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Legionella pneumophila occurrence in reduced-occupancy buildings in 11 cities during the COVID-19 pandemic†

Katherine S. Dowdell, ‡[a] Hannah Greenwald Healy, ‡[b] Sayalee Joshi,[cd] Marianne Grimard-Conea,[e] Sarah Pitell,[f] Yang Song,[g] Christian Ley,[hi] Lauren C. Kennedy,[bj] Solize Vosloo,[k] Linxuan Huo,[l] Sarah-Jane Haig,[fm] Kerry A. Hamilton,[cd] Kara L. Nelson,[b] Ameet Pinto,[l] Michèle Prévost,[e] Caitlin R. Proctor,[in] Lutgarde Raskin,[a] Andrew J. Whelton,[io] Emily Garner,*[p] Kelsey J. Pieper*[k] and William J. Rhoads§*[q]

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In spring 2020, numerous buildings were closed or operated at reduced occupancies to slow the spread of COVID-19. An unintended consequence of these social distancing measures was a reduction in water demand in many buildings. Concerns arose that contaminants associated with water stagnation, such as *Legionella pneumophila*, could become prevalent. To investigate the potential public health risk associated with *L. pneumophila*, samples from 26 reduced-occupancy buildings in 11 cities in the United States, Canada, and Switzerland were analyzed for *L. pneumophila* using liquid culture (Legiolert, n = 258) and DNA-based methods (qPCR/ddPCR, n = 138). *L. pneumophila* culture-positivity was largely associated with five buildings, each of which had specific design or operational deficiencies commonly associated with *L. pneumophila* occurrence. Samples from buildings with free chlorine residual disinfection had higher culture-positivity (37%) than samples from buildings with chloramine (1%). Additionally, 78% of culture-positive samples occurred when the disinfectant residual was ≤0.1 mg L^{-1} Cl_2 and only three free chlorine samples were culture-positive when the disinfectant residual was >0.2 mg L^{-1} as Cl_2. Although overall sample positivity using culture- and DNA-based methods was equivalent (34% *vs.* 35%), there was disagreement between the methods in 13% of samples (n = 18 of 138). Few buildings reported any water management activities, and *L. pneumophila* concentrations in flushed samples were

Water impact

Low building water usage can lead to water quality deterioration, but it was unclear how the widespread, extended occupancy reductions during the COVID-19 pandemic would influence *Legionella pneumophila* occurrence. In this study, *L. pneumophila* was measured in 26 buildings across three countries, concluding that building water system design issues and free chlorine systems were linked to higher *L. pneumophila* positivity.

[a] *Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, MI, USA*
[b] *Department of Civil and Environmental Engineering, University of California, Berkeley, Berkeley, CA, USA*
[c] *School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ, USA*
[d] *The Biodesign Center for Environmental Health Engineering, Arizona State University, Tempe, AZ, USA*
[e] *Department of Civil, Geological and Mining Engineering, Polytechnique Montreal, Montreal, QC, Canada*
[f] *Department of Civil and Environmental Engineering, University of Pittsburgh, Pittsburgh, PA, USA*
[g] *Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA, USA*
[h] *Department of Civil, Environmental, and Architectural Engineering, University of Colorado - Boulder, Boulder, CO, USA*
[i] *Division of Environmental and Ecological Engineering, Purdue University, West Lafayette, IN, USA*
[j] *Department of Civil and Environmental Engineering, Stanford, Palo Alto, CA, USA*
[k] *Department of Civil and Environmental Engineering, Northeastern University, Boston, MA, 02115, USA. E-mail: k.pieper@northeastern.edu*
[l] *School of Civil and Environmental Engineering, Georgia Tech, Atlanta, GA, USA*
[m] *Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA*
[n] *Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN, USA*
[o] *Department of Civil Engineering, Purdue University, West Lafayette, IN, USA*
[p] *Wadsworth Department of Civil and Environmental Engineering, West Virginia University, Morgantown, WV, 26506, USA. E-mail: emily.garner@mail.wvu.edu*
[q] *Department of Environmental Microbiology, Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Dubendorf, Switzerland. E-mail: rhoadswj@bv.com*

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‡ Co-first authors.

§ Current affiliation: Black and Veatch, Kansas City, MO 64114, USA.

occasionally greater than in first-draw samples. This study provides insight into how building plumbing characteristics and water management practices contribute to *L. pneumophila* occurrence during low water use periods and can inform targeted prevention and mitigation efforts.

1. Introduction

Closures of non-residential buildings to limit the spread of COVID-19 resulted in water demand reductions in many public, industrial, educational, and commercial buildings.[1–5] While short periods of reduced water use (*e.g.*, nights, weekends, school breaks) are common in certain buildings,[6] many buildings were closed or at reduced occupancy for several months during the COVID-19 pandemic.[7–10] Reduced water use is associated with decreased disinfectant residual levels, equilibration of hot and cold water temperatures with building temperature, and potential for increased concentrations of contaminants such as disinfection byproducts, metals, and opportunistic pathogens.[11–21] The bacterium *Legionella pneumophila*, the primary causative agent of Legionnaires' disease and Pontiac fever, was of particular concern because its occurrence has been associated with low water use,[22–26] and disease incidence is rising around the world.[27–30] While there are other species of *Legionella* that cause human infections, *L. pneumophila* is responsible for the majority of legionellosis illnesses with an identified causative agent.[31] Though previous studies have linked low water use to *L. pneumophila* occurrence, few studies have investigated its occurrence during extended periods of widespread reduced water use, such as during the onset of the COVID-19 pandemic.[22,26,32–34]

The quantification of *L. pneumophila* in potable water systems has traditionally been performed using plate culture methods. However, alternative methods such as the IDEXX Legiolert® kit, a liquid culture method that yields results in most probable number (MPN) per volume, has become more widely used due to its ease of use and reported specificity for *L. pneumophila*.[35–41] DNA-based methods (*e.g.*, quantitative polymerase chain reaction [qPCR] and droplet digital polymerase chain reaction [ddPCR]) have also become more common due to the fast turnaround time, the possibility to detect cells in the viable but non-culturable state, and the potential for increased specificity, as compared to culture-based methods.[42–44] However, comparisons of results obtained with different methods are generally lacking in the current scientific literature.[30,43] *L. pneumophila* monitoring is typically not conducted outside healthcare settings unless the location is associated with disease incidence. This lack of broad surveillance and method comparability has limited our understanding of typical *L. pneumophila* concentrations in commercial and public building plumbing, the impacts of water demand patterns on *L. pneumophila* occurrence, and the effectiveness of various interventions for *L. pneumophila* mitigation.[34]

Investigations into the impact of COVID-19 pandemic-related building closures have reported that building water quality overall was negatively impacted.[45–53] However, studies have reported mixed results with respect to *L. pneumophila*, including no or low detection of *L. pneumophila*,[45,50,52] no change in the occurrence of *L. pneumophila* as water use returned to normal levels,[46] a small increase (2×) in *Legionella* spp. relative abundance after two months of reduced water use,[48] and widespread detection of *L. pneumophila* that increased during closure.[47] Each of these studies focused on individual cities, buildings, or regions, with none conducting sampling across distribution systems in different regions. Regional differences such as climate, source water, and system operation (*e.g.*, water age, pH, residual disinfectant type) may also influence *L. pneumophila* occurrence in drinking water distribution systems.[36,54] For example, a residual disinfectant in drinking water distribution systems is required in most public water systems in the United States and Canada, but not in other countries, such as Switzerland.[55]

The objective of this study was to characterize the occurrence of *L. pneumophila* in drinking water collected from large, non-residential buildings at reduced occupancy due to the COVID-19 pandemic across 11 cities and three countries (United States, Canada, and Switzerland) using both culture-based (Legiolert) and DNA-based (qPCR or ddPCR) methods. Physicochemical water quality parameters, building characteristics, and details of water management practices were collected to identify factors that contributed to *L. pneumophila* occurrence. Sampling was initiated as a rapid response to assess a potential public health concern, conducted across multiple academic institutions. Site-specific investigations were conducted at several of the sampling locations, the results of which are reported elsewhere.[51–53,56,57] This study presents a cross-sectional analysis of potential *L. pneumophila* exposure risks in buildings with diverse uses, plumbing configurations, operation, and climate regions.

2. Methods

2.1 Sampling locations

Samples were collected (n = 258) from 26 buildings that were either closed or operating at reduced capacity in 11 cities ("sites") in the United States, Canada, and Switzerland between April and December 2020 (ESI† Fig. S1). All buildings were connected to underground municipal drinking water distribution systems and received water with free chlorine (free chlorine buildings) or monochloramine (chloramine buildings) as the secondary disinfectant, except the building in Switzerland (CH-1), which received water without a secondary disinfectant ("no residual" building). All buildings from the same site were supplied by the same distribution system. Source waters for municipal systems were either surface water (five sites), groundwater (two sites), or

mixed groundwater and surface water (four sites). Buildings were large, multi-story, recreational, educational, office, commercial, or mixed-use (*e.g.*, research labs and offices) facilities (Table 1). Samples were collected from showers (n = 101), manual faucets (n = 139), electronic (automatic) faucets (n = 13), and bottle-filling stations and drinking fountains (n = 5). Samples included hot water (n = 114), cold water (n = 43), or "mixed" water (n = 101; hot and cold water mixed prior to the fixture). Samples included 203 first-draw and 55 flushed samples. The results for these samples are presented together except where noted.

Relevant building information was collected from owners and maintenance staff, including estimated building occupancy levels, building plumbing characteristics, and details of water management plans and practices prior to, during, and after building water shutdowns (Tables 1 and S1†). Building-level monthly water usage data were obtained for nine buildings (Fig. S2†). Building occupancy levels were used as a proxy for water consumption because metered flow data were not available for every building. At Site PA, samples were collected from laboratory-scale shower rigs supplied by building water. The building plumbing at Site CH was known to contain *L. pneumophila* prior to 2020. Permission was obtained prior to sample collection and results of testing were communicated to building owners. While the investigations at each site followed the same overall study design, there were variations in fixture flushing and analysis methods due to the collaborative nature of the sampling campaign.

2.2 Sample collection

First-draw samples (n = 203) were collected from every fixture sampled. Afterwards, flushed samples (n = 55) were collected at a subset of locations, with flush times ranging from one to 30 minutes. Samples were collected without removing or sanitizing aerators/showerheads (except at Site MA), representing what users would experience upon opening fixtures. For both first-draw and flushed samples, 1 to 1.5 L of water was collected in a sterile container. Samples were immediately split onsite for physicochemical (100 mL), culture-based (100 mL; IDEXX Laboratories Inc., catalog number WV120SBST-20), and DNA-based (0.8 L to 1.1 L) analyses and processed the same day. Residual disinfectant was quenched using sodium thiosulfate in subsamples used for culture-based and DNA-based analyses. Sample totals by analysis method and site are provided in Table S2.† Sampling controls are described in Table S3.†

2.3 Physicochemical analyses

Chlorine species were measured using the *N*,*N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method and portable spectrophotometers (Hach, Loveland, CO, USA) with limits of detection (LOD) ranging from 0.02 to 0.05 mg L^{-1} as Cl_2. Chlorine residuals are presented as total chlorine for all sites except for Site WV, where only free chlorine was measured (Table S4†). Site-specific details of additional physicochemical methods (including pH and temperature) are included in Table S4.† Physicochemical parameters were not recorded for samples collected at Sites CH and OH, and most samples from Site IN.

2.4 Culture-based methods

Culturable *L. pneumophila* was quantified using Legiolert according to manufacturer instructions for 100 mL potable water samples (IDEXX Laboratories, Inc., Westbrook, ME, USA, catalog number WLGT-20). The LOD was 10 MPN L^{-1}, and the upper limit of quantification (ULOQ) was 22 726 MPN L^{-1}. Positive and negative controls were included per manufacturer guidelines (Table S3†). Additional confirmation of positive wells was not performed.

2.5 DNA concentration and extraction

DNA was filter-concentrated onto membrane filters with pore sizes of either 0.2 or 0.4 μm on the day of sampling (Table S5†). Filters were frozen at either −80 °C or −20 °C until extraction. Samples were extracted using the methods described in Table S5.† Filtration and extraction controls are described in Table S3.†

2.6 qPCR and ddPCR

Of the 258 samples analyzed for culturable *L. pneumophila*, *L. pneumophila* was quantified using PCR-based methods in 138 samples (n = 120 by qPCR and n = 18 by ddPCR) using previously optimized assays that were validated by the individual laboratories (referred to as Laboratories A to E; Tables S6 and S7†). DNA extracts from Sites CA, WV, IN, and OH were shipped overnight on dry ice to Laboratory A for qPCR analysis (Table S7†). Laboratories A, B, and E analyzed samples using qPCR with an assay targeting a single-copy macrophage infectivity potentiator (*mip*) gene.[58] Laboratory D, which analyzed samples from Site QC, used the proprietary iQ-Check® Quanti *L. pneumophila* real-time PCR quantification kit (Bio-Rad, Hercules, CA, USA, catalog number 3578103). Laboratory C analyzed samples from Site PA using ddPCR and a different *mip* gene assay, which was modified for use without a probe (Table S6†).[59] Additional qPCR and ddPCR details are included in Tables S6 and S7.† Samples from Sites CH and MA were not analyzed for *L. pneumophila* gene targets.

All laboratories followed the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) and Environmental Microbiology Minimum Information (EMMI) guidelines.[60,61] For qPCR, all samples, standards, and controls were analyzed using triplicate reactions on each plate, except for Laboratory D, which used duplicate reactions (Table S7†). Serial dilutions of synthetic DNA consisting of the 79 base pair (bp) amplicon with 30 bp neutral adaptors on each end (gBlock, Integrated DNA Technologies [IDT], Coralville, IA, USA; Table S6†),[58] ranging from 10^8 to 10^0 gene copies per reaction (gc per reaction)

Table 1 Summary of sampling locations and building characteristics

				Building characteristics					Shutdown conditions		Sampling totals		
Disinfectant type	Site	Bldg.	Source water	Use type	Number of floors	Approx. number of fixtures[f]	Approx. year built (renovation date)	Primary plumbing material	Approx. occupancy during shutdown	Days reduced occupancy at sampling	Number of fixtures sampled	Approx. percent of fixtures sampled	Number of samples
Free chlorine	IN	IN-1	G	Multifamily housing	4	23	1930	GS	0%	138–174	3	13%	4
		IN-2		Commercial	10	215	1962	C	0%	120	4	2%	4
		IN-3		Commercial	4	114	2002	C	0%	122	2	2%	2
		IN-4		Mixed1	5	193	1951	C	NR	122	2	1%	2
	OH	OH-1	G	Education	2	255	2012	C	0%	148–191	4	2%	4
	AZ[a]	AZ-1[e]	S & G	Mixed2	7	48	2018	C	30–50%	162	7	15%	7
	PA[b]	PA-1	S & G	Mixed2	15	9	1971 (2019)	C & PVC	5%	125	9	100%	18
	WV	WV-1	S	Mixed1	5	75	2015	PVC	5%	147	3	4%	5
		WV-2		Dorm	9	660	1966	NR	2%	147	6	1%	9
		WV-3		Dorm	3	315	1918	NR	0%	147	4	1%	7
		WV-4		Dorm	10	550	1966	C	0%	147	6	1%	9
	QC	QC-1	S	Mixed2	7	160	1959 (1989)	C	2%	62	21	13%	21
		QC-2		Mixed2	8	128	2005	C	2%	53	12	9%	12
		QC-3		Sport complex	5	290	1974 (2007)	C	0%	269	23	8%	23
Monochloramine	MI	MI-1	S & G	Recreation	3	95	1956–1978	C & GS	25%	160	6	6%	6
		MI-2		Recreation	2	37	1976 (2018)	C	25%	163	7	19%	7
		MI-3		Recreation	6	74	1928 (2016)	C	25%	165	6	8%	6
	VA	VA-1[e]	S	Mixed2	3	71	2005	C	15%	132	5	7%	10
		VA-2		Mixed2	2	25	2007	C	5%	134	4	16%	8
	MA[c]	MA-1	S	Mixed2	5	31	1963	NR	5%	74	2	6%	4
		MA-2		Mixed2	4	22	1984	NR	5%	74	2	9%	4
		MA-3		Mixed2	6	47	2017	NR	5%	74	2	4%	4
	CA	CA-1	S	Dorm	4	90	1990	C	0–4%	106	8	9%	8
		CA-2		Dorm	3	45	1940	C	0–2%	106	6	13%	6
		CA-3		Dorm	3	57	1950	C	0–2%	106	6	11%	6
None	CH[d]	CH-1	S & G	Mixed2	8	>200	1970s	SS	<5%	46	41	≤20%	62

Sites: IN: Indiana, USA; OH: Ohio, USA; AZ: Arizona, USA; PA: Pennsylvania, USA; WV: West Virginia, USA; QC: Québec, Canada; MI: Michigan, USA; VA: Virginia, USA; MA: Massachusetts, USA; CA: California, USA; CH: Switzerland. NR: not reported. Use types: Mixed1 – office, classroom, and commercial; Mixed2 – office and laboratory. Source water types – G: groundwater, S: surface water. Primary plumbing materials – C: copper, PVC: polyvinyl chloride, GS: galvanized steel, SS: stainless steel. [a] Water softening at building water inlet, varied hot water setpoint. [b] Laboratory-scale shower rig, no water use during closure. [c] Aerator removed prior to sampling. [d] Suboptimal hot water setpoint of 45 °C with two days per week at 60 °C. [e] Green building. [f] Approximate number of fixtures includes only the following potable fixtures: faucets in bathrooms and kitchens, drinking water fountains and bottle filling stations, and showers.

served as the standards for qPCR in all laboratories except Laboratory D, which used the proprietary standards included in the Bio-Rad kit. Efficiencies between 80% and 115% and R^2 values of at least 0.98 were required for all qPCR plates containing positive samples (Table S8†). Any plate that did not meet these criteria was re-run. For ddPCR, serial dilutions of synthetic DNA (gBlock, IDT)[59] were included on each plate, as were no-template controls (Table S7†). No-template controls were either negative or amplified at Cq values less than the LOD for each group.

The qPCR/ddPCR LOD and lower limit of quantification (LLOQ) were determined in each laboratory independently using serial dilutions of standards with at least 21 replicates across at least three plates, at concentrations ranging from 10^2 to 10^0 gc per reaction (Table S9†), except for Laboratory D which followed kit instructions. The qPCR/ddPCR LOD was defined as the lowest concentration at which at least 95% of the standard replicates were detected,[60] resulting in LODs ranging from 21 to 1×10^5 gene copies per liter (gc L^{-1}; 1 to 100 gc per reaction) across laboratories. The LLOQ was defined as the lowest concentration where the coefficient of variation was less than 25%,[62] resulting in LLOQs ranging from 3.5×10^2 to 2×10^5 gc L^{-1} (6.1 to 100 gc per reaction) across laboratories. Details of the conversion of LOD and LLOQ to gc L^{-1} are provided in eqn (S1).† Details of inhibition testing at each laboratory are provided in Text S1.†

Cross-laboratory validation of quantitative standards was performed using a single gBlock that was sent to all laboratories using the Nazarian *et al.* assay (Fig. S3†).[58]

2.7 Data analysis

Data analyses were performed using R (version 4.1.1) and RStudio (version 1.4.1717) using a custom R pipeline that included the packages *tidyverse*, *lubridate*, and *readxl*.[63,64] Because data were left-censored, imputation was performed, replacing results (physicochemical, qPCR/ddPCR, and Legiolert) less than the LOD with one half the LOD for plotting, calculating summary statistics, and non-parametric hypothesis testing. Legiolert results that were above the ULOQ were set at 30 000 MPN L^{-1} for non-parametric analysis and visualization. For qPCR/ddPCR data, values between the LOD and LLOQ were replaced with the average of the LOD and LLOQ to assign identical rank for non-parametric analyses. Samples were considered positive by qPCR/ddPCR if gene copy concentrations were above the LOD and quantifiable if concentrations were above the LLOQ. As data were non-normal (Shapiro–Wilk's, R function *shapiro.test*, $p < 0.01$), hypothesis testing was conducted using the non-parametric two-sample Wilcoxon rank sum test ("Mann–Whitney", R function *wilcox.test*) with a significance threshold of 0.05. The R package *stats* was used to calculate medians. Rank correlations were calculated using Kendall's tau-b using *cor.test*, which is well-suited for nonparametric, left-censored data.[65] Correlation analysis and principal component analysis (PCA) were performed using the R packages *vegan*, *Hmisc*, *GGally*, *forcats*, *corrplot*, *devtools*, and *ggbiplot*. The binomial generalized linear mixed-effects model used to investigate the relationship between *L. pneumophila* culture positivity, physicochemical parameters, and building characteristics was performed using *glmer*, with the input consisting of 112 samples from ten buildings. The equation used for model generation is provided in eqn (S2).† Figures were generated in R using the additional packages *ggPlot2*, *cowplot*, *scales*, *lubridate*, *repr*, *sf*, *rnaturalearth*, *maps*, *viridis*, *ggrepel*, and *ggnewscale*. The full R pipeline and associated data are available on GitHub (**https://github.com/kathdowd/Dowdell_and_Healy_2023**).

3. Results and discussion

3.1 *L. pneumophila* occurrence

3.1.1 *L. pneumophila* detection was building-specific. The overall *L. pneumophila* sample positivity was 34% (n = 88 of 258) by Legiolert and 35% (n = 48 of 138) by qPCR/ddPCR. However, *L. pneumophila* detection was limited to a few specific buildings and was heterogeneous among buildings within the same distribution system. Only seven of the 26 buildings yielded Legiolert-positive samples (IN-1, AZ-1, PA-1, QC-1, QC-3, MI-3, and CH-1; Fig. 1A), and the vast majority of positive samples (98%, n = 86 of 88) occurred in five buildings: four free chlorine buildings (IN-1, AZ-1, PA-1, QC-3) and the no residual building (CH-1). In these five buildings, the percentages of Legiolert-positive samples ranged from 65% to 100% (4–62 samples collected), and median *L. pneumophila* concentrations in Legiolert-positive samples ranged from 20 to >22 726 MPN L^{-1}. The highest concentrations of culturable *L. pneumophila* were observed in Buildings AZ-1 (median: >22 726 MPN L^{-1}) and QC-3 (median: 1198 MPN L^{-1}), both of which yielded at least one sample above the ULOQ (>22 726 MPN L^{-1}). Similar results were observed using qPCR/ddPCR: seven buildings had at least one positive sample (AZ-1, PA-1, QC-3, WV-1, WV-2, MI-1, CA-2), three of which (AZ-1, PA-1, QC-3) were also positive by Legiolert. The median positive sample concentrations in qPCR/ddPCR-positive buildings ranged from 158 to 3.1×10^6 gc L^{-1}, with the highest concentrations (>10^6 gc L^{-1}) occurring in Buildings WV-1, WV-2, and QC-3 (Fig. S4†).

There was heterogeneity in *L. pneumophila* occurrence among different buildings within the same distribution system. For instance, at Sites IN and QC, only one building yielded multiple Legiolert-positive samples while other buildings yielded either one or zero positive samples. Buildings that were positive for *L. pneumophila* tended to have multiple positive fixtures, which has also been commonly reported in the literature.[66–68] For example, though growth trends for *L. pneumophila* and *Legionella* spp. cannot be directly compared, a 2019 survey reported that buildings with *Legionella* spp. detection in the centralized hot water systems had an average distal positivity of 83% and had significantly higher average concentrations of *Legionella* spp. compared to buildings without *Legionella* spp. in the

centralized hot water system.[69] In that same study, the majority of systems with at least one positive sample showed *Legionella* spp. positivity rates of at least 30% at distal sites. It should be noted that distal site positivity does not necessarily predict incidence of Legionnaires' disease, which also depends on factors such as exposure route and population susceptibility.[70,71]

3.1.2 Building-specific features may have shaped *L. pneumophila* positivity. The buildings with multiple Legiolert-positive samples had identifiable building plumbing operational and/or design deficiencies that did not align with industry recommended practices. Building IN-1 (Legiolert positivity: 75% [n = 3 of 4]; qPCR positivity not measured) is a large, single-family home converted to a multi-family residence with an oversized water heater (100-gal heater with 115-gal insulated holding tank). Building AZ-1 (Legiolert positivity: 100% [n = 7 of 7]; qPCR positivity: 100% [n = 7 of 7]) uses a whole-building water softening system that treats water upon entry into the building. Samples collected from the water entry point revealed that the inlet water was Legiolert-negative and contained a chlorine residual of 0.59 mg L^{-1} as Cl_2,[53] whereas the sample from the water storage tank immediately after the softener was positive for *L. pneumophila* by both Legiolert (10 616 MPN L^{-1}) and qPCR (650 gc L^{-1}) and had a low chlorine residual (0.05 mg L^{-1} as Cl_2). These results indicate that residual disinfectant loss was occurring in the water softener and storage tank. Previous investigations in this building found that the softener was oversized for building water demand, leading to high water residence times in the softener.[72,73] Additionally, AZ-1 is a green building (LEED platinum-certified) equipped with low-flow fixtures. Buildings with low-flow water fixtures have previously been linked to high water residence times and, in certain cases, *L. pneumophila* occurrence.[12,72,73] Building PA-1 (Legiolert positivity: 94% [n = 17 of 18]; ddPCR positivity: 67% [n = 12 of 18]) is a laboratory-scale experimental shower rig consisting of three water heaters, each of which supplies three showers. The water heaters were operated at 49 °C and thermostatic mixing valves limited water temperature to 40 °C at the fixtures. After four months of no water use (while still maintaining the 49 °C heater set point), the median *L. pneumophila* concentration in first-draw samples was 66 MPN L^{-1}. Building QC-3 (Legiolert positivity: 83% [n = 19 of 23]; qPCR positivity: 91% [n = 21 of 23]) is a large sports building with a complex hot water system, which includes four water heaters in series. Issues identified included that a portion of the returned hot water was mixed with the hot water exiting the water heaters, resulting in a decrease of the distributed hot water temperature to below 60 °C; a single thermostatic mixing valve for many showerheads (>20) created large volumes of tepid water; and the third water heater was found to be off but still connected to the system, resulting in cooling of hot water to 30 °C.[51] Building CH-1 (Legiolert positivity: 65% [n = 40 of 62]; qPCR positivity: not measured) is a mixed-use building that was historically culture-positive for *L. pneumophila* and fluctuates the water heater set point between 45 °C (5 days per week) to 60 °C (2 days per week) to save energy.[56] Building CH-1 also reported significant heat losses in the primary recirculating line and passive recirculation on individual floor loops, leading to suboptimal temperatures for control of *L. pneumophila*.

3.1.3 *L. pneumophila* occurrence varied by secondary disinfectant type. Five free chlorine buildings yielded multiple Legiolert-positive samples, whereas only a single sample was Legiolert-positive from the chloramine buildings. Thus, the positive buildings strongly influenced the overall sample positivity by secondary disinfectant residual, resulting in free chlorine samples having a substantially higher Legiolert-positivity (37%, n = 47 of 127) than samples collected from chloramine buildings (1.4%, n = 1 of 69, Fig. 1B). Culturable *L. pneumophila* results were also significantly higher in free chlorine samples compared to chloramine samples ($p < 0.01$). The median *L. pneumophila* concentration in the Legiolert-positive samples from the free chlorine buildings was 126 MPN L^{-1}. The concentration measured in the single Legiolert-positive sample from the chloramine buildings was 133 MPN L^{-1}. Similar trends were observed in qPCR/ddPCR-positive samples, where positivity was 52% in free chlorine building samples (n = 45 of 86) and 6% in chloramine building samples (n = 3 of 52, Fig. S5†). Median concentrations in qPCR/ddPCR-positive samples were 5.3×10^3 gc L^{-1} (n = 45) in the free chlorine buildings and 1.1×10^5 gc L^{-1} (n = 3) in the chloramine buildings.

Drinking water systems using chloramine for secondary disinfection have previously been reported to have lower *L. pneumophila* occurrence and concentrations compared to free chlorine,[15,68,74–76] even in buildings with reduced occupancy.[45] This study supports these findings, as only one sample collected from chloramine systems was culture-positive for *L. pneumophila*. The low rate of *L. pneumophila* occurrence in chloramine systems was maintained despite 41% of the samples containing little to no chlorine residual (≤0.1 mg L^{-1} as Cl_2; n = 28 of 69). One reason for lower *L. pneumophila* occurrence in chloramine systems may be that monochloramine has been reported to better penetrate biofilms,[77–80] which could result in enhanced inactivation of *L. pneumophila* within drinking water system biofilms. Other possible explanations for lower *L. pneumophila* occurrence in chloramine systems include that chloramine can be more stable in building water systems compared to free chlorine, though the presence of nitrification can reduce chloramine stability.[77] Chloramine may also more efficiently inactivate *L. pneumophila* and its amoebal hosts compared to free chlorine, and one study proposed that chloramine may trigger encystment of amoebae, preventing *L. pneumophila* infection.[81–83] Regardless of the mechanism, these results suggest that drinking water systems that use chloramine for secondary disinfection may better control *L. pneumophila*. However, most buildings cannot choose which residual disinfectant is supplied in the water, and there are other concerns with monochloramine that should be considered when evaluating secondary disinfectants, such as the

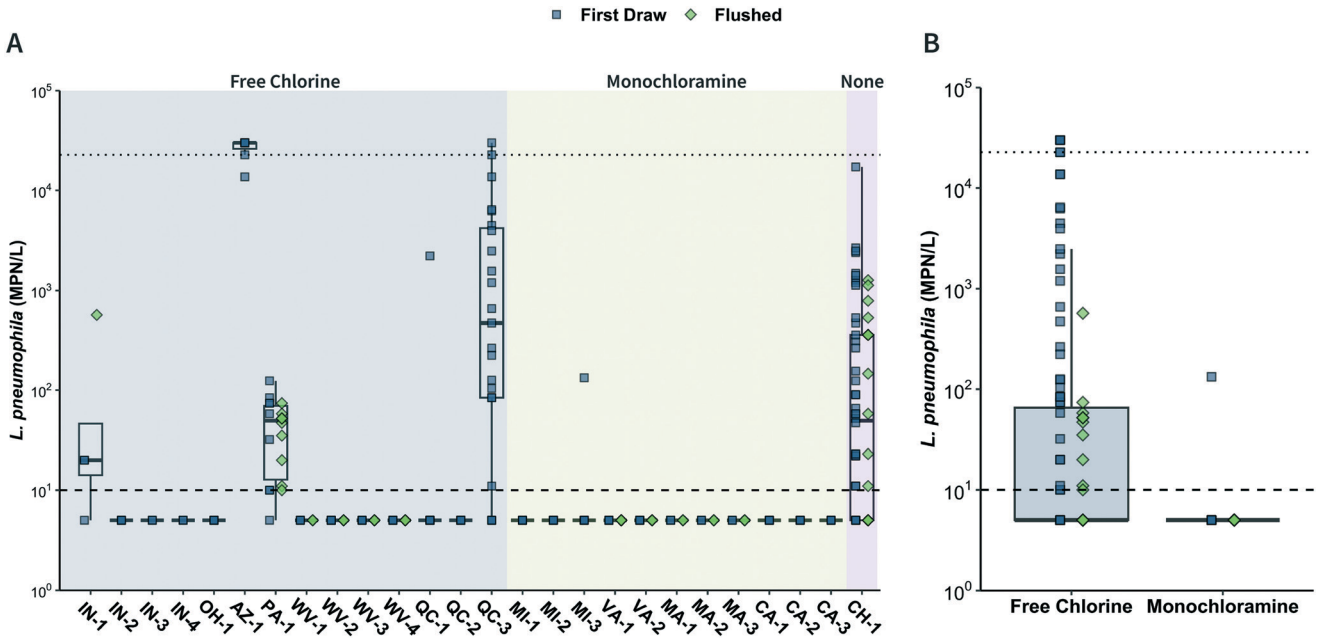

Fig. 1 Culturable *Legionella pneumophila* concentrations (MPN L^{-1}) by (A) building and (B) secondary disinfectant type. Marker color represents flush condition, where blue circles are first-draw samples and green circles are flushed samples. The dashed horizontal line indicates the LOD (10 MPN L^{-1}) and the dotted horizontal line indicates the ULOQ (22 726 MPN L^{-1}). Results below the LOD are plotted at one-half the LOD (5 MPN L^{-1}). Results above the ULOQ are plotted as 30 000 MPN L^{-1}.

potential for nitrification, formation of unregulated disinfection byproducts, and growth of other opportunistic pathogens.[84–86]

While samples obtained from the no residual building (CH-1) exhibited the highest Legiolert-positivity (65%, n = 40 of 62), this building was previously reported to contain *L. pneumophila* (*i.e.*, when water demand was typical).[87] The few previous studies that have examined *L. pneumophila* in distribution systems without a disinfectant residual generally report low prevalence of *L. pneumophila*,[59,88–91] though there have been reports of *L. pneumophila* occurrence in some building hot water systems.[92,93] A study conducted prior to the pandemic reported low occurrence of *L. pneumophila* in Dutch buildings, which were required to flush weekly.[89] Additional studies are needed to evaluate the impact of low water use resulting from the COVID-19 pandemic on buildings in distribution systems without residual disinfection.

3.2 Impacts of physicochemical parameters and flushing

3.2.1 Secondary disinfectant concentration influenced presence and concentrations of *L. pneumophila* in systems with residual disinfection. Despite reduced occupancies in the buildings and the limited use of water quality mitigation measures (Tables 1 and S1†), 69% (n = 101 of 146) of the first-draw samples analyzed for chlorine (from systems with residual disinfection) contained a detectable chlorine residual (≥0.02 or ≥0.05 mg L^{-1} as Cl_2) but the median concentrations were low (free chlorine buildings: <0.05 mg L^{-1} as Cl_2; chloramine buildings: 0.13 mg L^{-1} as Cl_2; Fig. S6A†). First-draw samples with detectable chlorine residuals were observed in buildings regardless of reported occupancy or preventative maintenance practices. Detectable chlorine in first-draw samples may be a result of water age being lower than expected due to water demand from leaks, maintenance activities, air conditioning systems or cooling towers, and/or operation of treatment systems (*e.g.*, water softeners that automatically regenerate). Though occupancy was low, recent use of fixtures may also have contributed to the presence of residuals, since access to study fixtures was not restricted at all sites prior to sampling. Flushed samples tended to have higher median residuals (free chlorine: 0.05 mg L^{-1} as Cl_2; chloramine: 0.88 mg L^{-1} as Cl_2; Fig. S6A†). The finding that many samples contained measurable disinfectant residuals differs from other investigations of COVID-19-related building closures, which have reported that measurable disinfectant residuals were lacking in most samples.[45,49] This difference in findings is likely linked to tap use prior to sampling.

L. pneumophila positivity in disinfected samples generally decreased with increasing disinfectant residual (Fig. 2A, S7 and S8†). In free chlorine buildings, approximately 80% (78%, n = 35 of 45 by Legiolert; 82%, n = 37 of 45 by qPCR/ddPCR) of *L. pneumophila* positive samples occurred when the chlorine residual was ≤0.1 mg L^{-1} as Cl_2 (Fig. S7C and D†). Only three free chlorine samples were Legiolert-positive with chlorine residuals above 0.2 mg L^{-1} as Cl_2, and the highest chlorine residual in the Legiolert-positive samples was 0.4 mg L^{-1} as Cl_2. The single Legiolert-positive sample from a chloramine building had a chlorine residual below the detection limit (<0.05 mg L^{-1} as Cl_2). However, the other

27 chloramine samples that contained little to no chlorine residual (≤0.1 mg L^{-1} as Cl_2) were Legiolert-negative.

The question of what concentrations of residual disinfectant are required to control *L. pneumophila* in building water systems is one of pressing importance for public health. In this study, only 7% (n = 3 of 45) of samples collected from free chlorine systems that contained free chlorine concentrations above 0.2 mg L^{-1} as Cl_2 were culture-positive for *L. pneumophila*. This finding is similar to the results from other studies, which have suggested that free chlorine concentrations ranging from 0.1 to 0.4 mg L^{-1} as Cl_2 may control *Legionella* spp. or *L. pneumophila* in distribution systems and building plumbing.[30,94–96] However, at least one study has reported no relationship between building water system free chlorine concentration and *Legionella* spp. occurrence.[69] While higher concentrations of chloramine have been found to decrease the occurrence of *L. pneumophila*, the concentration required for prevention of *L. pneumophila* varies in the literature.[30,75,94,97,98] The relationship between *L. pneumophila* occurrence and chloramine concentrations could not be characterized in this study due to the low overall positivity in chloramine systems. Additional studies are needed to elucidate the factors that influence the relationships between residual disinfectants and *L. pneumophila* to identify concentrations that may prevent occurrence.

3.2.2 Sample temperature and pH were not associated with *L. pneumophila* culture-positivity. Of the samples where temperature was measured (n = 182), first-draw sample (n = 146) water temperatures were similar to ambient building temperatures regardless of their source (hot, cold, or mixed), with an overall median of 25 °C (Fig. S6C†). Only 19% (n = 4 of 21) of cold water first-draw samples were below 20 °C (median: 23 °C), and only 5% (n = 2 of 39) of hot water first-draw samples were above 50 °C (median: 22 °C, Fig. 2C). In paired first-draw and flushed samples where temperature was recorded (n = 28 pairs), flushing significantly changed the water temperature, reducing the cold water temperatures and increasing hot and mixed water temperatures ($p < 0.05$, Fig. S9†).

Overall, 46 samples with corresponding temperature measurements were Legiolert-positive, including one cold water sample, five hot water samples, and 40 mixed water samples. Among these samples, temperatures ranged from 22 °C to 43 °C (median: 29 °C), which is within the suitable growth range for *L. pneumophila* (20 °C to 45 °C).[67,99,100] Within these samples there was no apparent trend between temperature and *L. pneumophila* occurrence or concentration (Fig. 2C). Culture-negative samples occurred at all temperatures measured (n = 136, 14 °C to 54 °C) and sample temperature types (hot, cold, and mixed). The majority of Legiolert-negative samples (82%, n = 111 of 136) were also at temperatures within the *L. pneumophila* suitable growth range. In samples analyzed using qPCR/ddPCR for which temperature was recorded (n = 130), the temperature in the positive samples (n = 48) ranged from 20 °C to 43 °C and included only hot (n = 10) and mixed (n = 38) water samples. In qPCR/ddPCR-negative samples (n = 82), temperatures ranged from 17 °C to 52 °C (Fig. S10†). Concentrations of *L. pneumophila* above 1×10^6 gc L^{-1} occurred in three samples, which were all at approximately room temperature (20 °C to 26 °C). These samples included two hot water samples from Buildings WV-1 and WV-2 and one mixed water sample from Building QC-3.

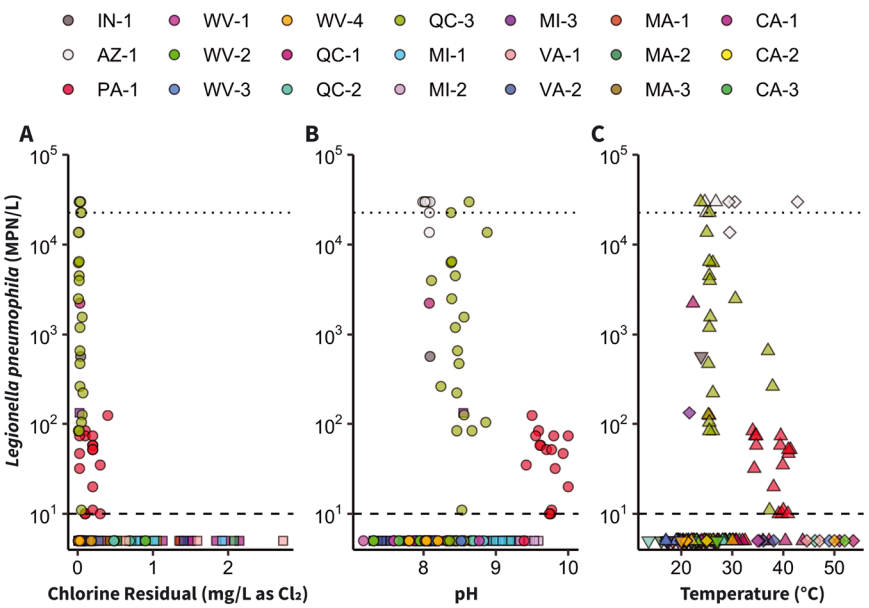

Fig. 2 Physicochemical parameters and culturable *L. pneumophila* (MPN L^{-1}) for samples by building. A) Chlorine residual (mg L^{-1} as Cl_2), B) pH, and C) temperature. For A and B, marker shape represents secondary disinfectant type, with circles being free chlorine and squares being monochloramine. For C, marker shape shows the water temperature type, with upside down triangles being cold water samples, diamonds being hot water samples, and triangles being hot and cold water mixed. The dashed line shows the LOD. The dotted line shows the ULOQ. Buildings CH-1, IN-2, IN-3, IN-4, and OH-1 are excluded because physicochemical parameters were not recorded.

It is recommended that hot water temperatures be at least 60 °C in water heaters and 55 °C at distal points to control *L. pneumophila*.[30,101] In this study, only four of the 52 hot water samples were at temperatures of at least 50 °C, and none were at or above the recommended temperature of 55 °C. Additionally, the median first-draw hot water temperature was 22 °C. *L. pneumophila* was not detected in samples with temperatures above 43 °C. The temperature range of *L. pneumophila* positive samples (20 °C to 43 °C) is in alignment with previous studies, which reported that temperatures between 20 °C and 40 °C were associated with increased *L. pneumophila* occurrence.[96,102,103] However, observed water temperature at distal fixtures may not always be helpful in predicting *L. pneumophila* concentrations because distal sampling locations acclimate to ambient temperature.[69,95,104] Therefore, approaches that incorporate whole-building temperature profiling may be more effective for assessing the influence of hot water temperature on *L. pneumophila*.[99] Intermittent heating, and other energy-saving methods have previously been linked to lower water qualities due to decreased hot water temperatures in building plumbing.[105,106] Consistent with this, two buildings with multiple culture-positive samples (CH-1 and AZ-1) practiced hot water temperature fluctuation for energy savings.

Additional physicochemical parameters, including pH (Fig. 2B and S11†), dissolved oxygen (Fig. S12†), and electrical conductivity (Fig. S13†) were measured in a subset of samples (Tables S4 and S10†). Sample pH in chloramine buildings (median: 9.1, n = 69) was higher than the pH of samples from free chlorine buildings (median: 8.2, n = 112; Fig. 2B and S6B†), most likely because higher pH is a strategy to reduce nitrification, which can lead to diminished chloramine residual, nitrate/nitrite formation, and reductions in pH and dissolved oxygen.[107] pH values in Legiolert-positive samples ranged from 8.0 to 10.0. Though the median pH was higher in Legiolert-positive samples (8.6, n = 46) than in Legiolert-negative samples (8.3, n = 135), pH tended to co-vary by building. In paired samples where pH was measured (n = 27), pH was significantly different between first-draw and flushed samples ($p < 0.05$). pH may influence the occurrence of *L. pneumophila* in distribution systems and building water,[108] though one laboratory study reported that *L. pneumophila* can grow in water with pH values ranging from 5.5 to 9.2.[103] A 2021 cooling tower study suggested that high pH (9.6) may be a technique for *L. pneumophila* control, though the authors noted this may be related to a reduction in protozoan hosts at high pH.[109] Ultimately, additional research is needed to investigate the pH sensitivity of *L. pneumophila* alone and within amoebae.

3.2.3 Fixture flushing did not consistently reduce *L. pneumophila* occurrence. The impact of flushing during sampling on *L. pneumophila* was investigated using paired samples from 48 fixtures (n = 96 samples from 12 buildings). While flushing (for two to 30 minutes, median: five minutes) tended to increase disinfectant residual (Fig. S14†) and shift water temperatures toward cold influent and hot water set points (Fig. S9†), the impact of flushing on Legiolert-positivity varied by fixture: 24 pairs were Legiolert-negative in both first-draw and flushed sample, 18 were positive in both, three fixtures went from negative to positive (PA-1 and CH-1), and three fixtures went from positive to negative with flushing (CH-1; Fig. S15†). With qPCR/ddPCR, nine fixtures were negative in first-draw and flushed samples, three fixtures were positive in both samples (PA-1), four fixtures went from negative to positive (PA-1 and WV-1), and four went from positive to negative (in PA-1 and WV-2; Fig. S16†). When only considering Legiolert-positive pairs (n = 18 pairs from three buildings [PA-1, CH-1, and IN-1]) flushing did not significantly change the concentration of culturable *L. pneumophila* (median decrease of 27 MPN L^{-1} with flushing, p = 0.24). However, at individual sampling locations, flushing was also observed to increase *L. pneumophila* concentrations as much as 549 MPN L^{-1} (IN-1) and decrease concentrations by as much as 1.6×10^4 MPN L^{-1} (CH-1). In the three qPCR/ddPCR-positive sample pairs (PA-1), the impact of flushing on *L. pneumophila* gene copies was similarly mixed (*L. pneumophila* concentrations increased in one fixture and decreased in two).

Most guidance on *L. pneumophila* prevention and building recommissioning recommends flushing to introduce fresh water from the distribution system into building plumbing.[110–115] Recommendations typically include volume- or water-quality-based flushing, usually of outlets at specific locations (*e.g.*, dead-ends) or the full building water system.[110,111,116] Previous studies have reported that flushing that is not performed systematically or that does not consider building plumbing characteristics may be ineffective for improving building water quality.[117,118] In practice, a variety of flushing strategies may be employed, which was observed in the reported water management practices of the study buildings. Only two buildings (MI-1 and MI-2) of the 17 buildings for which additional information was provided reported regularly flushing building plumbing while occupancy was restricted (Table S1†). Of these buildings, MI-1 tended to have higher first-draw disinfectant residuals (median: 0.8 mg L^{-1} as Cl_2, n = 6) than the disinfectant residuals in first-draw samples from other chloramine buildings (median: 0.09 mg L^{-1} as Cl_2, n = 48). Interestingly, samples from MI-1 were among the only samples from chloramine buildings where *L. pneumophila* was detected by qPCR, though the results were below the LLOQ (Fig. S4†). None of the samples from MI-2 (n = 7) contained measurable concentrations of disinfectant. While water management practices varied by building, only one (OH-1, of n = 21 respondents) reported having a formal building water management plan that predated the pandemic and one (CH-1) reported a pre-existing informal management plan. Though the effectiveness of flushing for reducing *L. pneumophila* concentrations was mixed, this study was not designed to specifically investigate flushing strategies and additional work is needed to determine optimal flushing methods after extended stagnation.

3.2.4 Fixture type was not associated with differences in *L. pneumophila* occurrence. Showers and other fixtures that generate droplets and aerosols are of particular concern for opportunistic pathogen respiratory infections because inhalation is the primary route of exposure.[119–121] Among showers and manual faucets, the primary fixture types in this study, overall culture-positivity was similar (showers: 38%, n = 38 of 101; manual faucets: 34%, n = 47 of 139). By qPCR/ddPCR, showers were 45% positive (n = 38 of 84) and manual faucets were 11% positive (n = 4 of 36). However, the distribution of fixture types sampled was not uniform across buildings. Positivity in electronic faucets (n = 13 samples across four buildings) was 23% by Legiolert (n = 3, AZ-1) and 46% by qPCR (n = 6, AZ-1 and WV-1). Electronic faucets and other water-saving fixtures have been previously linked to *Legionella* spp. occurrence.[12,14,105,122,123]

3.2.5 Source water type was not associated with differences in *L. pneumophila* occurrence. Source water type is another factor that might relate to *L. pneumophila* occurrence.[124] This study included 16 samples from buildings receiving treated groundwater (n = 5 buildings, Sites IN and OH), 136 samples from buildings receiving treated surface water (n = 15 buildings, Sites WV, QC, VA, MA, and CA), and 106 samples from buildings receiving mixed groundwater and surface water (n = 6 buildings, Sites AZ, PA, MI, and CH). The sites using groundwater received water with free chlorine as the secondary disinfectant. The surface water and mixed source water sites included systems using free chlorine and chloramine. Median concentrations of culturable *L. pneumophila* by source water type were below the method LOD (<5 MPN L^{-1}) for groundwater and surface water and 23 MPN L^{-1} for mixed source water (Fig. S17†). Nineteen percent (n = 3 of 16) of samples from groundwater systems and 15% of surface water samples (n = 20 of 136) were culture-positive for *L. pneumophila*, while culture positivity was 61% (n = 65 of 106) in mixed source water samples. Among surface water samples from free chlorine systems (n = 86), which included samples from Sites QC and WV, the median concentration was below the LOD (<10 MPN L^{-1}). For mixed water samples from free chlorine systems (n = 25), which included samples from Sites AZ and PA, the median was 58 MPN L^{-1}. Municipal drinking water systems using groundwater have previously been reported to have lower concentrations of *Legionella* spp.[59] and to be less associated with legionellosis outbreaks compared to those using surface water sources.[124] In contrast to previous studies, samples from groundwater and surface water systems in this study contained similar concentrations of *L. pneumophila*, with mixed source water having the highest *L. pneumophila* positivity. However, as this study was not designed to specifically test the influence of source water, differences between the groups, such as sample size, disinfectant type, and building-specific characteristics, prevented a more thorough evaluation of the role of source water type in *L. pneumophila* occurrence. Findings from this study and others support the need for additional investigation of the potential influence of source water type on *L. pneumophila* in drinking water systems.

3.2.6 Combined effects of quantitative physicochemical and building parameters in *L. pneumophila* occurrence. To investigate the impact of building characteristics and physicochemical parameters on *L. pneumophila* culture-positivity in free chlorine buildings, a binomial generalized linear mixed model (glmm) was developed. The model was developed using free chlorine samples from 10 buildings where associated physicochemical measurements and building characteristics were available (n = 112) because disinfectant type has been previously shown to influence *L. pneumophila* occurrence.[68,74,125] The model included the physicochemical parameters pH, temperature, and free chlorine concentration and the building-specific parameter building age as fixed effects; these parameters were chosen based on measured factors previously reported to influence *Legionella* spp. occurrence.[66,89,126–128] To account for clustering by building, building identity was included in the model as a random effect. This analysis showed that none of the parameters were significantly associated with *L. pneumophila* positivity ($p > 0.05$, Table S11†). While it was observed that culture positivity was significantly associated with lower disinfectant residuals overall, when looking at the relationship between culture positivity and disinfectant residual within specific buildings, this trend was not observed, likely due to building-specific factors, as discussed in section 3.1.2. Though no parameters met the significance threshold, pH was the model input with the greatest effect on the probability of *L. pneumophila*-positivity (p = 0.14, odds ratio: 10.9; see eqn (S2)† for equation). A principal component analysis (PCA) was also used to investigate the impact of multiple parameters on *L. pneumophila* occurrence. *L. pneumophila* positivity again could not be fully explained by the quantitative variables included, but most Legiolert-positive samples clustered in a region representing lower chlorine concentrations (Fig. S18†).

3.3 *L. pneumophila* quantification with Legiolert *versus* qPCR/ddPCR

L. pneumophila was quantified in samples from 17 buildings using qPCR (n = 120) and one building using ddPCR (PA-1; n = 18). Of these samples, 35% (n = 48 of 138) were positive by qPCR/ddPCR and 34% (n = 88 of 258) were positive using Legiolert, with an 87% (n = 120 of 138) agreement between qPCR/ddPCR and Legiolert (positive by both methods: n = 37; negative by both methods: n = 83). The 13% (n = 18 of 138) of samples where there was disagreement between the two methods included 11 samples (8%) that were positive only by qPCR/ddPCR (PA-1, QC-3, WV-1, WV-2, MI-1, CA-2) and seven samples (5%) that were positive only by Legiolert (PA-1 and MI-3). The samples that were only positive by Legiolert exhibited low concentrations (median: 32 MPN L^{-1}), and all but one (n = 6 of 7) were collected from PA-1 and analyzed using ddPCR, indicating that the ddPCR LOD and/or DNA extraction recovery efficiency may have impeded detection. Samples positive only by Legiolert (5% of samples) could

have been false positives, which have been observed with rates ranging from 3–4% in previous studies.[129–131] However, because confirmation testing of positive wells was not performed, the extent to which these samples were false positives is unclear. The majority of samples that were positive only by qPCR/ddPCR (n = 9 of 11) were quantifiable (above the LLOQ), spanning the full range of *L. pneumophila* concentrations (10^2 to 10^6 gc L^{-1}, Fig. 3A), indicating the presence of dead or non-culturable *L. pneumophila*.

Samples positive and quantifiable by both methods had a median 0.72 log more *L. pneumophila* measured by qPCR/ddPCR compared to Legiolert (n = 31, Fig. 3B). However, seven samples that were positive by both methods yielded higher results with Legiolert than qPCR/ddPCR (median difference: 0.32 log). Linear regression analysis of the quantifiable samples resulted in a line with a slope of 0.74 and y-intercept of 0.07 (R^2 = 0.47, Fig. 3B). This analysis was used to estimate the relationship between qPCR/ddPCR and Legiolert, but, as shown in Fig. 3B, this relationship may be influenced by location-specific characteristics. Among all samples analyzed by qPCR and Legiolert, there was a negative correlation (Kendall's tau = −0.277, $p < 0.001$, n = 138), likely due to the way non-quantifiable values were substituted. When only the quantifiable data are considered, the correlation is stronger and positive (Kendall's tau = 0.493, $p < 0.001$, n = 31).

Previous studies have reported higher *Legionella* spp. percent positivity by qPCR than culture-based methods, attributing this difference to higher sensitivity of DNA-based methods and the inclusion of cells that are non-viable and/or non-culturable.[132–134] However, the relatively high LODs of some of the laboratories (100 gc per reaction, translating to up to 10^5 gc L^{-1}; Table S9†), as well as losses during concentration and extraction, may have prevented the detection of low concentrations of *L. pneumophila* DNA in some samples. The higher concentrations observed with qPCR/ddPCR compared to Legiolert agree with previous studies, which have reported that qPCR results were 0.5 to 3 log higher than the corresponding culture-based results.[43,94,133] The correlations between quantifiable samples (Kendall's tau = 0.493; linear regression slope = 0.74; Fig. 3B) were similar to those reported in previous studies comparing qPCR to culture-based methods.[134,135]

Action levels for *L. pneumophila* are primarily based on plate culture methods, such as the International Organization for Standardization (ISO) method 11 731, which yield results in colony forming units (CFU) per volume.[30] However, liquid culture and DNA-based methods like those used in this study are increasingly being used for environmental monitoring due to their ease of use and reduced time to results.[42–44] *L. pneumophila* action levels that have been proposed by various publications and organizations vary widely: risk-based limits vary from 14.4 CFU L^{-1} (disability adjusted life year, 10^{-6} DALY target) to 1410 CFU L^{-1} (10^{-4} annual probability of infection target) in non-healthcare showers and from 12.3 CFU L^{-1} to 4670 CFU L^{-1} for total building water fixture exposures;[136] 1000 CFU L^{-1} is recommended by the European Working Group for *Legionella* Infections (EWGLI);[137] and

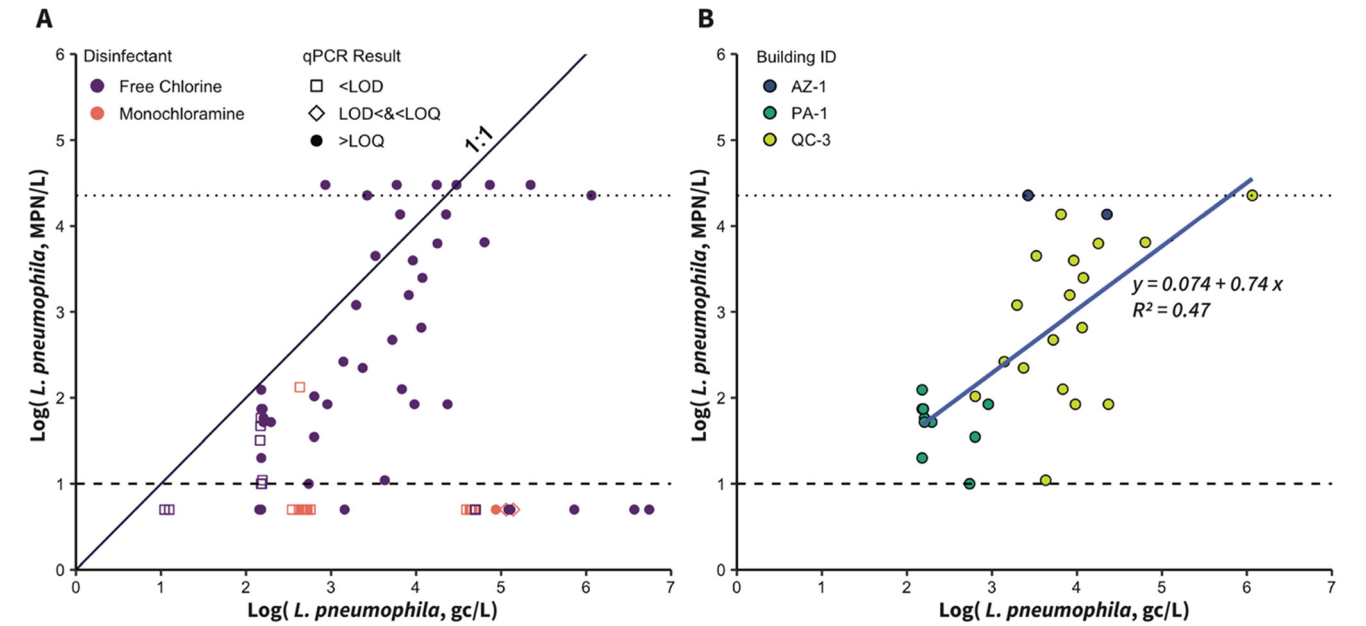

Fig. 3 Culturable *L. pneumophila* results (log(MPN L^{-1})) *versus* qPCR/ddPCR results (log(gc L^{-1})). A) Results for all samples analyzed by both Legiolert and qPCR/ddPCR (n = 138). The solid black diagonal line has a slope of 1 for reference. Samples that were below the LOD were plotted at one half the LOD (open squares). qPCR/ddPCR samples between the LOD and LLOQ were plotted as the average of the LOD and LLOQ (open diamonds). Samples above the LLOQ were plotted as filled circles. Results above the Legiolert ULOQ were plotted at 30000 MPN L^{-1}. B) Results for the subset of samples that were quantifiable by both methods (n = 31). The blue line is the linear regression. The dashed horizontal line indicates the Legiolert LOD (10 MPN L^{-1}) and the dotted horizontal line indicates the Legiolert ULOQ (22 726 MPN L^{-1}).

health outcome-based levels are as high as 50 000 CFU L^{-1}.[30] To place MPN and gene copy results in a health context given existing standards, they must be converted to approximate CFU. Previous studies have found MPN to be equivalent to approximately 1.2 times CFU concentrations.[129,138] Using this approximation, the number of samples in the current study that would exceed action thresholds ranged from 33 samples from five buildings using the adjusted Hamilton *et al.* 14.4 CFU L^{-1} threshold,[136] 25 samples from four buildings if using the adjusted EWGLI 1000 CFU L^{-1} threshold,[137] and up to three samples (samples above the Legiolert ULOQ) when using the adjusted 50 000 CFU L^{-1} threshold.[30] It should be noted, however, that none of the buildings included in the study were associated with any legionellosis infections. As suggested by others, a combination of routine monitoring using DNA-based methods to monitor trends and focused investigations with culture-based methods when issues arise may be ideal for balancing cost and public health goals.[30] Additional studies that compare the performance of these methods in environmental matrices and realistic building management scenarios and relate the results to health outcomes and risk assessment are needed to bridge the gap between results and action limits.

3.4 Limitations and future work

Although this study represents the most expansive drinking water sampling campaign conducted during the COVID-19 pandemic, the study sample size is limited and, therefore, cannot represent conditions in all non-residential buildings. Additional limitations of this study include that most sites were not monitored for *L. pneumophila* prior to COVID-19 pandemic building closures, which prevented investigation of how *L. pneumophila* concentrations changed over time with reduced water use in buildings. Differences in sample analysis methods between laboratories may also influence results, particularly the use of different DNA extraction methods, which have been shown to influence recovery of total DNA.[139–141] Detailed water use data were also only available for nine of the buildings. Also, other water quality issues that can result from low water use, such as occurrence of other opportunistic pathogens, metals, and disinfection byproducts, were not assessed in this study.[45,46,49,142] Additional work is needed to fully characterize the impact of reduced water use periods on building water quality and inform how buildings can prevent or react to reduced water use.

4. Conclusions

Significant attention was paid by researchers to the potential public health threat posed by exposure to *L. pneumophila* in building water systems during COVID-19 pandemic-related building closures.[24,26] This study found that in 20% of the large, low occupancy buildings sampled, building water contained significant concentrations of culturable *L. pneumophila* ($>10^2$ MPN L^{-1}). However, building water samples from the majority of sites did not contain substantial concentrations of *L. pneumophila*. This study is unique in that it included data from geographically and structurally diverse buildings in three countries. Results from smaller studies that have investigated the impact of the COVID-19 pandemic on *L. pneumophila* in building water have ranged from widespread contamination[47] to little to no occurrence.[45] This study reflects the range of results reported in the literature, as studies focused on only one or a few of the selected sites would yield vastly different results. Similar to previous reports, the choice of secondary disinfectant was the factor most strongly associated with sample positivity, with the overwhelming majority of *L. pneumophila* positive samples collected from buildings receiving drinking water disinfected with free chlorine compared to monochloramine. Heterogeneity was also observed between buildings within the same free chlorine distribution systems, indicating that building-specific factors, in addition to secondary disinfectant selection, impacted which buildings contained substantial concentrations of *L. pneumophila*. While the impact of COVID-19 pandemic building closures on legionellosis rates remains unclear, this study highlights that large buildings that receive chlorinated water and have building plumbing characteristics or management practices that are not in-line with best practices may be particularly vulnerable to *L. pneumophila* occurrence during low flow periods.[143–145]

Author contributions

WJR, KJP, EG, AJW, LR, CRP, MP, AP, KLN, KAH, and SJH: conceptualization and funding acquisition. KSD, HGH, SJ, MGC, SP, YS, CL, LCK, SV and LH: sample collection and analysis. KSD, HGH, and WJR: data compilation, analysis, and manuscript preparation. All authors: review and editing.

Conflicts of interest

IDEXX Laboratories, Inc. provided 140 free Legiolert tests and additional kits at a discount for this study. IDEXX Laboratories, Inc. did not contribute to the design, analysis, or writing of this manuscript. The interpretation of results and opinions presented in the manuscript are those of the authors and do not necessarily reflect those of IDEXX Laboratories, Inc.

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