# OCCURRENCE OF *Legionella pneumophila* IN DRINKING WATER DISTRIBUTION SYSTEMS

Select Results from Water Research Foundation Project 5156

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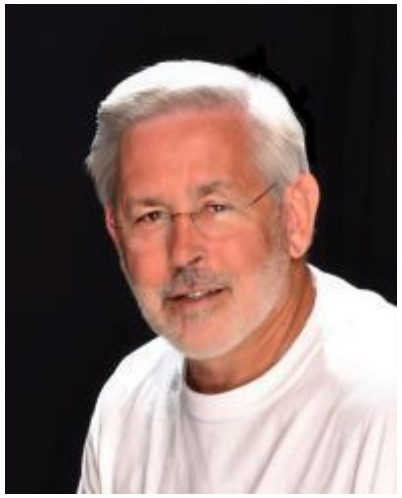


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# Other Contributors and Acknowledgments


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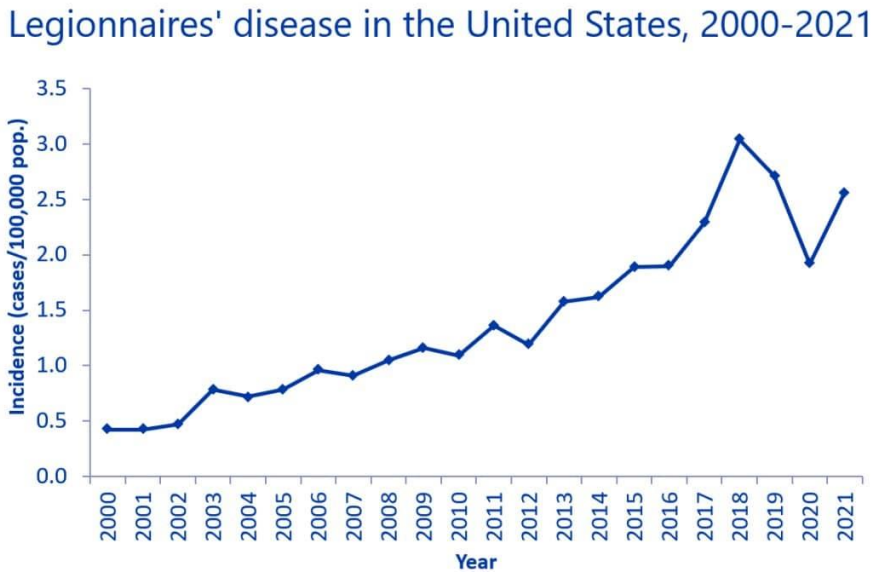
# INTRODUCTION AND PROJECT OVERVIEW

# Why *Legionella*?

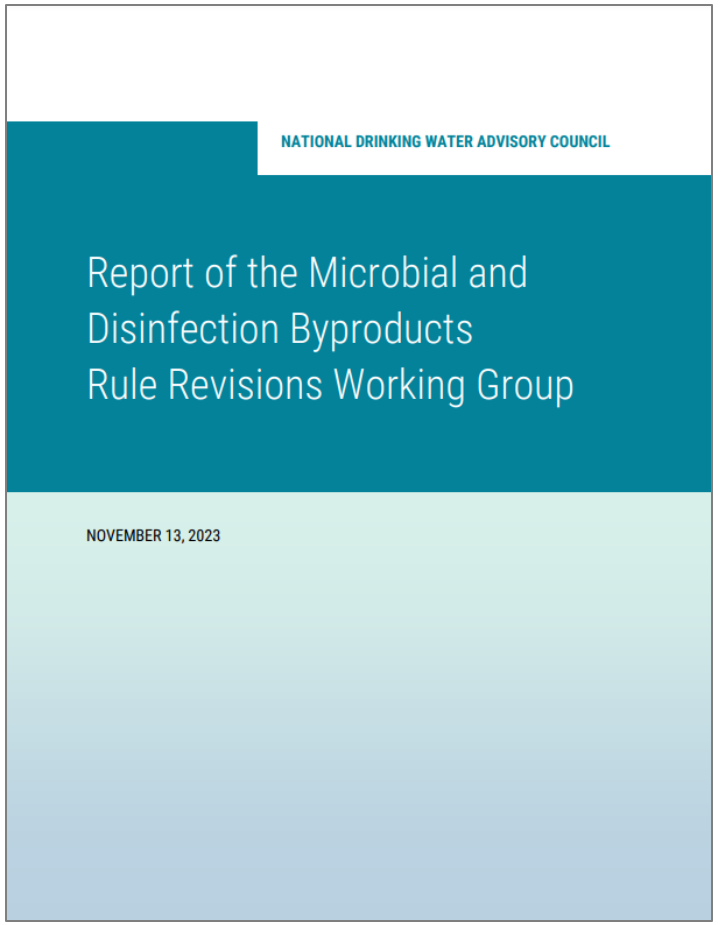
- Some *Legionella* species cause Legionnaires' Disease (LD), a serious disease with a high mortality rate, public health cost and economic impact.
  - Some *Legionella* species can also cause Pontiac fever, a milder disease whose prevalence is generally unknown.
  - US outbreaks are more prevalent in the eastern US
  - Reports demonstrate the incidence of LD is increasing in the United States, Canada, Europe and Japan
- Outbreaks have occurred in
  - Buildings served by both **surface & groundwater systems**,
  - Buildings supplied by **public & private utilities**, and
  - Buildings supplied by **large, medium, & small systems**; outbreaks in larger cities (with larger systems) may be more readily recognized due to
    - greater populations affected and
    - more engaged public health resources to investigate.




https://www.cdc.gov/legionella/php/surveillance/index.html

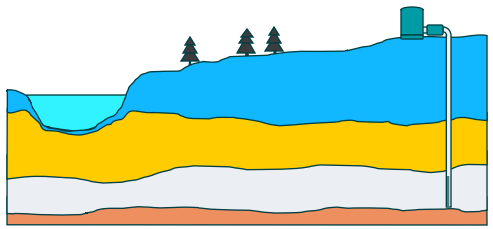
# Why Now?

- The USEPA has recognized the importance of *Legionella* and is reviewing microbial and disinfection byproduct (MDBP) rules to assess whether revised rules are needed
- The EPA's MDBP rule working group made *Legionella*-related recommendations, including:
  - A numeric minimum disinfectant residual requirement
  - A national building water quality improvement initiative
  - Addressing finished water storage tank vulnerabilities
  - Improving chloramination practices
  - Improving water quality and regulatory compliance rates for consecutive systems
- EPA's challenge: near-absence of *Legionella* data to inform revised regulations and better protect public health



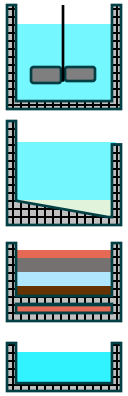
# *L. pneumophila* Exposure Pathway and Progressive Management

**Groundwater**
**Surface Water**



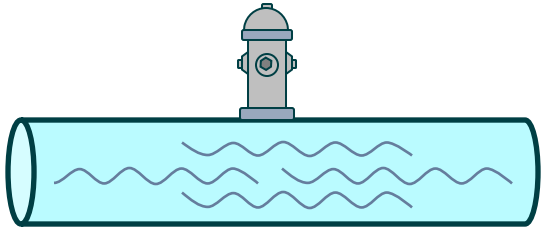
Natural occurrence

**Water Treatment**



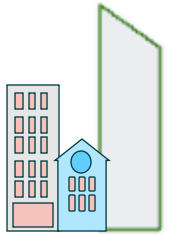
Reduction to below detection

**Water Distribution**



Baseline survival & growth

**Building Water Systems**



Ongoing survival & amplification

**Water Consumers**

Human exposure

**Public Water System**
Water quality management
Regulatory compliance

**Building Water System**
Water management program

**All buildings are *de facto* consecutive systems!**

# Project Objectives and Rationale

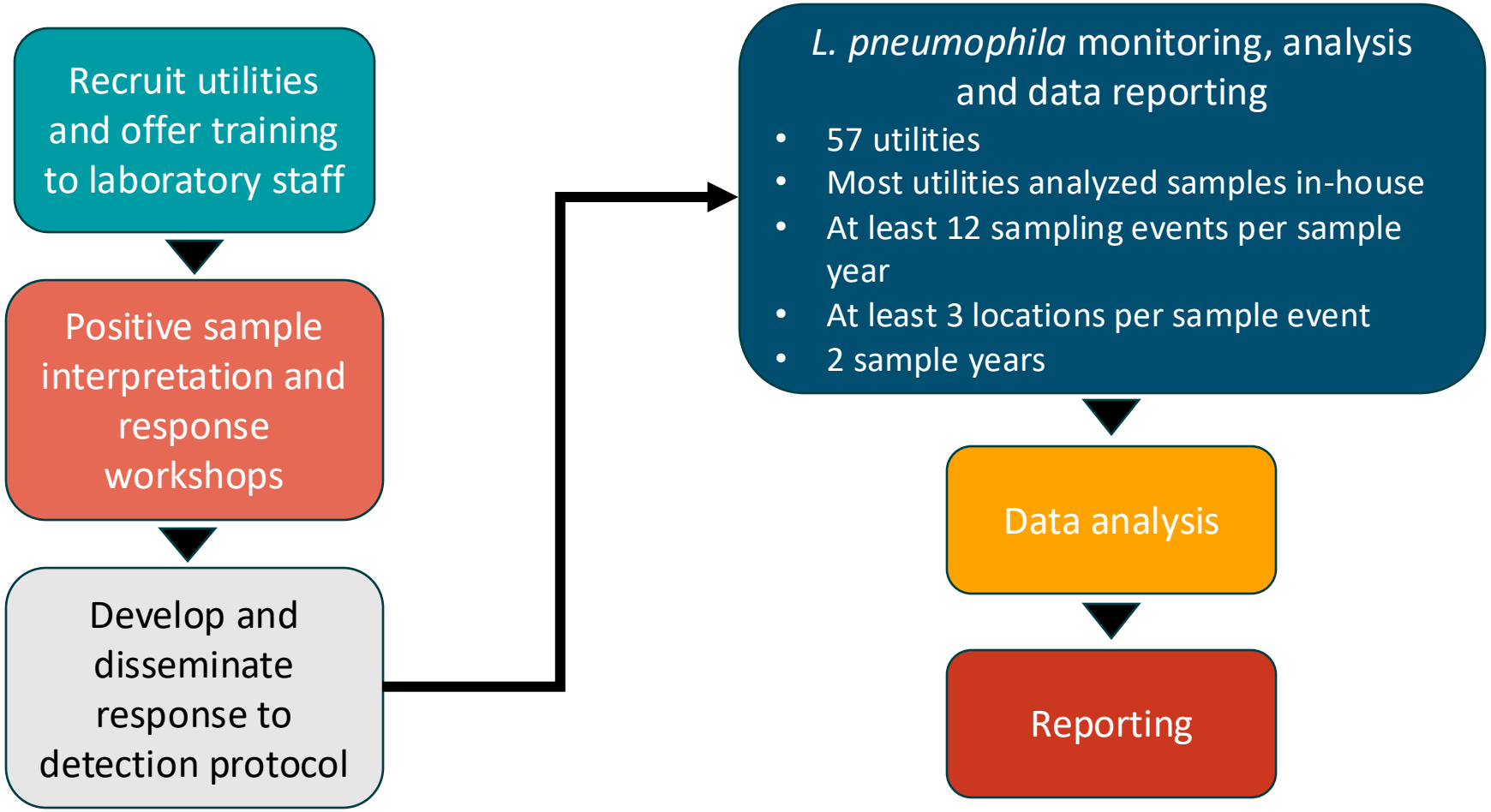
## Objectives

- Develop a response and communication protocol that utilities can use following *L. pneumophila* detections,
- Collect data to characterize the relationship between *L. pneumophila*, occurrence/concentration and disinfectant residuals in PWS DSs,
- Conduct analyses to:
  - Quantify the association between disinfectant residual (type and concentration), utility characteristics & occurrence and abundance of *L. pneumophila* in bulk water samples,
  - Identify locational characteristics associated with heightened likelihood of *L. pneumophila*, and
- Conduct a benchmark risk analysis of *L. pneumophila* in DSs

## Anticipated Outcomes & Benefits

- Assess the value of monitoring for *L. pneumophila*
- Develop a fully-characterized data set for use in MDBP rule revisions
- Identify approaches for utilities to provide the highest standard of care in *Legionella* management
  - What can utilities do?
  - What are the limits to utility contributions to *Legionella* management?
- Characterize occurrence nationally and between systems, define the low-level occurrence characteristic of distribution systems of well-run utilities.

# Project Overview

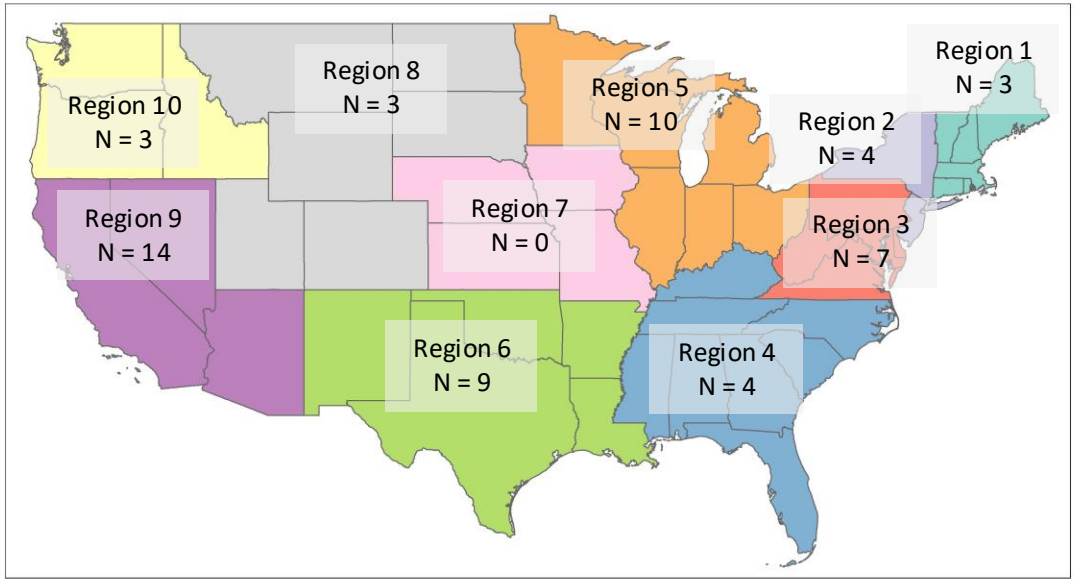


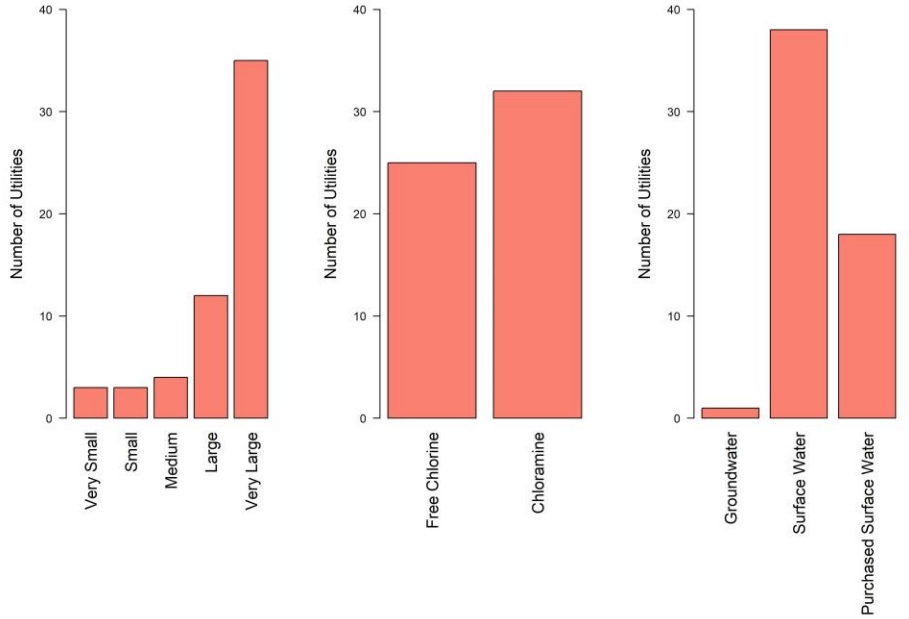
# Utility Selection & Characteristics

- 57 utilities volunteered to collect 3 samples per week for 12 weeks during warm water months (>15°C) for 2 summers (total: >9,000 samples)

- Includes:

  Various system sizes,
  Geographical distribution,
  Free and chloramine residuals,
  Groundwater, surface, and mixed supplies



# Sample Collection and Analysis Strategy

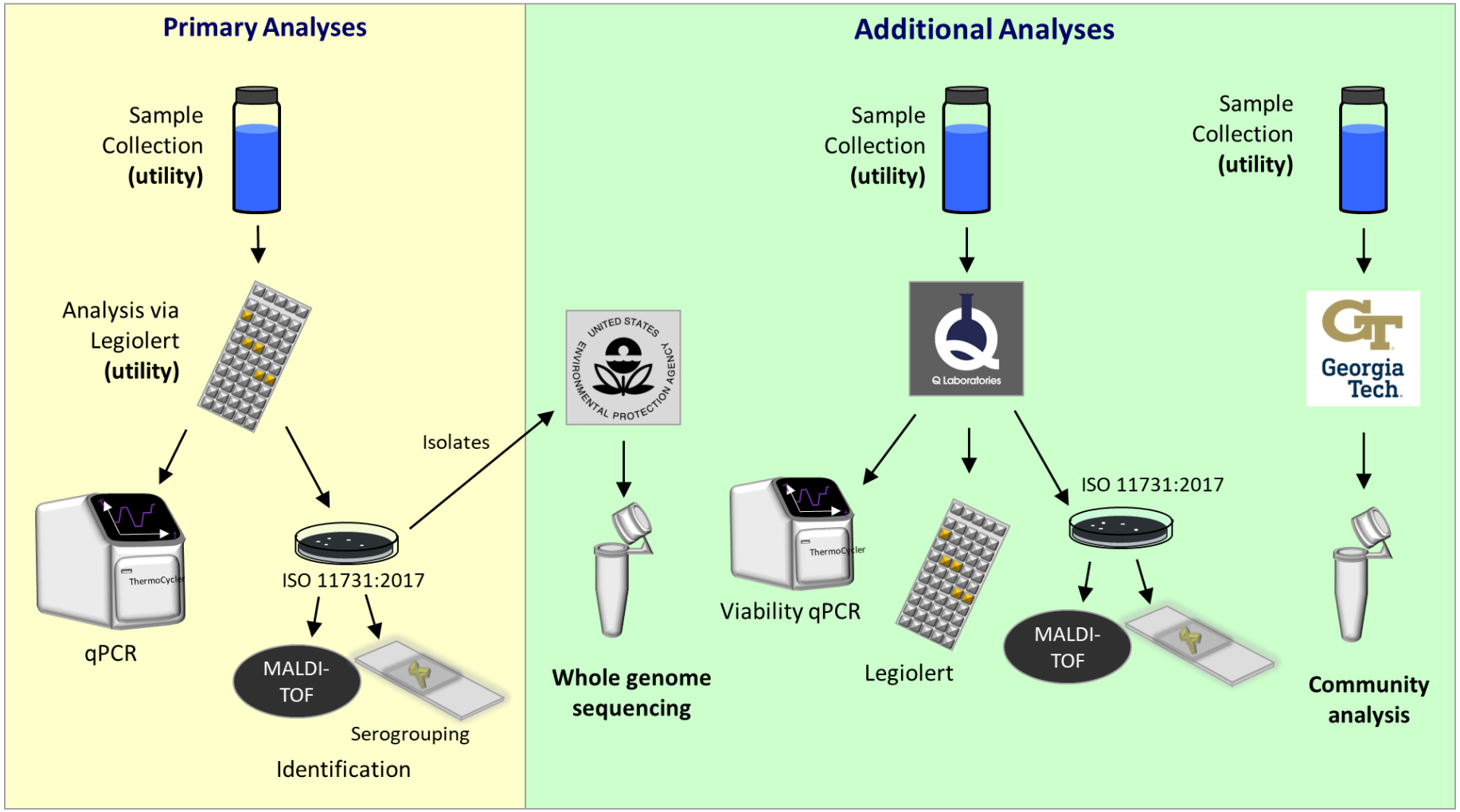

## Utilities without ongoing *L. pneumophila* monitoring

- Two-year study  period
- Samples were collected weekly for 12 weeks from 3 locations after water temperature exceeded 15°C
- Samples assayed by utility via Legiolert, except 2 utilities that sent samples to 3rd party lab for Legiolert analysis
- Sample locations were chosen at the locations with low disinfectant residuals or other features making them more likely to be *L. pneumophila* positive
- Follow-up sample analysis by Q Laboratories

## Utilities with ongoing monitoring programs

- Year-round data collection
- Samples analyzed via Legiolert by utilities
- Follow-up sample analysis by utilities

# Project Workflow Overview



# Legiolert platform

- Unique 100 mL “Quanti-Tray” device
  - 6 large wells (overflow)
  - 90 small wells (resolution)
  - Counts *L. pneumophila;* from 1-2272 MPN/ Quanti-Tray
- Blister pack reagent


Reaction with *L. pneumophila*

Negative Sample

# RESULTS AND ANALYSIS

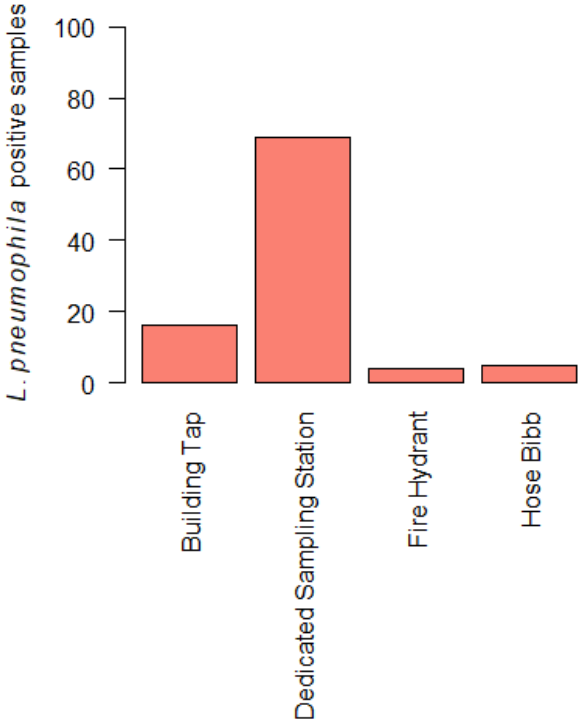
# Final Data Set Summary Statistics

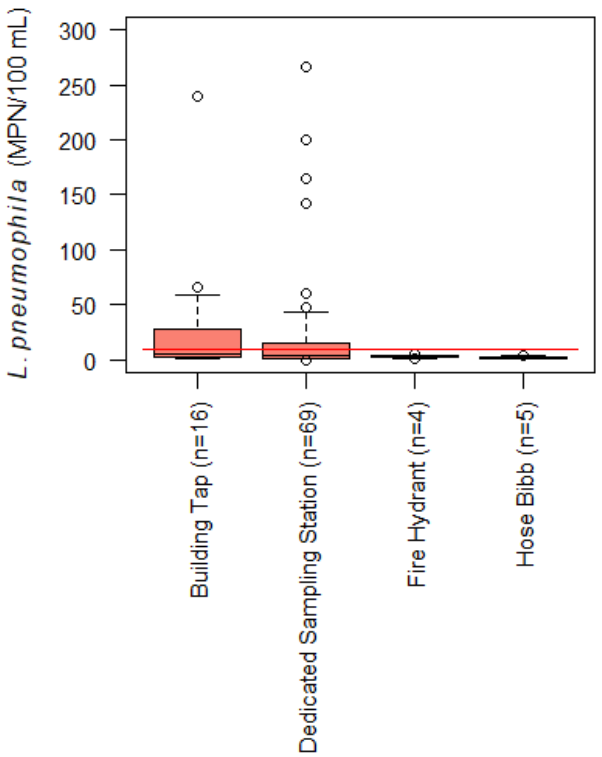
| Pooled data[†] | All systems | Free chlorine systems | Chloramine systems |
|---|---|---|---|
| **Included samples[‡]** | 9181 | 6680 | 2501 |
| ***L. pneumophila* positive samples** | 109 | 87 | 22 |
| **% positive *L. pneumophila* samples** | 1.19 | 1.30 | 0.88 |
| **Average disinfectant concentration (mg/L)** | - | 0.99 | 2.02 |
| **Average water temperature (°C)** | 20.3 | 20.3 | 20.3 |
| **Number of utilities** | 57 | 25 | 32 |
| **Utilities with at least one *L. pneumophila*+ sample** | 18 | 11 | 7 |

[†] Data from a prior study (LeChevallier, 2018) that employed similar methods were pooled with data from the current study

[‡] 292 out of 8323 samples collected in the current study were excluded from analysis because the sample bottle broke (2), *L. pneumophila* results were not reported (51), the analysis did not pass QA/QC checks (38), or the sample was for a location other than distribution system (201)

# Positivity by Sample Collection Location Type

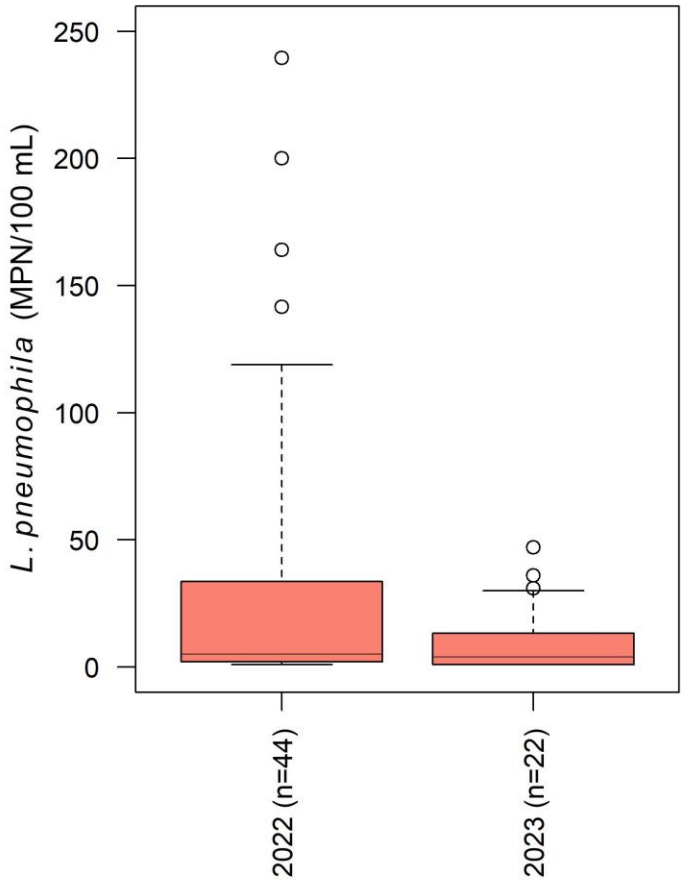



| Sample Tap Type | Number of Samples | No. *Lp* positive samples | % positive samples |
|---|---|---|---|
| **Building tap** | 1657 | 16 | 0.97 % |
| **Dedicated sampling station** | 6061 | 69 | 1.14 % |
| **Fire Hydrant** | 103 | 4 | 3.88 % |
| **Hose bibb** | 237 | 4 | 1.69 % |

# Occurrence and Abundance in Study Years 1 (2022) and 2 (2023)

| | 2022 | | | 2023 | | |
|---|---|---|---|---|---|---|
| | All | Free chlorine | Chloramine | All | Free chlorine | Chloramine |
| **Number of samples, total** | 2094 | 1951 | 953 | 2815 | 1812 | 1003 |
| **Number of positive samples** | 44 | 30 | 14 | 22 | 15 | 12 |
| **Percentage positive samples** | 1.5% | 1.5% | 1.4% | 0.78% | 0.83% | 1.2% |
| **Mean, + samples (MPN/100mL)** | 34.5 | 19.7 | 66.2 | 9.7 | 12.5 | 3.6 |
| **Geo mean, + samples (MPN/100mL)** | 7.5 | 3.8 | 32.4 | 4.1 | 5.8 | 2.0 |

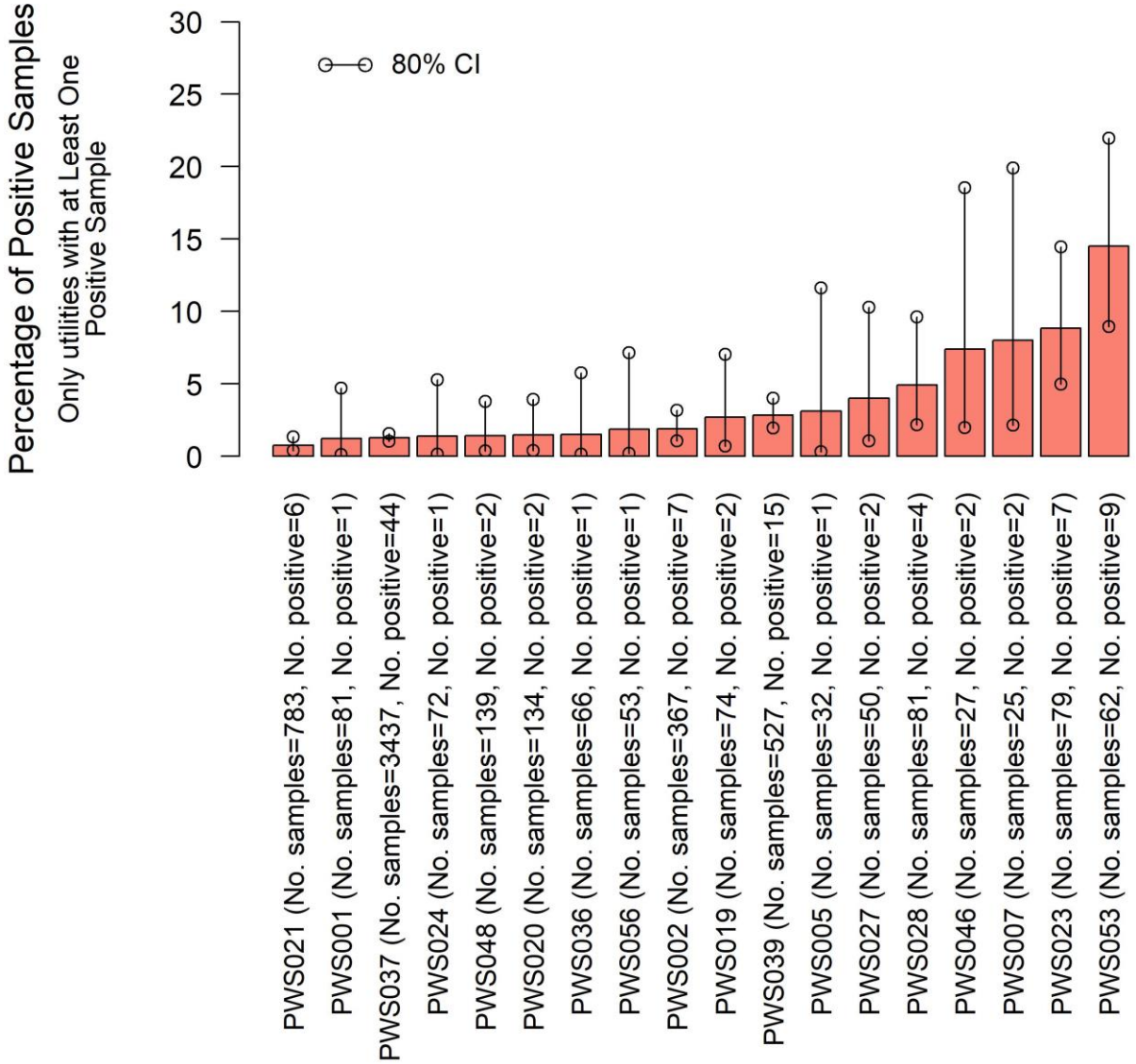


# Why the Decrease in Both Occurrence and Abundance in Year 2?

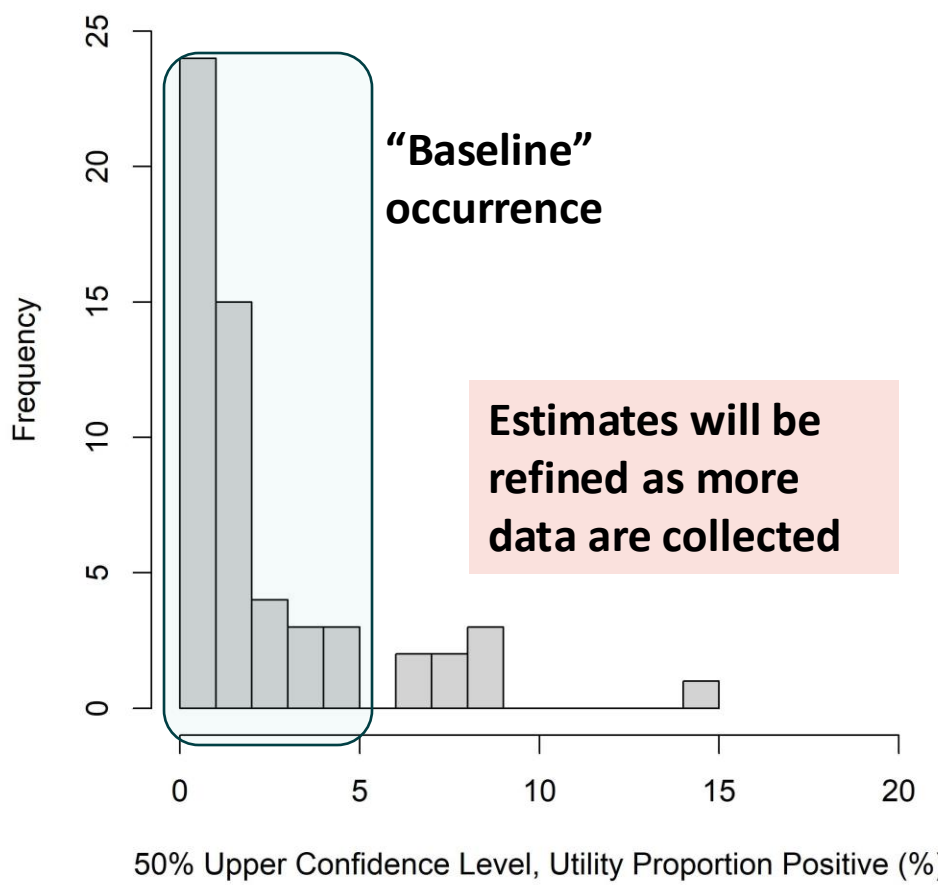
- **No statistical difference in year 1 and year 2 for**
  - the distribution of disinfectant concentrations
  - the distribution of water temperatures
- **Most utilities used the same sample locations in year 1 and year 2**
- **The most plausible explanation is that utilities reduced occurrence and abundance via their responses to positive detections** (monitoring and management of *L. pneumophila*)
  - Even in year 1, levels were never high enough to pose an unacceptable acute health risk (more on this later)
  - Utilities with positive detections conducted follow-up monitoring and instituted mitigative and protective practices such as flushing, cleaning, and improving disinfectant concentrations near the sample collection location with the positive sample
  - Other studies have shown similar improvement in *L. pneumophila* control when utilities have responded to positive detections with deliberate management strategies

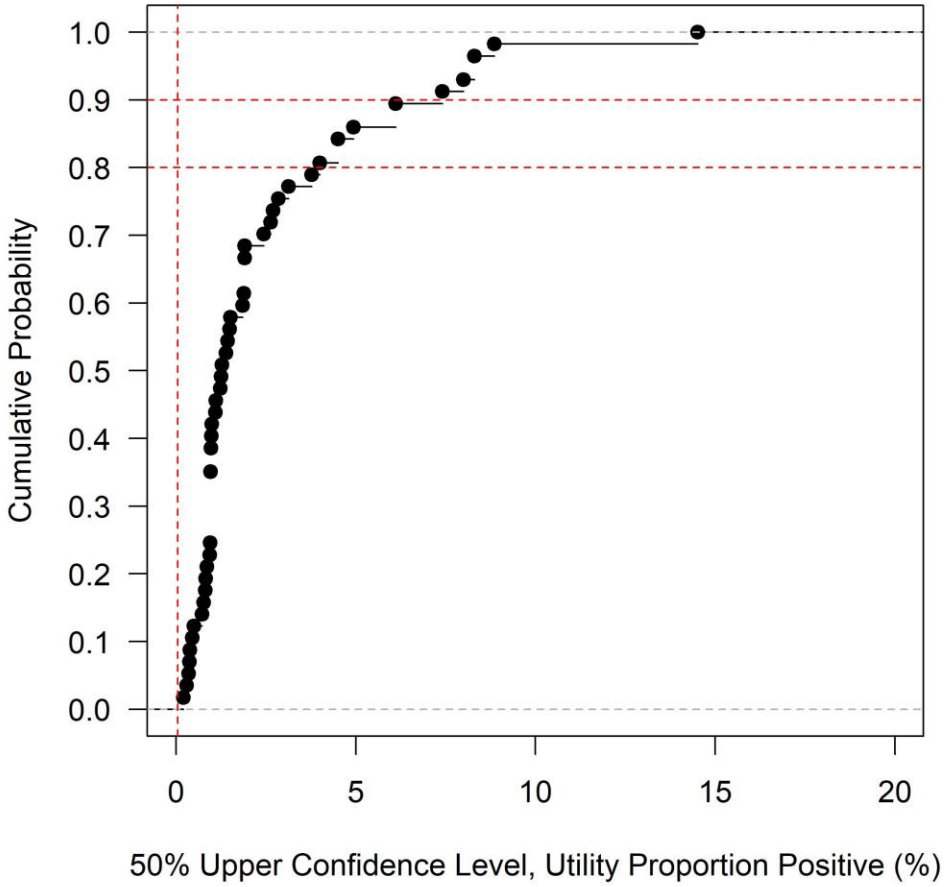
# Proportion Positive, by Utility

- 18 out of 57 utilities reported at least one positive sample
- Proportion positive among utilities reporting at least one positive sample ranged from 1% to 15%
- Generally, higher proportions positive were observed for utilities that collected fewer samples



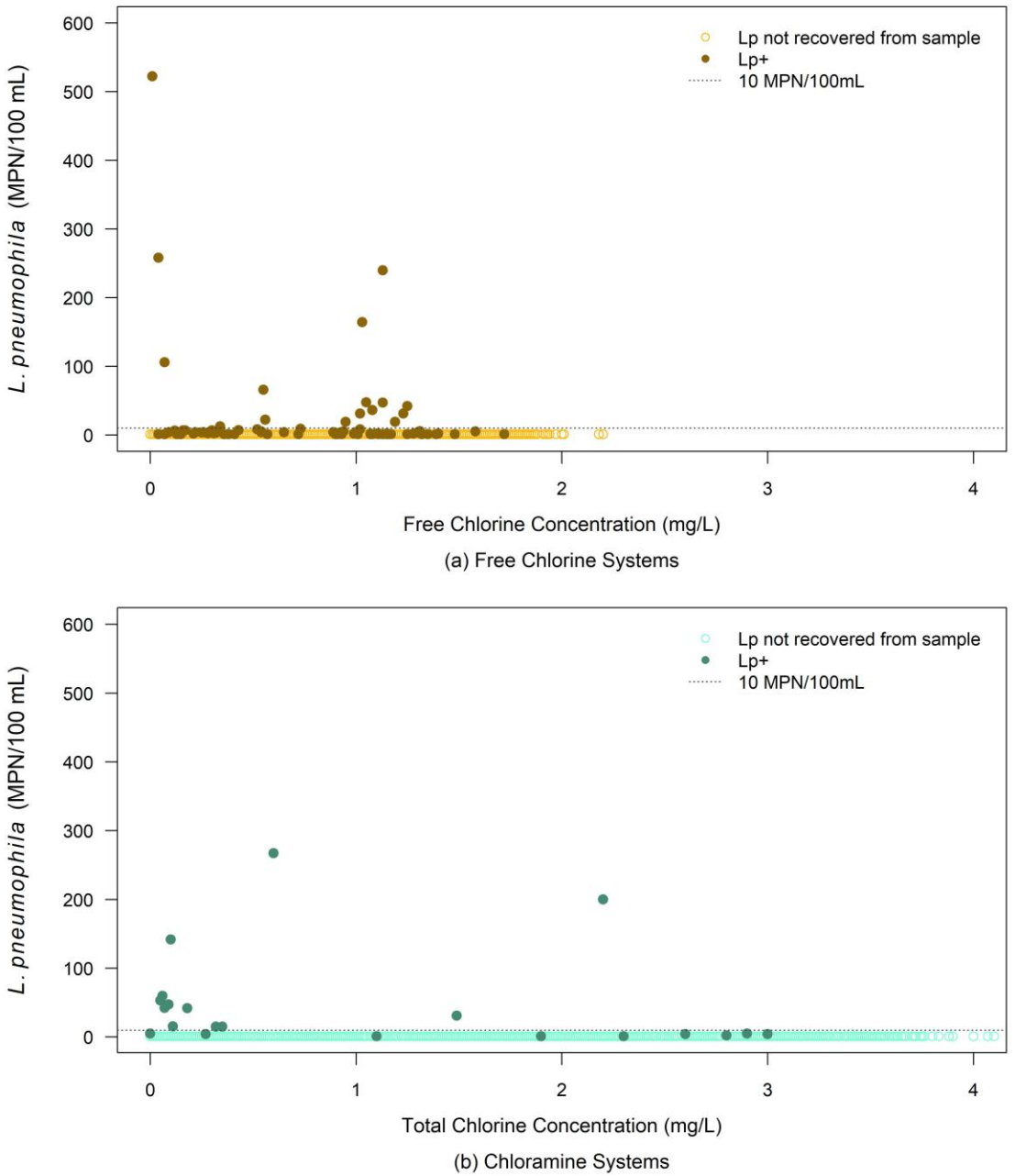
# An Estimate for Typical Proportion Positive for US Utilities Using Current Water Management Strategies?



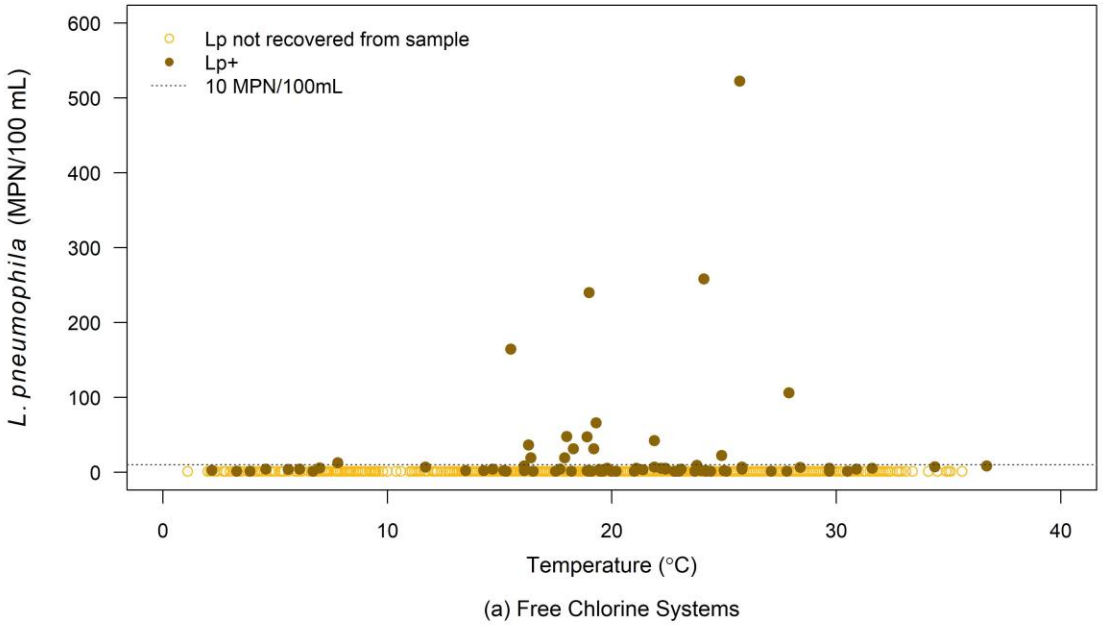
# *L. pneumophila* Concentration *v.* Disinfectant Residual Concentration

- No single sample had *L. pneumophila* concentration above a level posing significant public health concern (more on this later)
- Most high *L. pneumophila* concentrations were observed at lower disinfectant concentrations (both free chlorine and chloramines), but …
- Sporadic high *L. pneumophila* concentrations occurred above 1 mg/L (both disinfectants)
  - Disinfectant is not a silver bullet and multiple barriers remain the right approach

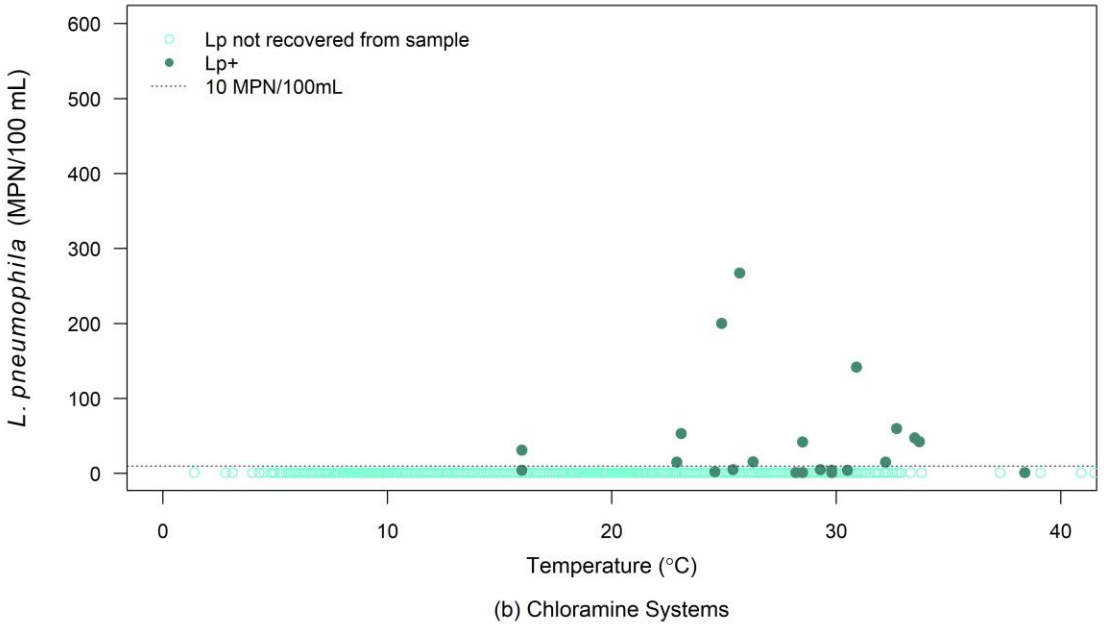


# *L. pneumophila* Concentration *v.* Temperature

- For both free chlorine and chloramine systems, most or all detections were at water temperature > 16°C
- The relatively few detections at low water temperature were at very low concentrations
- Very few samples had a temperature > 32°C; more data might demonstrate a trend of increasing occurrence and concentration



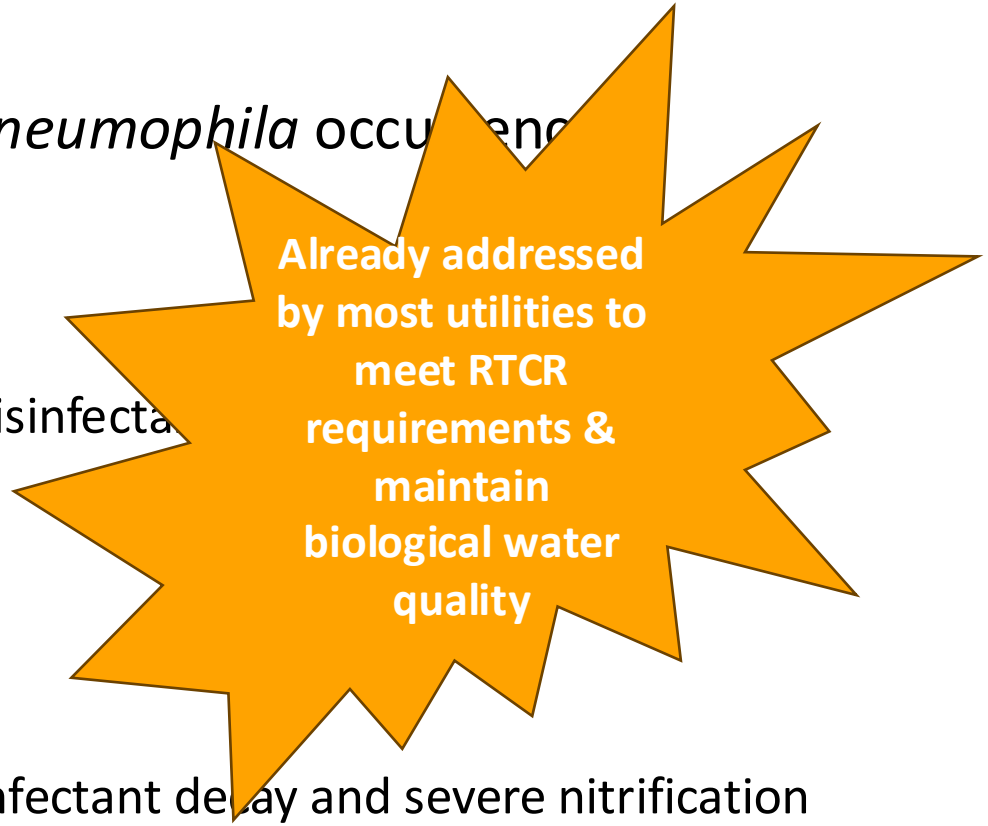
(a) Free Chlorine Systems



(b) Chloramine Systems

# *L. pneumophila* Occurrence and Control in Distribution Systems

- Primary factors known or suspected to impact *L. pneumophila* occu enc distribution systems:
  - Water temperature
  - Residual disinfectant type and concentration
  - Water age (related to water temperature and residual disinfecta
  - Pipe material and condition
  - Sediment accumulation
  - Distribution system integrity
- Factors are coupled
  - Higher water temperatures associated with greater disinfectant de ay and severe nitrification
  - Old, unlined cast iron pipes exert disinfectant demand and generate corrosion products
  - …



# Logistic Regression Model

- *L. pnuemophila* occurrence data fit to logistic regression model

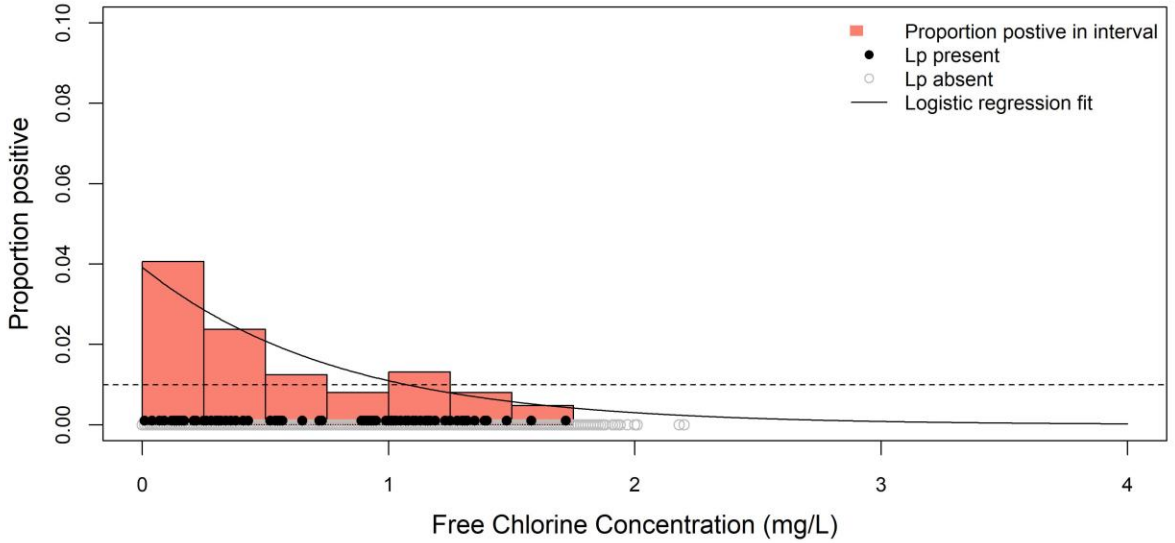
$$\log\left(\frac{P_{Lp}}{1-P_{Lp}}\right) \sim \beta_C C + \beta_T T$$

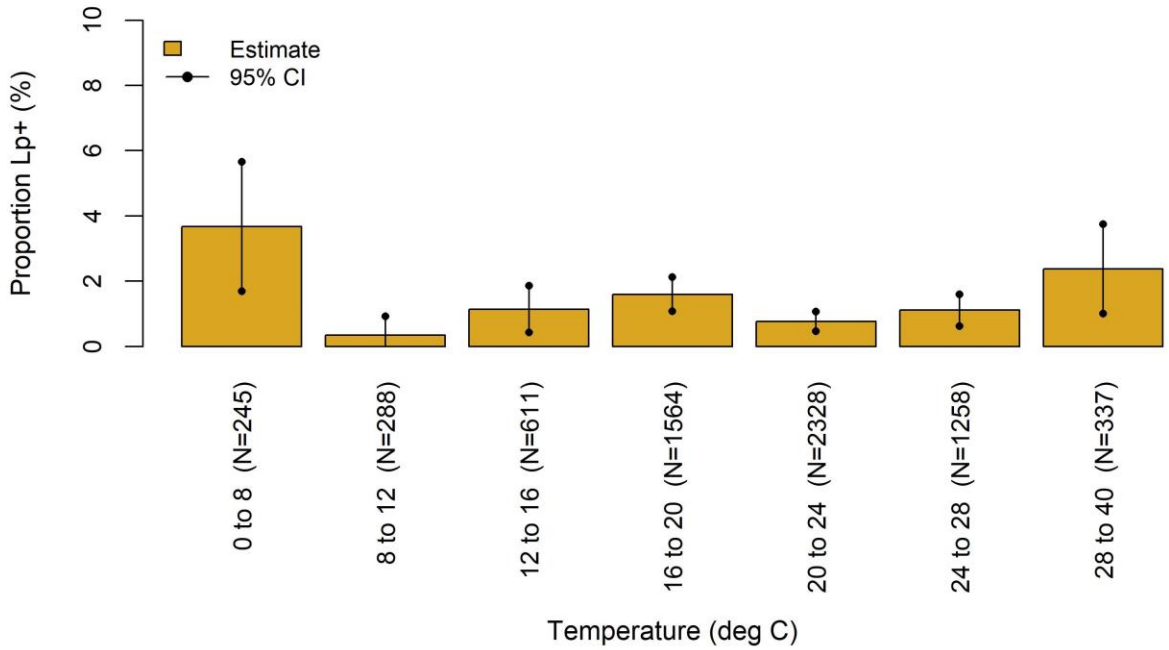
- Logistic regression model is the best approach because
  - It does not rely on arbitrary bin size selection
  - It includes both temperature and disinfectant residual concentration
- Mixed results
  - For free chlorine systems, only the disinfectant residual concentration term was significant
  - For chloramine systems, both terms were significant

| | Free Chlorine | | Chloramine | |
|---|---|---|---|---|
| **Parameter** | Estimate | Significance ($p$) | Estimate | Significance ($p$) |
| **Intercept** | -3.05 | $3.5\times10^{-12}$ | -3.86 | $<2\times10^{-16}$ |
| $\beta_C$ | -1.20 | $6.9\times10^{-6}$ | -0.75 | 0.00183 |
| $\beta_T$ | -0.0135 | 0.48 | 0.020 | 0.031 |

# Free Chlorine

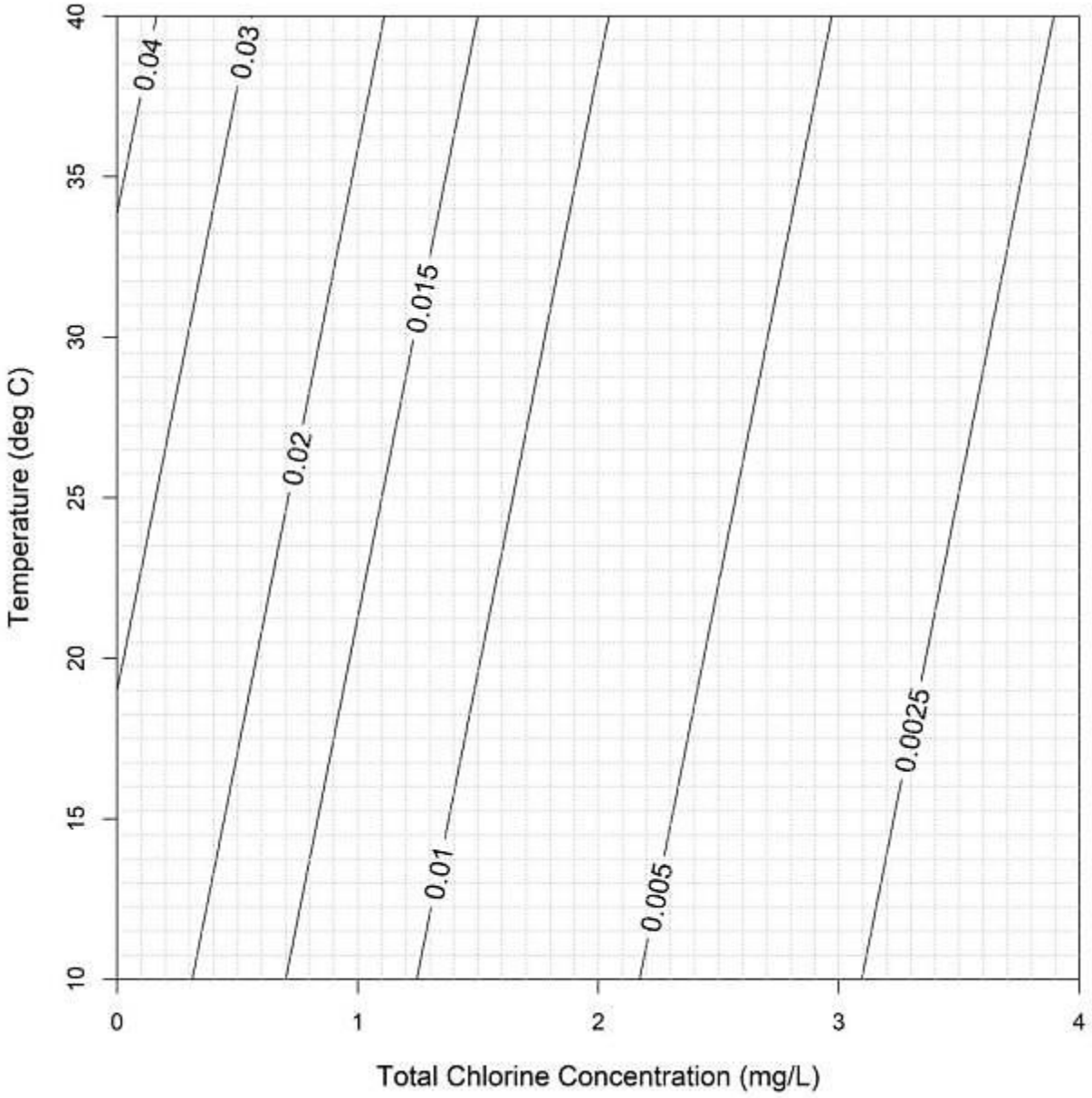
- Significant reduction in proportion positive when disinfectant concentration raised from 0 to 0.2 mg/L
- Diminishing returns with further increases
- Many positive samples when residual disinfectant concentration > 0.8 mg/L
- Low temperature detections might be confounding the analysis



# Chloramines

- The spacing between lines of iso-occurrence increases with increasing residual disinfectant concentration (diminishing returns for increases in disinfectant concentration)
- Temperature effects are less important than disinfectant residual (iso-occurrence lines have steep slope)
- Control to a target occurrence level requires higher residual disinfectant concentration as temperature increases (intuitive)



# CONCLUSIONS

# Big-Picture Conclusion

- Overall, this research project finds that *L. pneumophila* occurrence in the well-run PWS DSs that participated in this study is, on average, very low.
- Positive samples have concentrations far below any current level of public health concern.
- Conscious and consistent understanding and management of *L. pneumophila* by utilities can make *L. pneumophila* occurrence even rarer and reduce concentrations in positive samples even further.
- Among the utilities participating in this study,
  - Most had occurrence rates (the percentage of culturable *L. pneumophila* positive samples) of 5% or less and 68% had no detections of *L. pneumophila.*
  - The utilities with higher occurrence rates in year one conducted system assessments, reevaluated their controls, conducted remedial activities such as flushing, and saw reductions in their occurrence rates in the second year of the study.

# Monitoring Worked

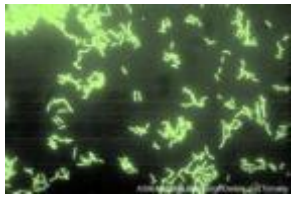
- More than 50 utilities monitored for *L. pneumophila* in their DSs over at least a 2-year period at times when distribution system conditions were, at least, generally opportune for *L. pneumophila* survival and growth.
- The collection of over 9,000 samples demonstrates that *L. pneumophila* monitoring is feasible by properly trained water utilities.
- Utilities had a high success rate (only a small number of assays did not meet QA/QC requirements) using the Legiolert test.
- Follow up analyses were able to recover and serotype the *L. pneumophila* strain in 96% of the Quanti-Trays when they were stored and shipped overnight on ice and promptly analyzed by qPCR.
- A protocol for responding to positive samples was a critical enabling element of the study; buy-in on a protocol by health agencies and regulators will promote more widespread monitoring.

# Residual Disinfectant is not a Silver Bullet

- Conscious and consistent management does not mean treating secondary disinfectant as a silver bullet.
- For both free and total chlorine systems, the greatest reduction in occurrence of *L. pneumophila* was achieved as disinfectant concentration increased from below 0.2 mg/L to above 0.2 mg/L. Further increases in disinfectant concentration yielded small or negligible reductions in *L. pneumophila* occurrence.
- Occurrences even at high disinfectant residual concentrations imply high disinfectant levels alone are not a guarantee of *L. pneumophila* control and that many factors contribute to *L. pneumophila* survival and amplification; *L. pneumophila* is not effectively managed if the factors beyond disinfectant are not identified and addressed.

# Comparison of Residual Disinfectant Types

- In this study, a minimum disinfectant residual concentration of 0.2 mg/L (as total chlorine) in chloramine systems provided the same benefit and level of control as a minimum disinfectant residual concentration of 0.2 mg/L for free chlorine systems.
- Chloramine utilities often opt for higher residual disinfectant targets to meet other water quality objectives
  - Nitrification control
  - Partnership program goals
  - Regulatory requirements (for some states with numeric minimum disinfectant residual regulatory requirements)
- Why do chloramines exceed expectations?
  - Biofilm penetration
  - Persistence
  - Impact on amoebae



# RECOMMENDATIONS

2 out of 6 project recommendations (the others are in ”extra slides” following the presentation)

# Utilities can and should proactively manage *L. pneumophila*

- Even without a specific *L. pneumophila* management program, **many utilities already manage *L. pneumophila*,** albeit indirectly, to achieve low occurrence and abundance in their DSs.
- Utilities would not have to start their *L. pneumophila* management programs from scratch – **Most of the activities that likely promote *L. pneumophila* management are already in place** as within RTCR compliance activities, good water quality management activities, nitrification control programs, and other regulatory and water industry programs.
- Effective control requires more than maintaining a secondary disinfectant residual throughout the DS. It requires **maintaining and managing the multiple barriers** with ***high reliability.***

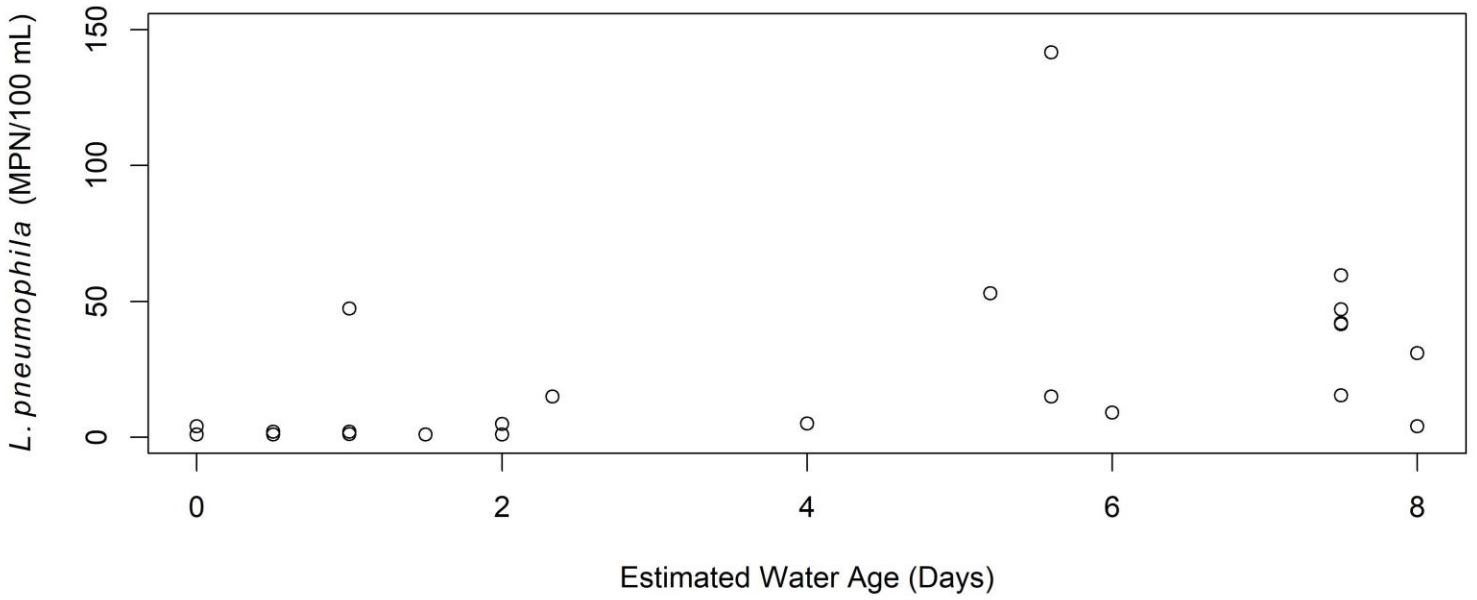
# For *L. pneumophila* management, utilities should maintain a minimum disinfectant residual concentration of at least 0.2 mg/L for both free chlorine and chloramine systems

- This recommendation is not for a national minimum disinfectant residual requirement – **it is a starting point** for utilities developing a *L. pneumophila* management program.
- Maintaining a residual is a good indicator of effective distribution system management.
- **Many utilities already target a higher disinfectant residual concentration** for other purposes:
  - Nitrification control
  - AWWA's Partnership for Safe Water, Distribution System Optimization Program
  - Other biological controls (e.g., *Naegleria fowleri* control in Louisiana)

# EXTRA SLIDES

# Water Age and *L. pneumophila* Concentration, Positive Samples Only

- Water age at sample collection sites was estimated for a relatively small proportion of samples
  - 22 out of 107 positive samples
  - Too few data to make statistically significant conclusions or account for factors such disinfectant type
- Apparent trend in *L. pneumophila* concentration with increasing water age
  - additional data collection and analysis recommended
  - Emphasize high water age locations in monitoring plans



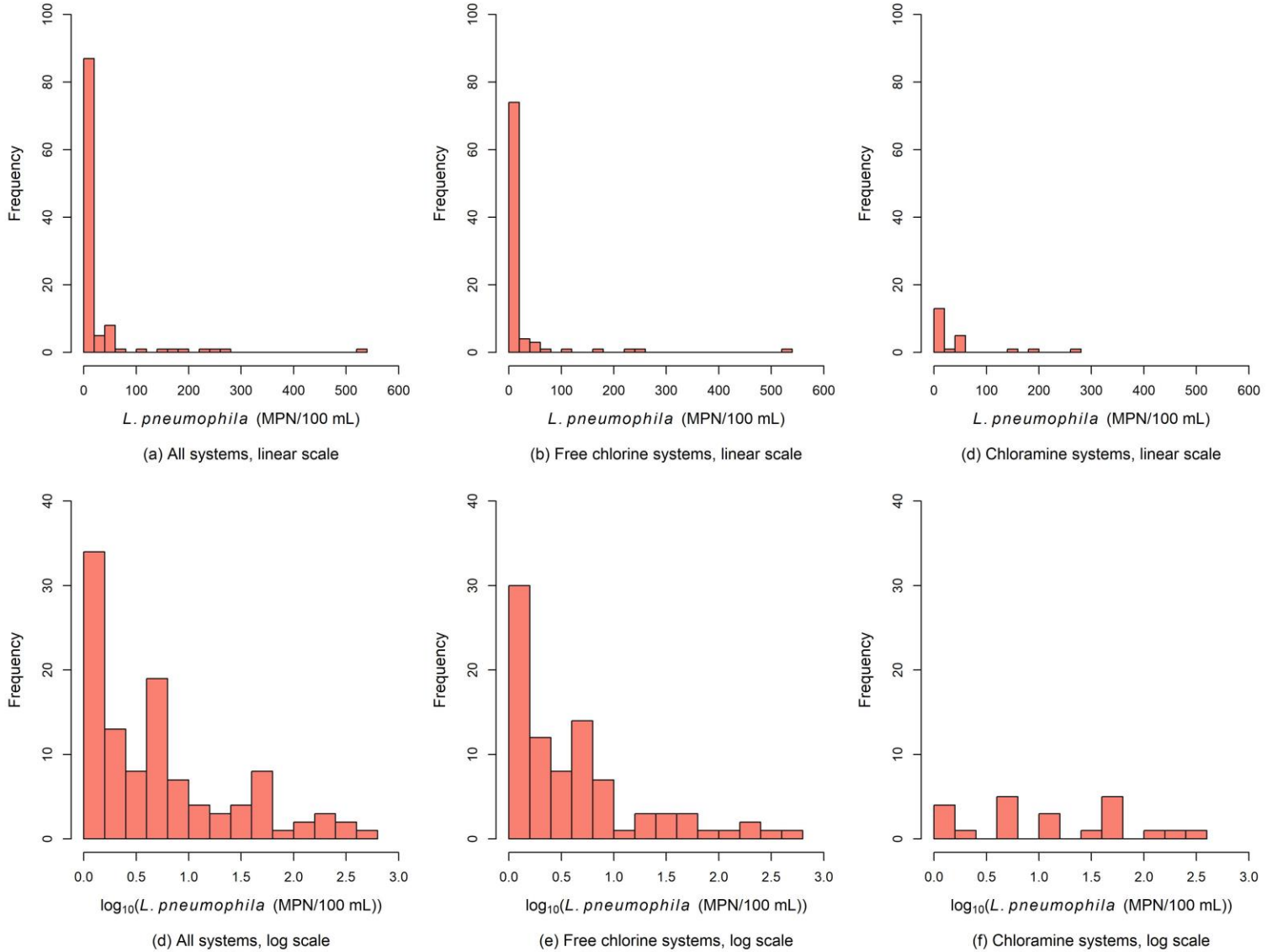
# Baselines

- A utility baseline for *L. pneumophila* is the occurrence (% positive locations) and range of concentrations among positive samples for samples from locations representative of the utility's distribution system
- Utilities have different baselines for *L. pneumophila* because of differences in their
  - source waters,
  - disinfection (both primary and secondary),
  - distribution system characteristics, and
  - other factors
- Differences in baselines between utilities do not mean that some utilities are inherently risky or poorly operated; it is a simple reflection of the reality that treated water is not sterile and that ecological conditions determine the degree to which *L. pneumophila* is present at low levels in distribution systems

# Distribution of *L. pneumophila* Concentrations by Disinfectant

- Concentrations from positive samples were fit to statistical distributions
- The lognormal distribution provided the best fit in all cases (all systems combined, free chlorine systems and chloramine systems)

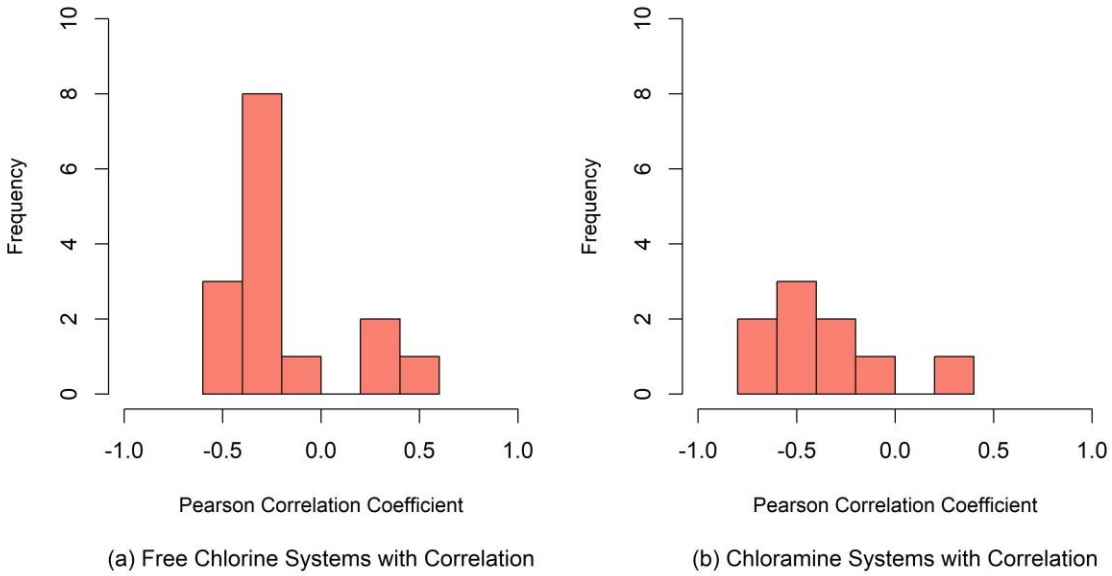
| Systems | $\mu_{\log C}$ | $\sigma_{\log C}$ |
|---|---|---|
| All | 1.6 | 1.6 |
| Free Chlorine | 1.5 | 1.5 |
| Chloramine | 2.5 | 1.8 |



# Disinfectant Concentration Correlation with Water Temperature

- Evaluated correlation between residual disinfectant concentration and water temperature
  - Important to establish before developing a regression model for *L. pneumophila* occurrence
  - Important consideration in development of *L. pneumophila* management strategies
- Weak or no correlation over the entire data set, both types of secondary disinfectants
- Significant correlation observed at the utility level, though not for all utilities

| | Pearson correlation coefficient estimate ($\hat{\rho}$) | *P* ($H_0$: the true correlation = 0) |
|---|---|---|
| **All samples, free chlorine systems** | 0.087 | $1.21\times10^{-12}$ |
| **All samples, chloramine systems** | -0.019 | 0.88 |




(a) Free Chlorine Systems with Correlation

(b) Chloramine Systems with Correlation

# Modeling Caveats and To-Do List

## Caveats

- Models were developed using data from all systems pooled
  - As seen earlier, baseline *L. pneumophila* conditions differ among utilities
  - Model results provide a general sense of the level of control but might not reflect control at a given utility
- System-by-system models need to consider correlation between residual disinfectant concentration and water temperature
- Many potentially important factors not present in the model (recall – disinfectant is not a silver bullet)

## To-Do List

- Collect and analyze more data, including contextual data
- Analyze data with system as a factor (Bayesian statistical analysis)
- Re-analyze data after removal of low-concentration positives
- Re-analyze the data with low-concentration *L. pneumophila* data excluded

# Utilities can and should monitor *L. pneumophila* in their DSs

- To date, **no surrogate parameter has been shown to predict *L. pneumophila*** occurrence or abundance reliably and, in the current study, *L. pneumophila* were detected in samples with high disinfectant concentrations, low water temperatures, and low levels of HPC bacteria.
- **Routine *L. pneumophila* monitoring is already in place at several utilities** that participated in this research project and proved both feasible and affordable for most of the utilities that participated in this research project.
- You can't determine whether you are performing well enough if you don't collect the data! **We need more data to better define our baselines and to verify that controls are effective**.

# Regulators should develop consensus on reporting and communication requirements for utilities detecting *L. pneumophila* in distribution system samples

- **Reporting requirements, or lack of them, can be a strong disincentive** against starting new monitoring programs for unregulated contaminants.
- At present, there is no pertinent national guidance, and most primacy agencies and health departments **lack guidance or infrastructure for assisting utilities** in appropriately addressing *L. pneumophila* positive samples.
- Thus, **many utilities are hesitant to monitor**, despite the likely benefits to the utility and for public health.
- This study provides a **starting point for developing reasonable consensus**.

# *L. pneumophila* data collected by utilities or regulators should be maintained in an analyzable database that is accessible to researchers

- Low positivity rates and many factors mean **very large data sets** of *L. pneumophila* concentration paired with contextual data are needed.
- To protect the utilities contributing data and promote data submission, utilities should be anonymized in the database, though contextual information (e.g., system size category, primary source water type, etc.) should be retained with sample data.

# Final Thoughts on Next Steps

- **More research is needed** to better define baseline occurrences, and what constitutes whether a distribution system is in or out of control.
- Data are needed on **case studies** where controls are used to mitigate elevated occurrences.
- The **RTCR provides a good example** of how a monitoring program for *L. pneumophila* could be designed.
- We need to **incentivize utilities to collect data**:
  - The current MCLG of zero for *L. pneumophila* is a disincentive and should be reconsidered.
- Knowing how little we still know **we need to advance in reasonable steps**, based on sound science, so that we don't paint ourselves into a corner.