

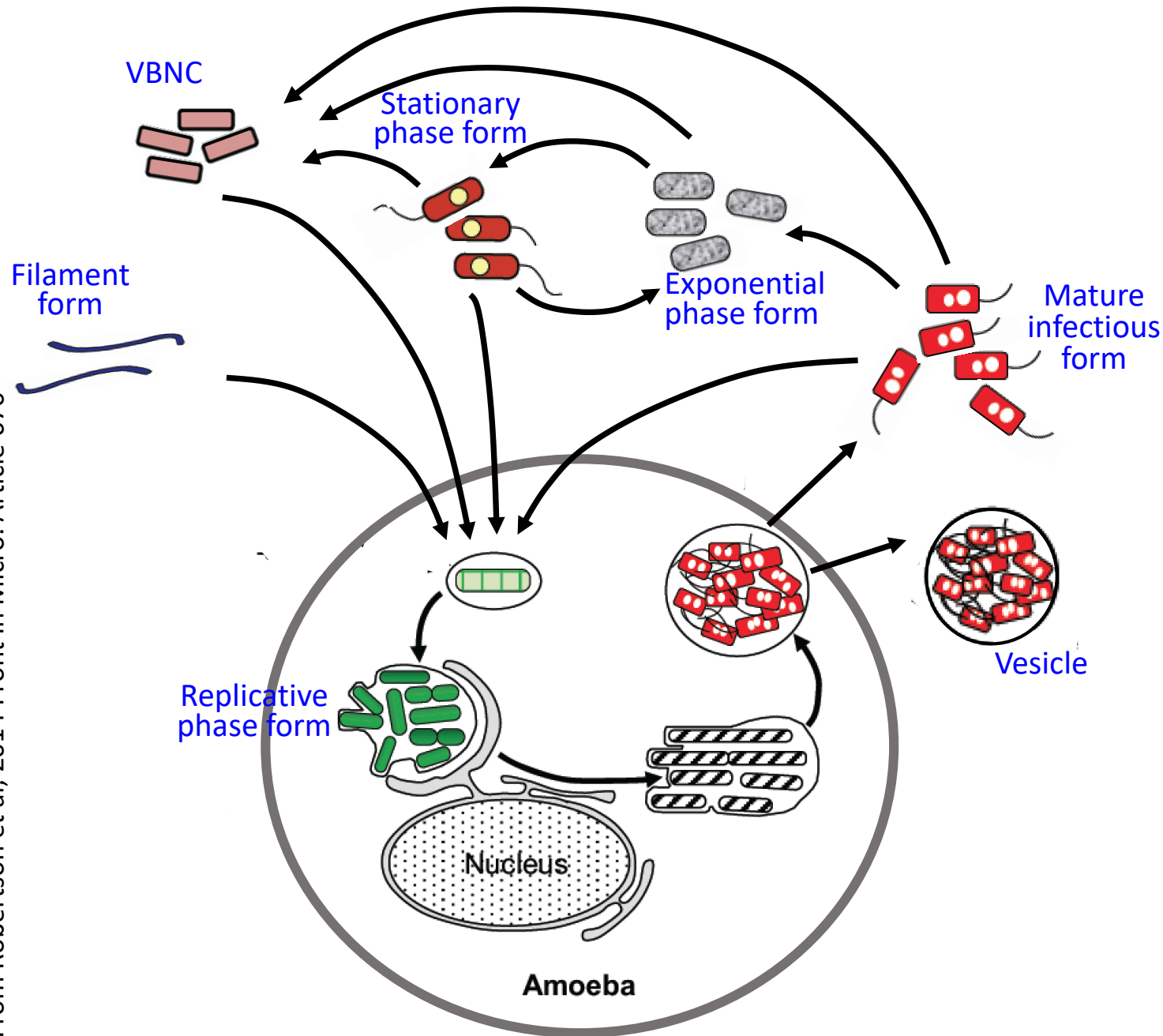
Legionella in premise plumbing systems: ecology and control

Dr Phil Bond

ARC Linkage Project Proposal



What do we know about Legionella?



- Has a complex life cycle
 - A range of forms includes VBNC state
- Cultured isolates studied in planktonic form
- Detection is by culturing from water samples (planktonic)



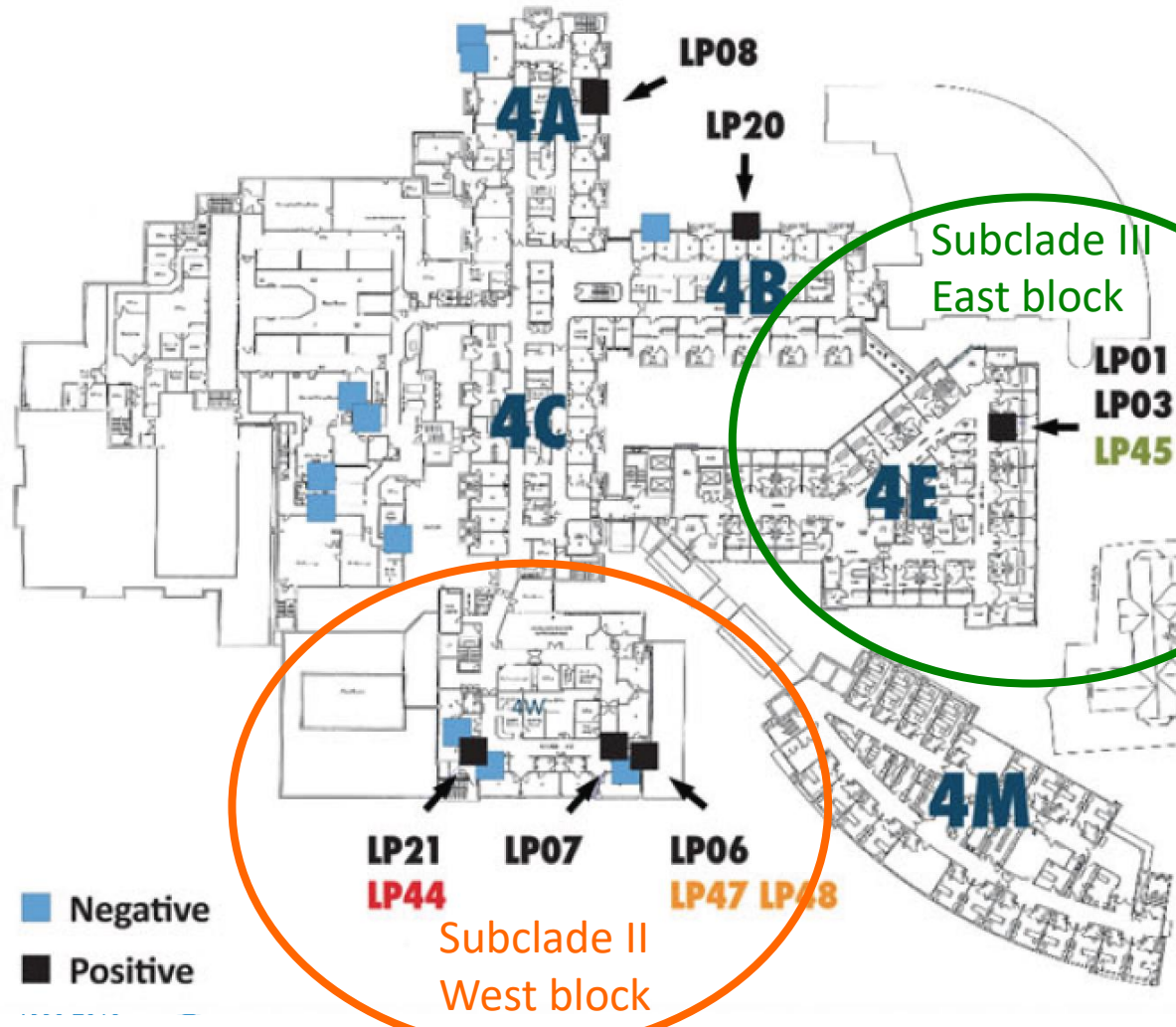
- In the environment will mostly exist in a biofilm!

The Wesley Hospital legionellosis occurrences

- 2011 outbreak, 73 year-old patient died
- 2013 outbreak, one patient died, another in intensive care
 - Tracked source to contaminated water taps
- 2016, a patient tested positive for legionellosis
 - Ice machine was possible source
- Plumbing systems were treated post 2013 outbreaks
 - Series of flushing with hot water, and hyperchlorination treatments
- *Legionella* isolates were available from patients and from water samples
- Used Whole Genome Sequencing (DNA) to characterise isolates

Genomic study of Wesley Hospital Legionellosis outbreaks

Wesley Hospital, Level 4 plan



- *L. pneumophila* SG1 isolates obtained from hospital tap water and patients
- Oct 2011, 73 year-old patient (LP44)
- 2013 patients (LP47, LP48, LP45)
- Characterised the isolates by sequencing their genomes (fine resolution)
- Very few differences between genome sequences of isolates

Strains of *L. pneumophila* SG1 maintained in system as persistent biofilms in plumbing

Premise plumbing – good environment for *Legionella*.

Pipe conditions:

- Disinfectants levels vary
- Temperature ranges - warm
- Pipe material: copper, iron, synth polymers, fittings
- Low dissolved oxygen levels, low nutrient
- Flow variations: dead end pipes
- Connections to various devices, taps, showers, ice machines
- High surface area:volume



Legionella:

- Survive well in low nutrient conditions, viable but non-culturable state (VBNC)
- Biofilm ecology suits their many growth stages
- Biofilm provides protection

Specific qualities and ecology of pipe biofilms

- Mixed microbial communities, diverse, interacting, structured
- Cells adhere by use of extracellular polymeric substances (EPS)
- Conditions within biofilm habitat varies
- Will support persistent/slow growing microbes
- Chemical disinfectants less effective
- Removal is difficult, if not impossible
- Difficult to monitor and study
- Does occurrence of *Legionella* correlate with other microorganisms?
 - Replicates within amoeba,
 - Likes *Pseudomonas* spp.
 - *Bacillus* spp. can be toxic

Establish ARC and Industry funded project

Aims

- Improve understanding of *Legionella* ecology in biofilms occurring in hospital premise plumbing (PP).
- Test and determine treatments that reduce levels of *Legionella* in premise plumbing biofilms.

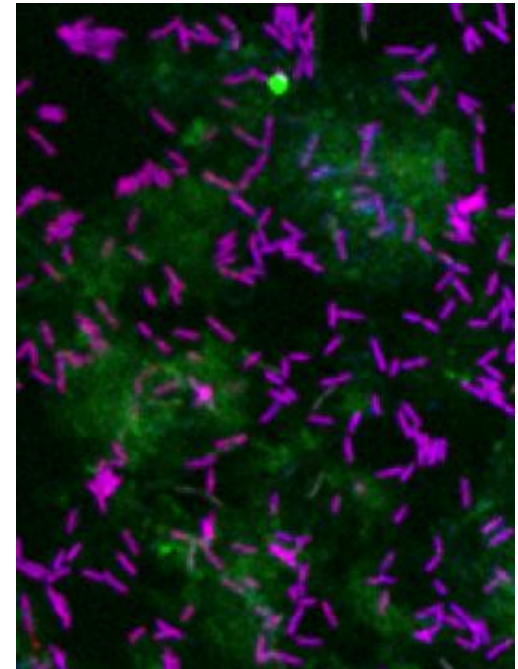
Approach to understand and control *Legionella* levels in plumbing pipe biofilms.



Task 1. Detect and understand *Legionella* types in pipe biofilm

- A range of methods can be used to detect *Legionella* in biofilms and determine the nature of the organism:
 - Specific detection of *Legionella*, e.g. standard culture method, FISH, qPCR, immunogenic separation,
 - Then general whole community analyses by DNA sequencing.
- Reveal the presence and nature of *Legionella* in the plumbing biofilms.
- Determine particular microbial-types and pipe conditions that either favour or are antagonistic towards biofilm *Legionella*.
- Examine questions:
 - are certain types of *Legionella* being released from biofilms?
 - are biofilm *Legionella* more infectious than water phase cells?

FISH: *Legionella*
& other Bacteria

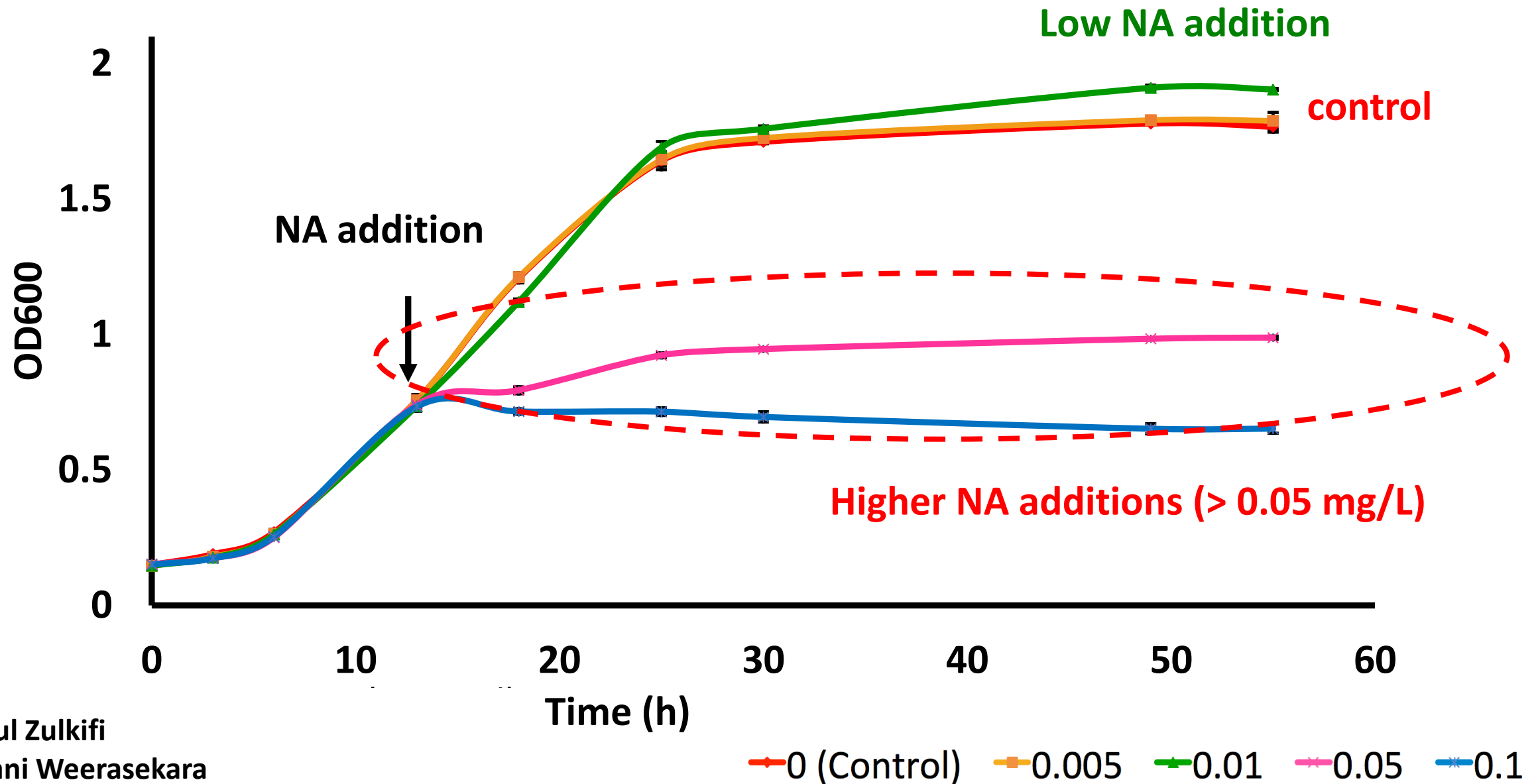


Approach to understand and control *Legionella* levels in plumbing pipe biofilms.

Task 2. Determine conditions that disfavour *Legionella* in pipe biofilms.

- Apply a range of antimicrobial agents and treatments to biofilms to detect the specific removal of *Legionella*.
 - real pipe biofilms
 - laboratory constructed biofilms.
- The range of treatments could include: chlorine, hydrogen peroxide, nitrite, copper-silver ions, silver nanoparticles, nano zero valent iron (NZVI), nitrous oxide, phage, and combinations
- Following the treatments, detect *Legionella* specifically and perform whole community analysis.
- Discover treatments that diminish *Legionella* abundance in the biofilm.

Legionella growth inhibited by nitrous acid (HNO₂)

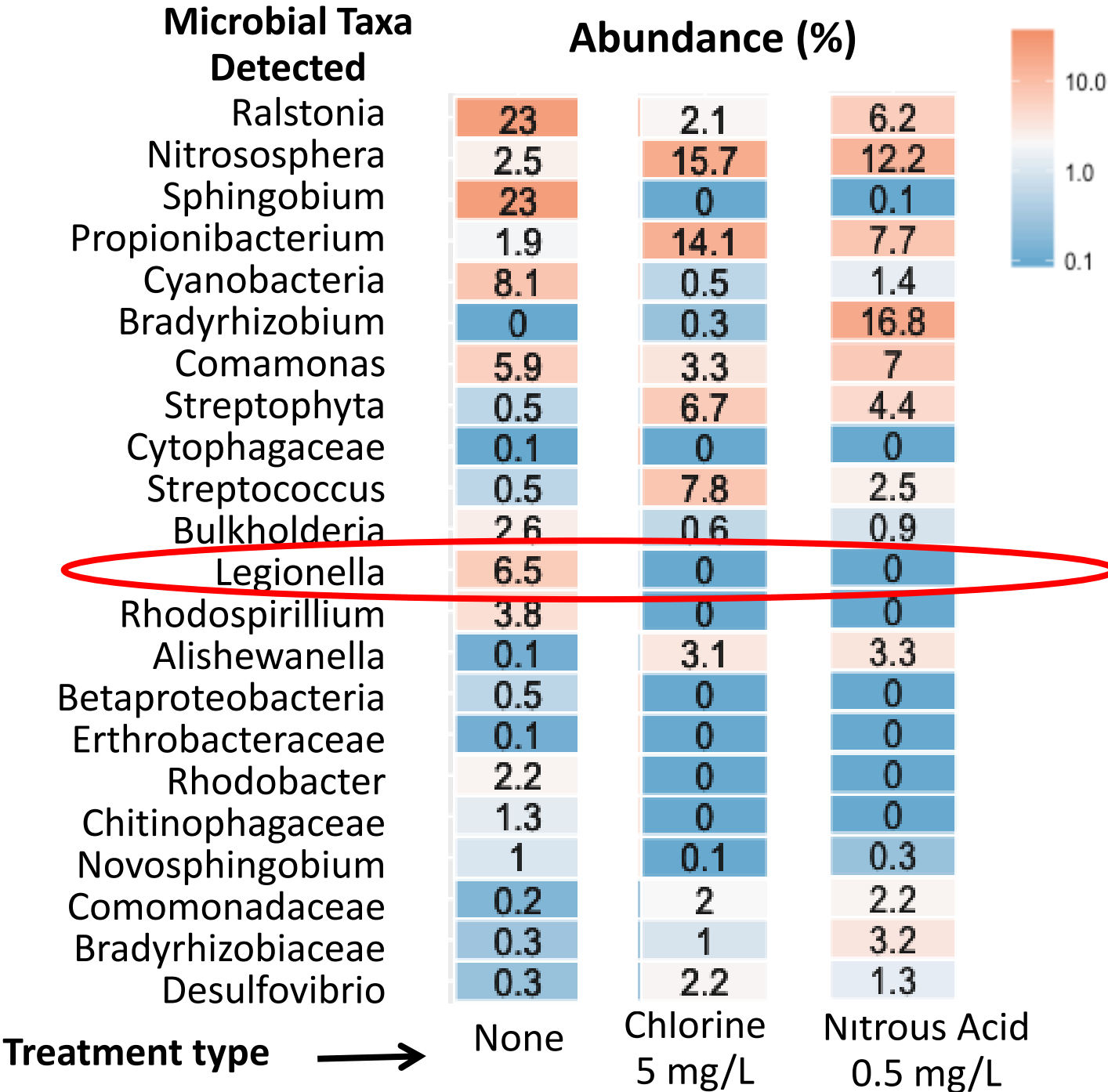


Microbial communities detected in pipe biofilms following treatment

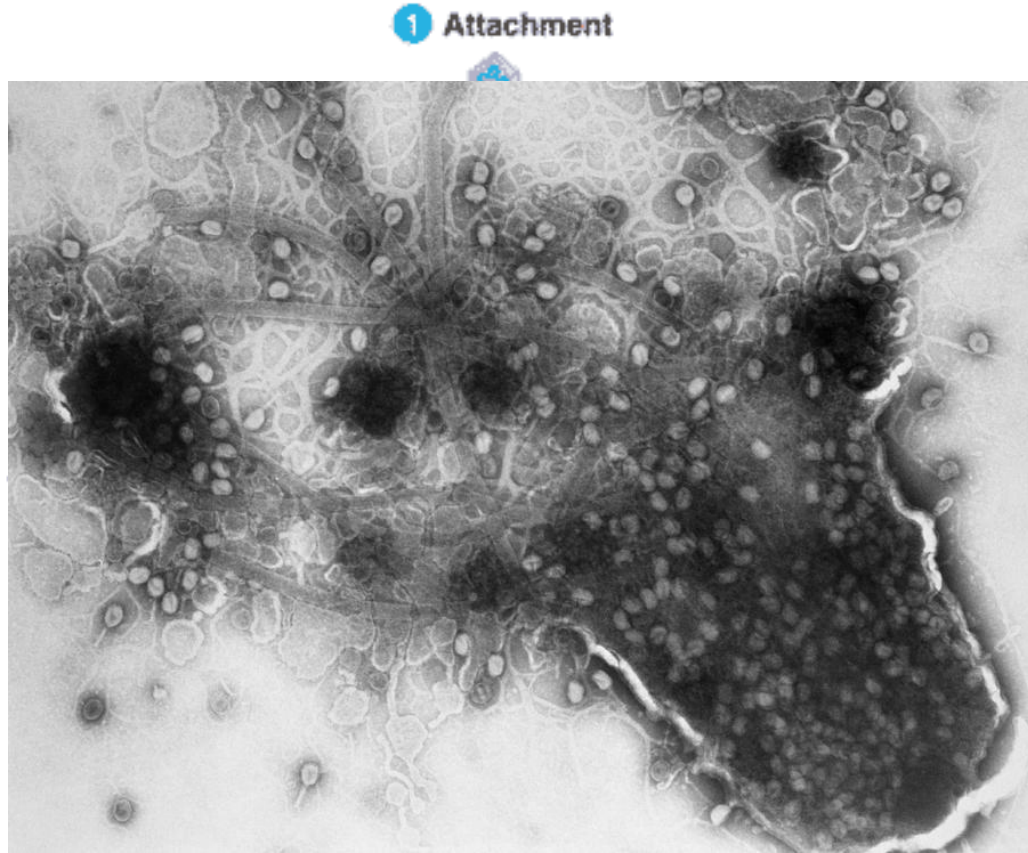
- Sections of copper pipe plumbing obtained from RCH, Herston
- In pipe biofilms treated 4 h/week over 6 weeks
- Microbial community composition determined by DNA sequencing (16S rRNA gene)



Lyman Ngiam
Anjani Weerasekara



Bacteriophage as a treatment to destroy *L. pneumophila* in pipe biofilms



- Phage (virus) can infect bacteria
- Replicate within bacteria and destroy cells.
- Phage can be species - strain specific
- Phage against *Legionella pneumophila* have been detected
- Isolate phage with strong/persistent infection capabilities of *L. pneumophila*
- Apply as a treatment to pipe biofilms

4 TEM of bacterial cell lysis due to phage infection

Approach to understand and control *Legionella* levels in plumbing pipe biofilms.



Task 3: To verify the use of the anti-*Legionella* treatment in a pilot-scale premise plumbing system.

- Testing of antimicrobial agents in the conditions of a real PP system for removal of *Legionella* from the biofilms
- Pilot-scale plumbing systems will be established at our large scale experimental facilities. These will contain removable pipe sections for biofilm analyses. Pipe water conditions will be typical of Queensland Health facilities.
- Outcome: provide a guide for treatment of premise plumbing to lessen the risk of *Legionella* infections from drinking water supply system.

ARC-Linkage Project proposal

- Proposal application to ARC due December 6th
- 3 Year project
- 5 Chief Investigators, Uni of Qld, Uni of Melbourne, 3 Partner Investigators, QH, CETEC, TWH
- 8 Industry Partners,
 - Queensland Health, The Wesley Hospital, CETEC, Beavis & Bartels, Metro South HHS, Metro North HHS, Master Plumbers Association QLD, Proteus Medical Services
- CIs, PIs and Industry Partners involved in project:
 - Preparation of Project Research Plan: includes research objectives, activities, outcomes and milestones.
 - Regular meetings (quarterly) with research team and Industry Partners during the project to discuss research activities, outcomes, progress on milestones.
 - Provide in-kind support to project

ARC-Linkage Project proposal

- Contract agreement between University of Queensland and Industry Partners
- Throughout the duration of the project the Industry Partners need to provide cash and in-kind support as detailed in the contract.
- Estimated ARC-LP funds to be requested ~ \$800k
- Industry Partner support currently is \$210k (cash)
- Seeking more Industry Partner support!

If interested, please speak to us! Phil.bond@uq.edu.au