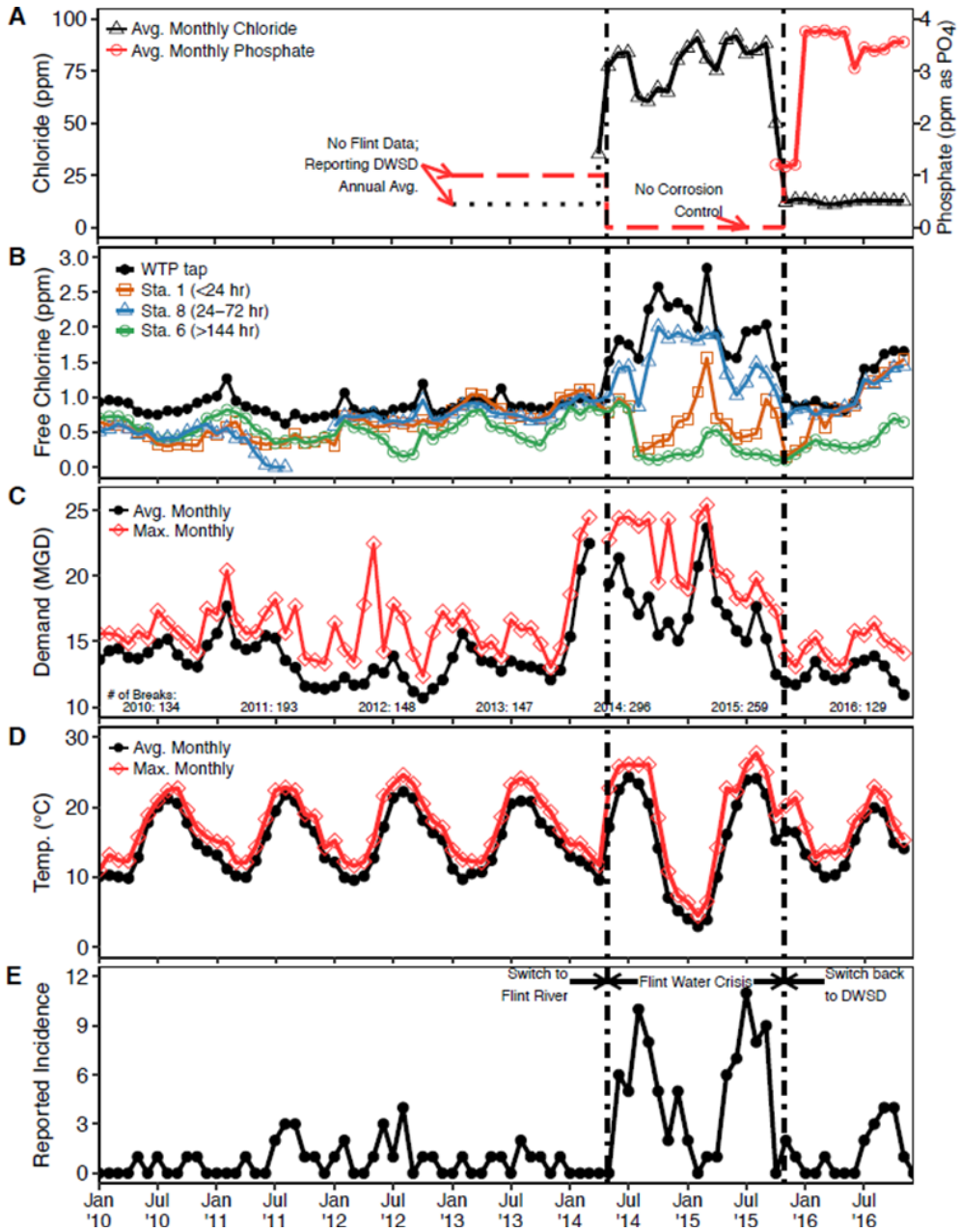


The National Academies of SCIENCES ENGINEERING MEDICINE — WATER SCIENCE AND TECHNOLOGY

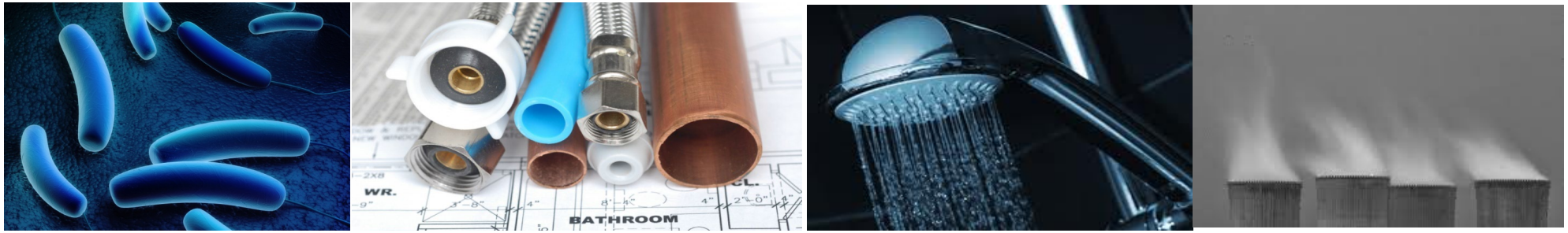
Management of *Legionella* in Water Systems

December 10, 2020 | 11:00 AM - 2:00 PM ET

Monitoring *Legionella* in Water Systems

Amy Pruden
W. Thomas Rice Professor
Virginia Tech

Rhoads et al. *Environ. Sci. Technol.* 2017 "Distribution System Operational Deficiencies Coincide with Reported Legionnaires' Disease Clusters in Flint, MI"



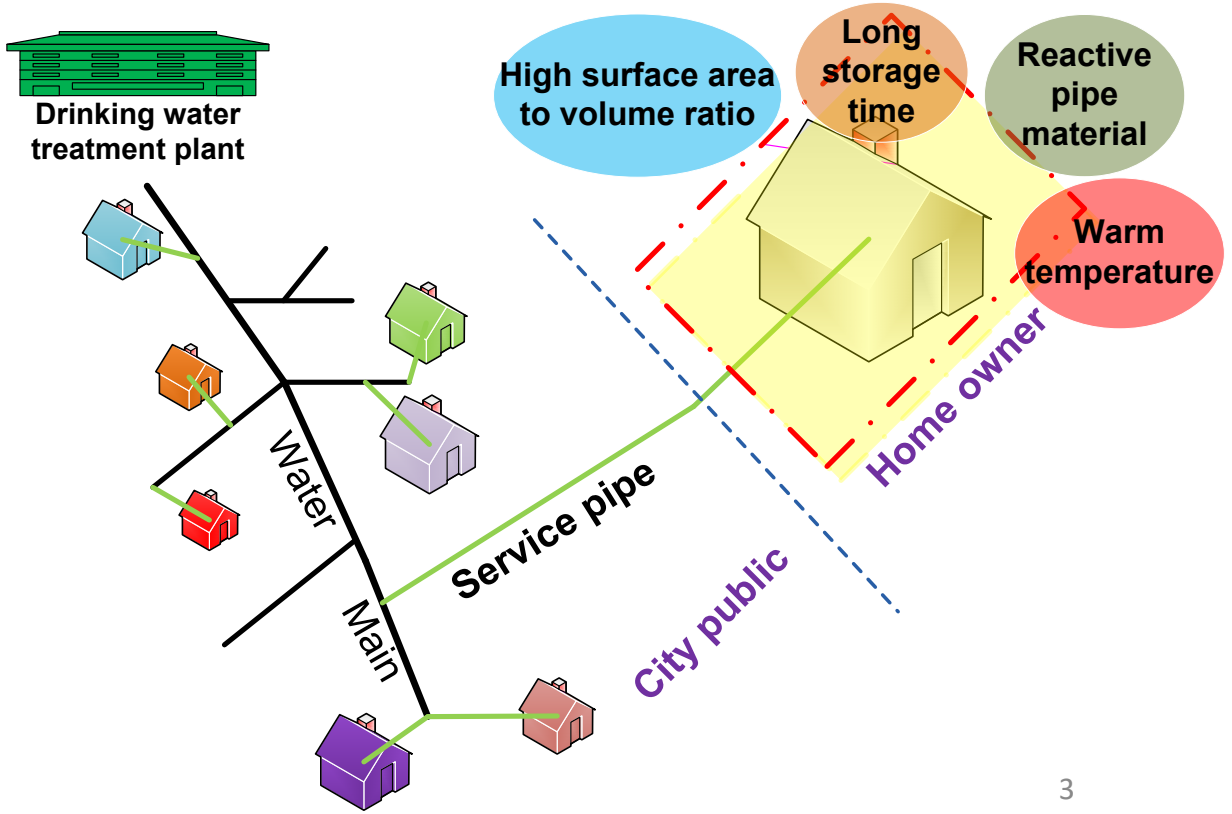
Why test *Legionella* in the water?

- **Outbreak response-** Find source of an outbreak and mitigate
- **Outbreak prevention-** Identify red flags, where is there vulnerability for transmission
- **Mitigation assessment-** Is the mitigation applied (e.g., disinfectant added, temperature elevated) resulting in reduced *Legionella* numbers?
- **Research-** Expand knowledge about the conditions that lead to *Legionella* amplification, and how to control it

*The Safe Drinking Water Act **does not** provide protection from Legionella*

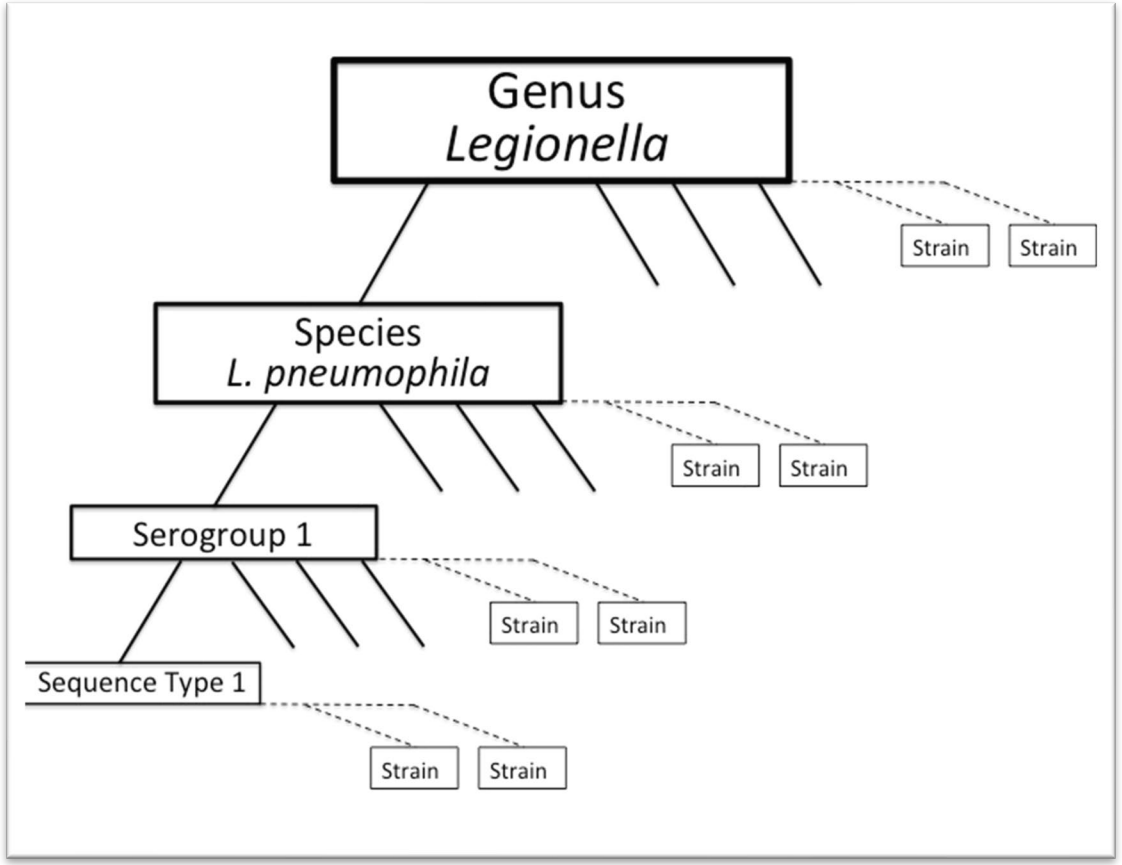
Cooling Towers:
Optimal *Legionella* growth Temp + aerosols

- The role of water utilities today:
  - Be responsive to SDWA
  - Provide distribution system disinfectant residual *to the property line*
  - *No requirement for* Legionella *monitoring*
- Premise plumbing distinct from water mains:
  - Surface/volume ratio- *biofilms*
  - Temperature
  - Water age
  - Poor persistence of disinfectant residuals
- Premise plumbing can create ideal environment for *Legionella* growth



What to test?

- There are 61 known species of *Legionella*
- Pathogenic species include: *L. pneumophila, L. micdadei, L. bozemanii, L. dumoffi, L. anisa, and L. longbeachae*
- *L. pneumophila* is the most dominant species isolated from patients and associated with risk
- 14 serogroups (SGs) of *Legionella*, clinical testing tends to only focus on serogroup 1, environmental more broad.
- Numerous sequence types (STs), determined through isolation and whole genome sequencing or sequencing 7 alleles



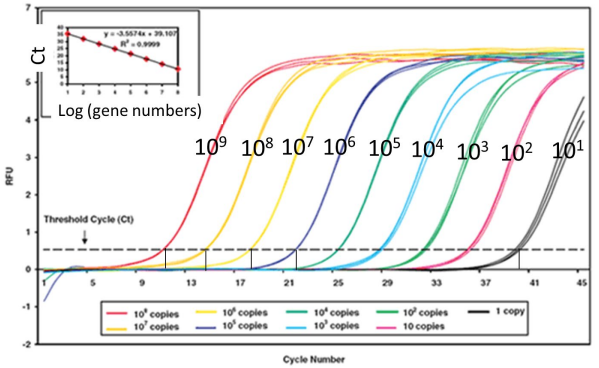
Ideal Method Attributes

- Specific- Exclusively detects *Legionella* target
- Sensitive- Can detect low levels
- Quantitative- Reports numbers, not just +/-
- Fast- Able to identify problem sites quickly
- High-throughput- Able to assess many sites in parallel
- Discriminant- Live, dead or VBNC
- Economical – Low $$
- Easy to use- No specialized skill required, in-field detection
- *In situ* - Reflective of actual situation in system when sampled
- *Indicative of risk* – Represents an infectious and transmissible target, informative to risk assessment
  - E.g., *L. pneumophila* more frequently associated with disease than other species

Culture

versus

Quantitative Polymerase Chain Reaction (qPCR)



- Specific- But, requires confirmation testing
- Sensitive- 10 CFU/mL
- Quantitative- But, have to get dilution right
- Discriminant- Exclusively reports live organisms (not VBNC)
- Not fast or high throughput (3-7 days minimum for result)

Suitable application:

- Outbreak response- to identify source and compare to clinical strains
- Baseline monitoring of highest risk sites
- Assess that mitigation actually *killing Legionella*

- Specific- Can target Lp, Lspp., SG1, etc.
- Fast- Results in < 1 day
- Sensitive- Depends on volume
- Quantitative- *more so* than culture
- High-throughput- 90 well plates
- Does not discriminate live/dead/VBNC

Suitable application:

- Broad outbreak response- identify sites to focus culturing
- Establish baseline for routine monitoring, quickly identify anomalies
- Long-term assessment of mitigation, coupled with culture to verify *Legionella* being killed

Legionella Culture Methods

ISO/BYCE Method

- Gold standard
- 3-7 days for result- + confirmation (by culture, MALDITOF or PCR)
- Biased towards *L. pneumophila*, but captures other *Legionella* too
- Provides an isolate that can be further characterized and genetically compared to clinical isolates in outbreak investigation

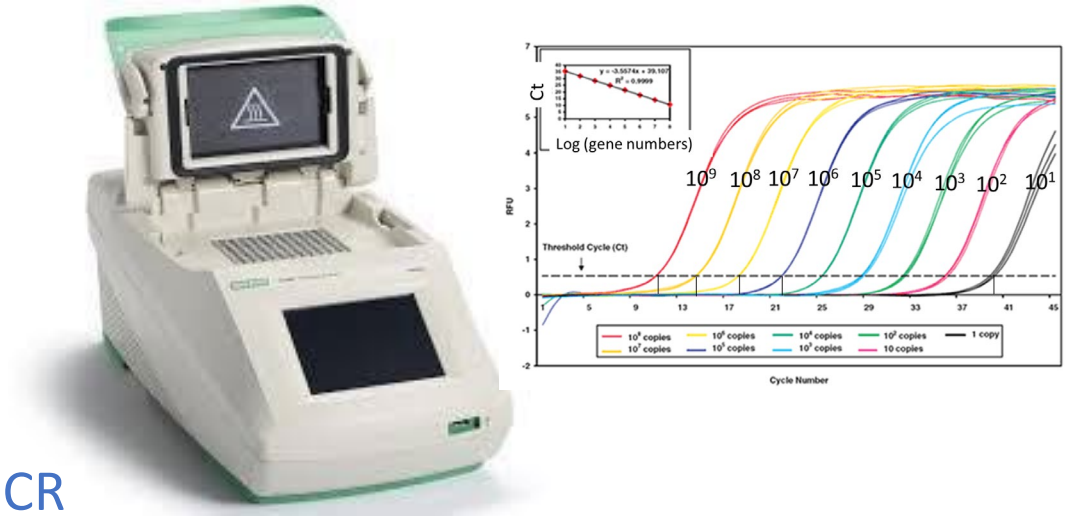
e.g., commercial option: LegioIert

- Easy to use- no need to send to outside lab
- Higher throughput than traditional culture
- Targets *L. pneumophila* only
- Results in 7 days
- Reports MPN/L
- Wells can be analyzed further..

Legionella qPCR methods

Example Chapter 3 Box 3-4: Methods. New York used PCR for rapid screening of cooling towers, followed by culture.

Commercial in-field qPCR



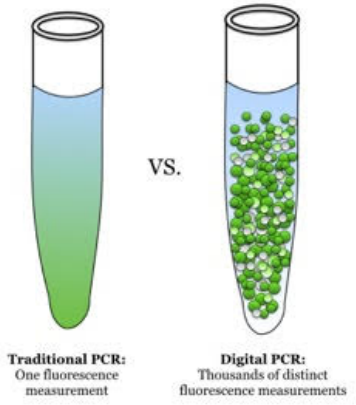
Traditional lab-based qPCR

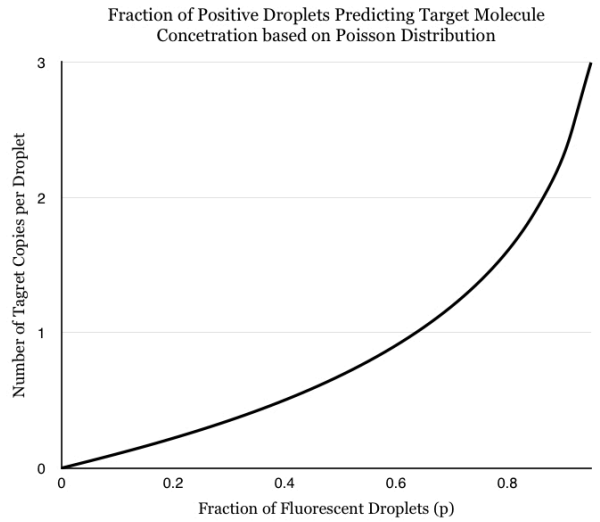
qPCR Examples

THE Water Research FOUNDATION

WRF project
4721 PI Raskin

Targeted organisms	Targeted gene	Amplicon size (bp)	Reference
***Legionella* spp.**	23S rRNA	92	Nazarian et al. 2008
L. pneumophila	*mip*	78	Nazarian et al. 2008
L. *pneumophila* SG 1	*wzm*	75	Merault et al. 2011

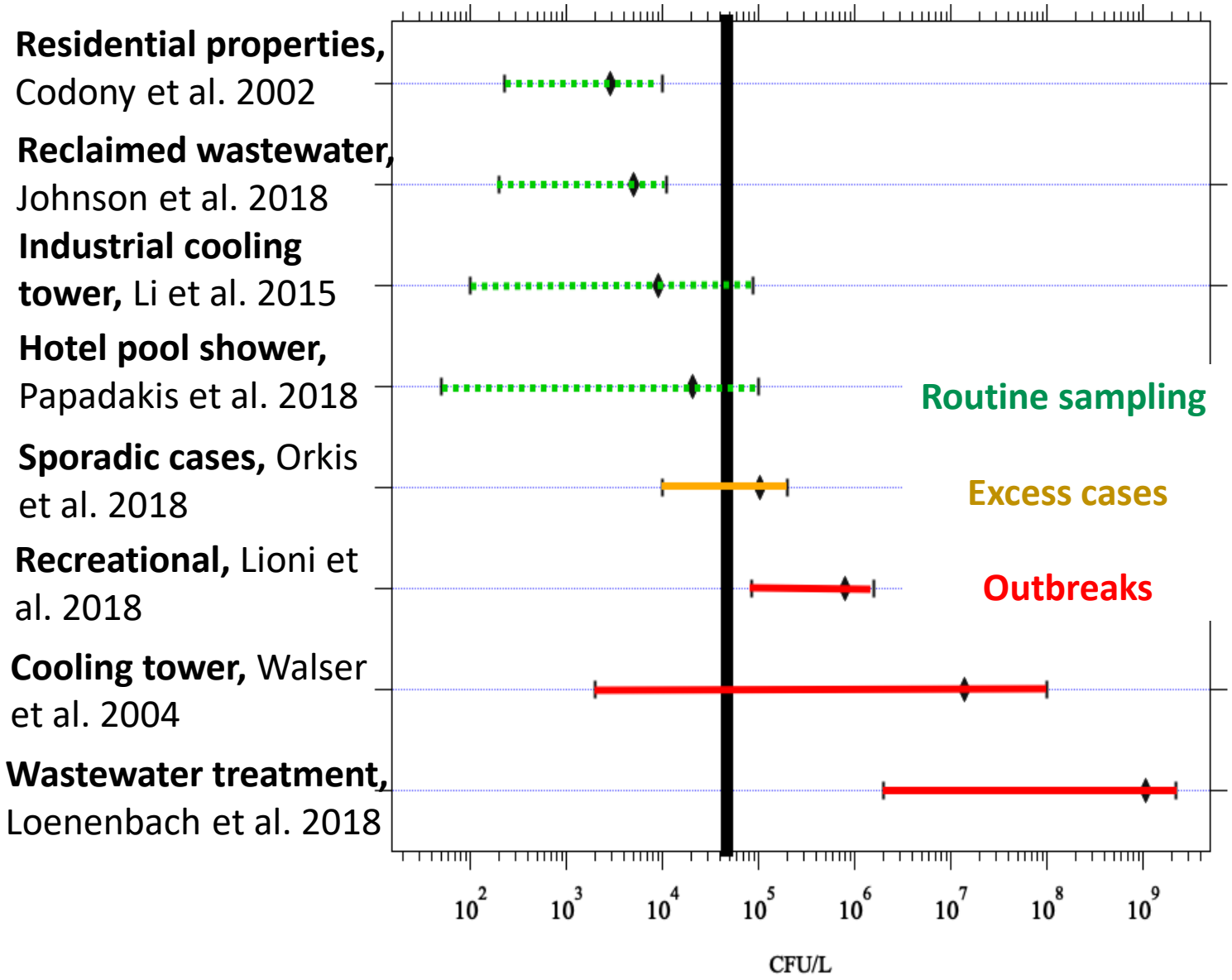




Droplet Digital qPCR

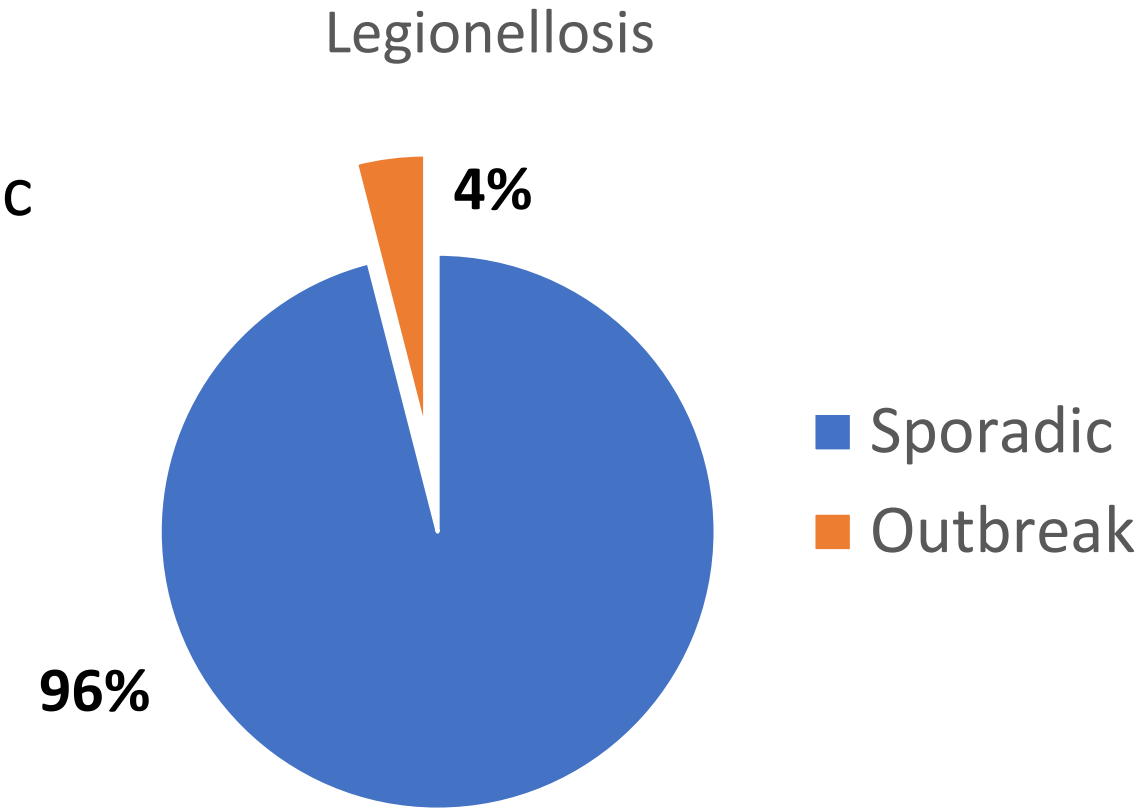
What Level Should Trigger Concern/Action/Remediation?

- **50,000 CFU/L** was identified as corresponding to imminent outbreak based on published studies
- Suitable "Action level," likely lower than 50,000 CFU/L, but greater than zero.
- EU Directive (Oct. 2020) 1,000 CFU/L
- Might also vary from building to building:
  - Lower levels for at risk individuals/facilities
  - Value to assessing baseline and noting anomalies



Most LD is Sporadic, not Outbreak

- Most of what we know is from outbreaks, but most disease is sporadic
- *Could a national survey of distribution systems and premise plumbing help us better understand sporadic sources?*
- *Could better inform long-term monitoring and regulatory requirements*



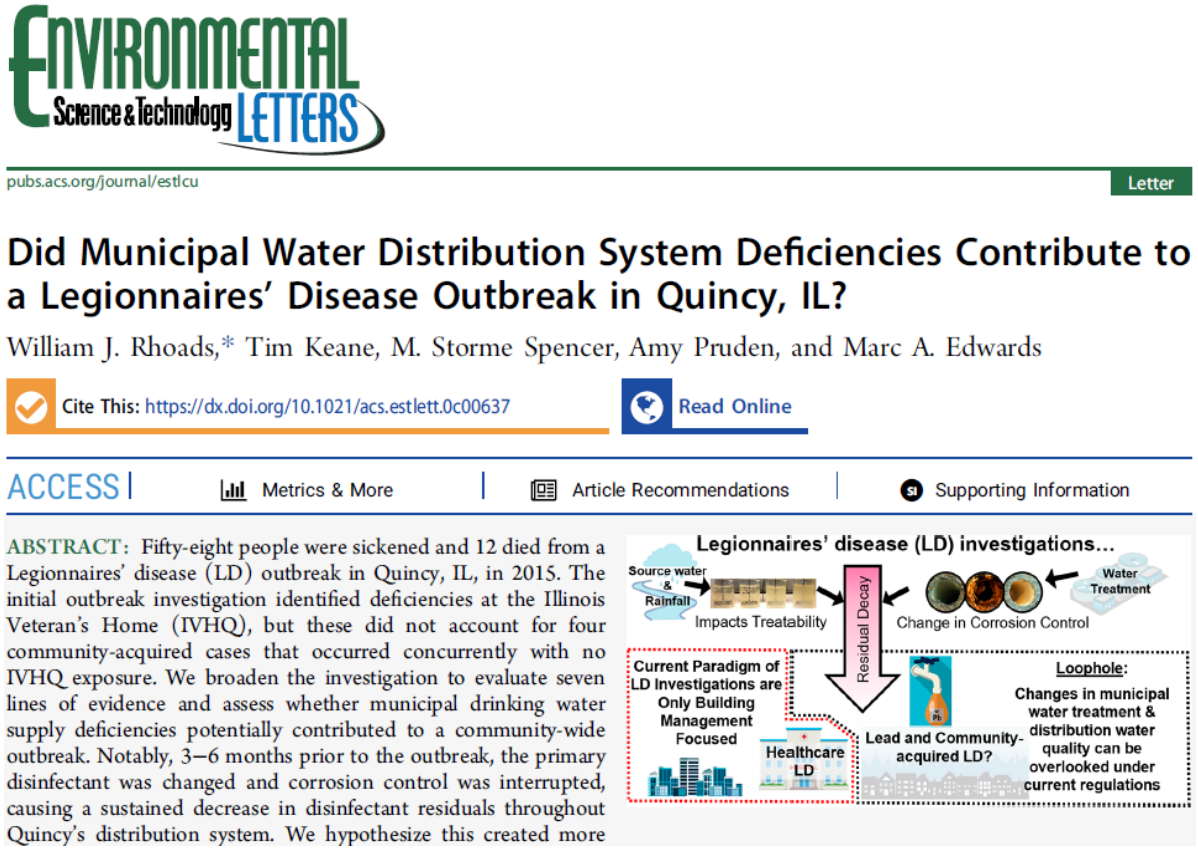
Hicks, L., L. E. Garrison, G. E. Nelson, and L. M. Hampton, 2011. Legionellosis—United States, 2000–2009. *Morb. Mortal. Wkly. Rep*. 60(32):1083-1086.

Distribution System Monitoring?

- Widespread distribution system monitoring will be costly and likely yield little *Legionella* detection
- Required reporting, elevated vigilance and monitoring around "events" could help focus *Legionella* monitoring:
  - Lapse in corrosion control
  - Storms
  - Watermain breaks
  - Construction
- Could also identify high risk portions of the distribution system with high water age / worst case scenario buildings
  - Analogous to Pb-Cu Rule?
  - Potentially gain insight into sporadic disease?

THE Water Research FOUNDATION

WRF project 4911
PI Burkharia



ENVIRONMENTAL Science & Technology LETTERS

pubs.acs.org/journal/estlcu Letter

Did Municipal Water Distribution System Deficiencies Contribute to a Legionnaires' Disease Outbreak in Quincy, IL?

William J. Rhoads,* Tim Keane, M. Storme Spencer, Amy Pruden, and Marc A. Edwards

Cite This: https://dx.doi.org/10.1021/acs.estlett.0c00637 Read Online

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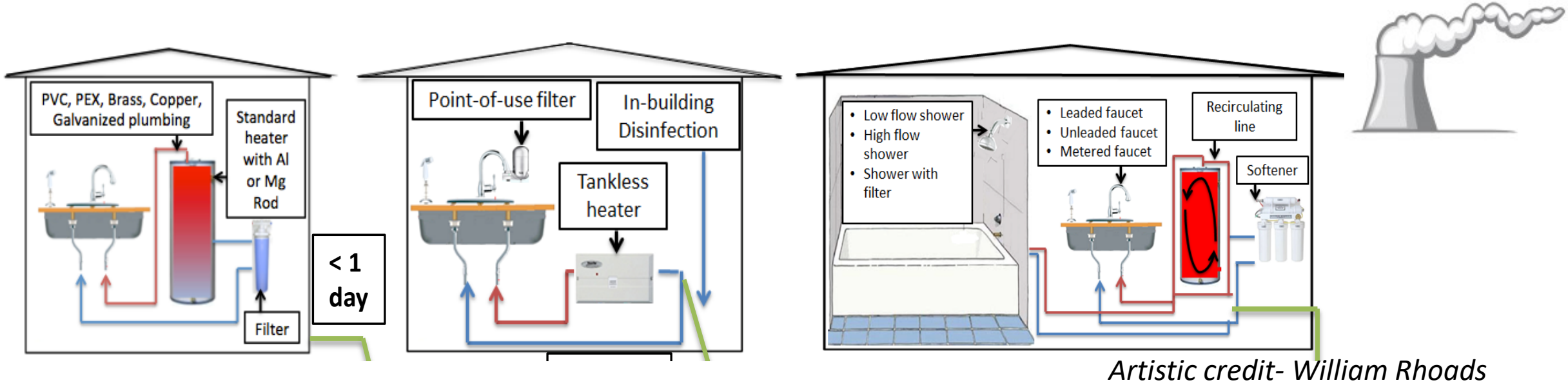
ABSTRACT: Fifty-eight people were sickened and 12 died from a Legionnaires' disease (LD) outbreak in Quincy, IL, in 2015. The initial outbreak investigation identified deficiencies at the Illinois Veteran's Home (IVHQ), but these did not account for four community-acquired cases that occurred concurrently with no IVHQ exposure. We broaden the investigation to evaluate seven lines of evidence and assess whether municipal drinking water supply deficiencies potentially contributed to a community-wide outbreak. Notably, 3−6 months prior to the outbreak, the primary disinfectant was changed and corrosion control was interrupted, causing a sustained decrease in disinfectant residuals throughout Quincy's distribution system. We hypothesize this created more

Premise Plumbing & Cooling Tower Monitoring



Artistic credit- William Rhoads

- Premise plumbing has multiple niches where *Legionella* can grow
- Sampling strategy must capture <u>representative</u> areas of risk
- Establish baseline, determine if levels rise above baseline- anomaly?
- Comparison to 1,000 CFU/L, a quantitative microbial risk assessment (QMRA)-derived goal equating to 10^{-6} DALY seen in Chapter 5 for faucets

Verifying Mitigation is Effective

qPCR Example

A *L. pneumophila* concentration (log gene copies/mL)

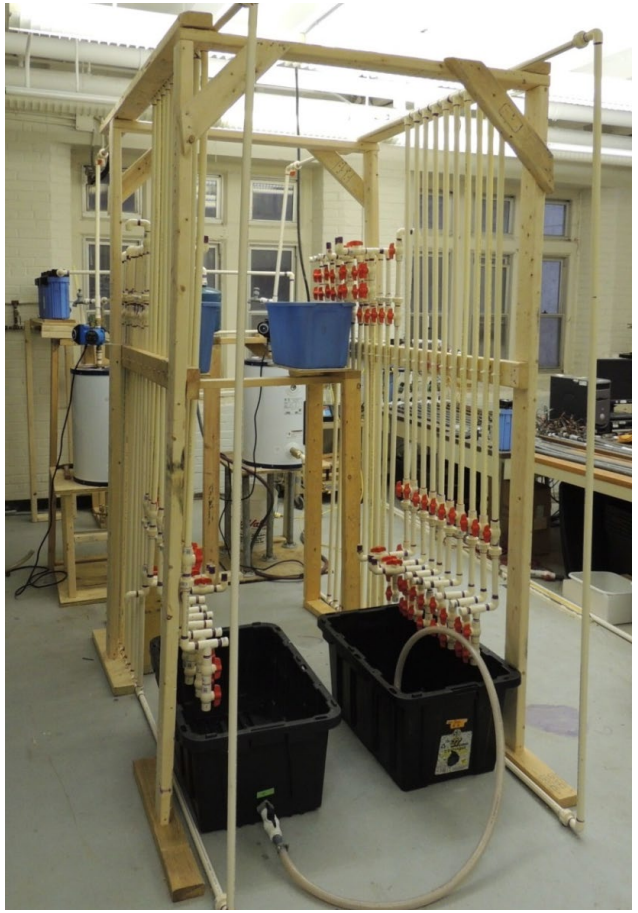
Control System (39° C)	Water Use Freqency Low	Medium	High
5 months	4.2	4.2	4.1
8 months	4.2	3.4	3.9
13 months	4.3	4.7	4.3
15 months	5.1	4.8	4.3

39°C- *Legionella* proliferates

Experimental System	Water Use Freqency Low	Medium	High
Baseline (39° C)	4.3	4.3	4.2
Exp.1 (42° C)	3.5	3.1	3.0
Exp. 2 (51° C)	4.3	2.3	2.2
Exp. 3 (58° C)	3.4	3.2	2.7

Increasing T 39°C - 58°C- *Legionella* killed

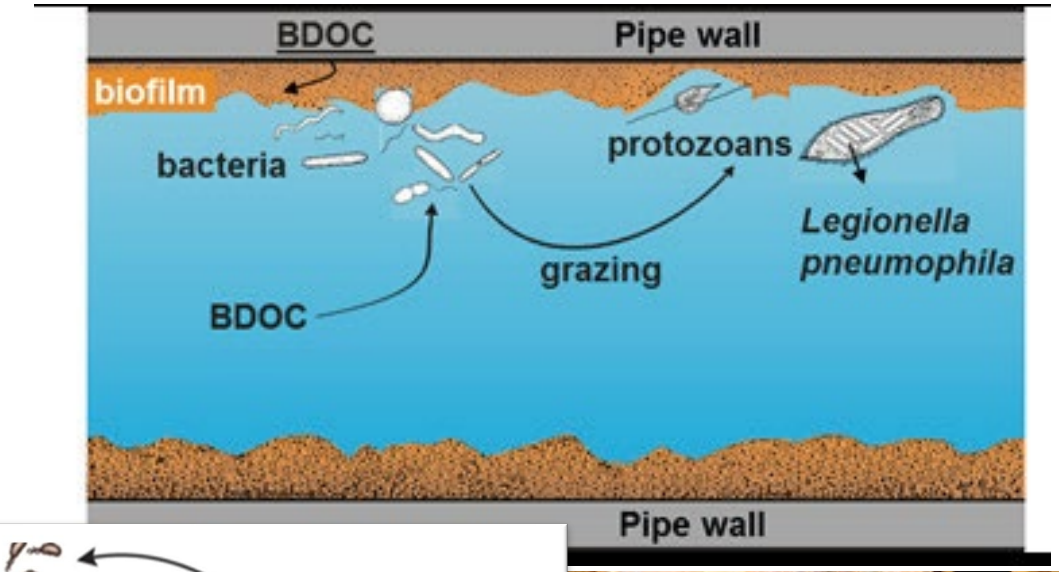
William Rhoads



Rhoads et al. Water Heater Temperature Set Point and Water Use Patterns Influence Legionella and associated Microorganisms at the Tap. *Microbiome* 2015

Biofilm versus Water

- *Legionella* proliferates in biofilms, participating as part of a complex ecology of organisms
- Growth in and release from free-living amoebae
- Sampling not easy!
  - Difficult to access
  - Difficult to quantify
- Water more appropriate for routine sampling, biofilm for outbreak investigation or research



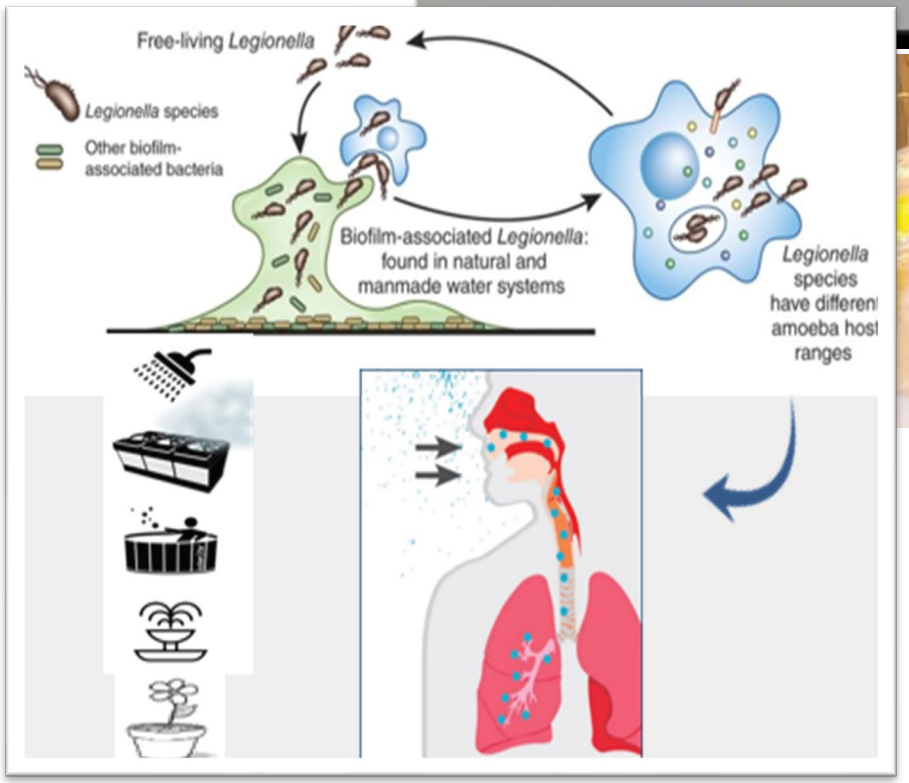


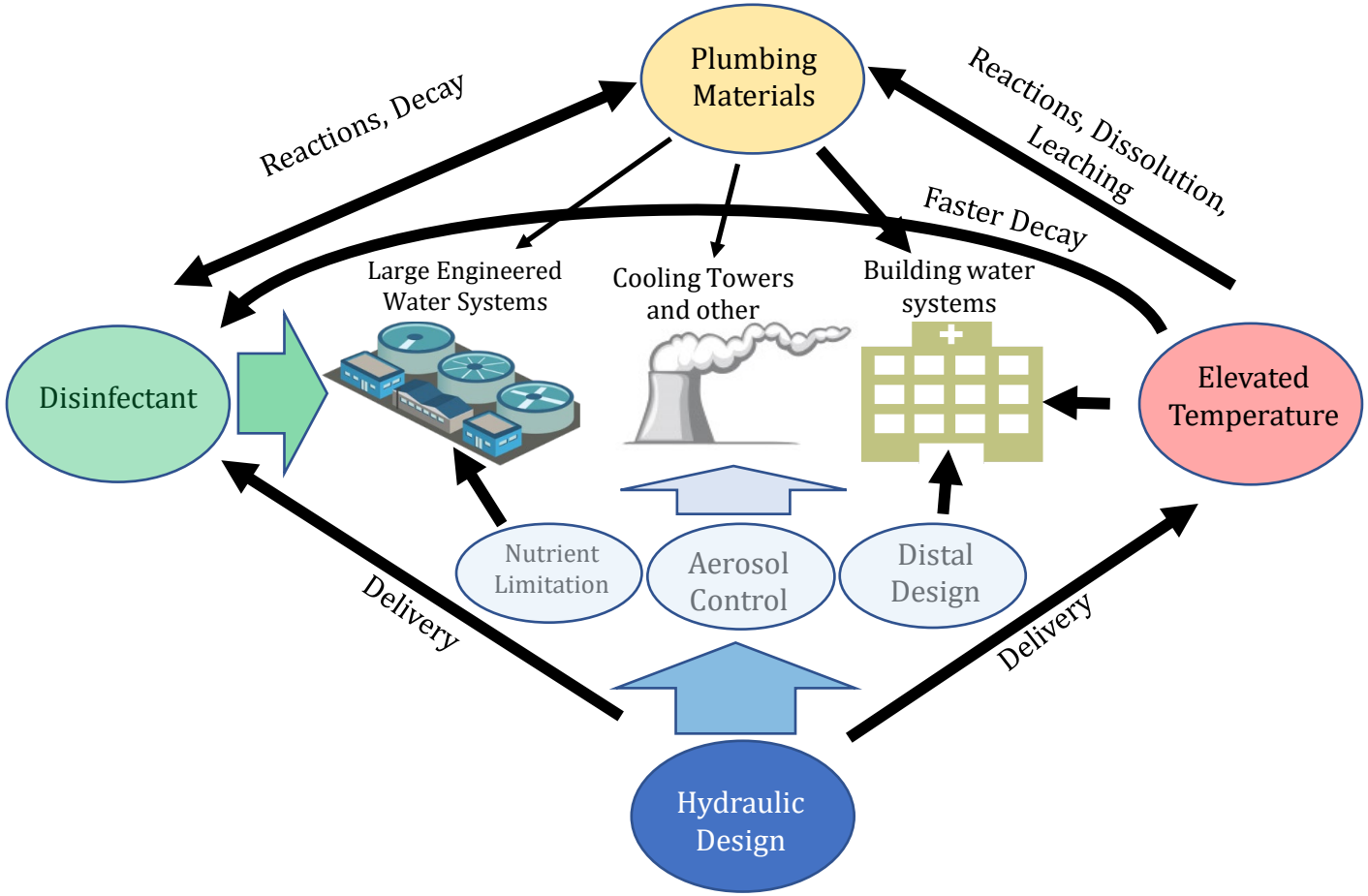
Fig 1-5: Courtesy of Paul van der Wielen

Some Relevant Recommendations from the Report

1. Expand the Centers for Medicare & Medicaid Services memo **to require monitoring for *Legionella*** in environmental water samples for all hospitals (Chap. 5)
2. **Register and monitor cooling towers** (Chap. 5)
3. Determining the most common sources of **sporadic disease** will require well-funded, population-based studies in multiple jurisdictions (Chap. 3)
4. **Regional Centers of Excellence** could serve as a backbone to strengthen the capacity of state health departments to detect and investigate cases of LD (Chap. 3)
5. **Systematic comparison** of culture methods for *L. pneumophila* (and other pathogenic legionellae) ddPCR, qPCR, viability-qPCR, and reverse transcriptase qPCR (Chap. 3)
6. **Quantitative microbial risk assessment** can help determine standards/targets for routine monitoring, mitigation, and regulation. How to incorporate qPCR-based measurements (Chap. 3)

Putting it all in context

- *Legionella* numbers will be most informative in the context of everything else happening in the system
  - What factors can be contributing to growth of *Legionella*?
  - What kinds of disruptions to system operation could be at play?



Summary-

- Environmental monitoring of *Legionella* is key to identifying problems, verifying system controls/mitigations are working, and preventing the spread of LD
- We need a framework- to support environmental monitoring of *Legionella*….
  - What to monitor?
  - Where to monitor?
  - Frequency of monitoring?
  - Action levels?
  - How to implement?