Pathogen Treatment Guidance and Monitoring Approaches for On-Site Non-Potable Water

Jay Garland[1], Mary Schoen[2], Brian Zimmerman[1], Scott Keely[1], **Nichole Brinkman**[1], Susan De Long[3], Sybil Sharvelle[3], **Michael Jahne**[1]

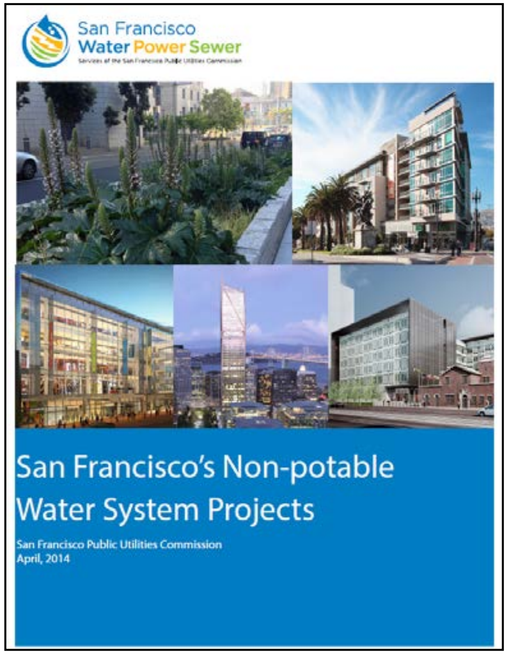
[1]USEPA Office of Research & Development

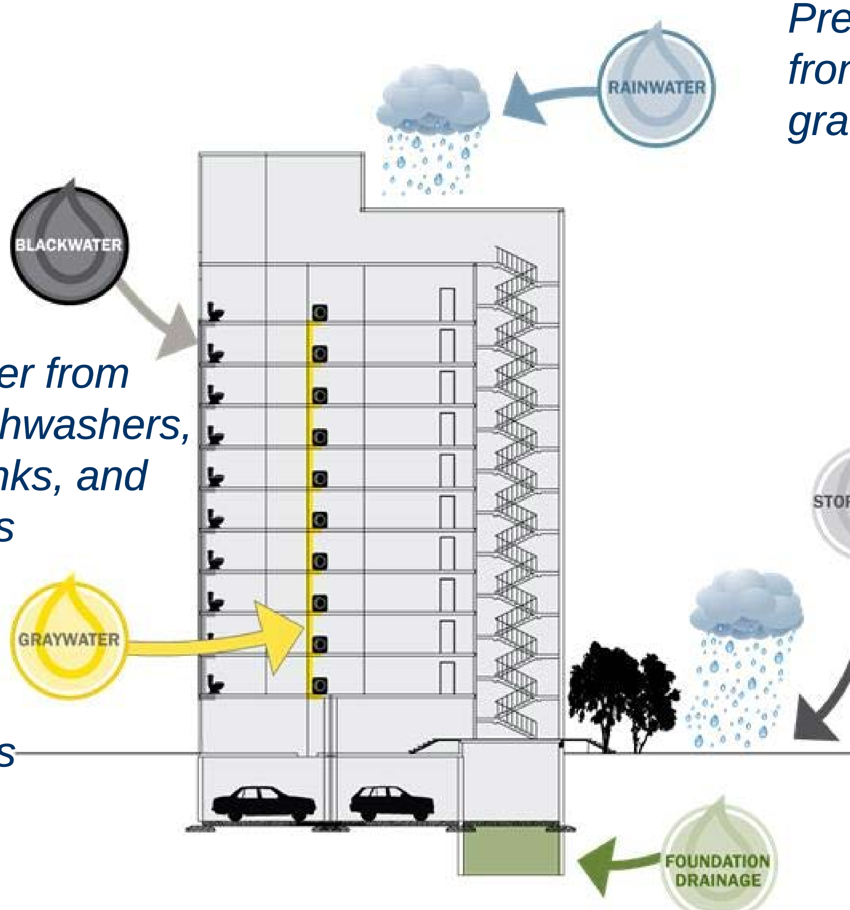
[2]Soller Environmental

[3]Colorado State University

Overview

- Context
  - Increasing interest in fit for purpose water reuse
  - Limits of conventional indicator organism approaches
- Approach
  - Quantitative Microbial Risk Assessment (QMRA) to define treatment requirements
  - Performance monitoring approaches
    - Rationale for moving away from traditional microbiological indicators
    - On-line, non-biological surrogates linked to treatment requirements
    - Alternative microbiological targets (infrastructure microbiome?)





Precipitation collected from roofs and above-grade surfaces

Wastewater from toilets, dishwashers, kitchen sinks, and utility sinks

Precipitation collected at or below grade

Wastewater from clothes washers, bathtubs, showers, and bathroom sinks

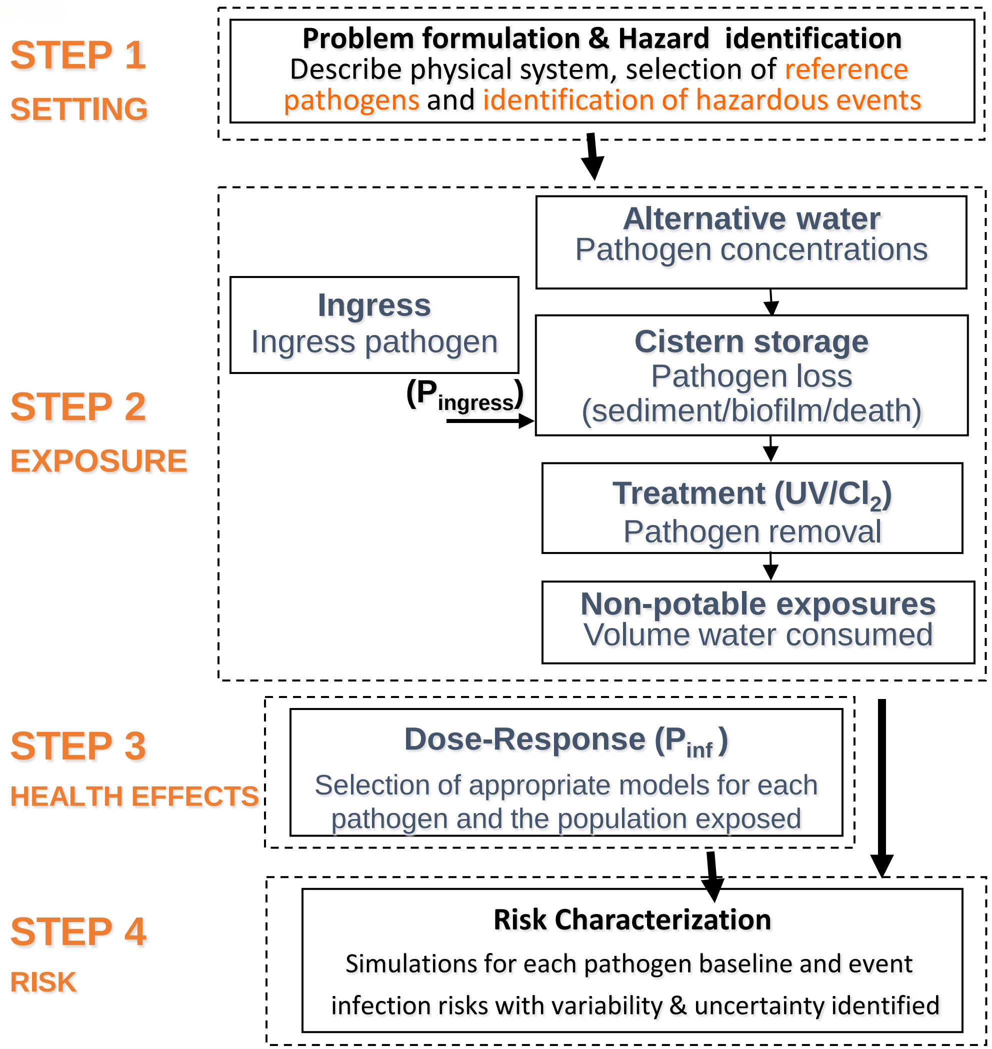
Nuisance groundwater from dewatering operations

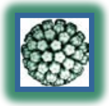
Traditional Indicators Are Not Predictive of Pathogen Levels in Alternative Waters

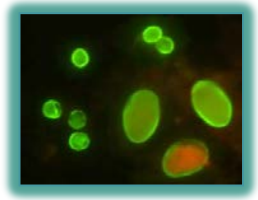
- **Graywater**
  - O'Toole et al. (2012)
    - A total of 185 greywater samples (laundry, bath) from 93 households in Australia
    - Analyzed for fecal indicator E. coli, pathogenic E. coli, and key viral pathogens (enterovirus, norovirus, rotavirus)
    - No association between the presence of indicators and the presence of pathogens
    - Norovirus was detected when the fecal indicator bacteria was not (7% of samples)
    - ***Not surprising given the fact that pathogen shedding is highly variable***
- **Rainwater**
  - Ahmed et al (2012)
    - Event driven, non-human fecal sources –lead to highly variable pathogens detections
  - Simmon et al. (2008)
    - Legionella outbreak from rainwater drinking water system
    - Importance of "environmental" pathogens (rather than host associated)
    - To add to the complexity, source of the Legionella was linked to aerosols from a pressure washer at a nearby marina

So Need to Start By Defining the Necessary Treatment To Meet Acceptable Risk

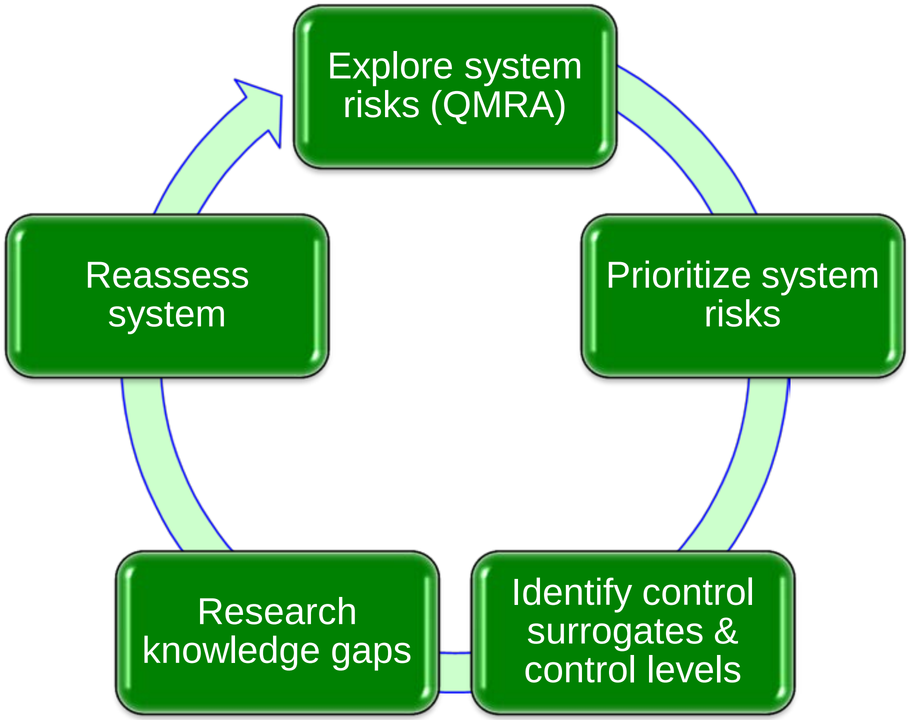
Quantitative microbial risk assessment (QMRA)








QMRA – Analytic Framework



QMRA of Non-Potable Reuse of Alternative Water Sources: A Literature Review

- Focused on on-site domestic and commercial systems (not centralized systems)
- Review publications that a) recommended technology performance standards or b) estimated health risks from microbial exposures
- Evaluated graywater, rainwater, stormwater, foundation drainage, and blackwater (using SF PUC definitions)
- Focused on non-potable uses, but not agricultural production

<table>
<tr><th rowspan="2">Water</th><th colspan="3">Scale[a]</th><th>Mis-</th><th rowspan="2">Event</th><th rowspan="2">Pathogen[c,d]</th><th colspan="12">Log Reductions</th><th rowspan="2">Ref</th></tr>
<tr><th>S</th><th>M</th><th>L</th><th>use</th><th><1</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>>10</th></tr>
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<tr><td rowspan="6">Stormwater</td><td></td><td>NA</td><td></td><td></td><td></td><td>R,Cj, C</td><td colspan="2">municipal</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>7</td></tr>
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<tr><td></td><td>NA</td><td></td><td></td><td></td><td>R,Cj, C</td><td colspan="3">non-food crops</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>7</td></tr>
<tr><td></td><td>NA</td><td></td><td></td><td></td><td>R,Cj, C</td><td colspan="2">irrigation</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>29</td></tr>
<tr><td>Rainwater</td><td></td><td>NA</td><td></td><td></td><td></td><td>Cj</td><td>all uses[e]</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>7</td></tr>
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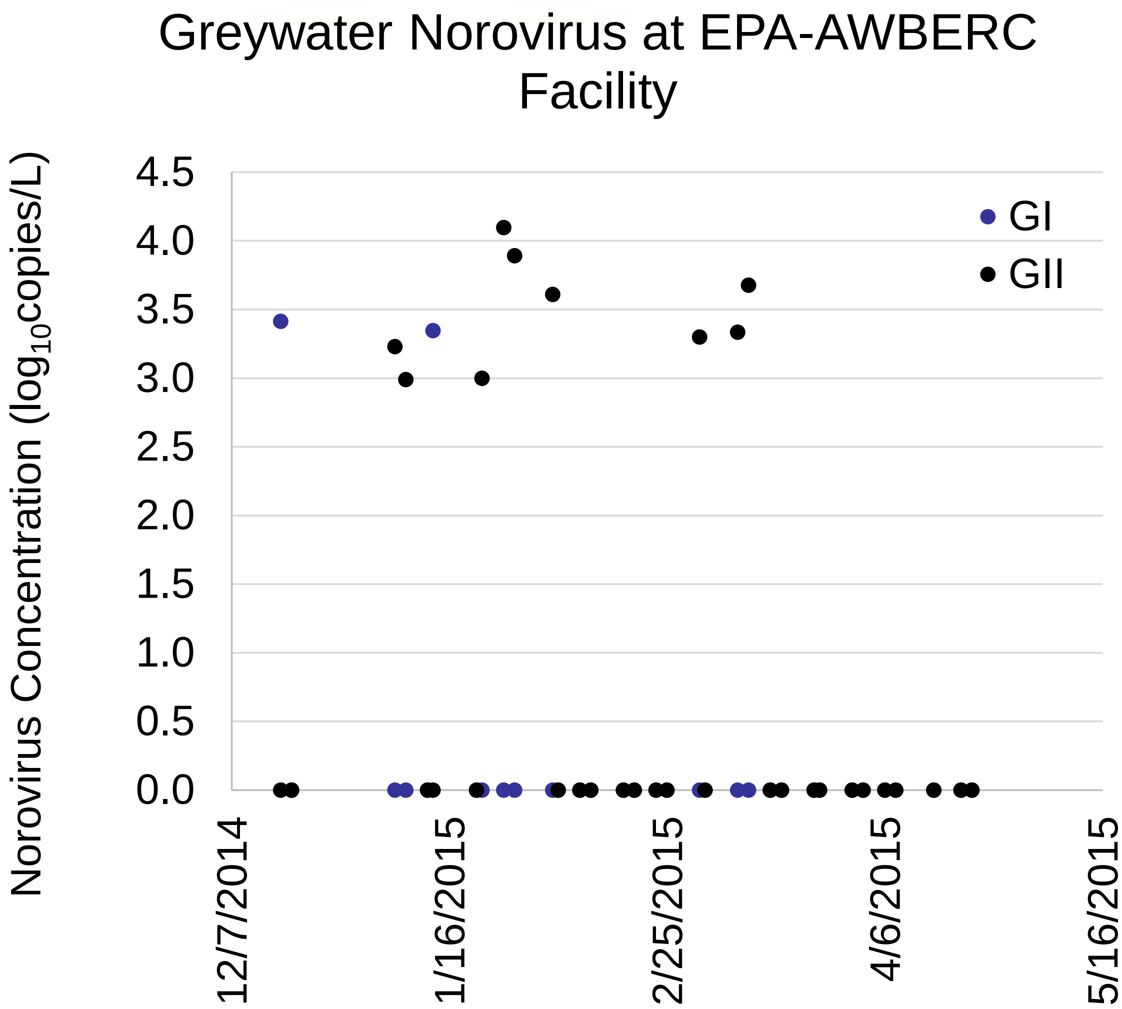
a. Small (S) is single household, Medium (M) is multi-home systems, Large (L) is community-wide
b. NA is not applicable
c. V is enteric virus, C is Cryptosporidium, R is Rotavirus, N is Norovirus, A is Adenovirus. Cj is Campylobacter jejuni
d. Pathogens ordered from highest to lowest required log reduction
e. municipal, indoor and outdoor, firefighting, agriculture, non-food crops

Conclusions of QMRA Literature Review

- Each water and use combination requires a unique pathogen reduction so that the water can be considered “safe”
- There are reuse applications for which the human health risk has not been characterized
  - On-site blackwater or mixed wastewater, Foundation drainage reuse, etc.
- Adoption of previously calculated pathogen reductions for on-site systems requires careful consideration so that waters can be considered safe
  - Differences in pathogen densities and occurrence between centralized and on-site systems
  - Need to account for sporadic nature of pathogen occurrences and treatment performance variation
- Review has been published *(Schoen and Garland 2015, Microbial Risk Analysis)*

Current work on-going to refine models/estimates

- Characterize pathogen density in on-site collection systems
  - Distinction from municipal wastewater/failure of indicator paradigm
  - Direct monitoring data needed
- Incorporate pathogen intermittency
  - Important for small-scale systems where pathogens may not be routinely present
  - Implications for determination of annual risk
- Improve exposure models
  - Are people really exposed to 0.01 mL from toilet flushing? (NRMMC 2006)
  - Need realistic science-based assumptions, but also need to consider failure/accidental exposure
- Independent Advisory Panel: Technical Requirements for Public Health Standards for Onsite Water Systems
  - Working with the expert advisory group so that this information, contained in separate publication(s), will be referenced in the framework document



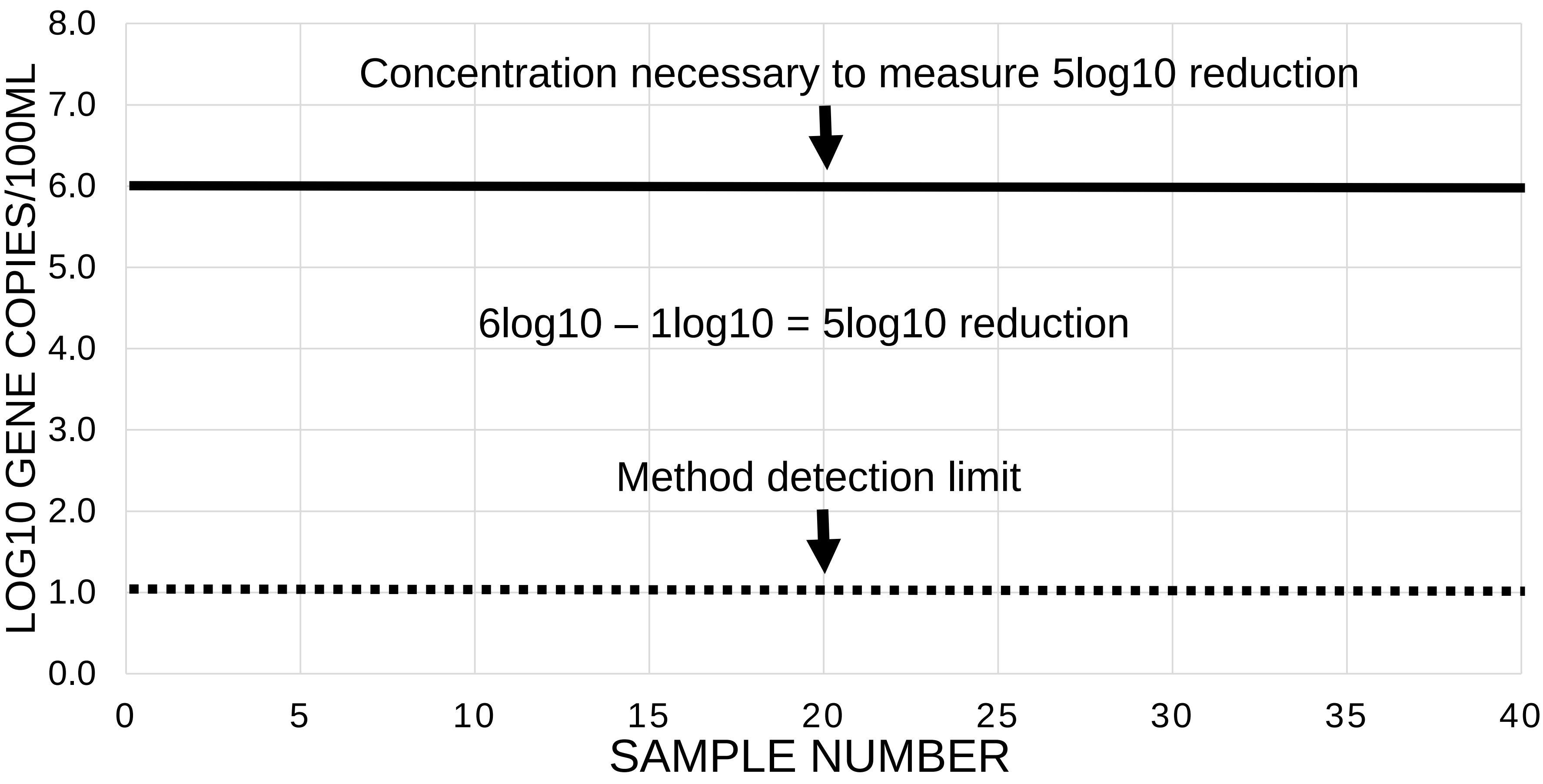
- 8-story, 800-person "office building"
- 33 greywater samples collected from sinks, water fountains, and showers (combined)
- Detection Rate
  - GI 6.1%
  - GII 27.3%
- Average Concentration
  - GI 3.38 log copies/L
  - GII 3.47 log copies/L

How do we quickly and effectively monitor the treatment performance of a system?

What About Monitoring?

- Process indicator
  - Demonstrates efficacy of a process (treatment)
- Could use common water quality parameters
  - Preferably using real or near real-time sensors
  - Need to be validated as an accurate predictor of treatment performance
- But what about biologically based process indicators?
  - Consistently present in sufficiently high numbers to measure necessary dynamic range required by log removal estimates

MEASURING A 5LOG10 REDUCTION



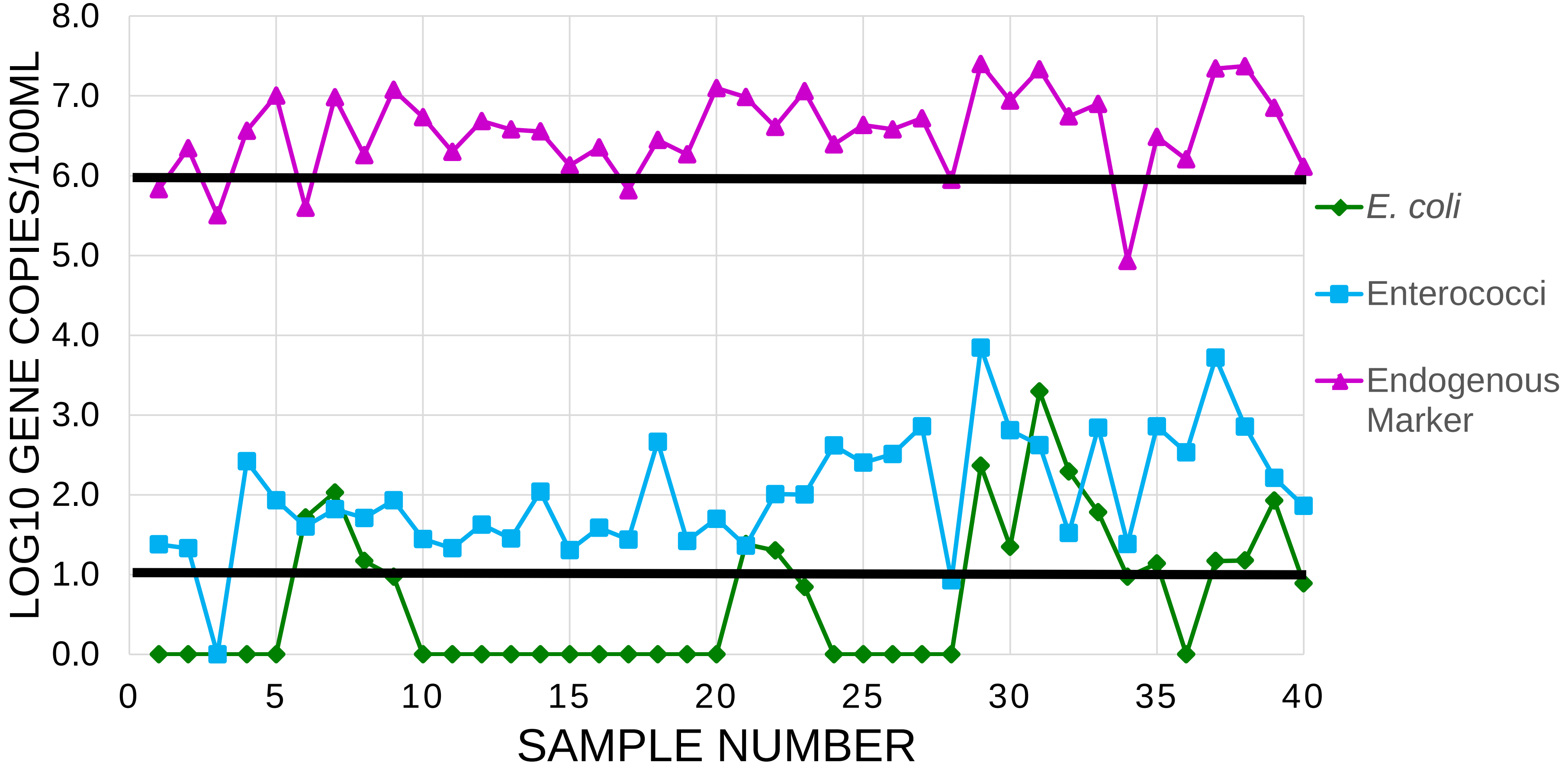
Indicator Organisms (IO) in Graywater

Indicator	Graywater log_{10}/100mL	Wastewater log_{10}/100mL
E. coli	0 - 6	4 - 6
Enterococci	0 - 4	4 - 6
Sulfite-reducing clostridia	0 - 3	3 - 6
Coliphage (Somatic and F-RNA)	0 - 3	6 - 7

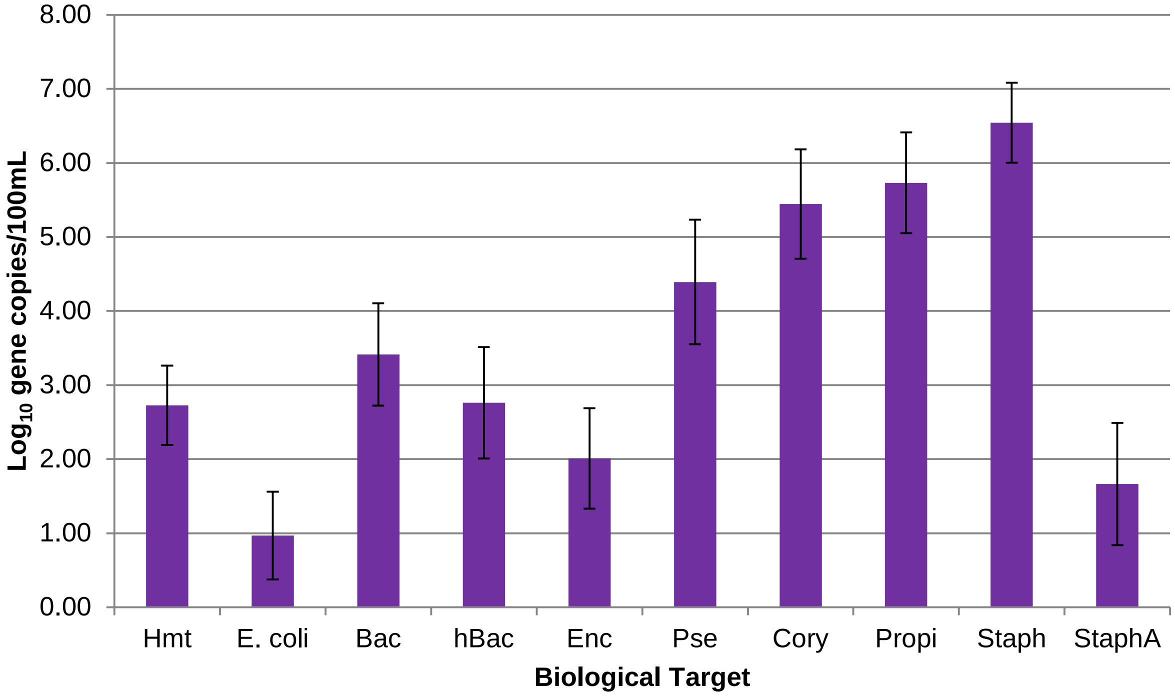
From: Ottosson (2003), Gilboa (2008), Winward (2008)

- No correlation between *E. coli* and gastroenteritis or *E. coli* and Norovirus occurrence (O'Toole, 2012)
- IO can grow ~1-2log_{10} in graywater (Ottosson, 2003)

INDICATOR BACTERIA IN GRAYWATER



Quantification of Select Targets In Laundry Water



Mean $\log_{10}$ copies ± SD of qPCR targets (Hmt = HmtDNA, Bac = *Bacteroides* spp., hBac = human-specific *Bacteroides*, Enc = *Enterococcus* spp., Pse = *Pseudomonas* spp., Cory = *Corynebacterium*, Propi = *Propionibacterium,* Staph = *Staphylococcus* spp., StaphA = *S. aureus*) in laundry graywater.
Adenovirus not found in any sample

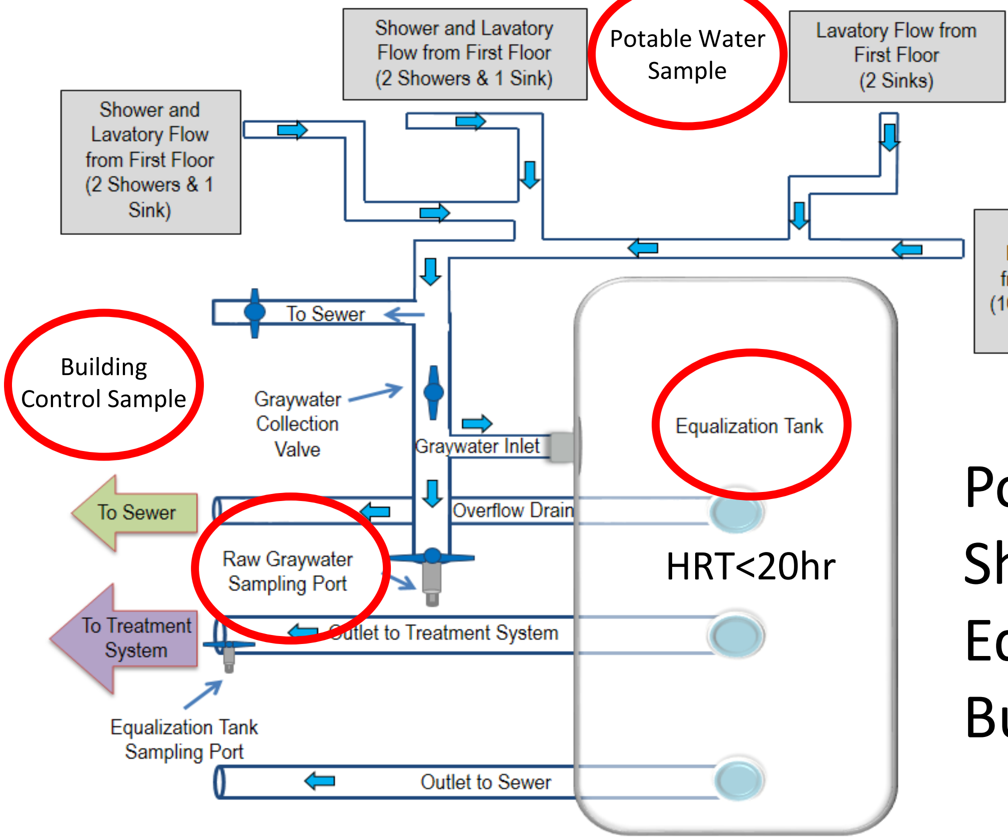
Log10 Reduction In Graywater Summary

- Enterococci and *E. coli* levels not sufficiently high to quantify 5log10 reduction
  - Can only measure average of 1-2log10 reduction
  - Measure 0log10 reduction 30% of the time
- Endogenous marker can measure up to 6.4log10 reduction
  - Can measure ≥5log10 reduction 85% of the time
  - Can measure average of 5.5log10 reduction

In Search of Endogenous Bacterial Markers in Graywater

- 52 graywater samples from two distinct graywater sources
  - Colorado State University (CSU) system (Ft. Collins, CO)
    - Dormitory including 14 residence halls
    - 14 showers and 14 sinks (28 person capacity)
    - Composited in 946L equalization tank
  - University of Cincinnati (UC) athletic department's commercial washing machine (Cincinnati, OH)
    - Launder ~10-30 garments per wash
- Analyzed by pyrosequencing 16S rRNA gene
  - Classification to genus level of characterized bacteria

CSU Recycling System Schematic



Potable Water (PW): n=1

Shower/Handwash (SH): n=18

Equalization Tank (ET): n=6

Building Control (BC): n=3

UC Commercial Washer

Laundry (LA): n=24

Conclusions from Bacterial Metagenomics of Graywater

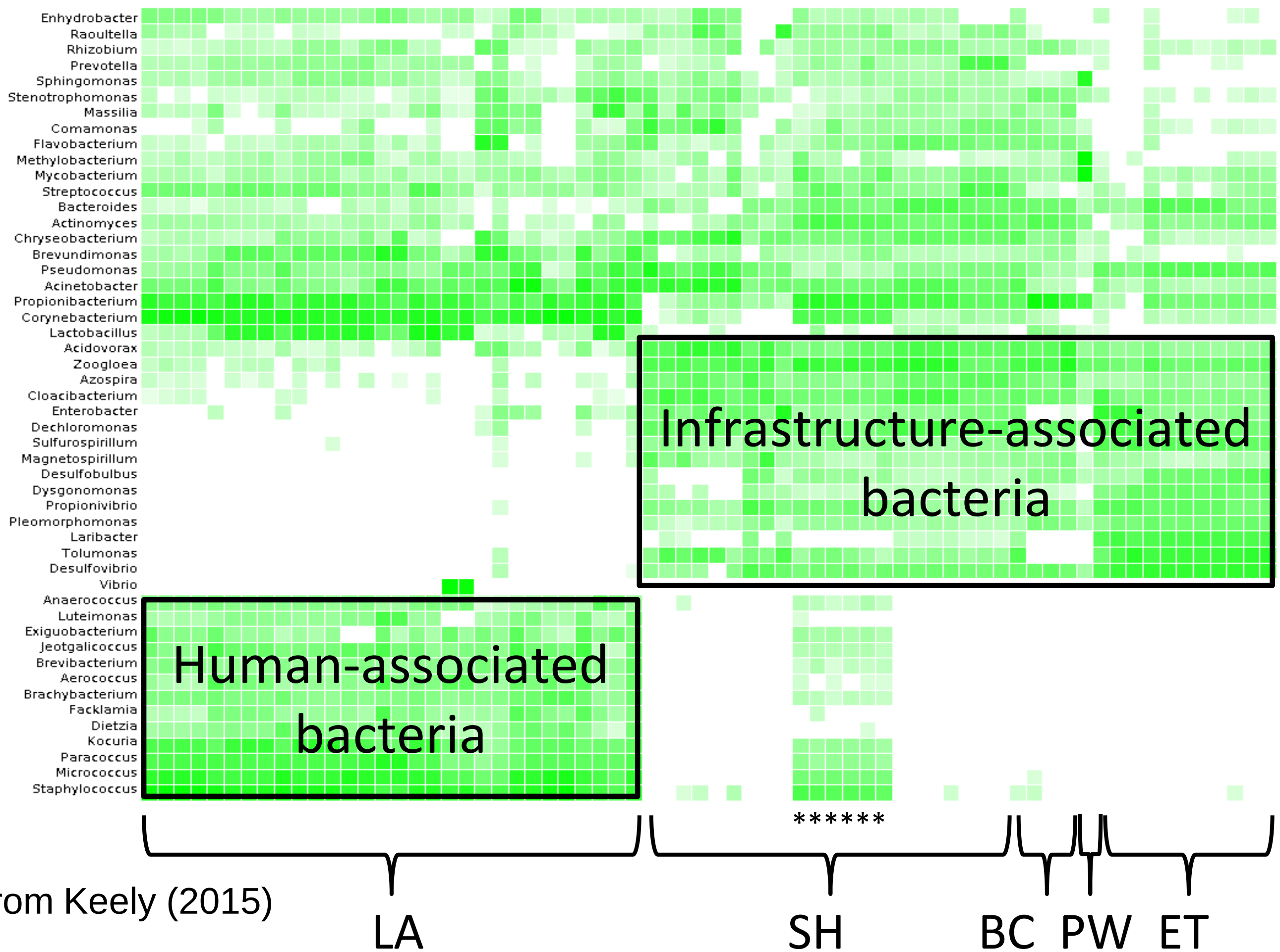
- Infrastructure-associated bacteria are the most abundant bacteria in graywater recycling systems
  - Suspended/attached growth or persistence of organisms in plumbing drain lines/equalization tank
- Skin-associated bacteria are the most abundant bacteria shed from humans
  - Most abundant in laundry
  - Present but variable in graywater recycling system

Sequence Statistics

- Over 1.8 million raw reads generated
  - Average over 35,000 raw reads per sample

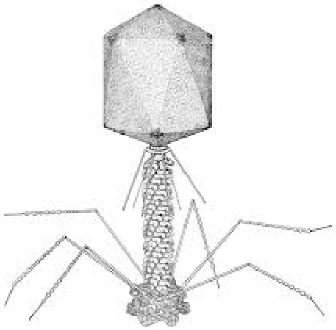
Sample Type	Number of Samples	Average Number of Genera Detected	Total Number of Genera Detected
SH	18	86	191
ET	6	53	90
BC	3	82	107
PW	1	37	37
LA	24	105	295

Log$_{10}$-scale Heat Map of Genera Detected

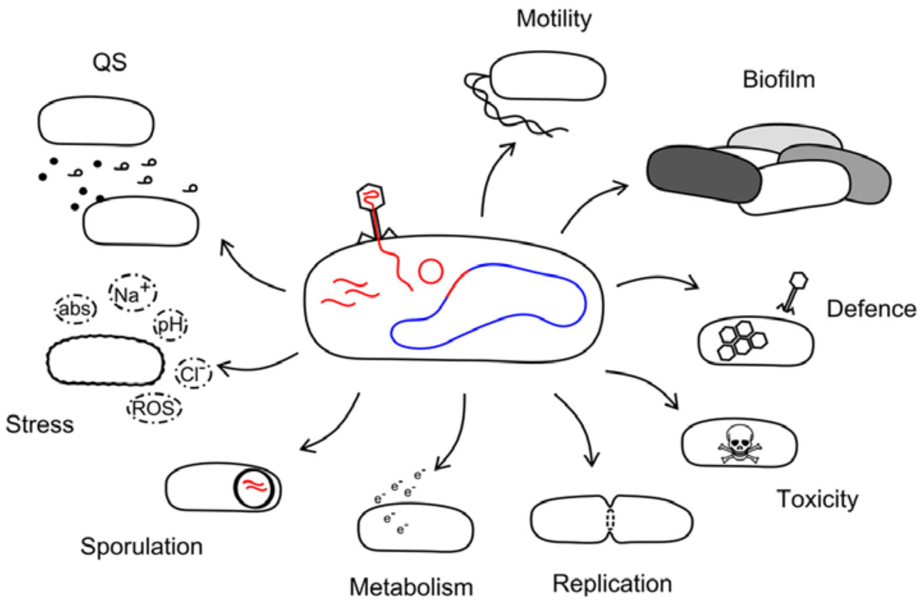


Adapted from Keely (2015)

Are Bacteriophage Better Targets?

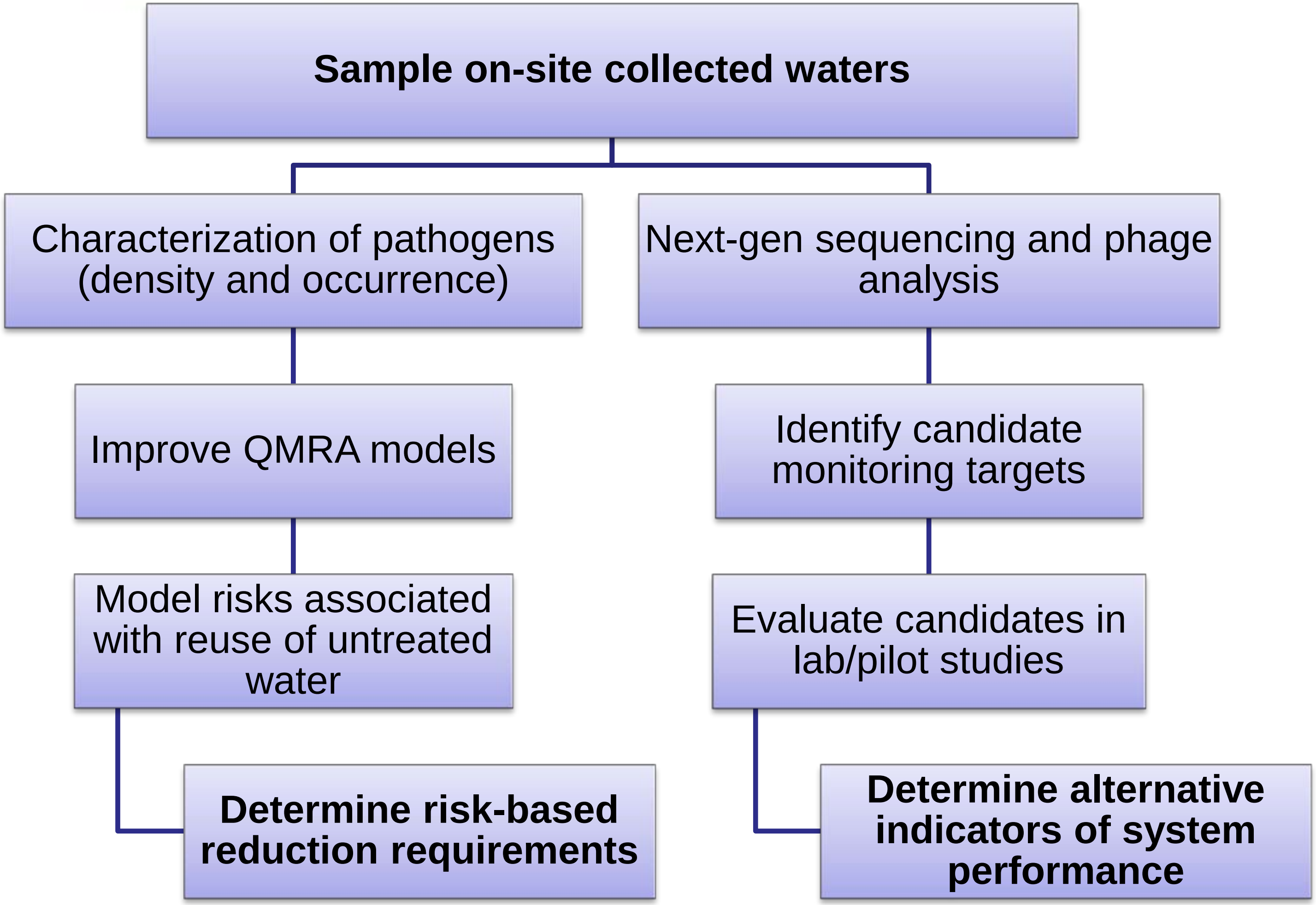


- Viruses that infect bacteria
- Abundant – 10x more than bacteria
- Relevant – biologically similar to viral pathogens
- Challenges for Characterizing "Phageome"
  - No universal gene
  - Need to remove prokaryotes, archaea and eukaryotes



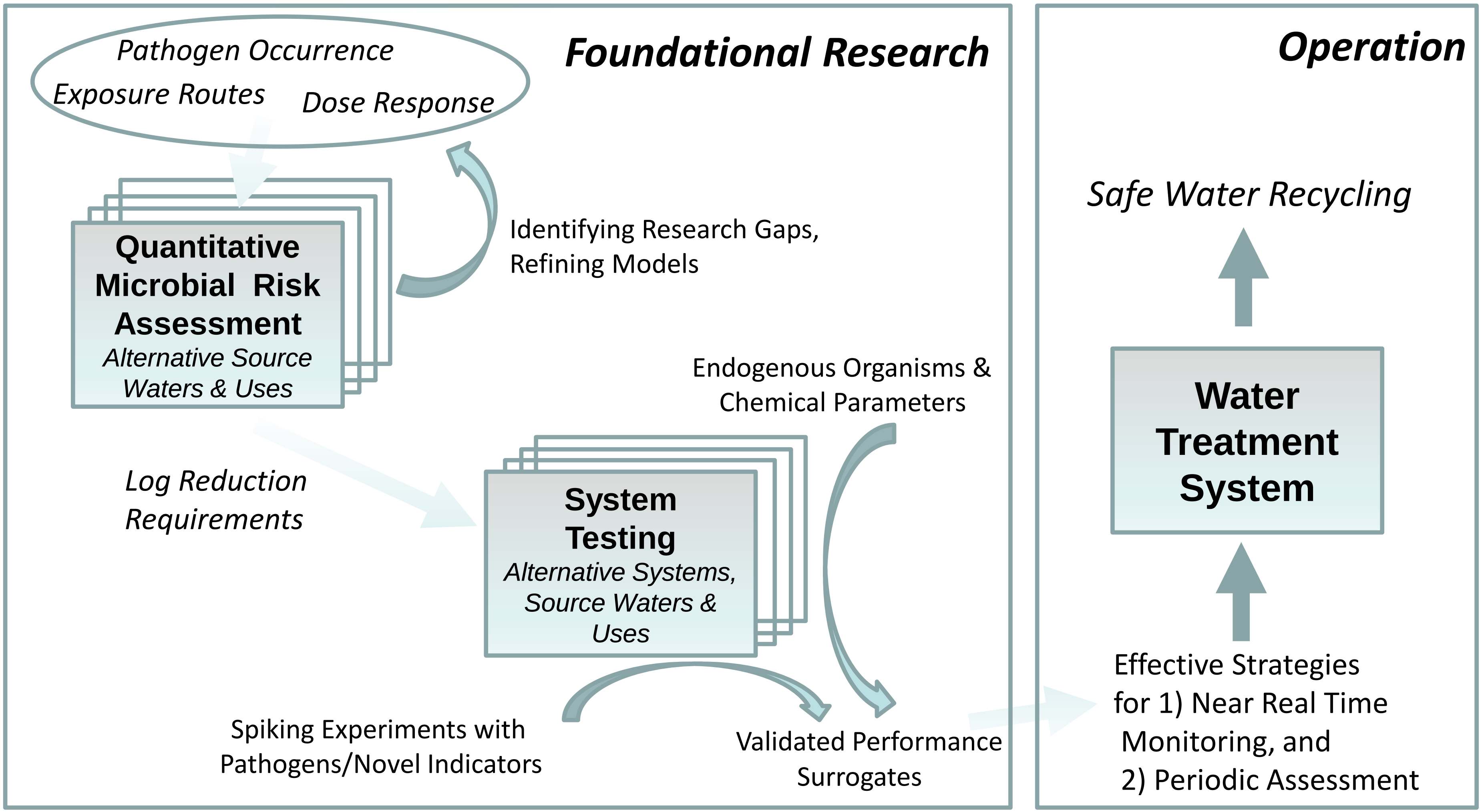
From Hargreaves et al. 2014. Bacteriophage 4:e29866, doi: 10.4161/bact.29866

Working With Partners



So….

Putting this all together


Pathogen Occurrence
Exposure Routes
Dose Response
Foundational Research
Quantitative Microbial Risk Assessment
Alternative Source Waters & Uses
Identifying Research Gaps, Refining Models
Log Reduction Requirements
Endogenous Organisms & Chemical Parameters
System Testing
Alternative Systems, Source Waters & Uses
Spiking Experiments with Pathogens/Novel Indicators
Validated Performance Surrogates
Operation
Safe Water Recycling
Water Treatment System
Effective Strategies for 1) Near Real Time Monitoring, and 2) Periodic Assessment