suburban street water samples. These results presented a strong initial indication of wet weather contamination from street water discharge into the estuarine environment. This is the first study to document the widespread occurrence of *Legionella pneumophila* in street water and to establish a clear pattern of increased concentrations of *Legionella pneumophila* during wet weather in an urban estuarine environment.

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INTRODUCTION

Legionella pneumophila, the causative agent in Legionnaires Disease (LD), is a bacterium with widespread distribution and is of increasing concern especially in densely populated environments where components of the built environment influence its distribution and exposure patterns. The recognition of *Legionella* came about after a large outbreak at an American Legion conference hotel in Philadelphia, PA, in 1976. Following the convention, 82 attendees became ill with serious forms of atypical pneumonia, and 29 died (McDade et al. 1977). The bacterium was isolated a few months later and named after the event that caused the outbreak (McDade et al. 1977). It wasn't until over a year later when the cooling tower atop the hotel was identified as the culprit. Since then, there have been several outbreaks of LD across the United States involving man-made water systems. As *Legionella* has been increasingly tied to sources such as cooling towers and HVAC systems, there has been limited research into other potential reservoirs in built environments that may also harbor the bacteria.

Although primarily studied in the built environment, *Legionella* has been reported in literature to have a widespread naturally occurring presence in lakes, river systems, and soils (van Heijnsbergen et al. 2015; Declerck et al. 2010; Steele et al. 1990; Fliermans et al. 1981). A 2010 study detected *Legionella* in 42% (185 out of 388) of Mt. Hope Bay, Massachusetts estuarine samples, indicating the *Legionella* can be found in widely different saline environments and grown in saline conditions (Gast et al. 2011). A 2014 study documented the growth of *L. pneumophila*, and associated amoeba biofilm, in subsurface water layers in three separate Poland lakes (Żbikowska et al. 2014). In soils, the presence of *Legionella* was detected in six garden soils that were mixed with composted materials (Hughes and Steele 1994). Furthermore, there is evidence that natural soil is a reservoir and source of *Legionella* (Wallis and Robinson 2005). There are currently more than 58 species that have been described in published articles (Prussin et al. 2017). Of these, approximately 25 are linked to disease, including *Legionella pneumophila* species serogroup 1, 3, 4, and 6. *Legionella pneumophila* serogroup 1 is the most virulent strain causing the majority of infections (Walser et al. 2014). Urban environments are perhaps the key centers where exposures of *Legionella pneumophila* occur. One relevant source in the context of urban waterways is wastewater.

Wastewater treatment plants (WWTPs) have been confirmed to contain *Legionella* (Buse et al. 2012; Caicedo et al. 2018; Vantarakis et al. 2016) which can play an important role in community cases and outbreaks of LD. Studying *Legionella* in WWTPs is noteworthy as the quantity of municipal wastewater produced worldwide is drastically increasing as a result of growing population numbers. This, coupled with the discharge of inefficiently treated wastewater, particularly during rain events, into surrounding surface water sources serves as a direct threat to water quality, marine life, and humans. The persistence of *Legionella* in aeration tanks and wastewater treatment plants, is complicated by the bacterium's ability to interact with a variety of protozoan species. Once infiltrated, *Legionella* can hide, repair, and replicate within its host organism. The host cell protects *L. pneumophila* from harsh environmental conditions while providing a nutrient rich replicative niche (Abdel-Nour et al. 2013; Boamah et al. 2017). This ability is likely what causes *L. pneumophila* to survive despite water disinfection procedures.

In highly dense urban centers like New York City, coastal water quality has been clearly linked to wet weather-related discharge and bacterial contamination. For example, previous research in the Hudson River Estuary (HRE) has shown an increased concentration of antibiotic resistant bacteria following rainfall (Young et al. 2013), increased estuarine greenhouse gas emissions following nutrient addition (Montero et al. 2015), and high levels of fecal indicator bacteria (FIB) delivered from urban street water to coastal waterways (Montero and O'Mullan 2018). While there has been plenty of evidence linking bacteria of concern to degradation of coastal water quality, there is less known about the distribution of *Legionella* in relation to water quality and sewage pollution.

The prior literature has not established an expected distribution in urban stormwater sources, and there is no clear expectation for how the distribution of *Legionella* will change along estuarine gradients and between urban and suburban environments. The goals of this study were to: 1) determine if *Legionella pneumophila* can be detected in urban and suburban Hudson River watershed environments; 2) determine if the concentration of *Legionella pneumophila* in coastal water is influenced by wet weather events; and 3) examine the distribution of *Legionella pneumophila* in urban and suburban street water. The hypothesis tested was that *Legionella* would be detected in estuarine and freshwater Hudson River environments and that concentrations would increase following wet weather due to precipitation linked pollution discharge from urban and suburban environments.

METHODS

In the summer of 2019, a total of 22 New York City sites were sampled during a 4-month period from June 2019 to October 2019 (Figure 1a). Of these 22 sites, fifteen were estuarine sites in western Long Island Sound and East River tributaries of New York City. Two sites were combined sewer overflow sites (BB08 and BB06) located in Flushing Bay (40.761858 N, 73.845919 W; 40.760250 N, -73.854587 W). Five street water sites (Figure 1a) were also sampled proximal to the Queens College campus. The chosen estuarine sites were a subset of sampling locations sampled at the time through the Riverkeeper water quality monitoring program conducted by the O'Mullan laboratory at Queens College. Urban estuarine sites shown in Figure 1a were accessed by boat. Once on site, 50 mL centrifuge tubes were triple rinsed with sample water before collection and then immediately stored on ice in a cooler to protect from sunlight until processing (Young et al. 2013). Samples were then returned to the lab shortly after where Legiolert, a cultivation method (IDEXX Laboratories, Westbrook, ME) based on a most probable number approach, was utilized to enumerate total *Legionella pneumophila* in water samples. The 1.0 mL protocol for non-potable water was used before transferring sample into a 96-well Legiolert Quanti-tray (IDEXX Laboratories, Westbrook, ME) and placed into an incubator at 37° C. After a 7-day incubation period, samples were taken out and analyzed for positive wells, which is indicated by a change in color as compared to the negative control tray. Results are given in most probable number (MPN) of *Legionella pneumophila* cells per 100 ml. The detection limit for the 1.0 mL assay is ≥100 organisms in 100 mL or ≥1 organism per mL. In parallel, Enterolert, an assay also developed by IDEXX Laboratories, was used to assess enterococci concentrations in all water samples (Young et al. 2013). Samples were transferred into Quanti-tray/2000 and incubated at 41° C. After 24 hours, trays were taken out and analyzed under a UV light. Any samples that presented a blue fluorescence were counted as positive.

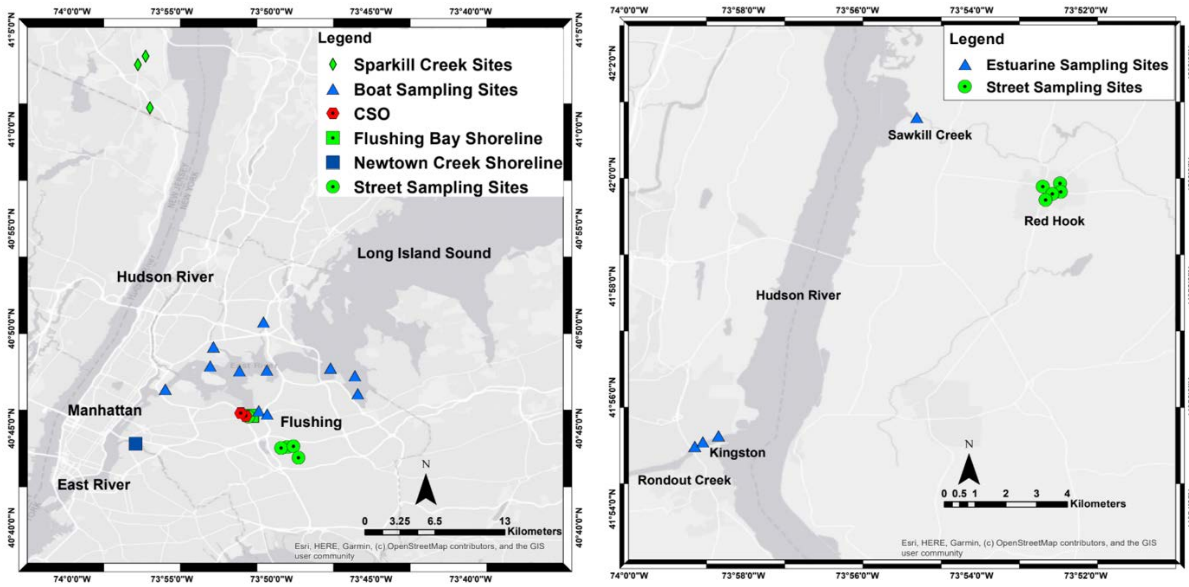

Figure 1. Map of Study Sites: a) 22 sites were regularly sampled in the New York City area. b) 9 sites sampled regularly in mid-Hudson Valley tributaries (Kingston, NY) and street water in Red Hook, NY.

During the fall of 2020 (September 2020 to November 2020), a total of nine sites and 44 samples were collected in the mid-Hudson River estuary watershed (Figure 1b) near Kingston, and Red Hook, NY. Four estuarine sites were sampled; three were located in the tidal reach of Rondout Creek, one was located at the tidally influenced mouth of the Sawkill Creek. Five street water sites located in the small suburban community of Red Hook, NY, were also sampled during wet weather events. Three additional samples were taken at Sparkill Creek sites, near Piermont, NY, above the dam and, therefore, not influenced by tide or salinity. Samples were sent to the O'Mullan laboratory at Queens College and were tested for *Legionella pneumophila*, with parallel samples analyzed in the Dueker Laboratory at Bard College for fecal indicating bacteria (FIB): enterococci, coliform, and *E. coli*.

Statistical analyses were run using Prism statistical analysis software (Version 6). Non-parametric tests were performed on the *Legionella pneumophila* and enterococci data to evaluate differences between the abundance of wet and dry weather bacteria because microbial data were non-normally distributed. Specifically, the Mann–Whitney and Kruskal–Wallis tests were used on microbial counts. Spearman's coefficient was used to evaluate the correlation between the fecal indicator, *Enterococcus*, and *L. pneumophila*.

RESULTS

During the summer 2019 sampling period, *Legionella pneumophila* was detected in 53% of estuarine samples. Many of the positive detects were mid-level detections (<=500 organisms/100mL) relative to the maximum detection level except for one sample, FB5 (110,970 organisms/100mL), which was taken during a wet weather event and is located near BBO8, one of New York City's largest combined sewage overflow (CSO) outfalls. In suburban estuarine samples, taken during fall 2020, *Legionella pneumophila* was detected in 26% of samples, mostly at low levels (<=110 organisms/ 100mL) near the assay's minimum detection limit apart from one Sparkill Creek sample (<=740 organisms/ 100mL). Urban estuarine samples had significantly higher concentrations (*Mann Whitney p=0.0086***)** of *L. pneumophila* than suburban estuarine samples (Figure 2).

To investigate the association of *Legionella* abundance with fecal indicating bacteria, enterococci concentrations were compared to *Legionella* concentrations in paired samples across all estuarine sites (Figure 3) and were found to be correlated (Spearman $r = 0.4571$, $p<0.001$). Although a similar relation was found when examining only the urban estuarine samples

(Spearman r =0.5380, p<0.001), the suburban estuarine samples on their own were not significantly correlated (Spearman r = 0.04728, p = 0.8304).

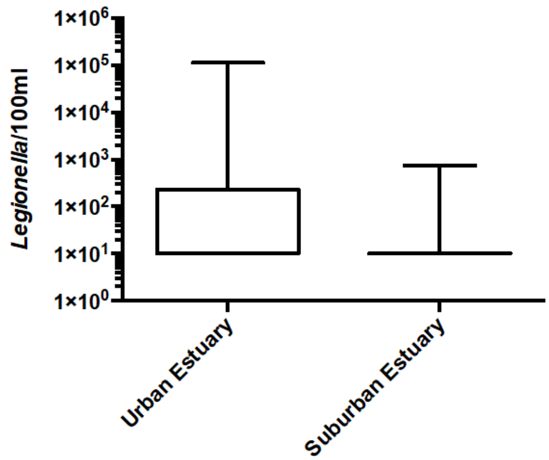

Figure 2. *Legionella* MPN in estuary box plot- Urban (boat + shore) vs. Suburban.

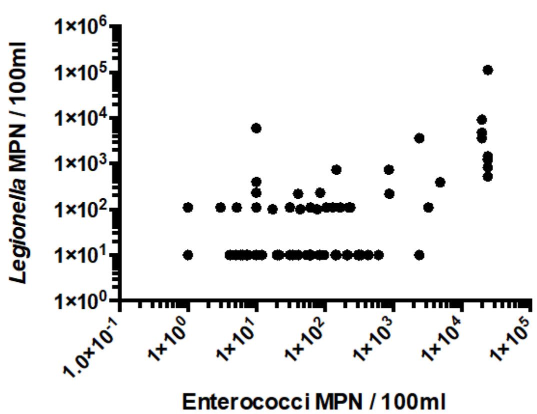

Figure 3. Enterococci-*Legionella* correlation for all estuary samples urban (boat and shore) and suburban (includes Sparkill Creek and mid-Hudson River estuary).

Wet vs dry weather results

The influence of rainfall on *Legionella* concentration was assessed by comparing samples collected in both wet and dry weather conditions. 102 samples were taken in wet weather events, while 38 samples were taken in dry weather events across all sampling sites: urban and suburban.

Enterococci concentrations during wet weather events were significantly higher than the dry weather counts in the urban environments, but not the suburban environment (Figure 4; Mann Whitney, p = 0.0136, p = 0.3171, respectively) when compared across sites. Conversely, *Legionella* concentrations were observed to be significantly higher during wet weather sampling in the urban environment (Figure 5; Mann Whitney, p = 0.003) but not in the suburban environment.

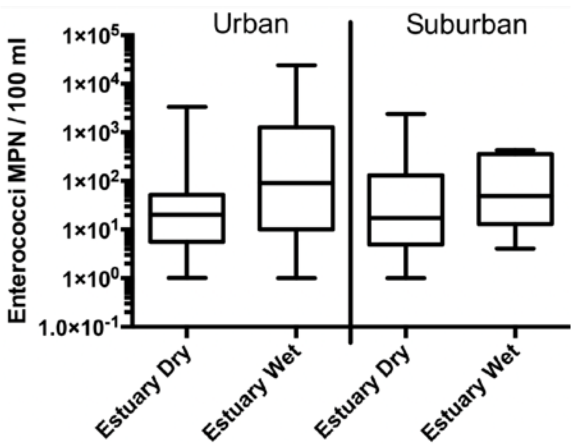

Figure 4. ***Enterococcus*** **wet vs. dry urban estuary and suburban estuary (urban wet, n= 46; urban dry, n=18; suburban wet n= 15; suburban dry, n=15)**

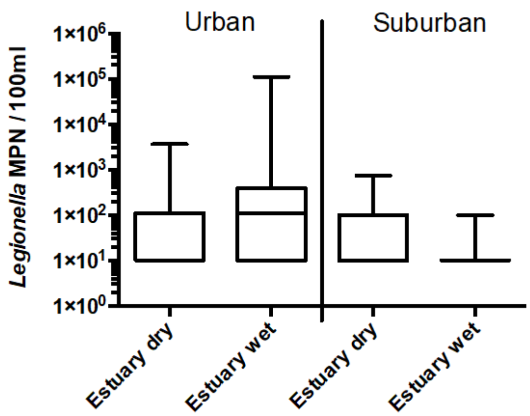

Figure 5. ***Legionella*** **wet vs. dry urban estuary and suburban estuary (urban wet, n= 46; urban dry, n=18; suburban wet n= 15; suburban dry, n=15).**

Street water results and comparisons

Although the concentration of enterococci was significantly higher in urban than the suburban street water (Mann Whitney, p <0.001, Figure 6), the concentration of *L. pneumophila* did not differ across urban and suburban street environments (Kruskal Wallis p = 0.691, Figure 7). Concentrations of *L. pneumophila* at CSO sites also did not significantly differ from urban and suburban concentrations of the bacteria in street water. A paired comparison of *Legionella* and enterococci across all sites was found to be weakly correlated (Spearman r = 0.4171, p= 0.01); however, a strong positive correlation was found between *L. pneumophila* and enterococci concentrations in the urban estuarine and street water environment (Figure 8b; Spearman r =0.8613, p<0.001).

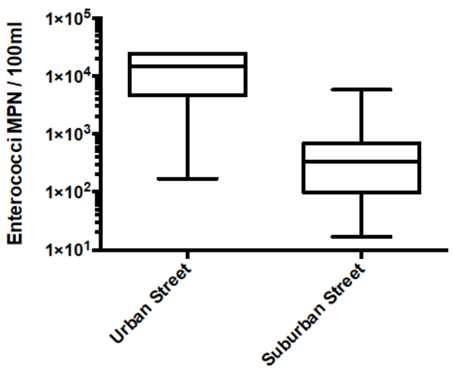

Figure 6. *Enterococcus* Street Urban vs Suburban.

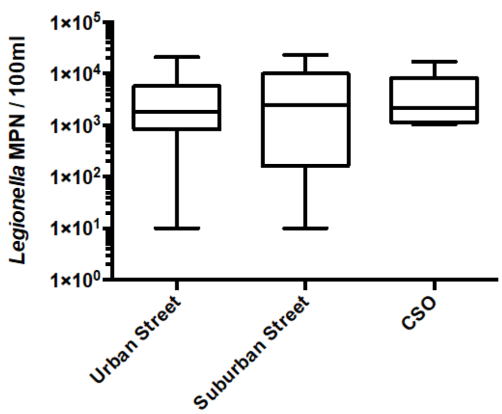

Figure 7. *Legionella* Street Urban vs. Suburban vs. CSO.

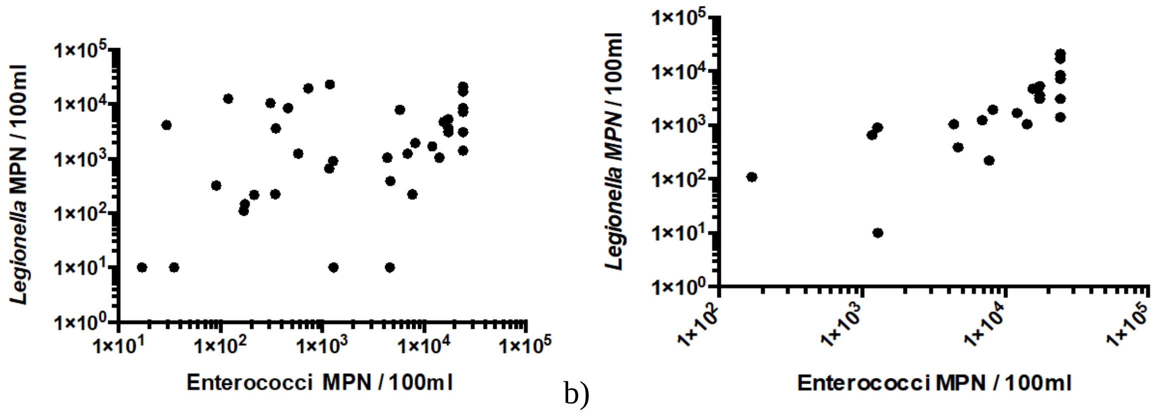

Figure 8. (a) Enterococci-*Legionella* correlation for all samples urban and suburban (b) Enterococci-*Legionella* correlation for urban samples only.

DISCUSSION

Urban vs Suburban Estuarine Results

The abundance of *L. pneumophila* in the estuarine environment was found to be significantly higher in urban proximal waterways than the less-saline suburban estuarine environment (Figure 2). Of the 15 suburban estuarine samples, only 4 samples were positive for *L. pneumophila* (26% of samples). This result is consistent with findings of the bacterium in similar aquatic environments (Walczak et al. 2013; Dutka and Ewen 1983; Fliermans et al. 1981). Walczak et al. 2013 reportedly detected *L. pneumophila* species in 12.42% of surface waters in five lakes in Poland. The lower detection of *L. pneumophila* in wet weather conditions in suburban estuarine was an unexpected result. This could relate to the lower number of samples collected/sampling locations, or it could do with a variety of physio-chemical parameters not directly assessed in this study. This result requires further investigation. As far as the detection of *L. pneumophila* in the urban estuarine environment, prior studies did not contain results from an urban waterway such as the lower Hudson River estuary. The results in this study detected *L.*

pneumophila in 51% of urban estuarine samples. This higher detection at the urban level is most likely due to wet-weather related discharges from the high density of outfalls in New York City. Tracking wet-weather discharges often involve FIB such as enterococci, which has been linked to both sanitary sewage and stormwater inputs (Suter et al. 2011; Montero and O'Mullan 2018).

Enterococci connection

Levels of *L. pneumophila* followed a similar distribution pattern to enterococci in the urban estuarine environment ($p > 0.001$) but did not follow that pattern in suburban estuarine waters ($p > 0.8304$). This pattern was also evident between wet and dry weather events as there was a significantly higher concentration of *L. pneumophila* observed in urban estuary wet weather samples versus urban dry weather samples. The suburban estuary wet versus dry weather events did not show a difference which most likely indicates a lower influence of wet-weather related discharge in less densely populated environments. The positive correlation between *L. pneumophila* and *enterococci* in the urban environment does not necessarily indicate a connection to sanitary sewage. Although the waterways in the urban environment had higher concentrations than suburban waterways, the levels of *L. pneumophila* in the suburban street samples were not significantly different than in urban streets (Figure 2 and 4); therefore, the difference between urban and suburban waterways may be more related to relative abundance and proximity to urban high-density outfalls. Anthropogenic sources then are likely contributors to higher levels of both FIB and *L. pneumophila.*

Highest abundance of Legionella pnuemophila found in stormwater

Prior literature has reported a clear connection between stormwater and elevated levels of enterococci (Montero and O'Mullan 2018). Similarly, this study found a clear connection between street water and elevated levels of *L. pneumophila*. The surfaces of the built environment, whether that is an urban or suburban environment, are primarily impervious where little infiltration occurs. Contaminants such as metals, chemicals, and pathogens can all accumulate on street surfaces. In this study, the highest levels of *L. pneumophila* were observed in street water. Coupled with the uniformly high levels of *L. pneumophila* in urban, suburban and CSO samples (Figure 7) this suggests that stormwater is likely the major source to both CSO discharge and to waterways. Moreover, it is likely the quantity of stormwater runoff, relative to waterway volume, is a determinant of *L. pneumophila* concentration in the coastal environment.

Ecology and Possible Sources

There is not much literature on whether the persistence of *L. pneumophila* differs in freshwater versus saline waters. Carvalho et al. (2007) did link a higher diversity of *Legionella* species with downstream, sewage-impacted waters when compared to lesser detection of *Legionella* in an upstream freshwater aquatic environment. The study was conducted along the Itanham River system in the Atlantic Forest of Brazil and did not definitively pinpoint salinity as the parameter affecting elevated detections. Rather, Carvalho et al. 2007 pointed to several factors related to anthropogenic sources such as the high level of organics and the presence of amoeba which allow for intracellular reproduction of *Legionella.* The host cell environment is known to protect *L. pneumophila* from harsh environmental conditions (Abdel-Nour et al. 2013). In the context of the urban environment and man-made water systems, this relationship between *Legionella* and protozoa does contribute to its overall persistence. Moreover, this relationship speaks to the importance of further investigation into the distribution of *Legionella* in the built environment and on street surfaces.

Management Relevance

Wet-weather contamination is closely connected to coastal water quality in the Hudson River Estuary. In New York City, the primary mechanism of sewage contamination is attributed to combined sewer overflow (CSOs) delivering a mixture of stormwater and untreated sanitary sewage to waterways. New York City has 426 CSO outfall pipes lining the city's coast which release approximately 20 to 25 billion gallons of untreated sewage and stormwater every year (NYC 2016). There are both national and regional monitoring programs that have been implemented to curtail this contamination. In 2012, NYC implemented a Long-Term Control Plan to deal with CSO contamination and related violations of the Clean Water Act (NYC 2012). This initially included a $1.7 billion-dollar investment in engineering measures and another $187 million toward green infrastructure to capture stormwater (NYSDEC 2012). Another management connection to bacterial pollution to coastal waterways, as pointed out by Montero and O'Mullan 2018, was the Municipal Separate Storm Sewer System (MS4) regulations (NYC 2016). MS4 is a system that transports stormwater in pipes separated from the sanitary wastewater system. Wastewater is delivered to a WWTP where it is treated, while untreated stormwater from separated sewers is discharged into a waterbody without treatment (NYC 2020). NYC's MS4 plan emphasizes preventing illicit discharges to stormwater pipes, controlling pollution in stormwater, and green or grey infrastructure initiatives. Additional management of stormwater under the MS4 permitting process may provide mechanisms to reduce both FIB and other microbes of concern, including *Legionella*. Currently, New York City does have legislations requiring action limits of *Legionella* concentrations in non-potable water systems. The New York state action limit is ≥ 20 per mL and the New York city action limit is ≥ 10 per mL (IDEXX 2019). The management of *Legionella* is especially important given the spike in associated outbreaks. During 2013-2014, drinking water reports showed a widespread distribution of *Legionella* contamination (Benedict et al 2017). While *Legionella* is not considered a major groundwater contaminant, it does account for many CDC-reported drinking water illness outbreaks. *Legionella* was responsible for 57% of water–associated outbreaks and 13% of illnesses (Benedict et al 2017).

Aside from stormwater management, aerosolization management is also vital in preventing the spread of *Legionella*. New York City, thus far, has been one of the leading municipalities with legislation requiring regular screenings of large HVAC systems atop buildings across the city. A potential area of concern that remains is aerosolization sources in waterways such as Newtown Creek. Increased culturable bio-aerosols were reported in the near-shore environment of Newtown Creek (Dueker and O'Mullan 2014) when the aeration process was occurring. Bioaerosol contaminants are of increasing importance to society. The SARS-CoV-2 pandemic will undoubtedly have an impact on the biocontrol management solutions used in built environments. It is important to better understand aerosolization sources and the consequences of airborne transmission of microbial contaminants, especially in densely populated areas.

CONCLUSION

Higher levels of *L. pneumophila* were detected in urban estuarine waterways compared to suburban waterways. The lower-level detection of *L. pneumophila* in wet weather conditions in the suburban environment was an unexpected outcome, and further analysis of this result is required. Although there was very little signal of *L. pneumophila* in the suburban estuarine samples, the suburban street environment contained high levels of *L. pneumophila,* comparable to the urban street environment and urban CSO samples. Thus, the difference between urban and suburban waterways is most likely related to the quantity of stormwater input from the built environment into an associated waterway. In highly dense urban centers like New York City, controlling the quantity of stormwater input into waterways is of management relevance given the public health consequences of aquatic pollution. While efforts have been made to manage *L. pneumophila* occurrence in cooling towers, there is not yet adequate information to minimize the occurrence and transmission of *Legionella* from other aerosolization sources such as WWTPs and aerated waterways. There is evidence to suggest that bio-aerosols can transmit pathogens a considerable distance. Thus, to minimize risk from Legionnaires' disease outbreaks, it is important to understand and manage environmental sources. Additional study is needed to evaluate the public health consequences of the widespread distribution of *Legionella* in urban and suburban water systems.

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