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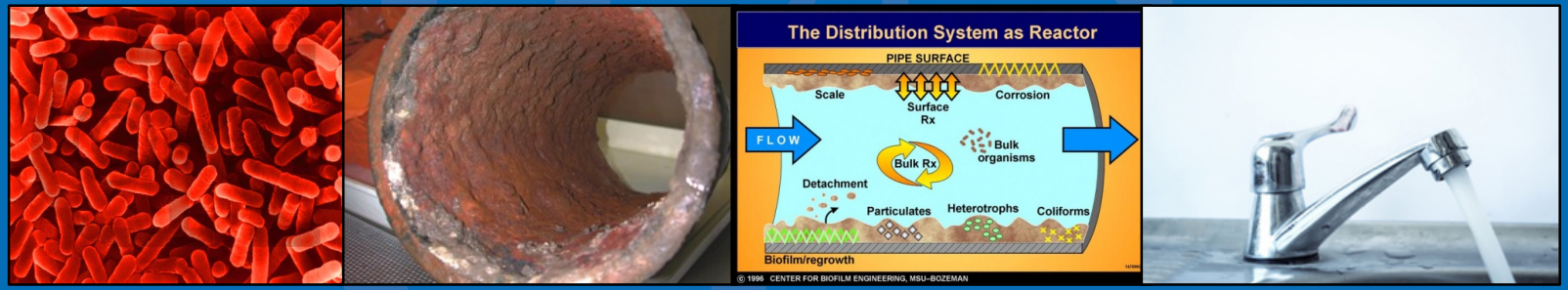
Water Quality and Microbial Dynamics in a Large Building Hot Water System and Managing Potential Risk Associated with *Legionella*

V. Gomez-Alvarez, M. Berberich†, L. Boczek, D. King, A. Pemberton†, S. Pfaller, M. Rodgers, J. SantoDomingo and R. P. Revetta

U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH 45268; †Pegasus Technical Services, Inc. c/o US EPA, Cincinnati, OH 45268



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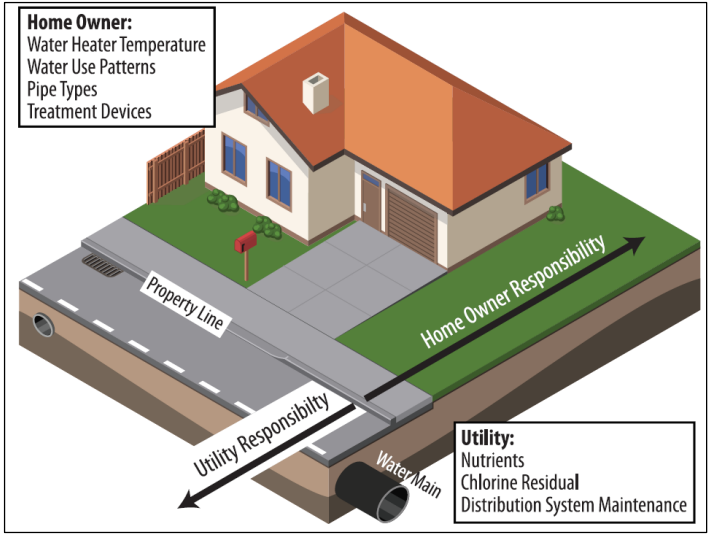
Why Premise Plumbing?

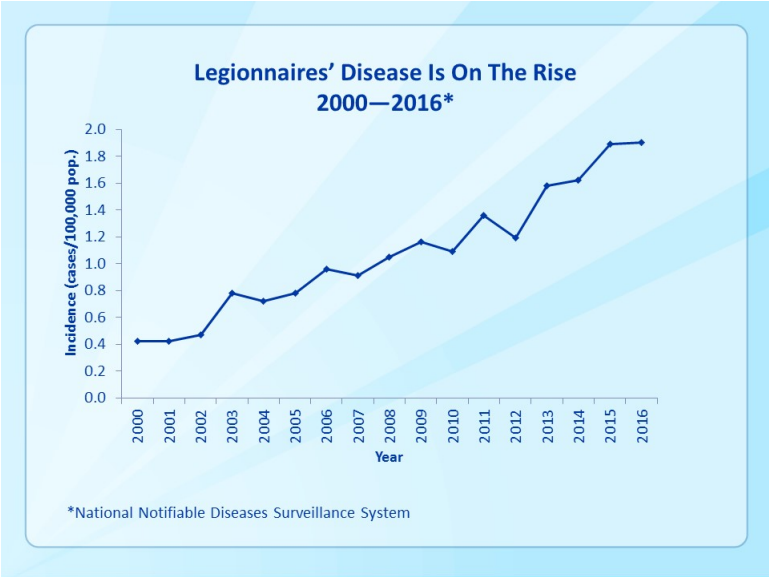
Premise plumbing (PP) includes that portion of the drinking water distribution system (DWDS) connected via the service line to houses and other buildings.



Current delineation of public versus private responsibility with respect to the DWDS (Adapted from "Pathogens in Premise Plumbing" by Water Research Foundation 2014, *AWR*, **24**(1):24).

- *Public health data shows that a significant fraction of the nation's* ***waterborne disease outbreaks*** *are attributable to PP systems.*
- *It is important to understand the characteristics of these systems which amplify the* ***potential public health risk*** *relative to the DWDS.*

Why *Legionella*?



In the United States, reported cases of Legionnaires' disease have grown by nearly four and a half times since 2000. (From the National Notifiable Diseases Surveillance System (CDC) https://www.cdc.gov.

Legionella pneumophila

It is unclear whether this increase represents:

- artifact (due to increased awareness and testing)
- increased susceptibility of the population
- increased *Legionella* in the environment
- or some combination of factors

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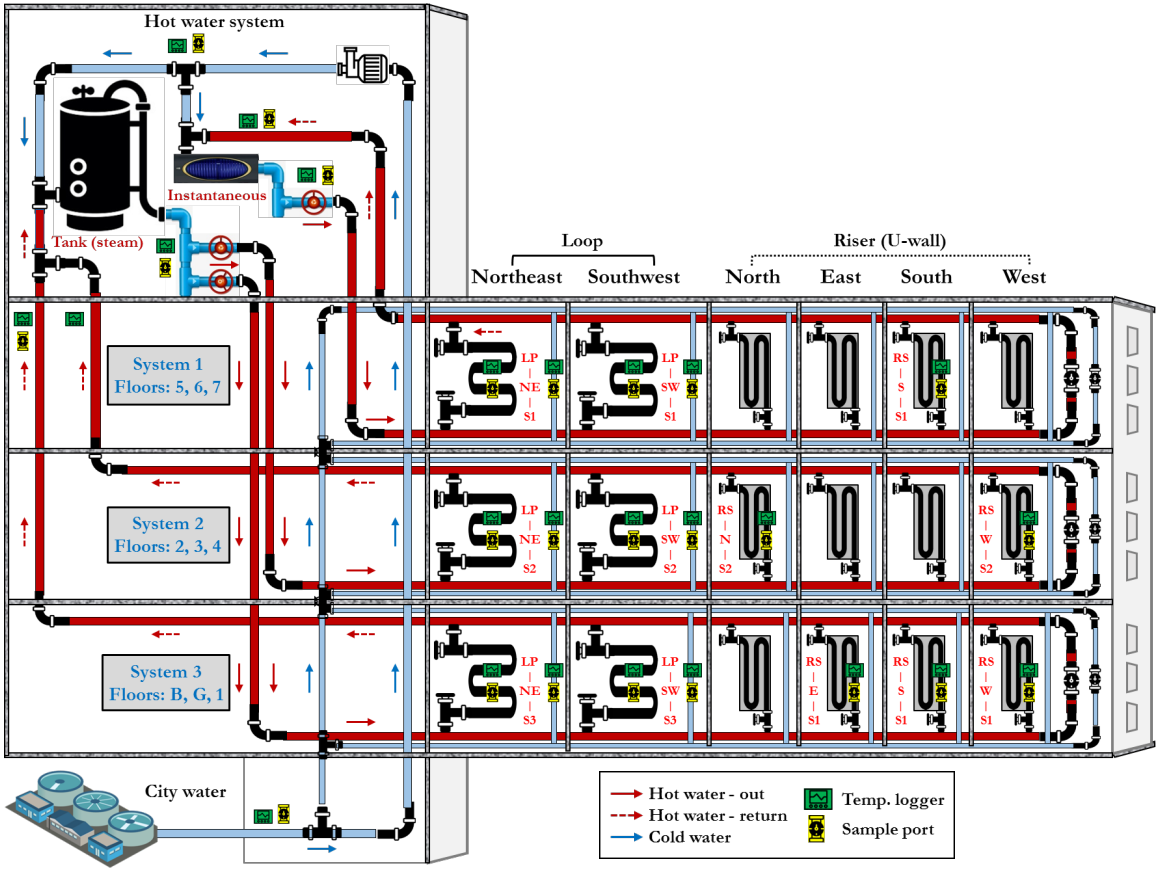
Objective

The current research examined the Bacterial population in a 40-year-old large building using NGS technology and culture-dependent assays to:

- Determine the extent to which the microbial community structure varies temporally and spatially within the build environment drinking system.
- Detect and enumerate waterborne pathogens in the hot water system.
- Perform genome-wide analysis of *Legionella pneumophila* serogroup 1.

Built Environment (drinking water system)



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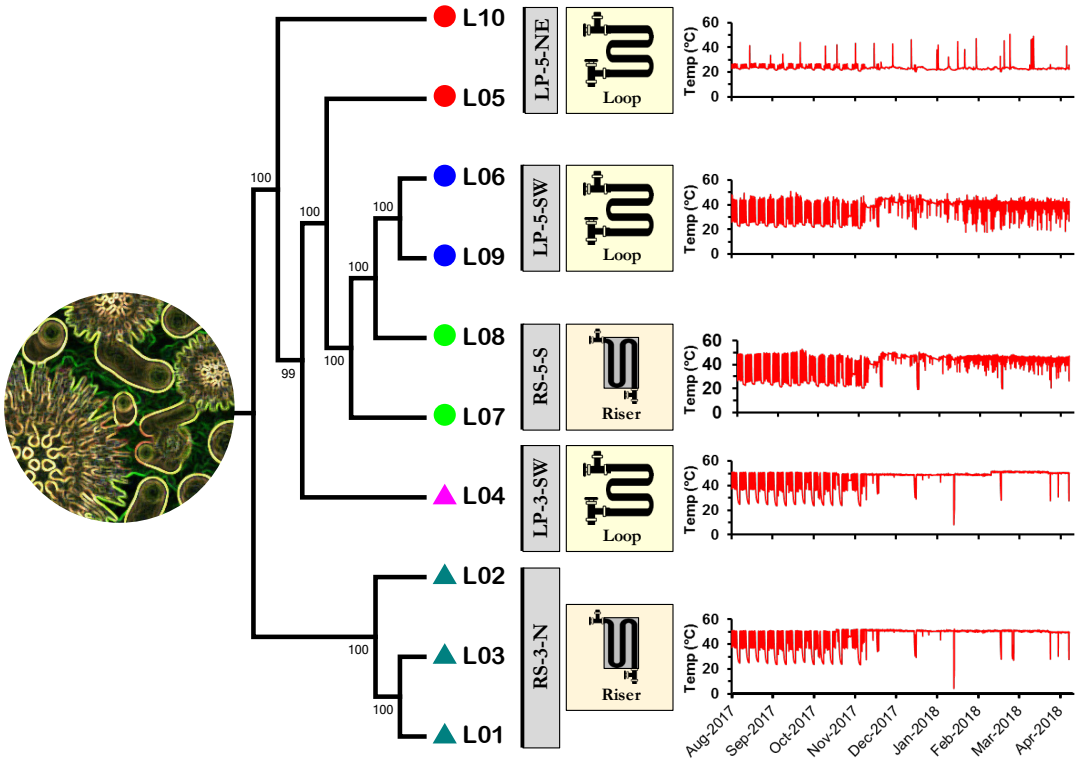
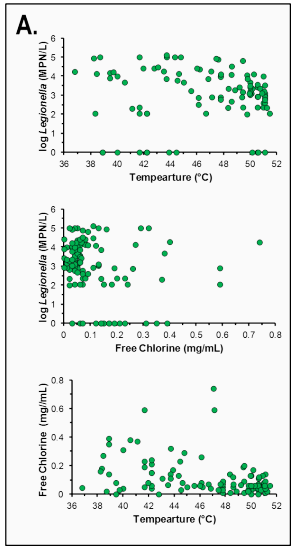
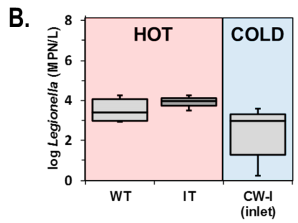
Microbial Community

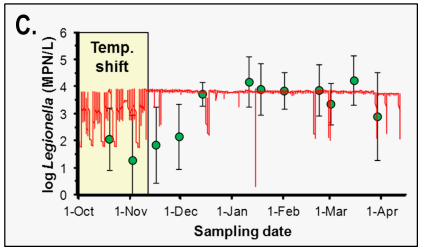


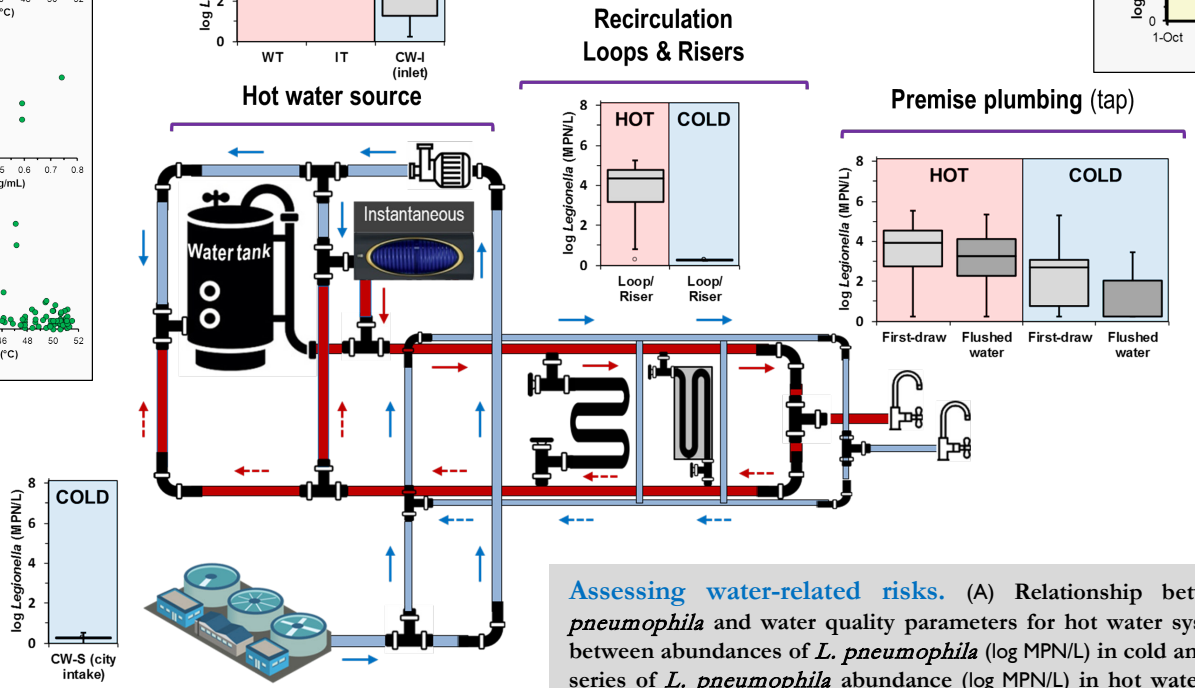
Community profile. Cluster analysis of 16S rRNA libraries based on Jensen-Shannon dissimilarity. Samples were identified by room location and grouped in their corresponding hot water systems.

Water Risk Assessment







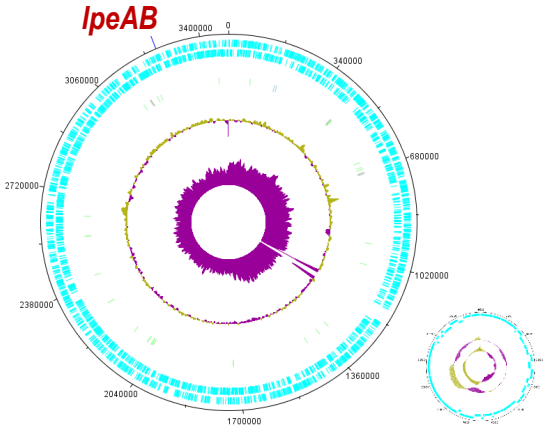


Assessing water-related risks. (A) Relationship between the abundance of *L. pneumophila* and water quality parameters for hot water system samples. (B) Comparison between abundances of *L. pneumophila* (log MPN/L) in cold and hot water systems. (C) Time-series of *L. pneumophila* abundance (log MPN/L) in hot water system samples. The water temperature (red line —) in the recirculation loop/riser is plotted together for comparison.

Genomic Characterization of *L. pneumophila* Isolates

Legionella pneumophila

Phylogenetic tree of *L. pneumophila* subspecies. Evolutionary distance was computed using the Maximum Composite Likelihood method of 48 aligned *gyrB* gene sequence (≈2.4kbp); nodes with a bootstrap value ≥50% of 500 replicates are identified.



Presence of the *lpeAB* genes encoding a macrolide efflux pump, which appears to be specific to ST1.

Identification *L. pneumophila* subspecies. Average nucleotide identity (ANI) values (in %) calculated for each *L. pneumophila* subspecies.

Subspecies	Isolates	*fraseri*	*raphaeli*	*pascullei*
pneumophila	99.96	91.70	91.39	90.63
fraseri	91.86	---	96.49	93.52
raphaeli	91.24	96.49	---	93.72
pascullei	90.55	93.52	93.72	---

Conclusions

- **Exploratory analysis of the water community** (16S rRNA-encoding gene) **showed clustering of samples based on** section/zones **in the hot water network.**
- **Variations in waterborne and opportunistic pathogen populations** (*Legionella*, *Mycobacterium* and *Pseudomonas*) **were observed among** section/zones**.**

- **First draw samples showed the** highest (↑) **counts of HPC and** *Legionella* **with a** decrease (↓) **in disinfectant residual and temperature. HPC and** *Legionella* **tended to** decline (↓)**, and the temperature and disinfectant residual** increased (↑) **with flushing.**
- **The** *Legionella* **population was dominated by** *L. pneumophila* **subsp.** *pneumophila* **and identified as** serogroup 1 **by agglutination and genomic analysis.**
- **The presence of** *LpeAB* **was correlated with** reduced sensitivity to azithromycin (*i.e.* macrolides) **in previous studies, which appears to be specific to** Sequence Type 1**.**

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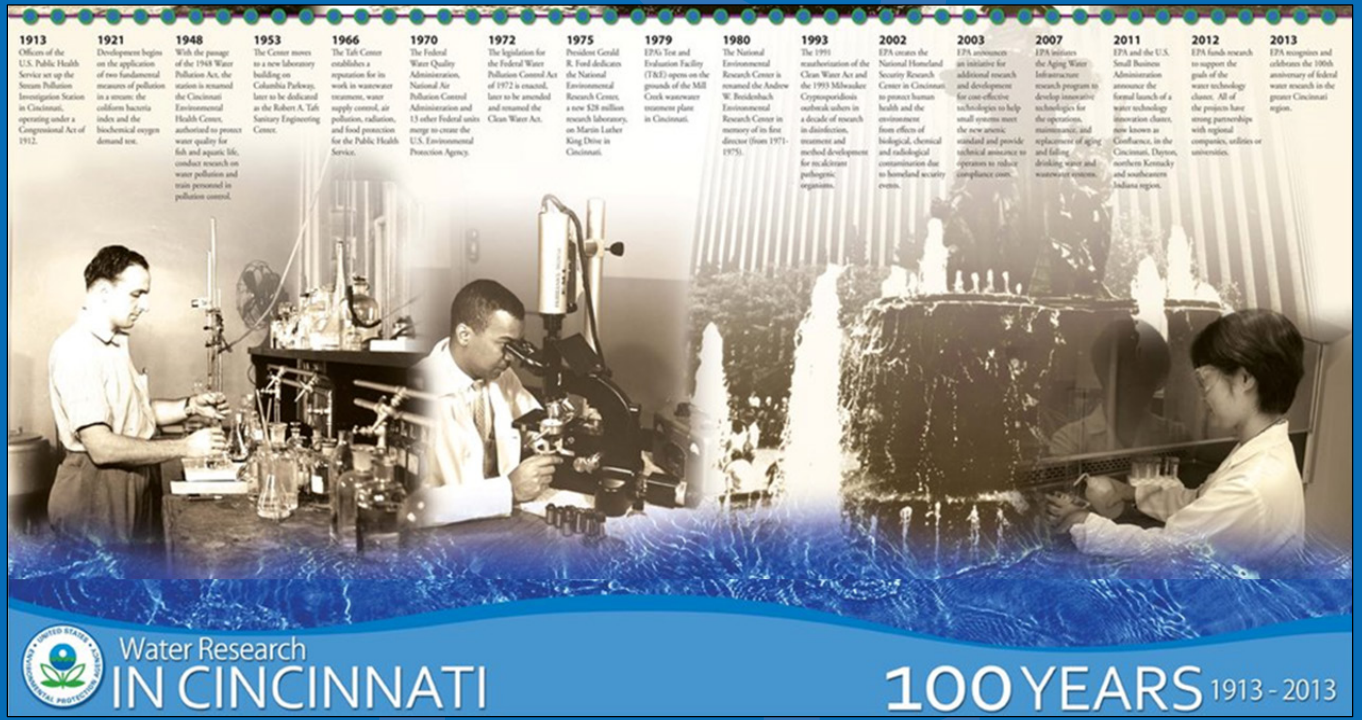

Questions?