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Investigating Distribution of Legionella Pneumophila in Urban and Suburban Watersheds

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INVESTIGATING DISTRIBUTION OF *LEGIONELLA PNEUMOPHILA* IN URBAN AND SUBURBAN WATERSHEDS

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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 (≥1 organism/ mL). In contrast, it 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. Lastly, *Legionella pneumophila* was 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 source. 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 not been as much research into other potential reservoirs in urban 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 human 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 (Abu Kwaik et al. 1998). 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 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 and 74 total samples were gathered 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. Samples were taken in both wet and dry weather events. There were 8 wet weather events and 3 dry weather sampling events. Wet weather events were characterized by any sampling event in which the cumulative past three days of rainfall is greater than 0.635 cm. If the cumulative rainfall was less than this, the event was characterized as a dry weather event.

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 (MPN) 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 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.

The Legiolert assay was evaluated against the CDC plating method in a recent study by assessing sensitivity and specificity of the Legiolert assay (Rech et al. 2018). Sensitivity was defined as the probability of obtaining a true positive result from a known positive sample whether that was from the Legiolert method or the CDC plating method. Specificity was defined as the probability of obtaining a true negative result from a known negative sample (if both methods fail to detect Legionella). Results showed that the Legiolert assay had a very similar analytical sensitivity in detecting *L. pneumophila* with the traditional CDC plating method (Legiolert sensitivity = 0.84 ± 0.08 and CDC sensitivity = 0.86 ± 0.07). This was also the case for analytical specificity, as Legiolert specificity = 0.97 ± 0.02 and the CDC specificity = 1.00 (Rech et al. 2018).

To independently confirm detections of *L. pneumophila*, a subset of water samples was sent to EMSL Analytical Laboratory (Carle Place, NY) for confirmation of positive detects of *L. pneumophila*. Initially, three water samples collected in July 2019 from sites with known sewage contamination locations (two CSO and 1 shoreline Flushing Bay samples) were sent straight to the EMSL laboratory within 24 hours of sample collection. The EMSL M342 ISO 11731 (ISO 2017) culture method was used to confirm and enumerate *L. pneumophila* serotypes 1-14. Additionally, three Legiolert Quanti-trays with both positive and negative wells of *L. pneumophila* were sent to EMLS laboratory in November 2019 for confirmation and serotyping. The first method performed involved cultivation on an agar plate. 3 mL of sample were sub-sampled from eight wells across three trays (5 positive wells and 3 negative wells). 0.1 mL of the sub-samples were streaked for isolation on BCYE Agar and BCYE W/O Cysteine for *L. pneumophila* (Supplemental Table 2). EMSL lab associates then incubated isolation plates at 35°C for 72 hours before observation. Post observation *L. pneumophila* isolates were suspended

in 2% formalin and subsequently stained with direct fluorescent antibody stains. *L. pneumophila* (1-14) panvalent stain and L species (B-M) were used for confirmation before doing individual serotyping. The results are shown in Supplemental Figure 1 and Table 2 (Appendix section).

To identify possible sources to urban street surfaces, soil samples were collected from soil beds adjacent to three different street water sampling sites: Kissena Boulevard, Melbourne Avenue, and Main Street in Flushing, NY. Approximately 5mL of soil was collected in 50 mL centrifuge tubes at each location and then brought back to the lab for analysis. Soil samples were all weighed and recorded. 20mL of sterile DI water was then added to each tube to create a soil slurry. Following this, the soil slurry samples was vortexed. Finally, the 1.0mL Legiolert protocol was performed utilizing an aliquot of the soil slurry and transferred to a Legiolert Quanti-tray for the 7-day incubation period. Soil results are reported in MPN per gram.

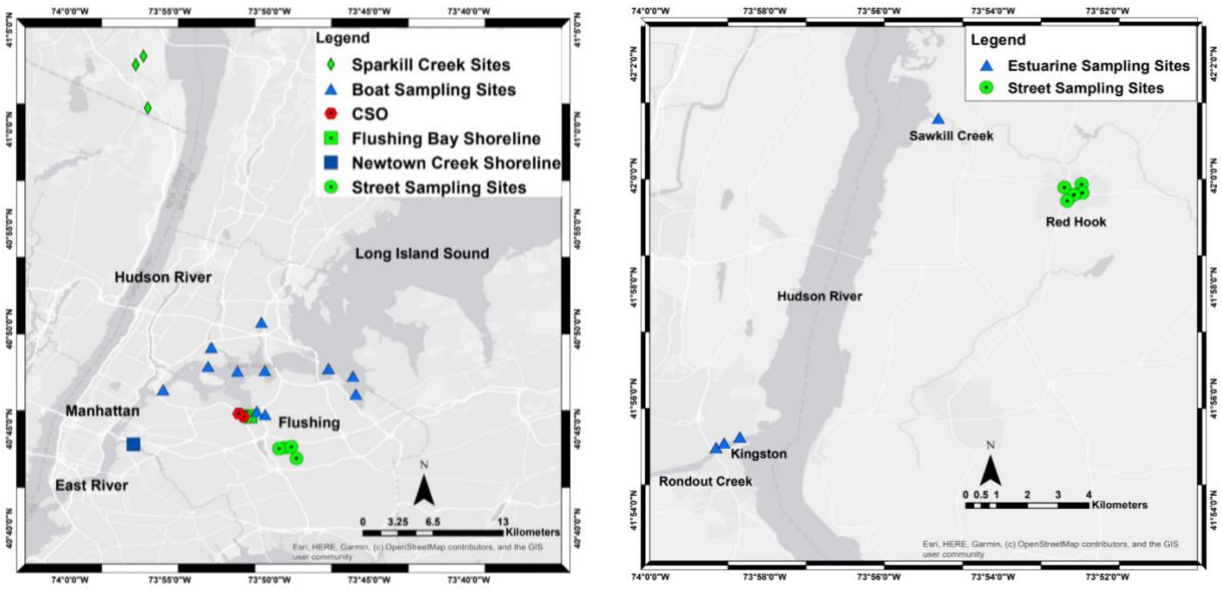


Figure 1. Map of Study Sites: a) 22 sites were regularly sampled in the New York City area. b) 9 sites sampled regularly in the mid-Hudson Valley tributaries Kingston, NY and street water in Red Hook, NY.

During the fall of 2020 (September 2020 to November 2020), a total of twelve sites and 44 samples were collected in the mid-Hudson River estuary watershed (Figure 1b) near Red Hook, NY and Kingston, NY. Four estuarine sites: three located in Roundout Creek tidal portion of the tributary, one 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 are not tidally influenced sites. There was a total of 3 wet weather sampling events and 3 dry weather sampling events. 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 the fecal indicating bacteria (FIB), *enterococci*.

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 *enterococci* and *Legionella pneumophila*.

RESULTS

During the summer of 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 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).

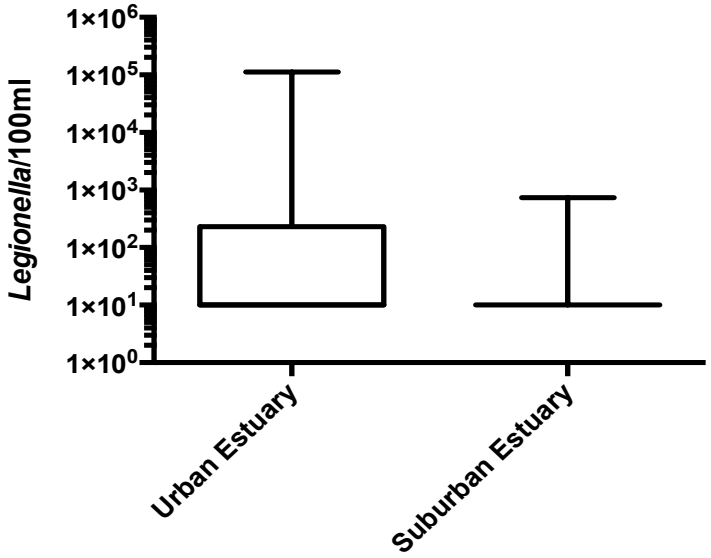


Figure 2. *Legionella* MPN in estuary box plot- Urban (boat + shore) vs. Suburban.

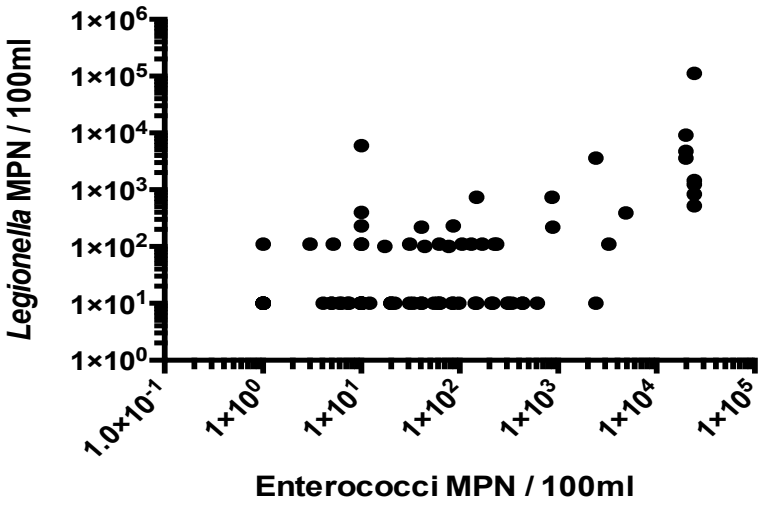


Figure 3. Enterococci-*Legionella* correlation for all estuary samples urban (boat and shore) and suburban (includes Sparkill Creek and northern).

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 difference 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.

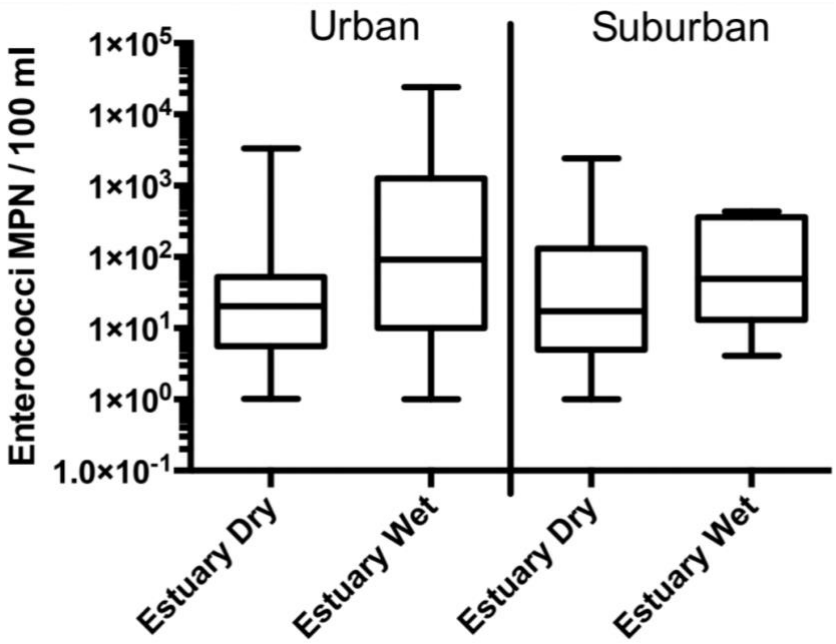


Figure 4. ***Enterococcus*** **wet vs. dry urban estuary and suburban estuary (urban wet, n= 46; urban dry, n=18; suburban wet n= 15; suburban dry, n=15)**

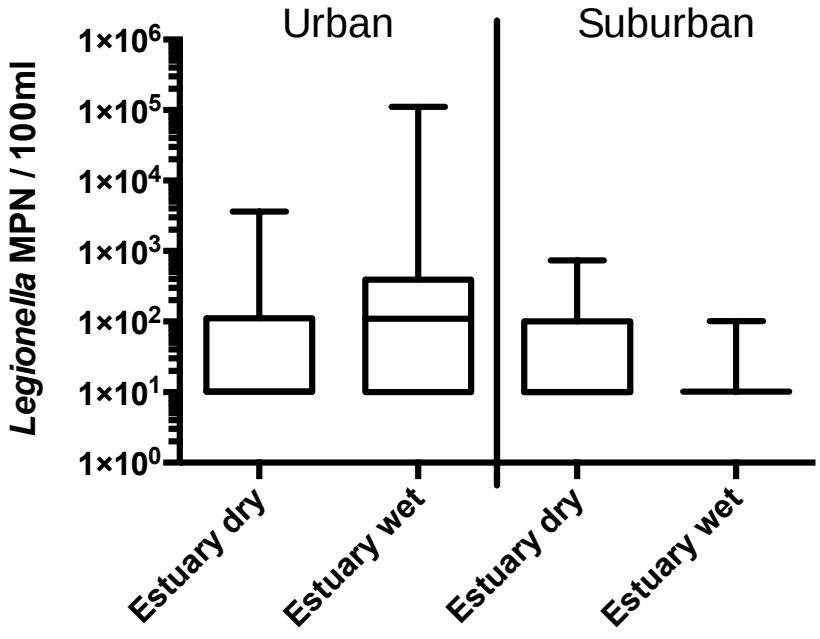


Figure 5. ***Legionella*** **wet vs. dry urban estuary and suburban estuary (urban wet, n= 46; urban dry, n=18; suburban wet n= 15; suburban dry, n=15).**

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 CSOs also did not significantly differ from urban and suburban concentrations of the bacteria in street water. A paired comparison of *L. pneumophila* and *enterococci* across all sites was found to be weakly correlated (Figure 8a; Spearman r = 0.4171, p= 0.01); however, a strong positive correlation was found between *L. pneumophila* and *enterococci* concentrations in the urban street water environment (Figure 8b; Spearman r =0.8613, p<0.001).

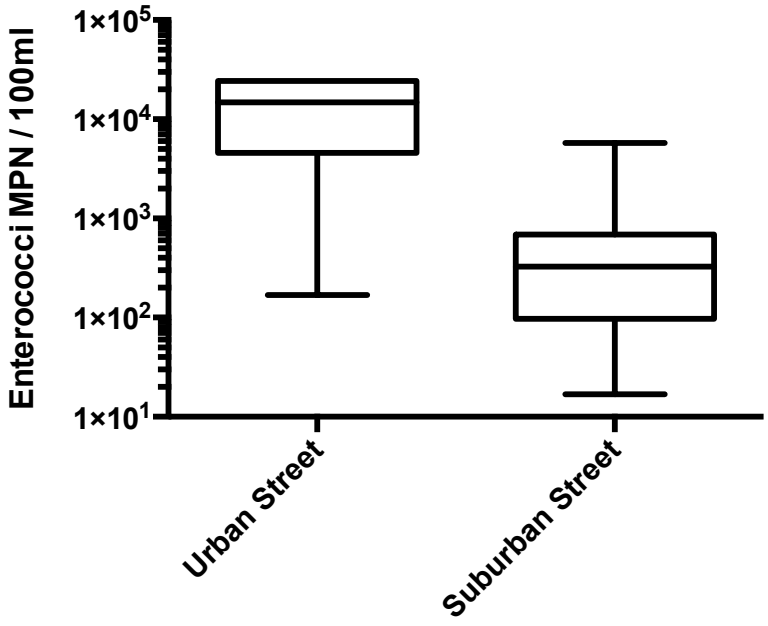


Figure 6. *Enterococcus* Street Urban vs Suburban.

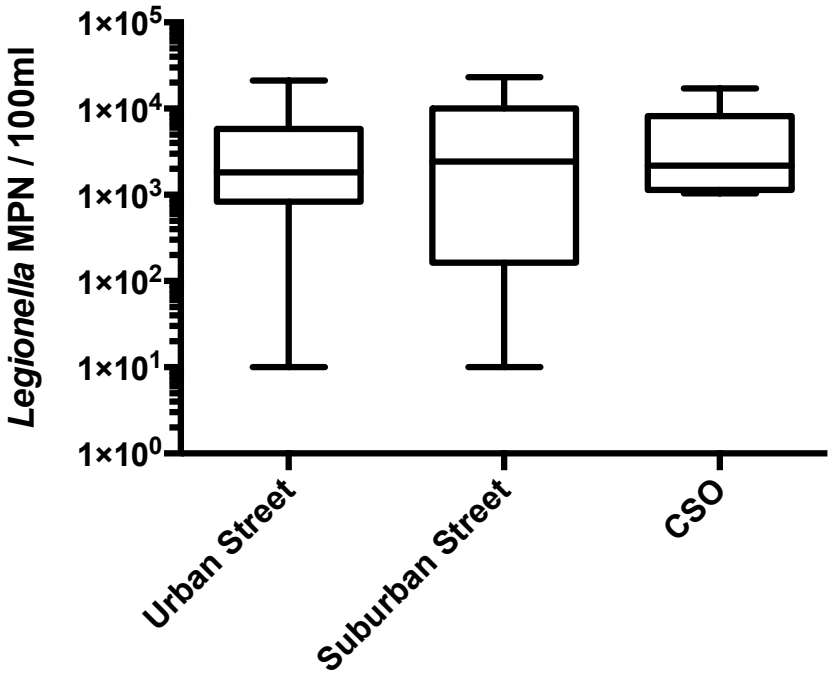
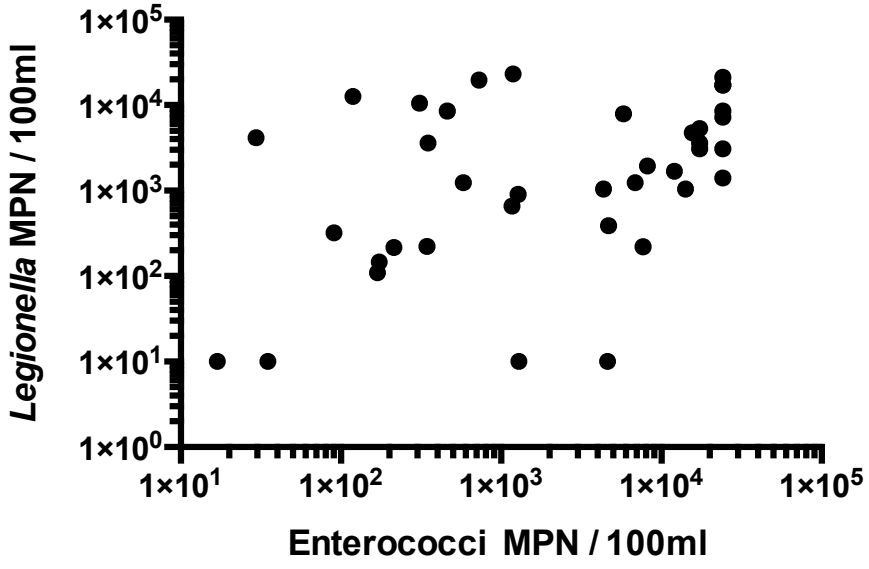
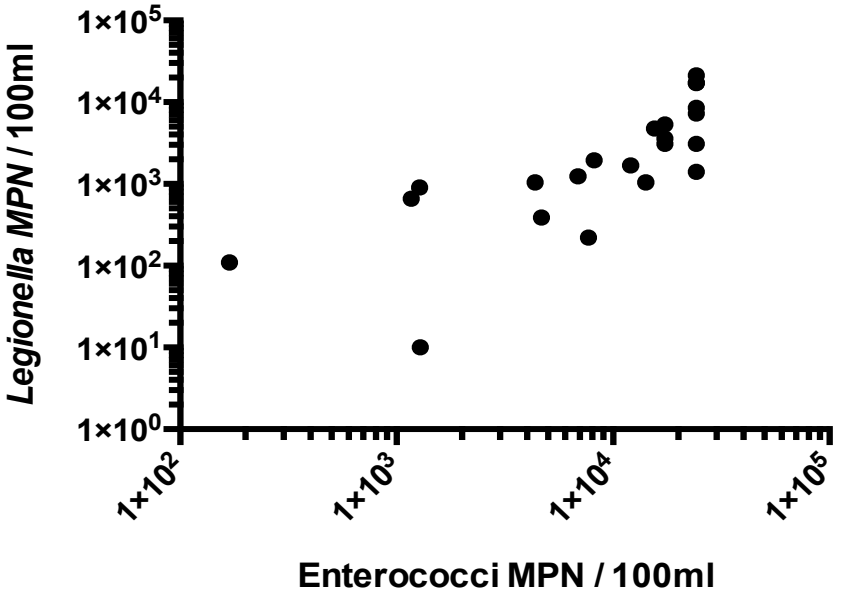


Figure 7. *Legionella* Street Urban vs. Suburban vs. CSO.



a)



b)

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

Soil analysis and EMSL confirmation results

Soil samples collected adjacent to three of the urban street sample sites were all positive for *L. pneumophila* with concentrations ranging from an MPN of 225 organisms/gram to an MPN of 3,915/gram. In June 2019, three Flushing Bay water samples (from the mixing zone of CSO BB06, CSO BB08, and from the Flushing Bay Pier Dragon boat dock) were sent for independent confirmation of *L. pneumophila* detections at EMSL laboratory. The results for these three water samples were inconclusive because initial enrichments were plagued by non-*Legionella* bacterial overgrowth issues. In order to more directly confirm results of Legiolert assays showing positive and negative results, in November 2019, three *L. pneumophila* Legiolert Quanti-Trays were sent to EMSL laboratory for another confirmation analysis based on wells from these trays. Of the 5 positive Legiolert wells and 3 negative wells that were sub-cultured on

BCYE plates, *L. pneumophila* colony growth was observed in all 5 of the positive confirmation assays. All 3 BCYE plates subsampled from negative Legiolert wells showed no *Legionella* growth (Supplemental Table S2). An analysis of *L. pneumophila* isolates from positive cultures using direct fluorescent antibody staining confirmed the presence of *L. pneumophila* serotypes 1, 5, and 6 (Supplemental Table S3).

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 2.013; 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 ($p > 0.8304$) (Figure 3). 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 ($p = 0.003$) (Figure 5). 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.* It is interesting to note that while analysis of sewage-impacted estuarine samples with traditional plate culturing methods (BCYE) were plagued by bacterial overgrowth complicating interpretation of these results, the Legiolert method was successful in enumerating positive and negative wells which could be confirmed using plate-based approaches (Supplemental Tables S2-S3 and Figures S1-S4**).**

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. Resuspended soil particles from soils beds can be a potential source to street surfaces following wet-weather events. (Wallis and Robinson 2005). *L.*

pneumophila was cultivated from soil samples collected directly from soil beds adjacent to urban street sampling sites (Table S1). All three of the soil samples collected returned positive Legiolert signal. While the soil sample slurry results aren't intended to be quantitative, they do confirm that *L. pneumophila* could be detected in suspended soils adjacent to locations where elevated stormwater samples were collected from the street. This confirms soil as one of the possible environmental reservoirs in the urban street environment.

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. 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 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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APPENDIX OF SUPPLEMENTAL TABLES AND FIGURES

Table S1. List of all study sites including minimum and maximum detection values at each site for *L. pneumophila* and *Enterococci*.

Site Name:	Urban / Suburban	# of Samples	Wet / Dry	Min/Max *L. pneumophila* MPNs	Min/Max *Enterococci* MPNs
Flushing Bay Pier 1 East	Urban	6	Both	10 - 840	9.8 - 24,196
Dragon Boat	Urban	4	Both	110 - 4740	31 – 24,196
World's Fair Marina Puddle	Urban	4	Wet	10 – 390	169 - 7701
BB08 - CSO	Urban	2	Wet	1240 - 17220	24,196
BB06 - CSO	Urban	2	Wet	1040 - 8540	24,196
Melbourne Puddle	Urban	4	Wet	660 – 21330	1166 – 24, 196
Main St. Puddle	Urban	4	Wet	900 - 17220	6867 – 24, 196
Kissena Blvd. Puddle	Urban	4	Wet	1040 - 7230	14136 – 24,196
Parsons Blvd. Puddle	Urban	4	Wet	1690 - 8540	8164 – 24,196
Newtown Creek	Urban	4	Both	10 - 400	10 - n/a
East River Hell Gate Bridge (ER3)	Urban	3	Both	10 - 220	10 -86
East River Hunt's Point (HP-WPCP)	Urban	3	Both	10 -110	41 - 169
Flushing Creek Mouth (FC1)	Urban	6	Both	10 - 3610	31 – 2419
Flushing Creek North (FC3)	Urban	3	Both	10 - 3610	10 – 24,196
East River mid-channel ER4	Urban	3	Both	10 - 110	10 -238
Bronx River Riverside Park BR2	Urban	3	Both	10 - 220	62 - 882
Westchester Creek Inner WC3	Urban	3	Both	10 -390	282 - 4884
Tallman Island (TI-WPCP)	Urban	3	Both	10 - 230	10 - 86
East River-Throgs Neck (ER6)	Urban	3	Both	10 - 110	10 - 148
Little Neck Bay Outer LN1	Urban	3	Both	10 - 110	10 - 20
Little Neck Bay Inner (LN2)	Urban	3	Both	10 - 5960	10 -20
Kingston Point 1	Suburban	5	Both	10	1 – 24,196
Kingston Point 2	Suburban	5	Both	10 - 110	2 – 17.3
Kingston Point 3	Suburban	5	Both	10 - 10	5 - 24196
Red Hook Street 1	Suburban	4	Wet	10 - 8540	344.8 - 4611
Red Hook Street 2	Suburban	4	Wet	10 -3610	16.9 - 350
Red Hook Street 3	Suburban	4	Wet	216 - 7880	214.3 - 5794
Red Hook Street 4	Suburban	4	Wet	320 - 10570	29.5 - 309

Red Hook Street 5	Suburban	4	Wet	146 - 23070	118.7 - 1187
Sawkill Watershed Site 2	Suburban	5	Both	10 - 100	4.1 – 44.1
Sparkill Creek 10	Suburban	1	Dry	10	388
Sparkill Creek 11	Suburban	1	Dry	110	131
Sparkill Creek 14	Suburban	1	Dry	740	480

Table S2. Positive and negative Legiolert from street water samples were sub-sampled (0.1mL) were streaked for isolation on BCYE Agar and BCYE W/O Cysteine plates. Initial Legiolert results from the O'Mullan laboratory are highlighted against the EMSL culture isolation plates.

Source	Well#	Legiolert Result	EMSL BCYE	EMSL BCYE W/O	Non-Legionella colonies Present
Main Street 1	1	Positive	Growth	No Growth	No
Main Street 1	2	Positive	Growth	Non-Legionella Growth	Yes
Main Street 1	3	Positive	Growth	No Growth	No
Main Street 1	4	Positive	Growth	No Growth	No
Main Street 2	5	Negative	No Growth	No Growth	N/A
Main Street 2	6	Positive	Growth	Non-Legionella Growth	Yes
Neg	7	Negative	No Growth	No Growth	N/A
Neg	8	Negative	No Growth	No Growth	N/A

Table S3. Results for Direct Fluorescent Antibody Staining

Source	Well#	Positive	Serotype
Main Street 1	1	Yes	L. pneumophila 1 & 5
Main Street 1	2	Yes	L. pneumophila 1 & 5
Main Street 1	3	Yes	L. pneumophila 1 & 6
Main Street 1	4	Yes	L. pneumophila 1 & 5
Main Street 2	6	Yes	L. pneumophila 1

Control L.p 1	-	Yes	L. pneumophila 1
Control E. coli	-	No	None Detected

Figure S1. Legiolert trays with corresponding sample # labels

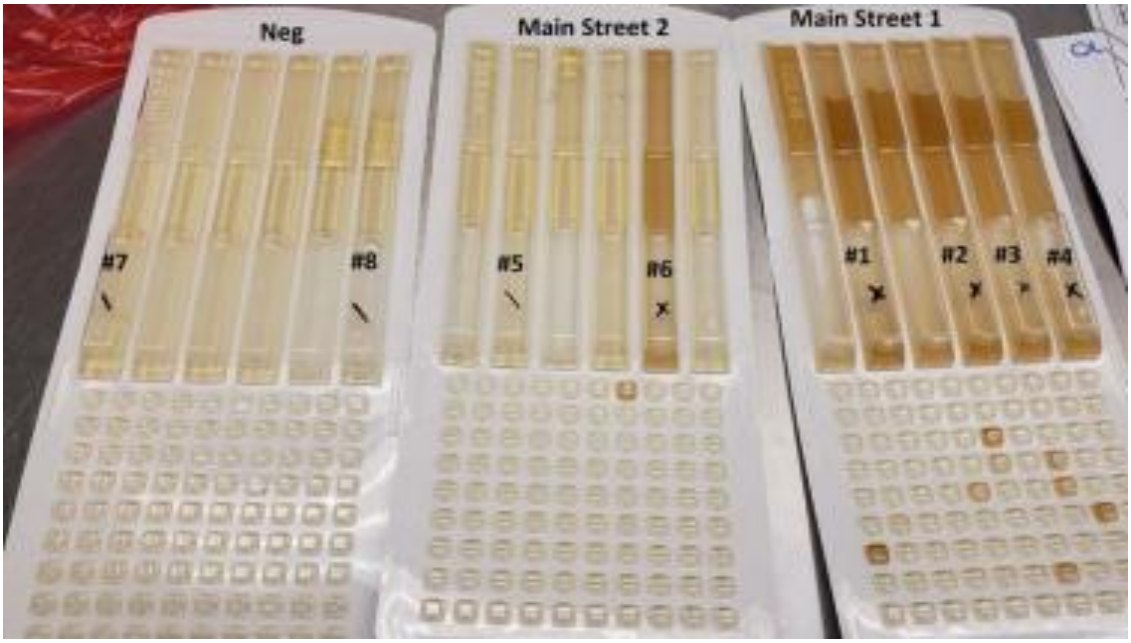

Figure S2. Samples 1-3 showing growth on BCYE with non-Legionella colonies on W/O for Sample #2. (BCYE plates on left, BCYE W/O on right).

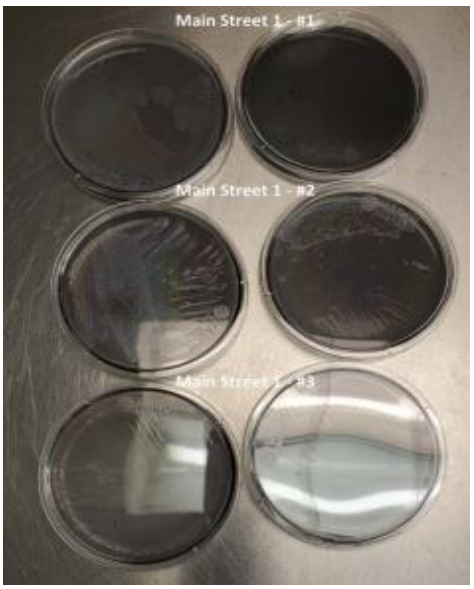

Figure S3. Samples 7 and 8 showing no growth. (BCYE plates on left, BCYE W/O on right).

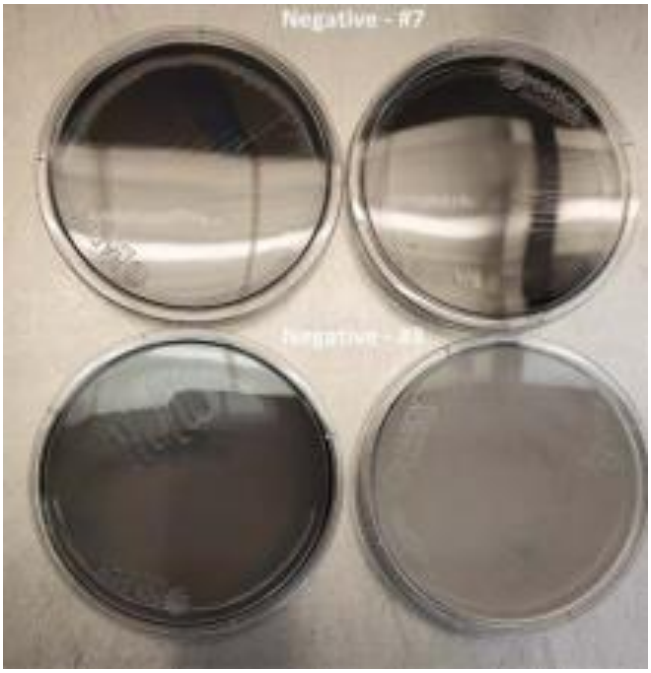

Figure S4. Sample 1 L. pneumophila 1 stain.