

Presence and treatment of potential opportunistic pathogens that can vary across groundwater geology

Dr. Natalie Hull, P.E.

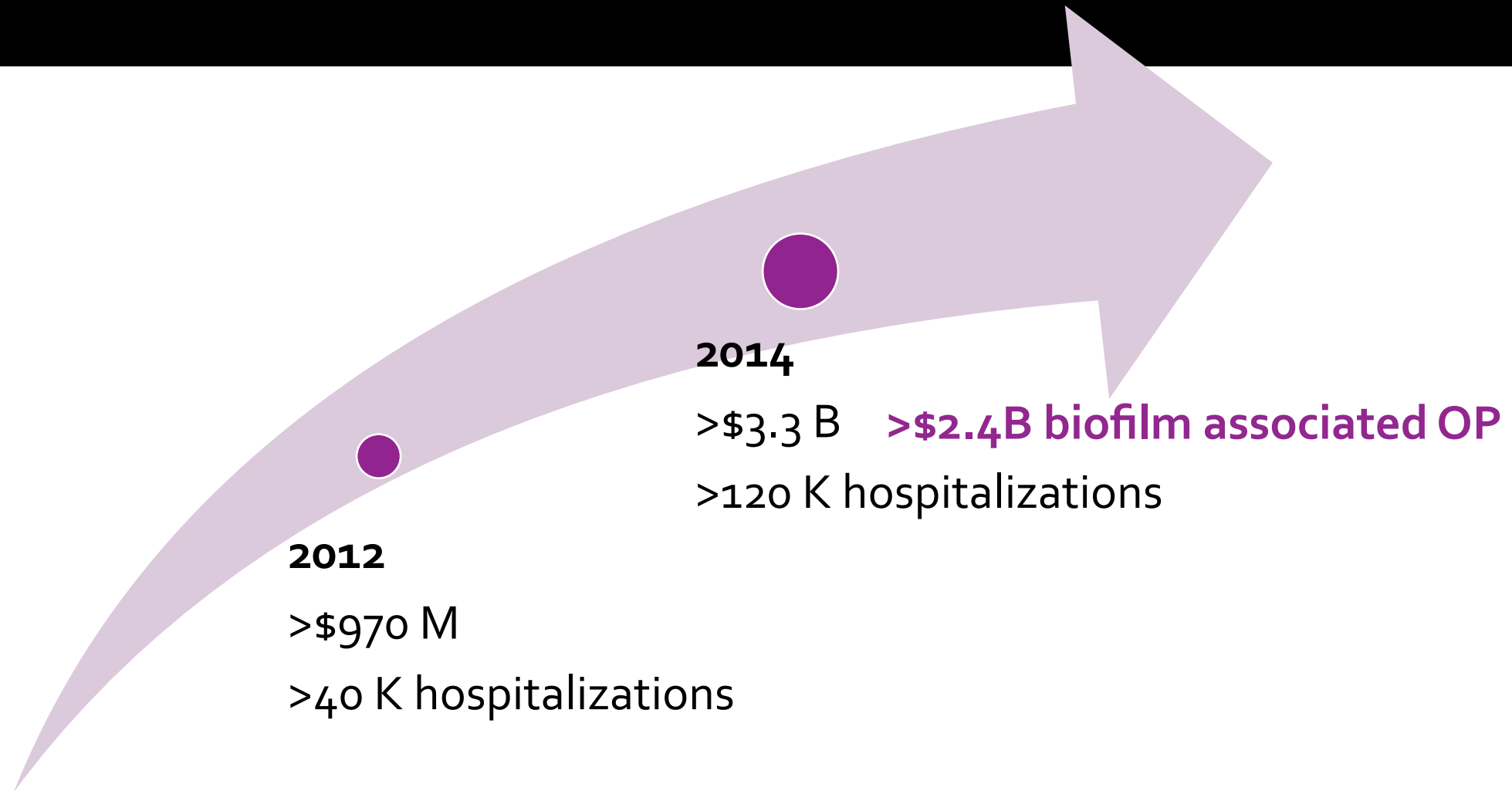
May 9, 2025



Waterborne pathogens: Primary vs opportunistic

	Primary Pathogen	Opportunistic Pathogen
Infects	Anyone	Immunocompromised
Source	Fecal matter	Environment
Exposure route	Ingestion	Inhalation, contact, ingestion
Monitoring	Indicator bacteria	Direct
Habitat	Planktonic, sessile	Sessile, amoebae, planktonic

Opportunistic pathogens are biofilm associated and contribute to a growing waterborne disease burden



Opportunistic pathogens cause a huge waterborne public health burden

	Mycobacteria	Legionella	Pseudomonas
Cases	97,000	11,400	57,800
Hospitalizations	51,400	10,800	21,090
Deaths	3,800	995	1,425
Cost (\$)	1.53 B	402 M	667 M

Mycobacteria spp.

- >190 recognized species
- Many species cause NTM infection
 - primarily lung infection

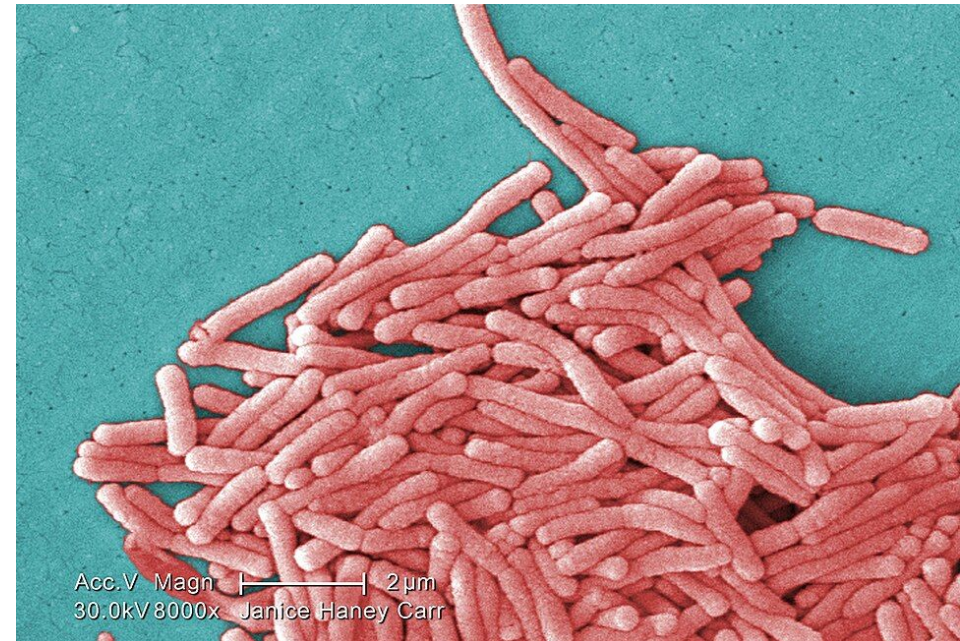
Non-tuberculous mycobacteria		
Rapidly growing mycobacteria	Slowly growing mycobacteria	
<i>M. chelonae</i> – <i>abscessus</i> complex <ul style="list-style-type: none"> • <i>M. abscessus</i> subsp. <i>abscessus</i> • <i>M. abscessus</i> subsp. <i>bolletii</i> • <i>M. abscessus</i> subsp. <i>massiliense</i> • <i>M. chelonae</i> <i>M. fortuitum</i>	<i>M. marinum</i> <i>M. ulcerans</i>	<i>M. tuberculosis</i> complex <i>M. leprae</i>
<i>M. smegmatis</i> <i>M. vaccae</i>	<i>M. avium</i> complex <ul style="list-style-type: none"> • <i>M. avium</i> • <i>M. intracellulare</i> • <i>M. chimaera</i> <i>M. haemophilum</i> <i>M. xenopi</i> <i>M. kansasii</i> <i>M. simiae</i>	
	<i>M. terrae</i> complex <i>M. goodii</i>	

True pathogens
Opportunistic pathogens
Saprophytes*

*can be detected in clinical samples and need retesting to confirm infection

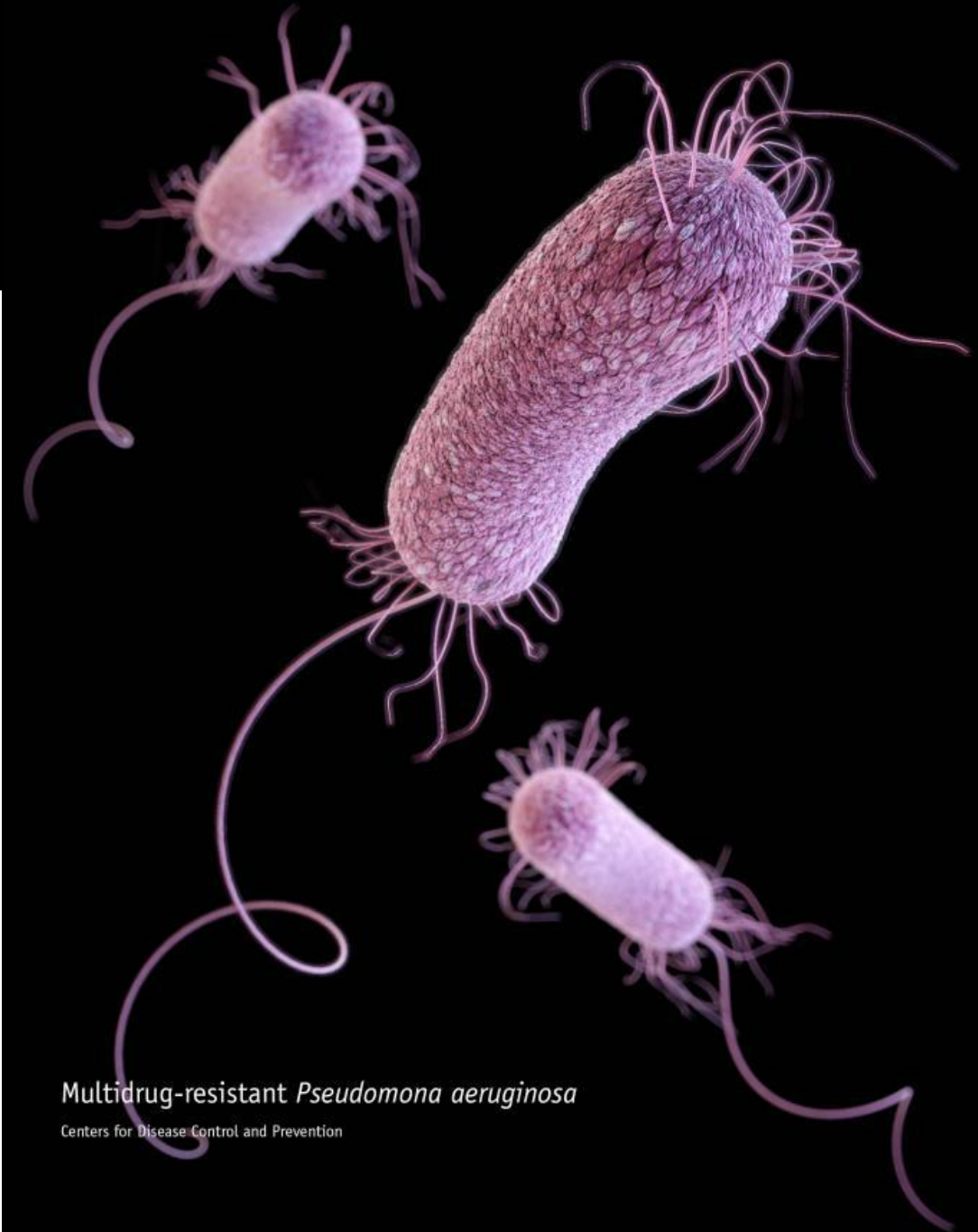
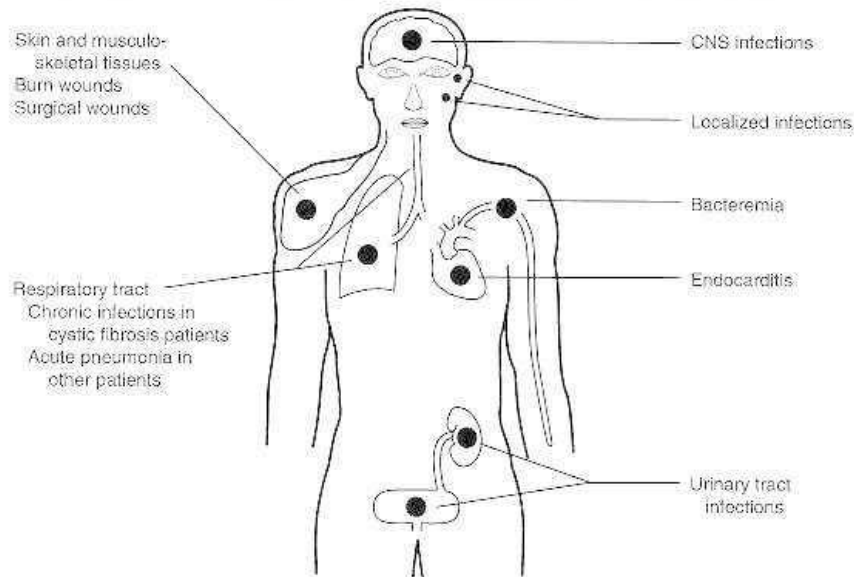
Legionella spp.

- >60 recognized species
- *Legionella pneumophila* (particularly serogroup 1) causes most legionellosis
 - Primarily lung infection with flu-like symptoms

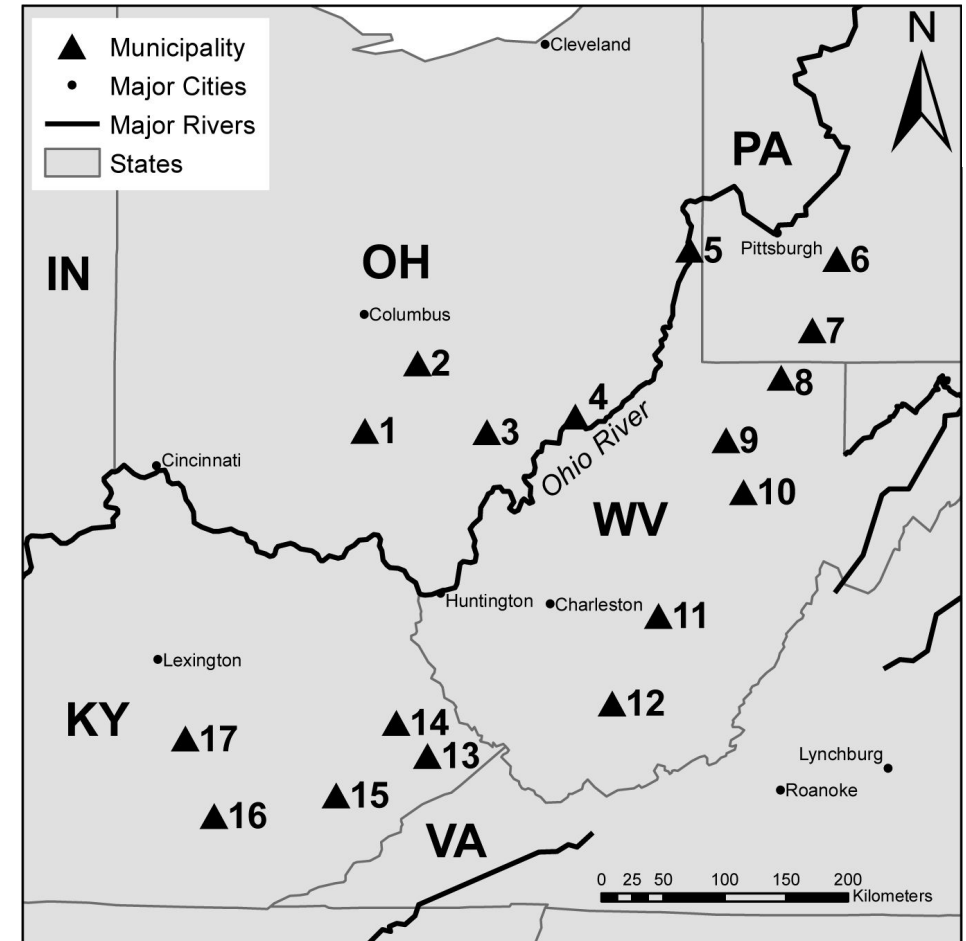
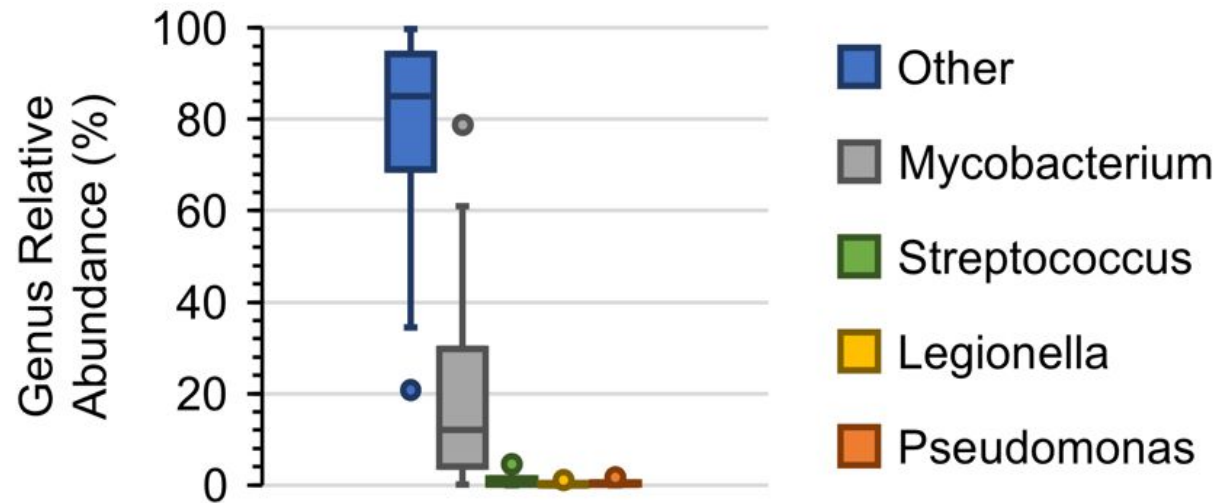


Pseudomonas spp.

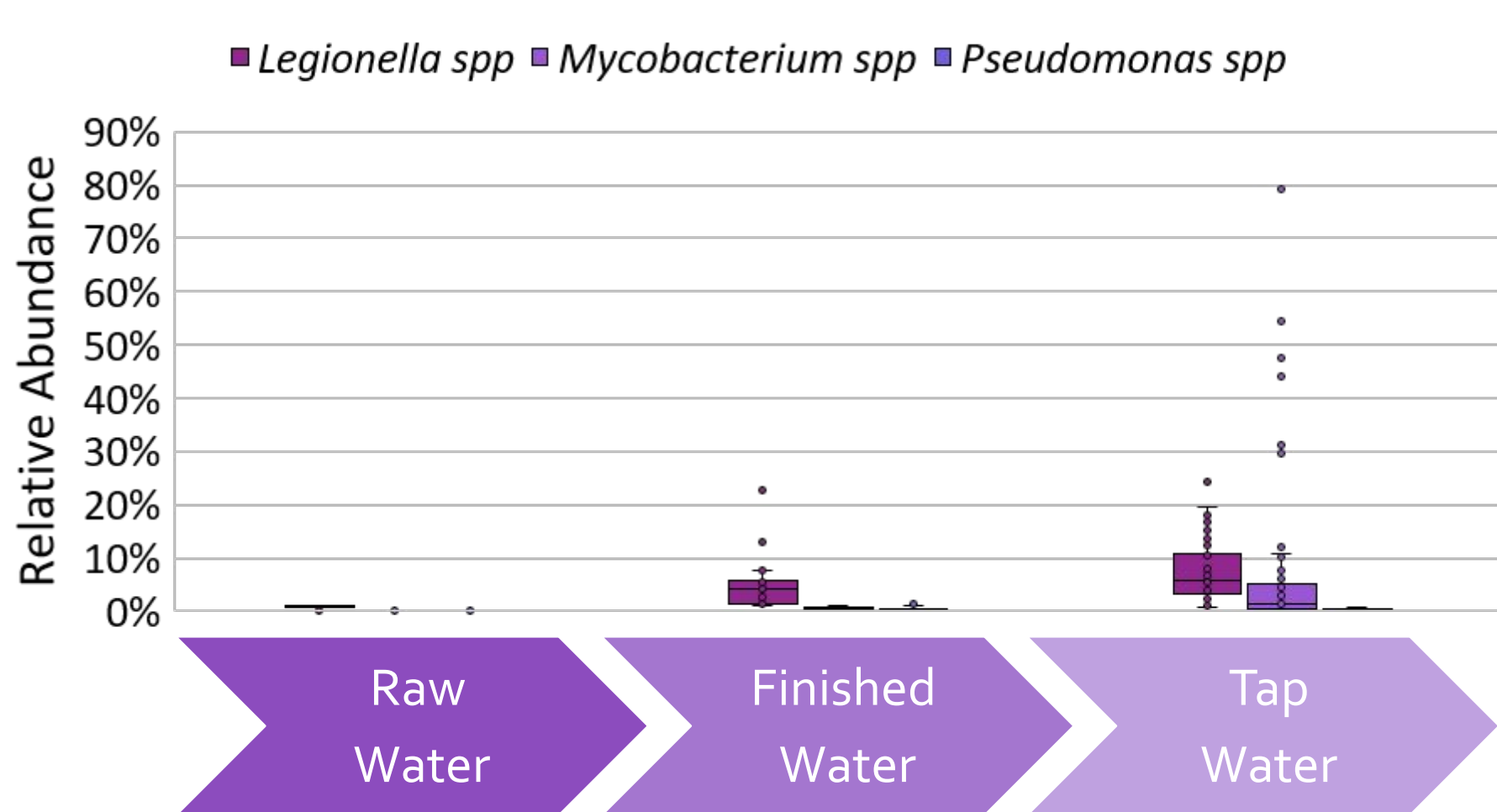
- >140 recognized species
- *Pseudomonas aeruginosa* and *P. maltophilia* cause most opportunistic infections
 - Infects almost any tissue



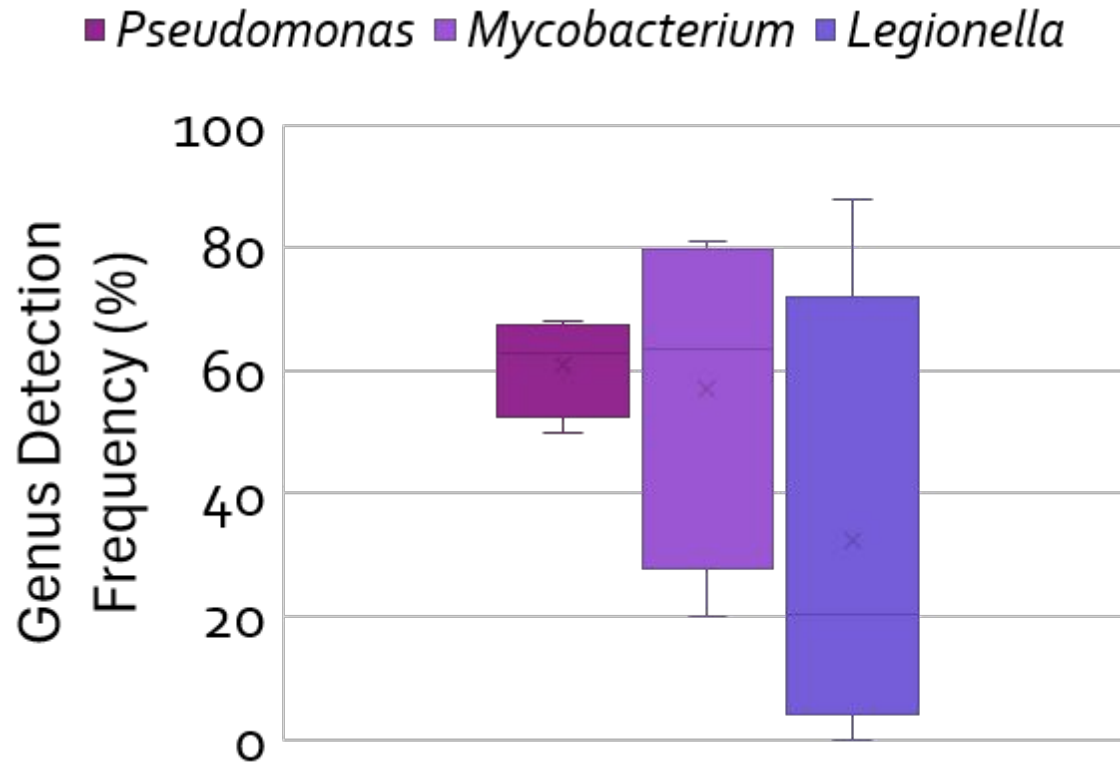
Potential opportunistic pathogens are ubiquitous in chlor(am)inated tap water



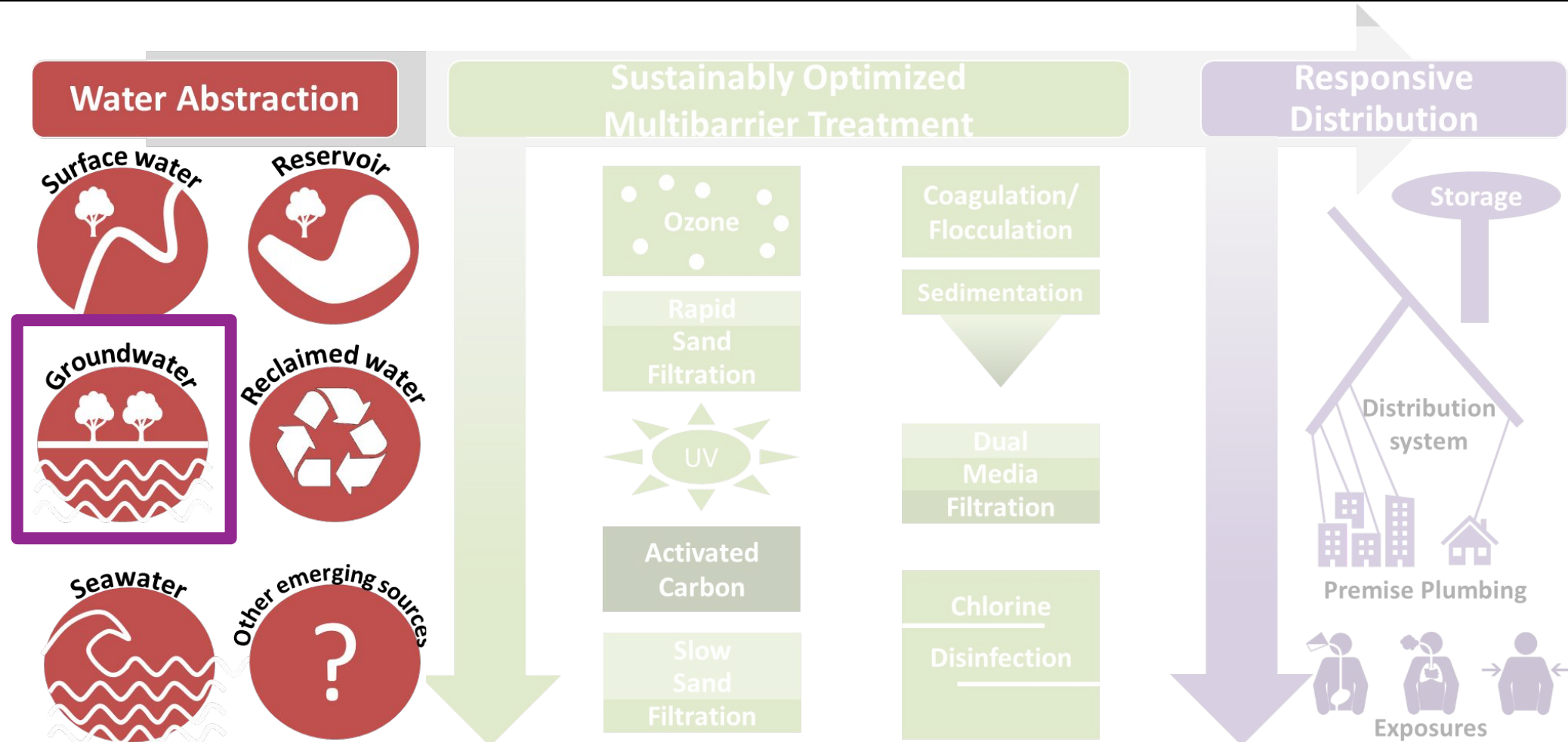
Chlorine treatment and distribution issues can select for potential opportunistic pathogens



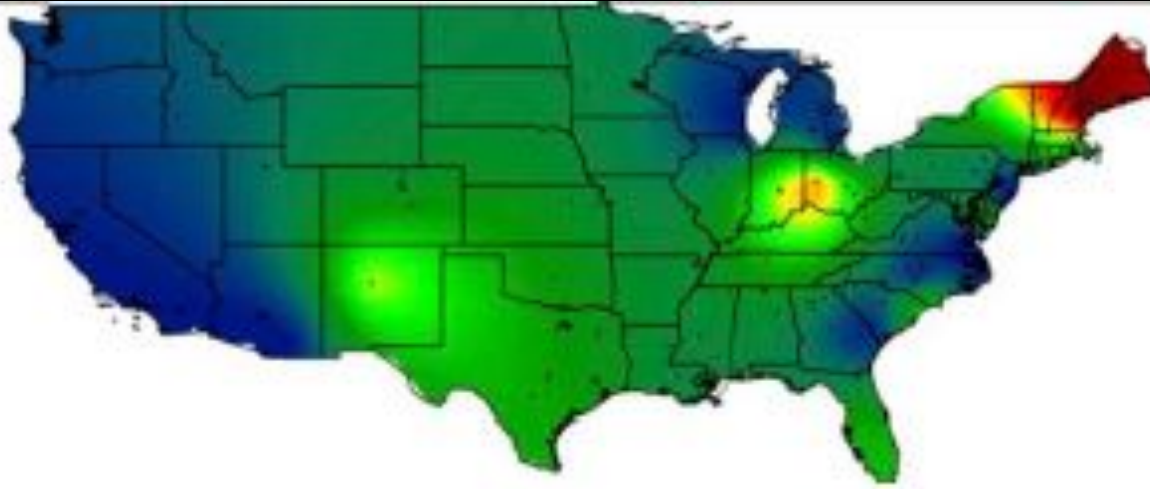
Potential opportunistic pathogens detected frequently in humidifiers



Water microbiomes must be optimized for multiple water sources, treatments, and exposure routes



NTM infection hotspots vary regionally by species;
NTM treatment (and disinfection?) varies by species

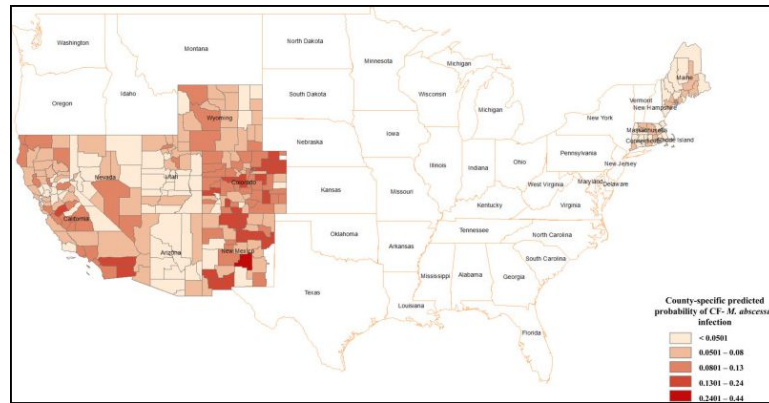


M. abscessus

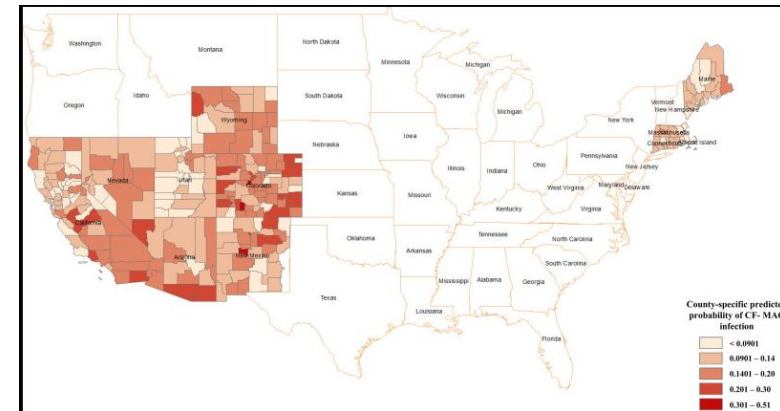


Mycobacterium avium complex or MAC

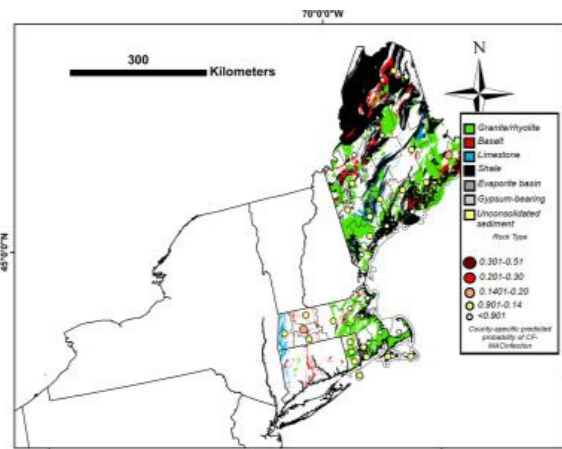
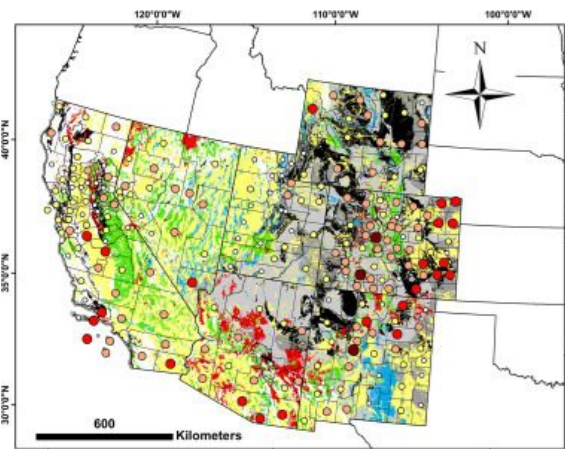
NTM infection hotspots are linked with geology and groundwater



M. abscessus molybdenum

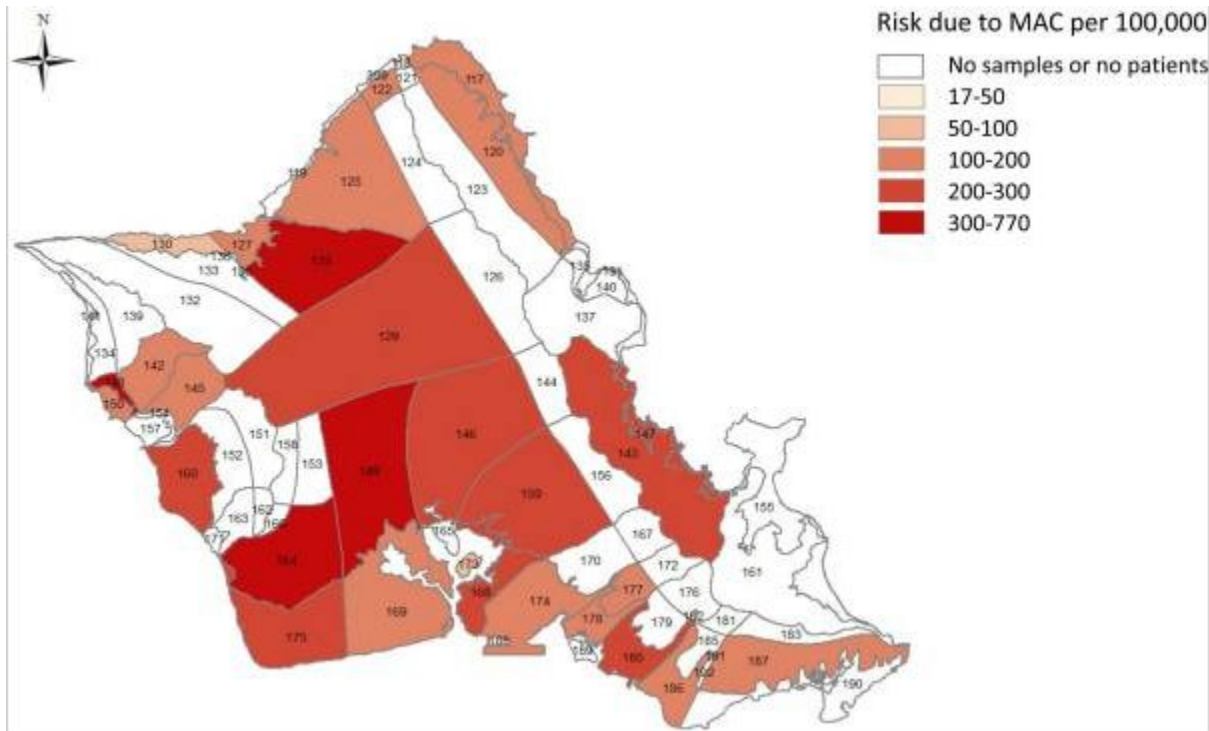


Mycobacterium avium complex or *MAC* vanadium



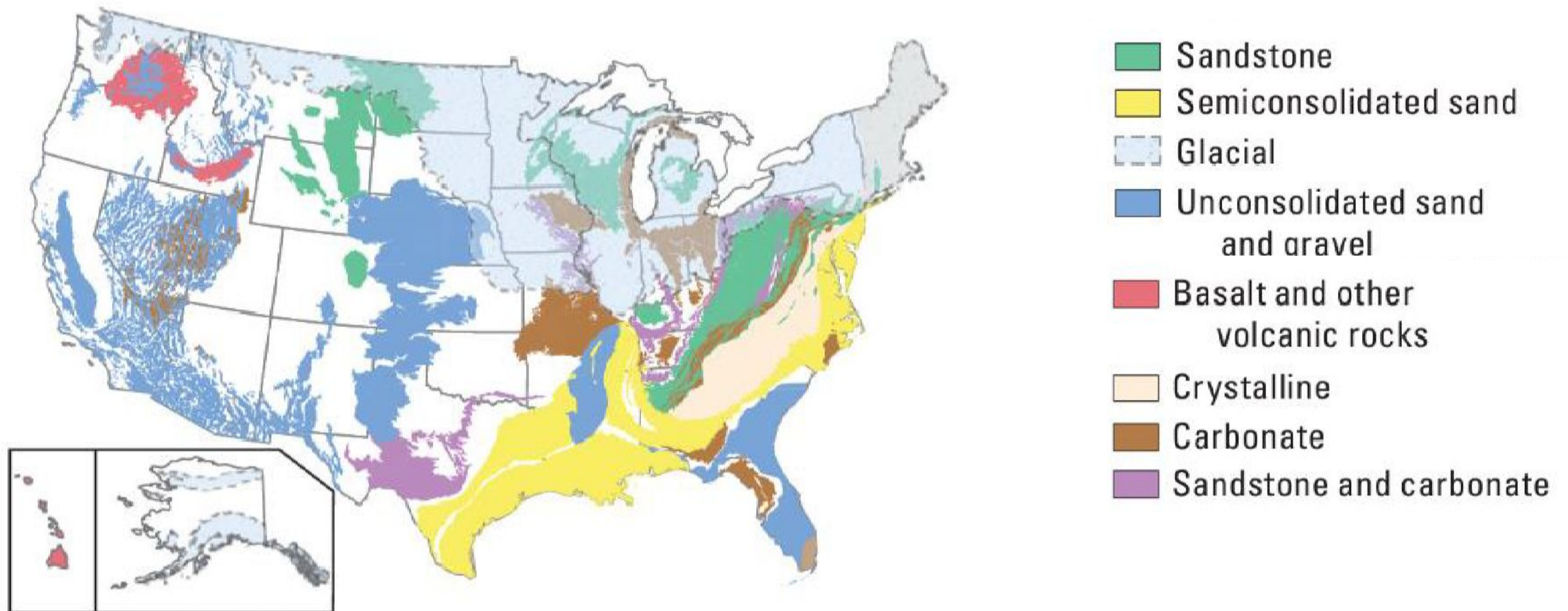
Rocks that impart **vanadium**, **molybdenum**, and **sulfate** to surface and groundwaters

NTM infection hotspots are linked with geology and groundwater – including in basaltic Hawaii

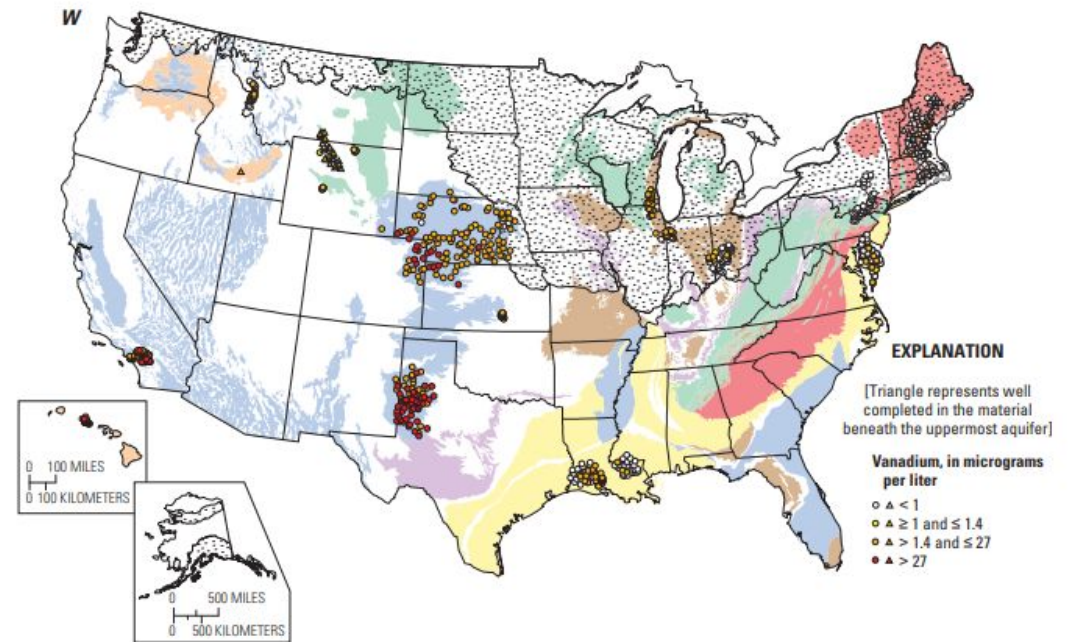
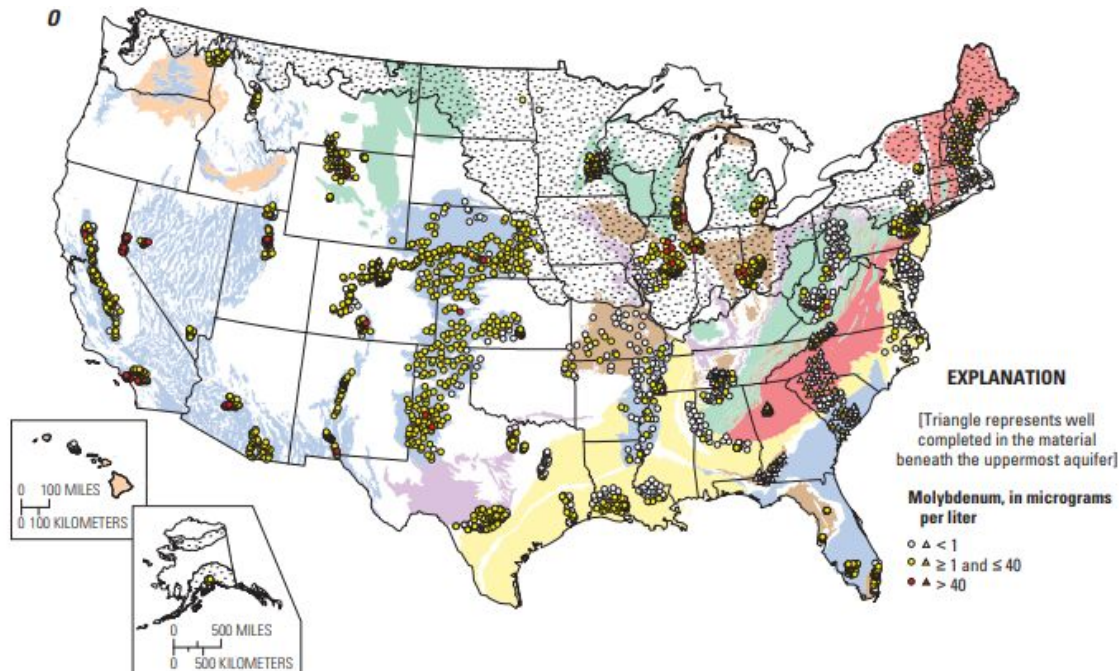


Mycobacterium avium complex or MAC
vanadium

Primary aquifers used for various sources including drinking water through public or private wells vary widely across the US



Molybdenum and Vanadium vary but are not widely studied in primary aquifers across the US



Research question

- How do potential opportunistic pathogens vary across groundwaters with different geology and water quality?

Other sample site selection considerations

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- Alabama
 - Soil and wastewater management issues

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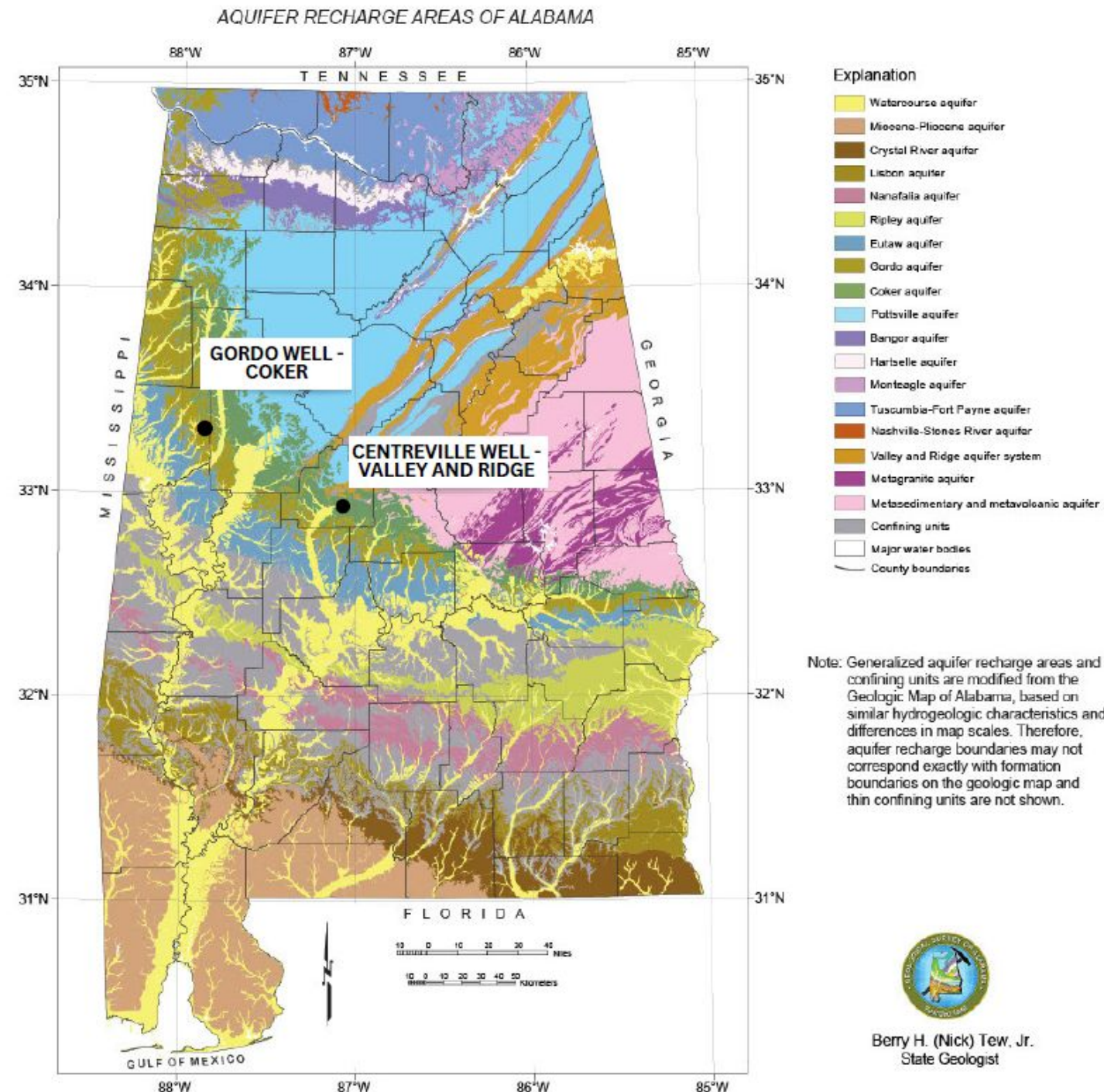


- Idaho
 - Heavy reliance on GW



Alabama aquifers

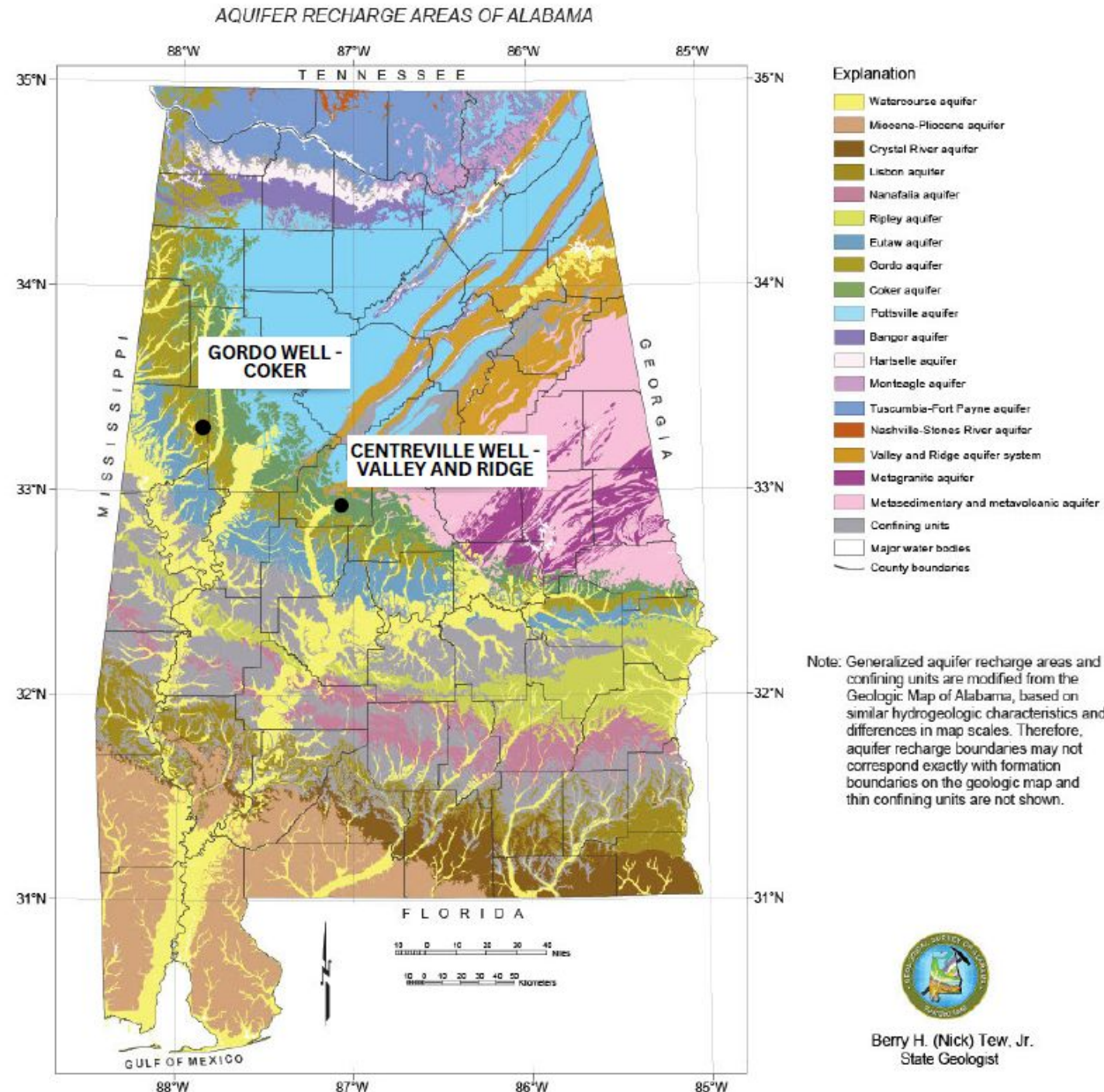
- Semi consolidated sand, soluble and porous carbonate, crystalline and sandstone rocks



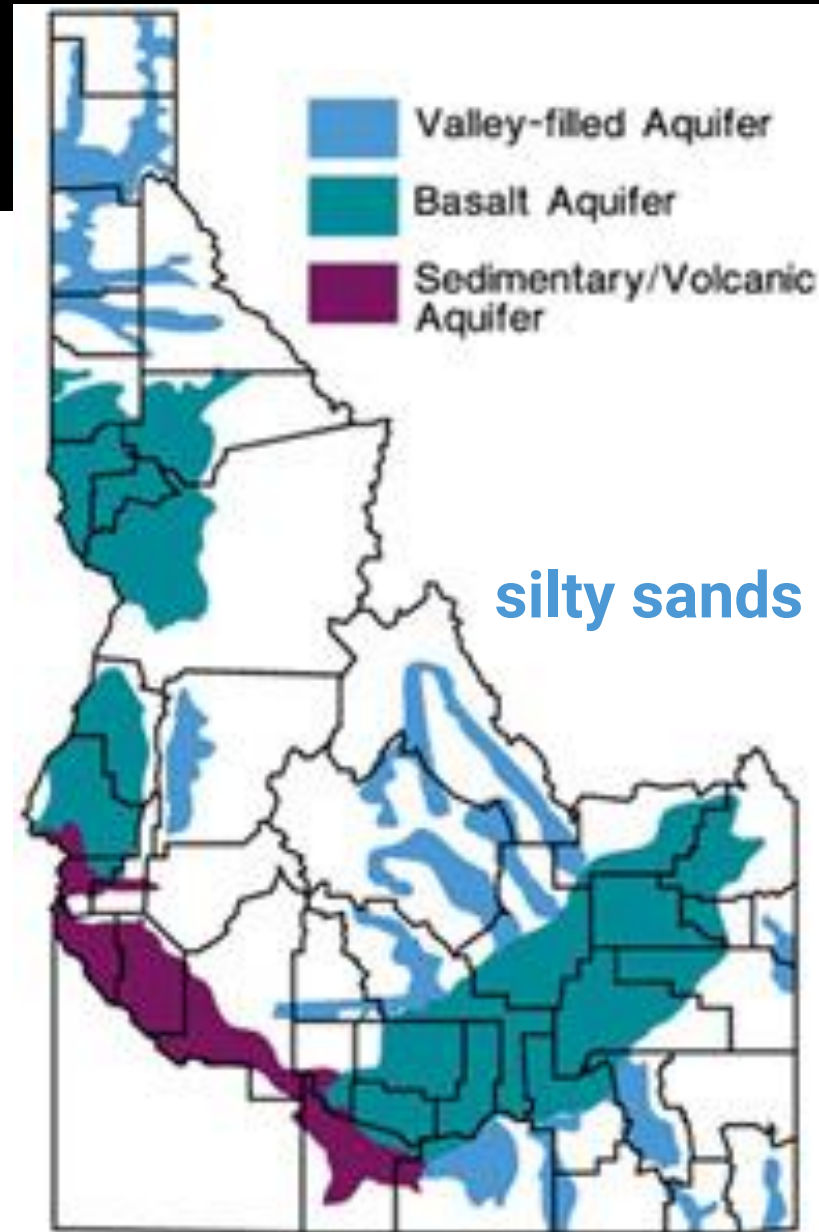
Alabama samples

IGM Summer 2024 sampling:

- 2 aquifers
- 1 water utility per aquifer
- 4 timepoints per utility
- 2L per sample



Idaho aquifers



silty sands and gravels; quite hard

Susceptible to surface water contamination

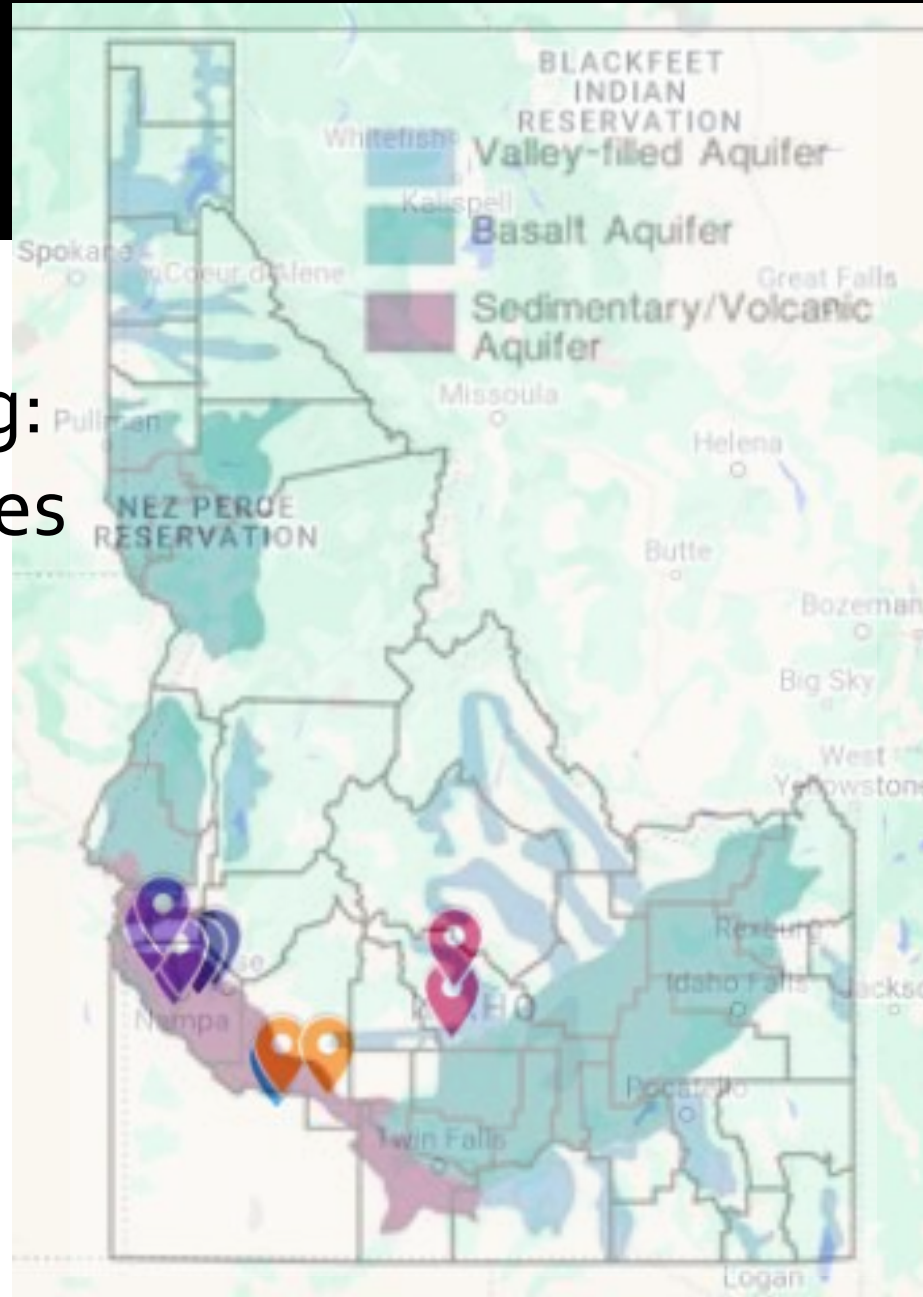
lower hardness and dissolved minerals; very high flow rate

lower hardness and dissolved minerals; very high flow rate

Idaho samples

IGM Summer 2024 sampling:

- 13 samples from 5 counties
- 1 timepoint per sample
- 4L per sample



Planned analyses

Sample collection □ ship to OSU □ filtration □ DNA extraction & quantification □ qPCR

- Targets:

- Total bacteria

- *Mycobacteria spp.* + MAC

- *Legionella spp.* + *L. pneumophila*

- *Pseudomonas spp.* + *P. aeruginosa*

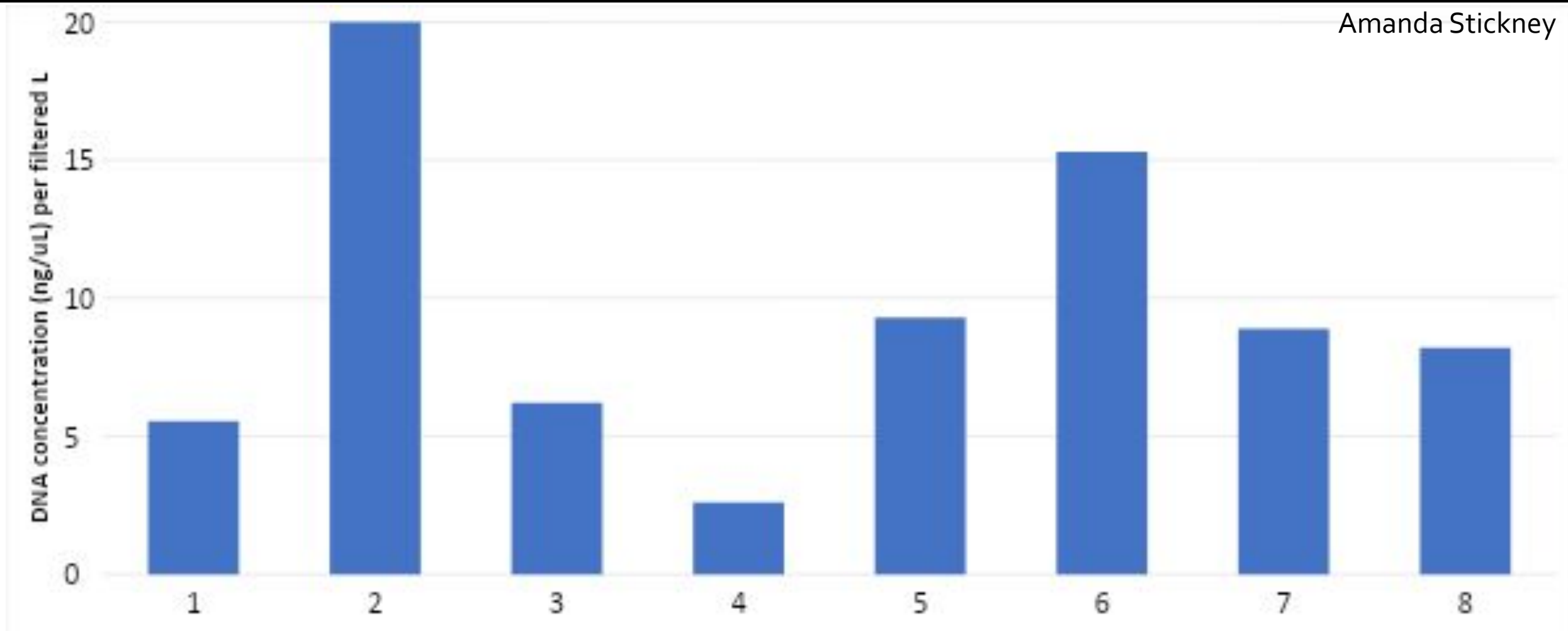
qPCR assay design

OP.	Target	Oligo name	Sequences (5'-3')	Reference
Total Bacteria	16S rRNA	515F	GTGCCAGCMGCCGCGGTAA	Hull et al 2017
		1391R	GACGGGCGGTGWGTRCA	
Mycobacteria	Mycobacterium spp.	23Smyco F	GGG GTGTGGTGTGTTGAG	Bruijnesteijn van Coppenraet, 2004 – 23S gene
		23Smyco R	GGG GTGTGGTGTGTTGAG	
		23Smyco Probe	6-carboxyfluoresceinTGGATAGTGGTTGCGAGCATCBHQ1	
	M. avium	Forward primer	GGGTGAGTAACACGTGTGCAA	Chern et al. (2014) – 16S gene
		Reverse primer	CCAGAAGACATGCGTCGTGA	
		Probe	FAM-TGCACTTCGGGATAAGCCTGGGAAA-TAMRA	
	M. intracellulare	Forward primer	GGGTGAGTAACACGTGTGCAA	Chern et al. (2014) - 16S gene
		Reverse primer	CCACCTAAAGACATGCGACTAAA	
		Probe	FAM-TGCACTTCGGGATAAGCCTGGGAAA-TAMRA	
Legionella	Legionella spp.	LegF	GATTAGCCTGCGTCCGATTAG	Lu et al. 2015
		LegR1	GAAATTCCACTACCCTCTCCCA	
		LegR3	AGTGTCAGTATTAGGCCAGGTAGC	
	Legionella spp.	Forward	AGGCTAATCTTAAAGCGCC	http://dx.doi.org/10.1016/j.microc.2013.09.017
		Reverse	CCTGGCTCAGATTGAACG	
	Legionella spp.	LegF	CTAATTGGCTGATTGTCTTGAC	https://doi.org/10.1007/s11356-013-1534-z
		LegR	GGCGATGACCTACTTTTCG	Kao et al. 2013
	L. pneumophila	Forward	TGGTGACTGCAGCTGTTATG	http://dx.doi.org/10.1016/j.microc.2013.09.017
		Reverse	CATTGCTCCGGATTAACAT	
Pseudomonas	Pseudomonas spp.	Forward primer	GGGTGGTGGAATTCCTGTGT	10.3389/fmicb.2020.00989
		Reverse primer	TTCCTTGTGGTCACCGCTTC	
		Probe	GTGAAATGCGTAGATATAG	
	P. aeruginosa	Forward primer	GGTGTTTCGAGGTGGTGGATA	https://doi.org/10.7717/peerj.787
		Reverse primer	TGGTGATGCTGATTCGCTG	

DNA concentration in Alabama



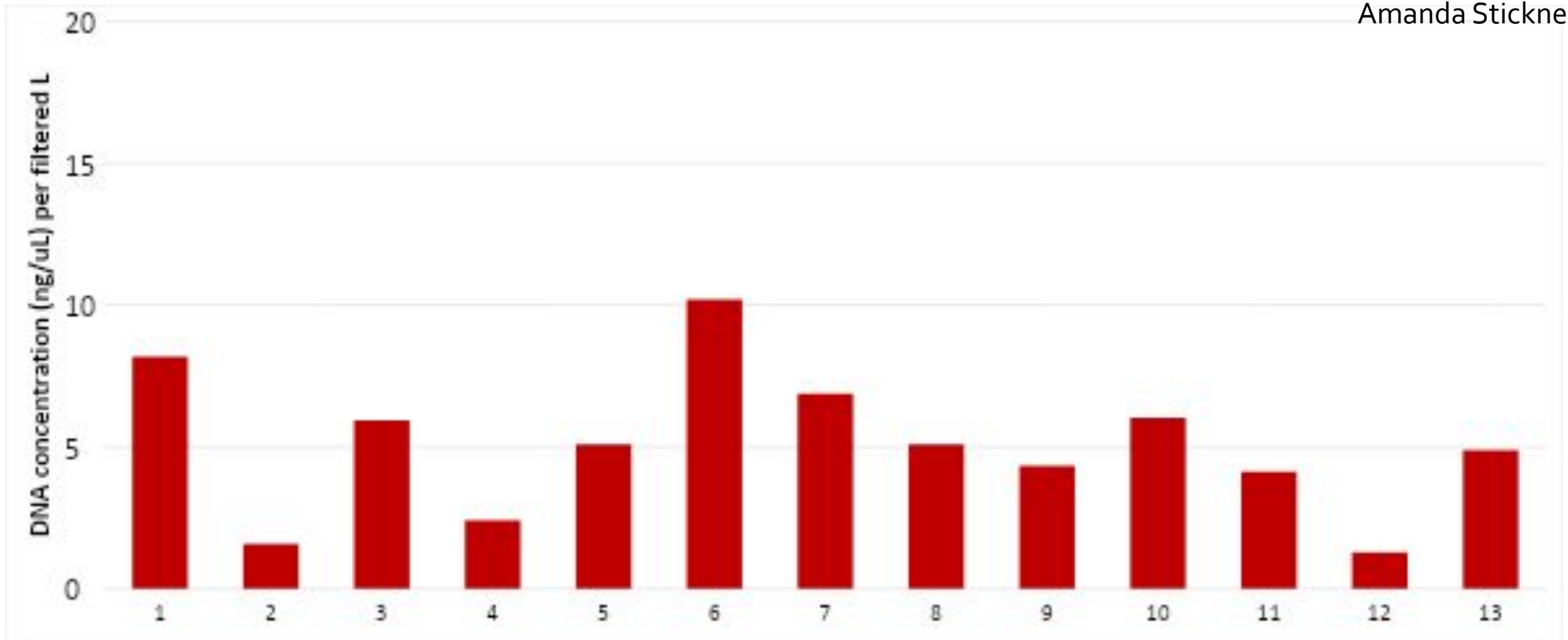
Amanda Stickney



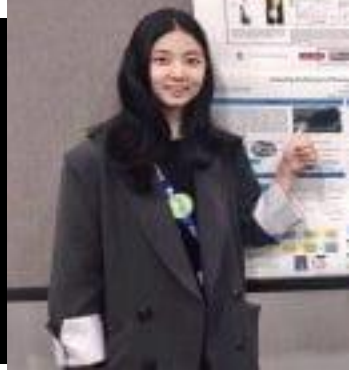
DNA concentration in Idaho



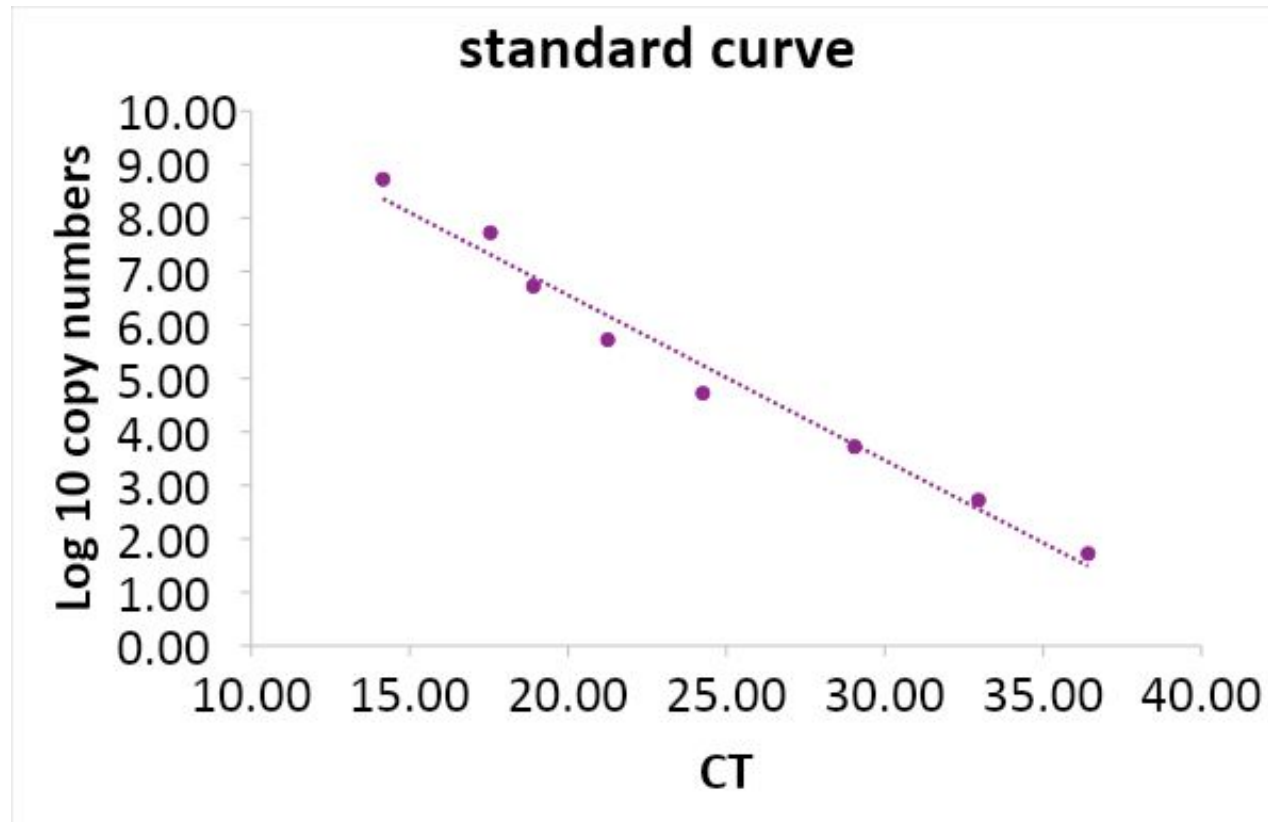
Amanda Stickney



qPCR results - *M. avium*



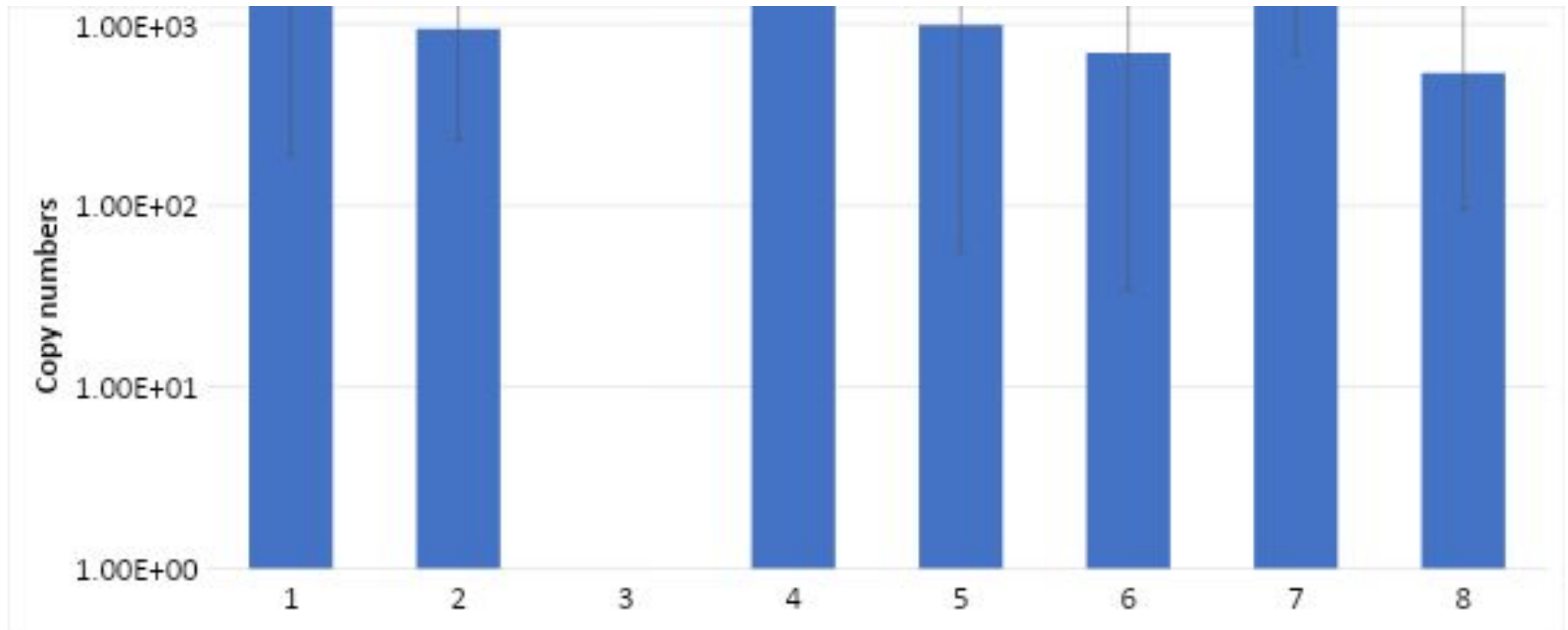
Yijing Liu



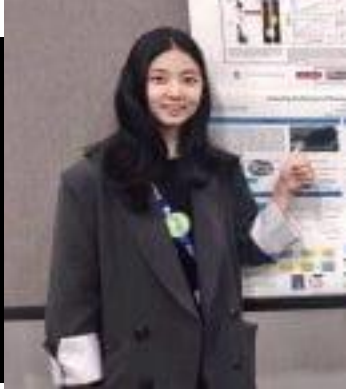
qPCR results - *M. avium* in Alabama



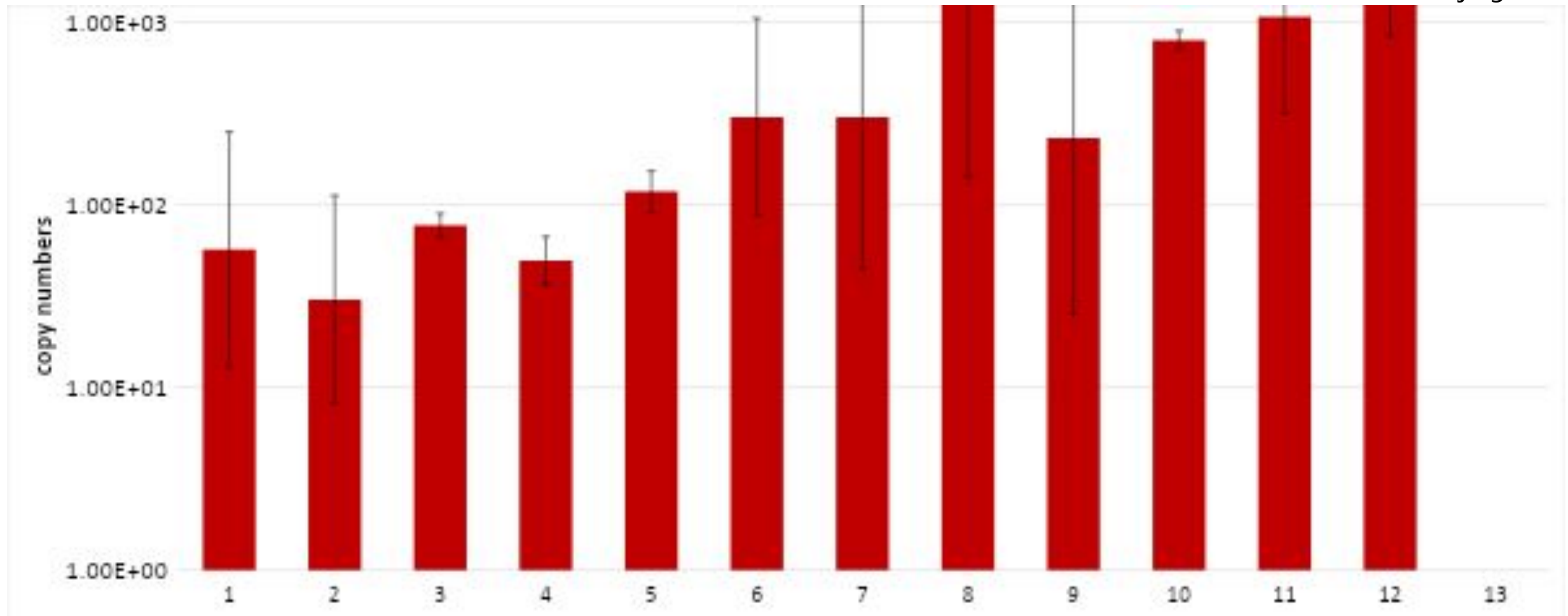
Yijing Liu



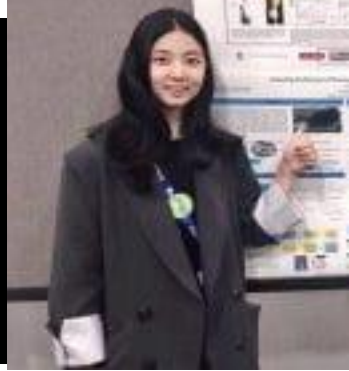
qPCR results - *M. avium* in Idaho



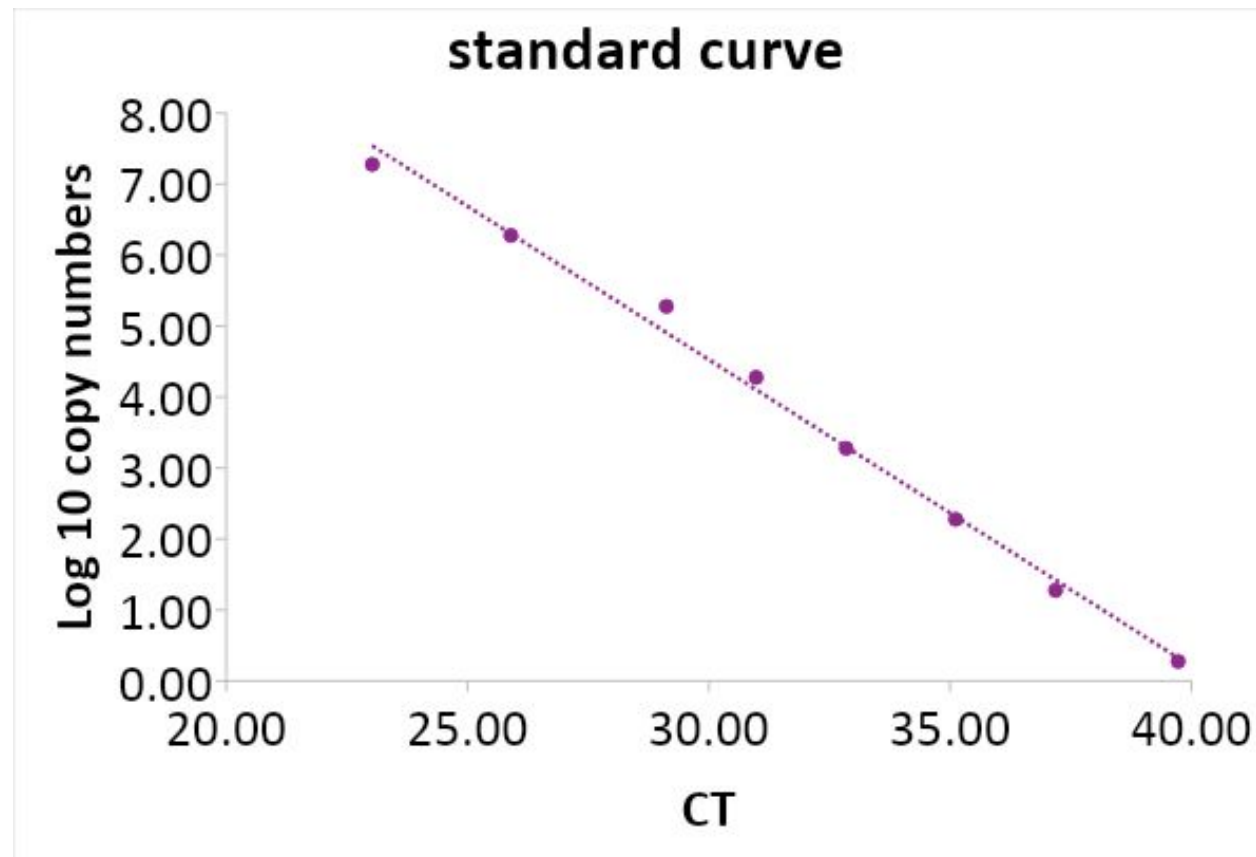
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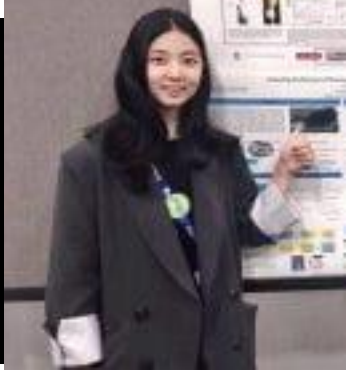
qPCR results - *M. intracellulare*



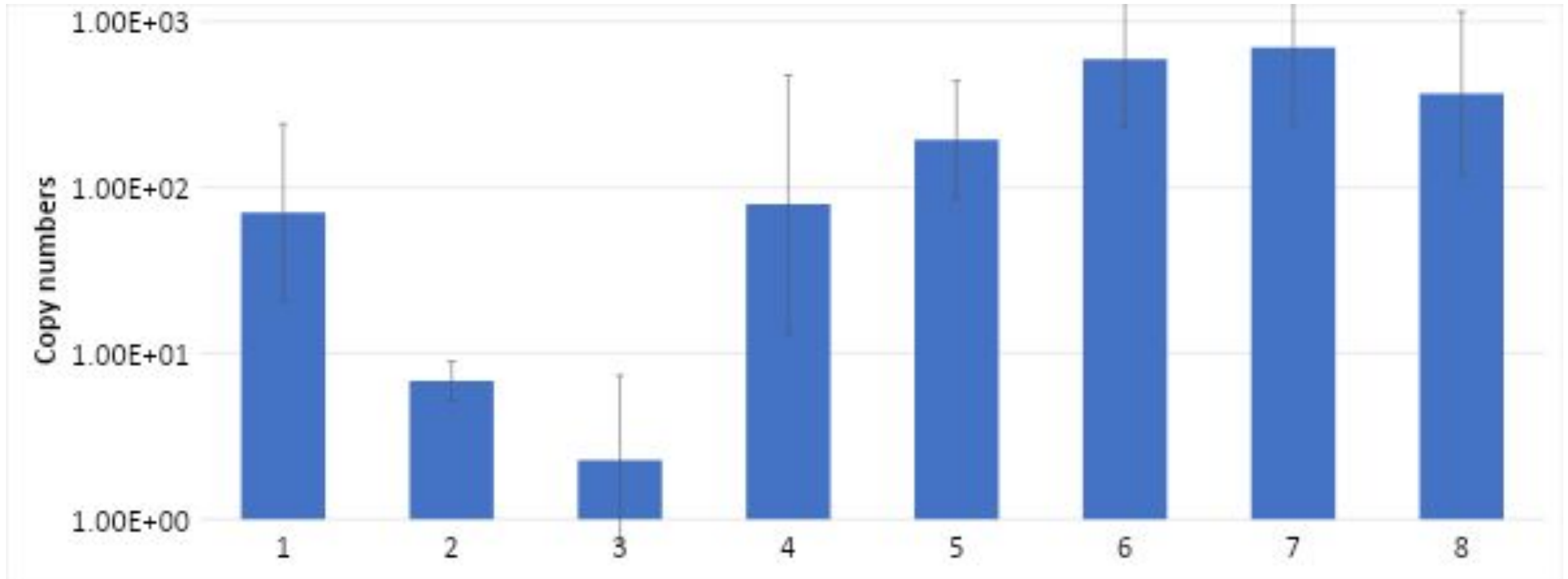
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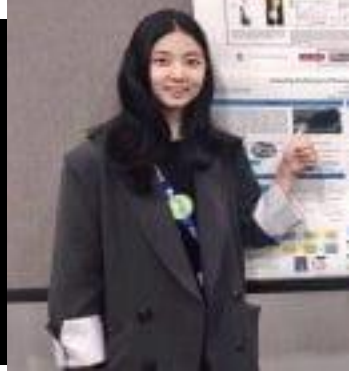
qPCR results - *M. intracellulare* in Alabama



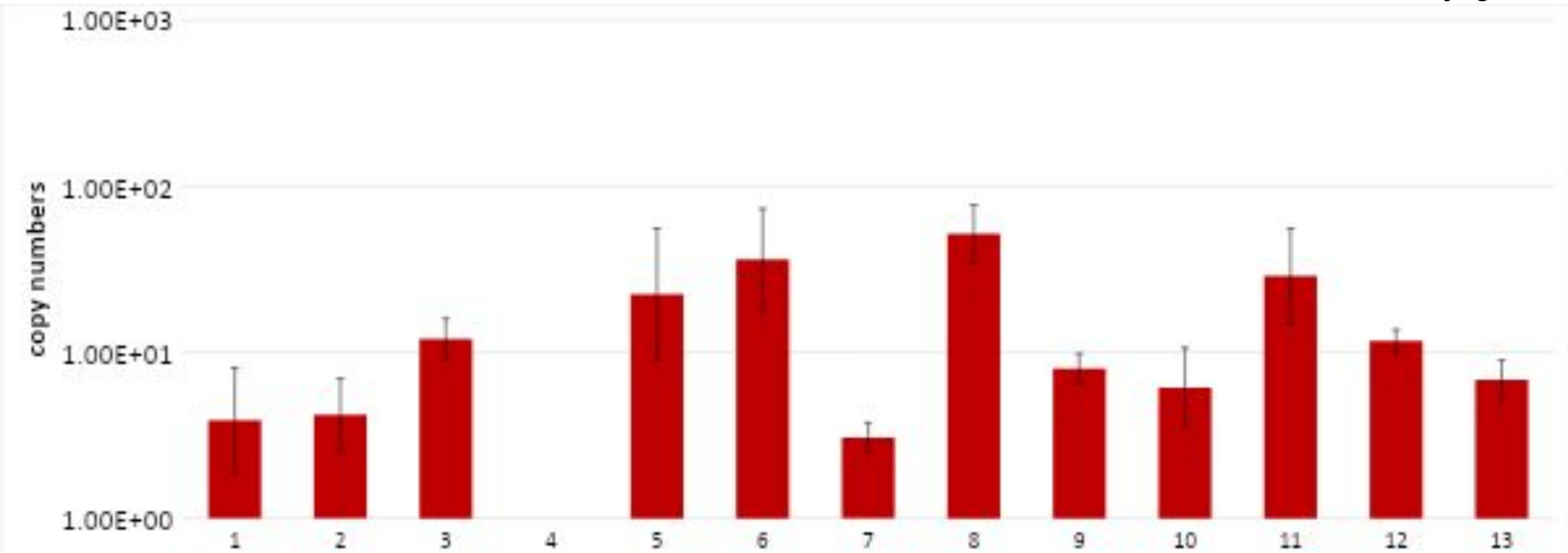
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qPCR results - *M. intracellulare* in Idaho



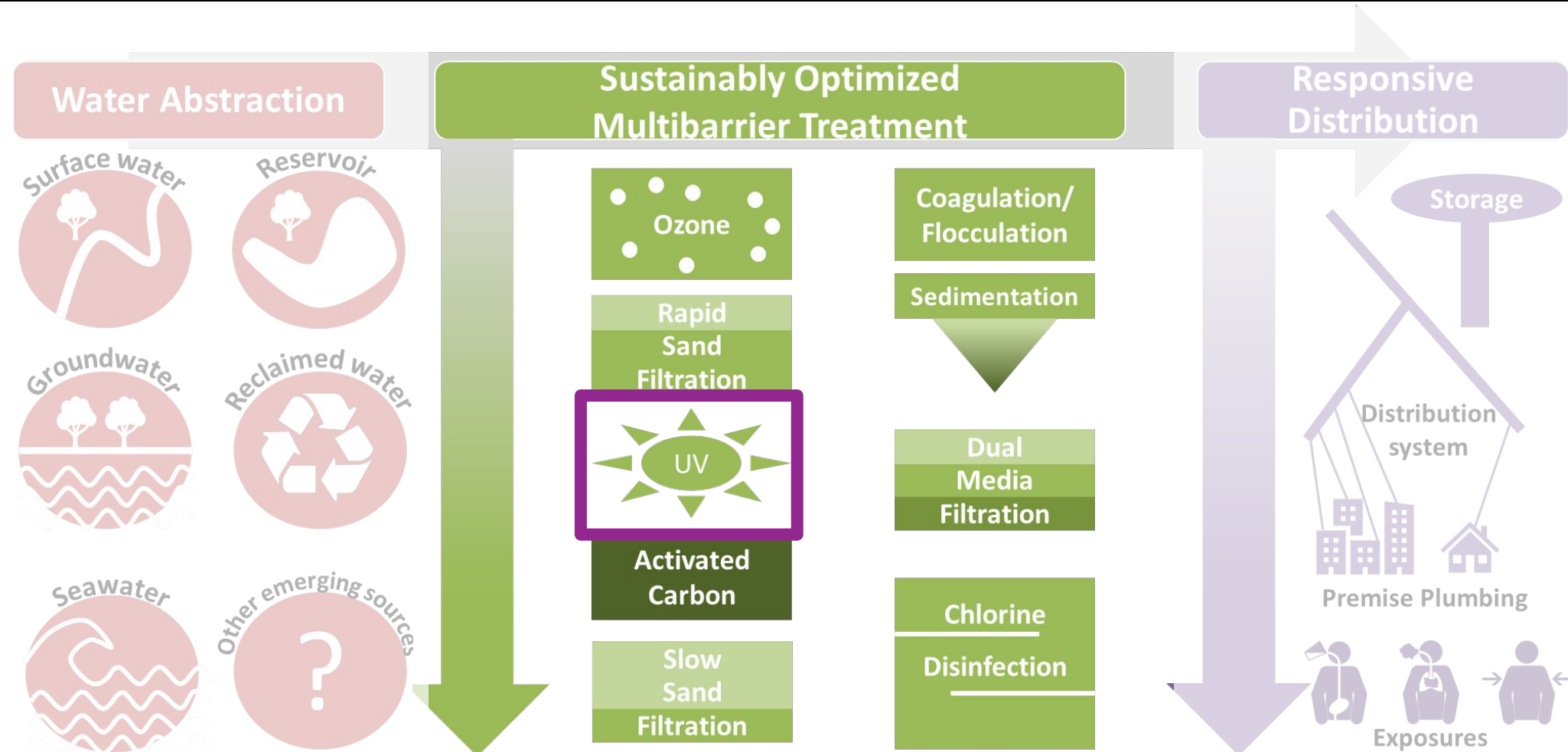
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Preliminary observations and next steps

- qPCR assays successfully detected mycobacterial targets in nearly all samples
- Differences observed between species, states, and wells
- Compile sample molecular, water quality, and other data
- Stats and reporting

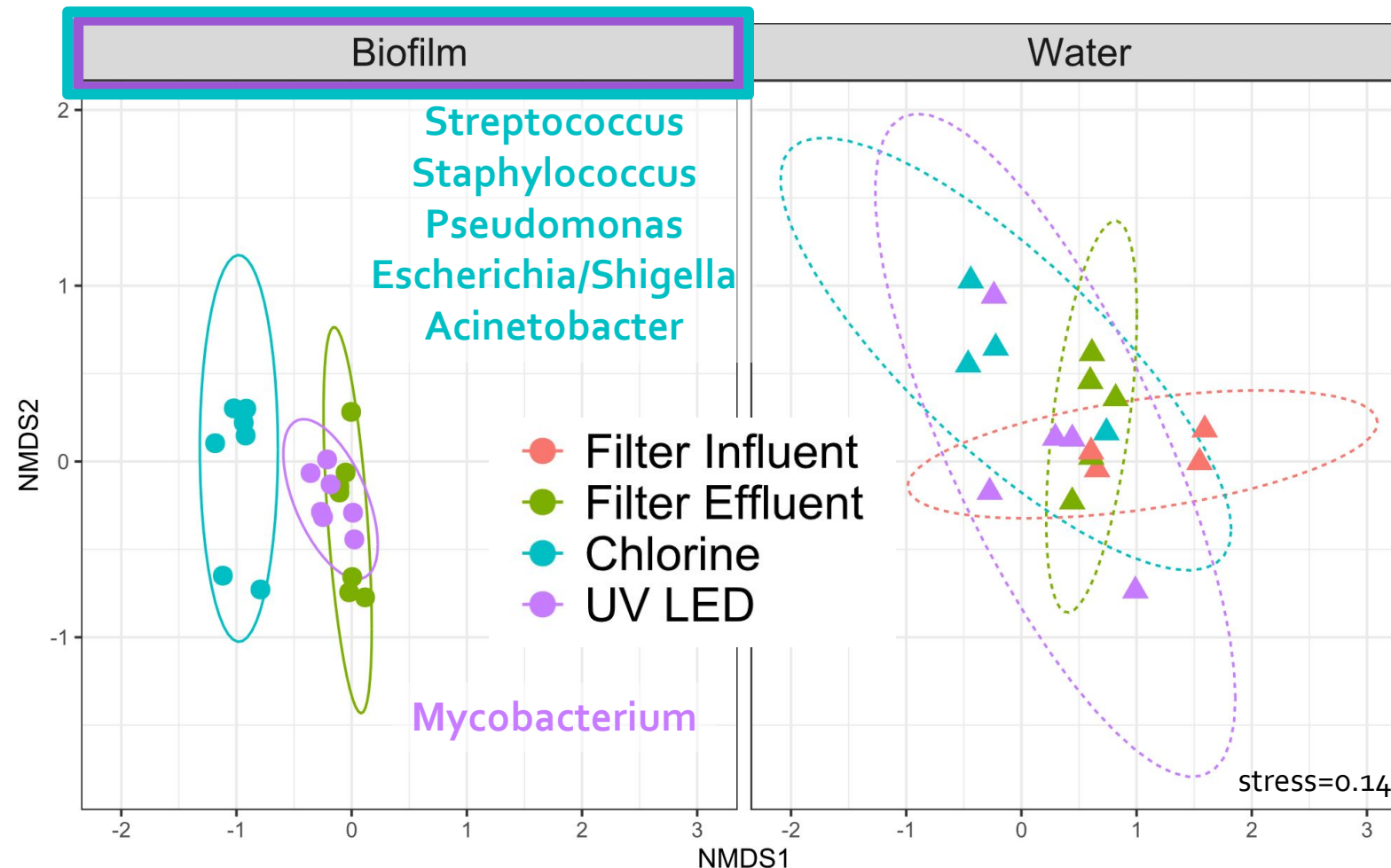
Water microbiomes must be optimized for multiple water sources, treatments, and exposure routes



Mercury-free UV LED had different selective pressure than chlorine disinfection on biofilm microbiomes

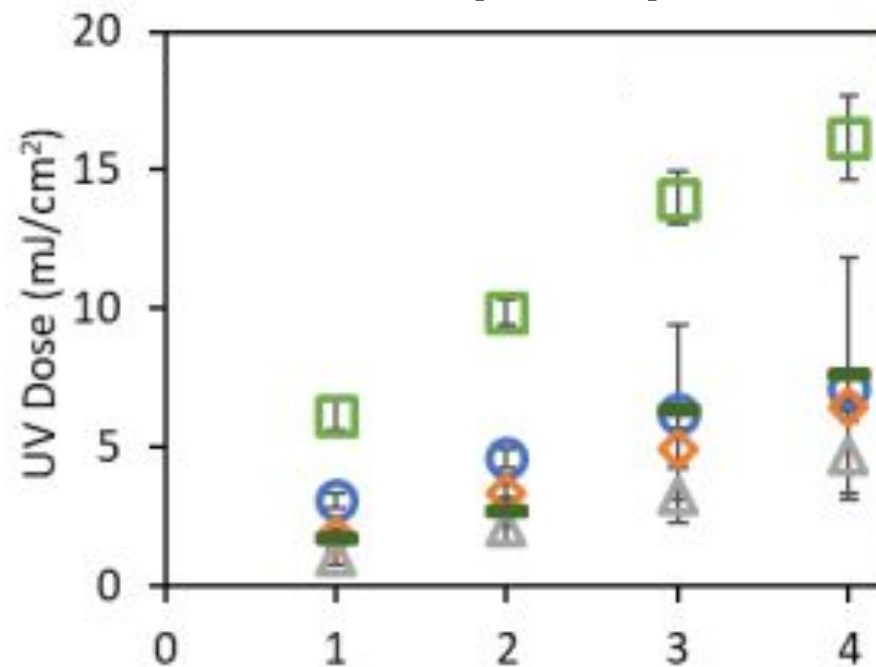


USEPA funded

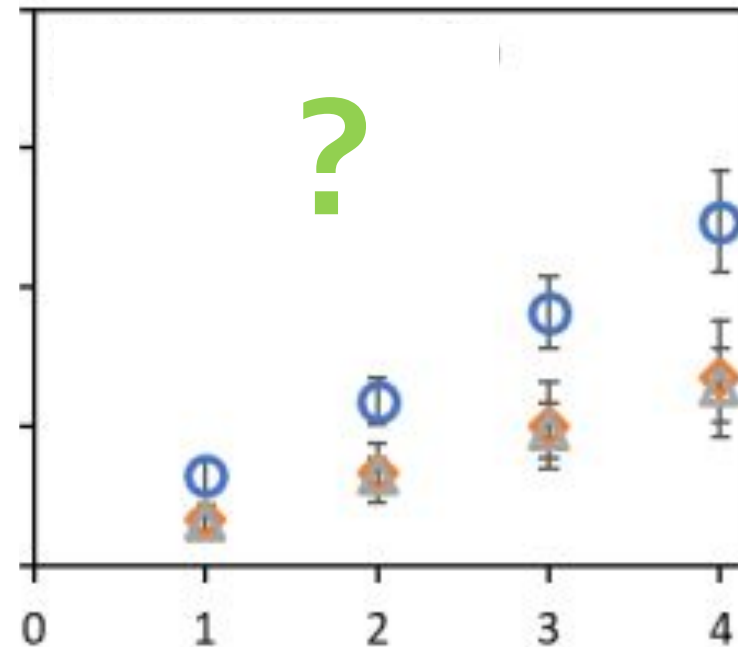


More research is needed to determine impacts of mercury-free UV on mycobacteria

Mercury lamps



Mercury-free UV



- *Escherichia coli*
- ◇ *Legionella* spp.
- △ *Pseudomonas aeruginosa*
- *Mycobacterium* spp.
- *Cryptosporidium parvum*

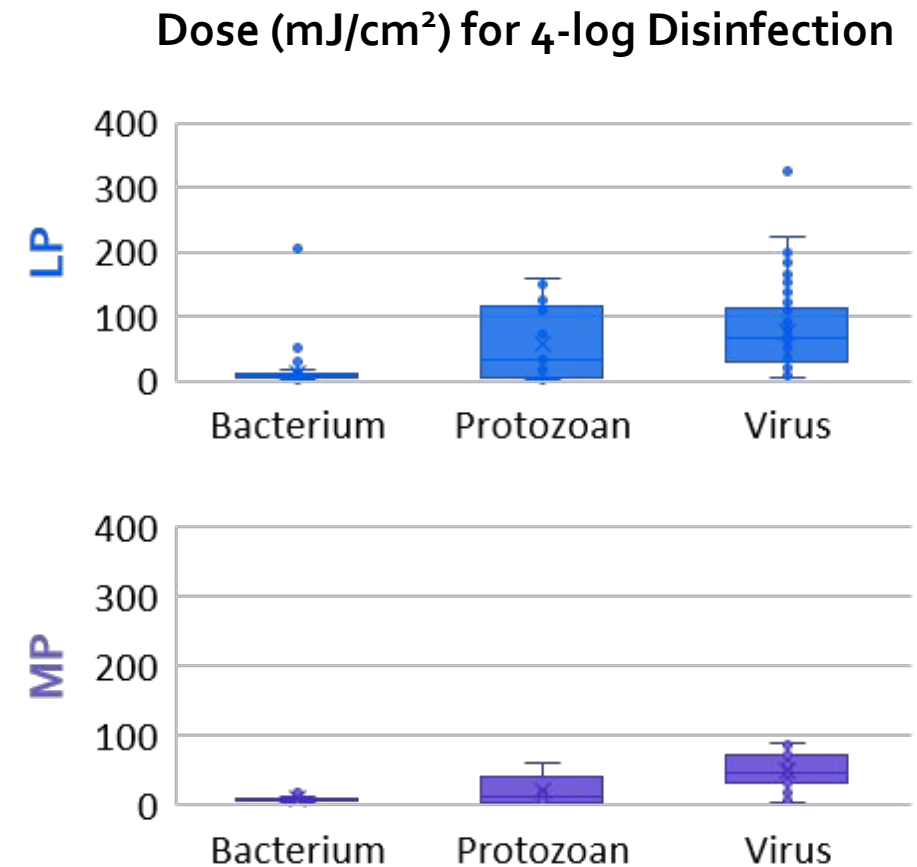
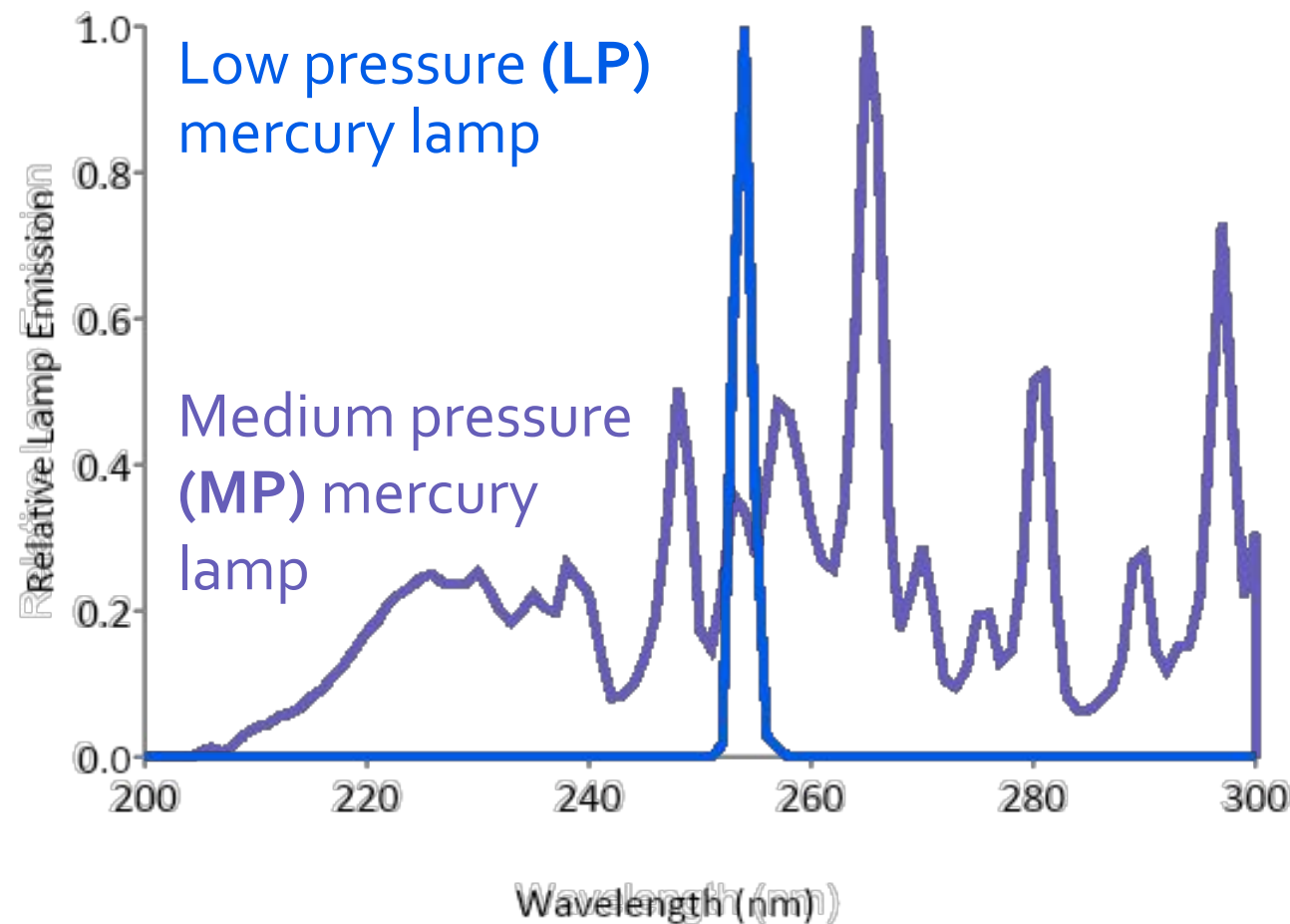
Average Log Reduction +/- Standard Error

Klever, A.M.; Alexander, K.; Almeida, D.; Anderson, M.Z.; Ball, R.L.; Beamer, G.; Boggiatto, P.; Buikstra, J.E.; Chandler, B.; Claeys, T.A.; Concha, A.E.; Converse, P.J.; Derbyshire, K.M.; Dobos, K.M.; Dupnik, K.M.; Endsley, J.J.; Endsley, M.A.; Fennelly, K.; Franco-Paredes, C.; Hagge, D.A.; Hall-Stoodley, L.; Hayes Jr, D.; Hirschfeld, T.K.; Hofman, C.A.; Honda, J.R.; Hull, N.M.; Kramnik, I.; Lacourciere, K.; Lahiri, R.; Lamont, E.A.; Larsen, M.H.; Lemaire, T.; Lesellier, S.; Lee, N.R.; Lowry, C.A.; Mahfooz, N.S.; McMichael, T.M.; Merling, M.R.; Miller, M.A.; Nagajyothi, J.F.; Nelson, E.; Nuernberger, E.L.; Pena, M.T.; Perea, C.; Podell, B.K.; Pyle, C.J.; Quinn, F.D.; Rajaram, M.V.S.; Rosas-Mejia, O.; Rothoff, M.; Sago, S.A.; Salvador, L.C.M.; Simonson, A.W.; Spencer, J.S.; Sreevatsan, S.; Subbian, S.; Sunstrum, J.; Tobin, D.M.; Vijayan, K.K.V.; Wright, C.T.O.; Robinson, R.T. (2023). The Many Hosts of Mycobacteria 9 (MHM9): A conference report. *Tuberculosis*.

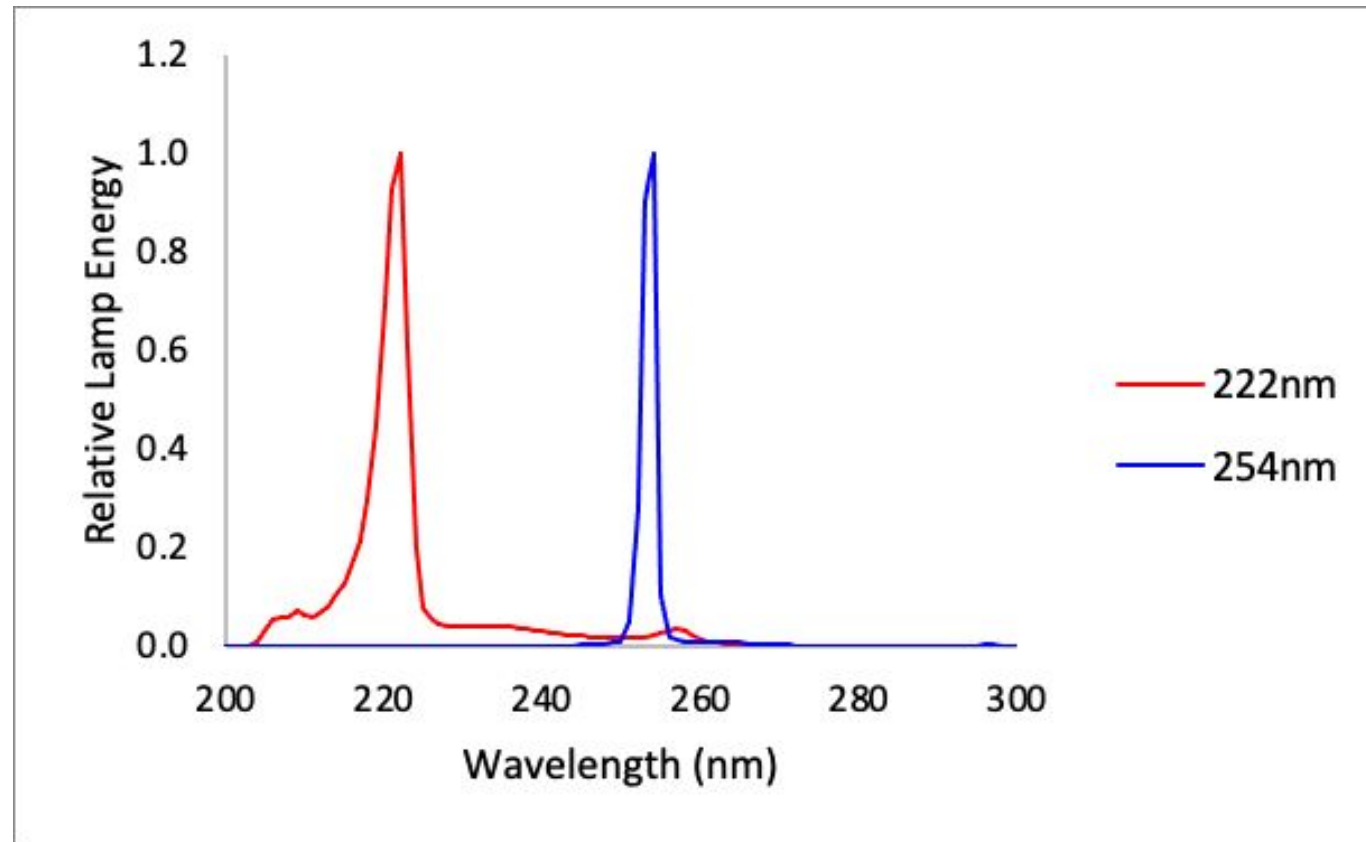
Claeys, T.A.; Rosas Mejia, O.; Marshall, S.; Jarzembowski, J.A.; Hayes, D.; Hull, N.M. Liyanage, N.; Chun, R.; Sulman, C.; Huppler, A.; Robinson, R. (2019). Attenuation of Helper T Cell Capacity for TH1 and TH17 Differentiation in Children with Nontuberculous Mycobacterial Infection. *Journal of Infectious Diseases*.

Linden, KG, Hull NM, and Speight, V (2019). Thinking outside the treatment plant: UV for water distribution system disinfection. *Accounts of Chemical Research Special Issue "Water for Two Worlds: Urban and Rural Communities"*.

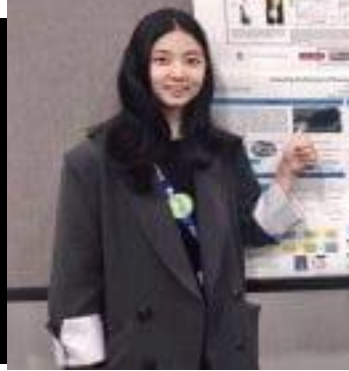
Mercury based UV has a proven history of disinfection



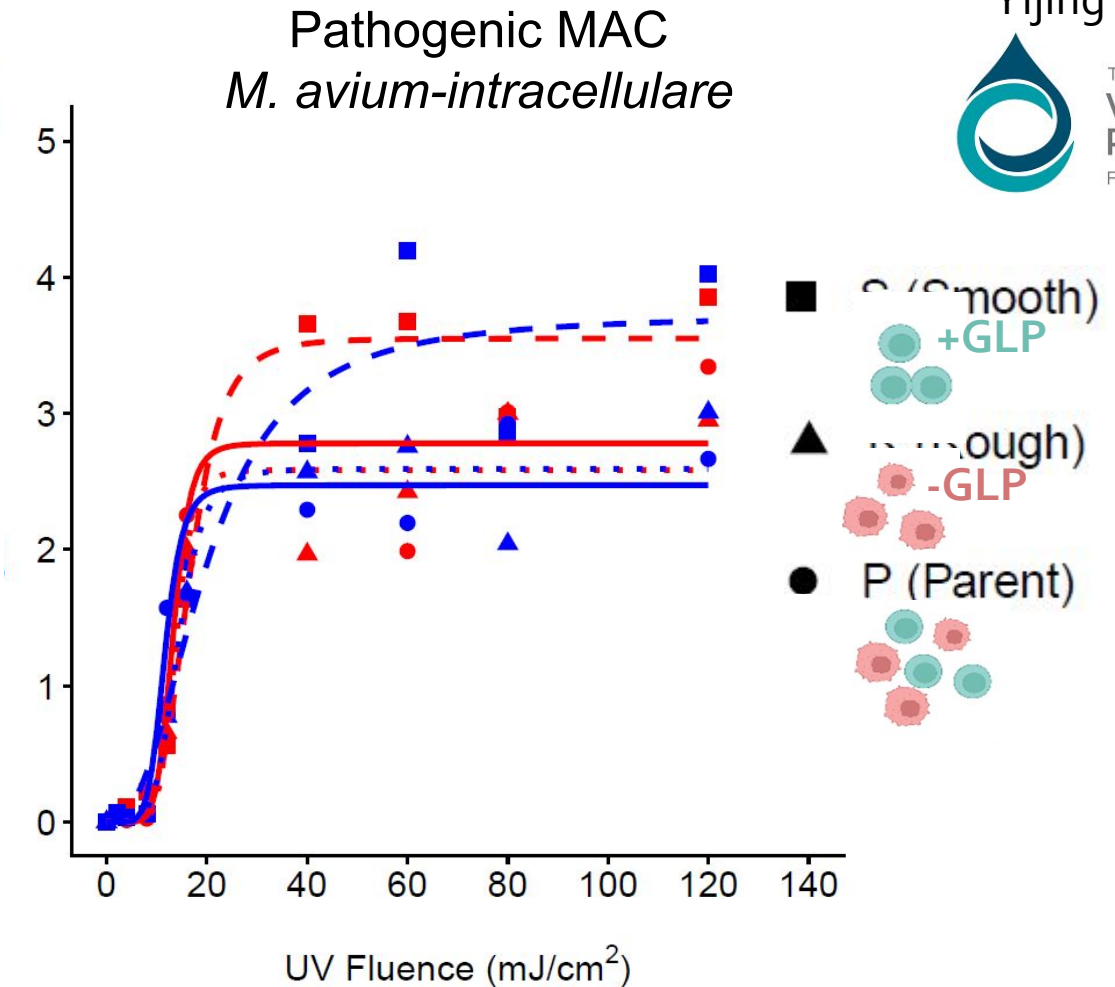
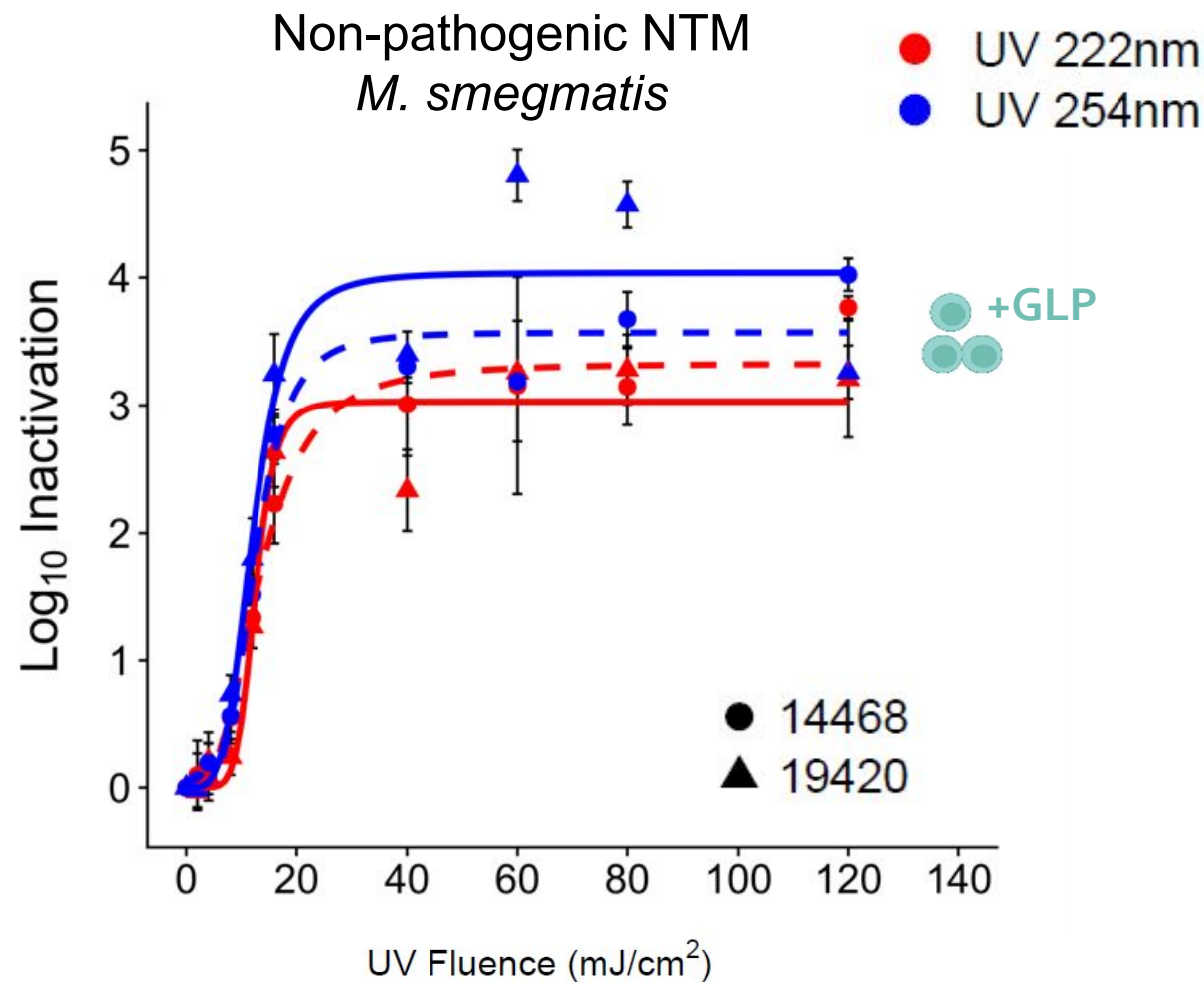
Mercury free far-UV is an emerging disinfection option



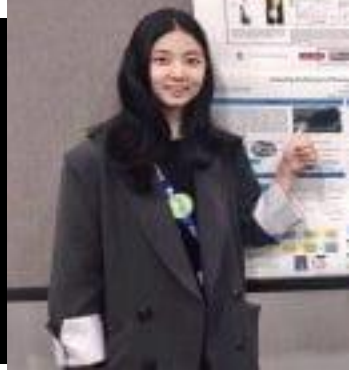
Mycobacteria virulence factors seem to impact UV disinfection resistance



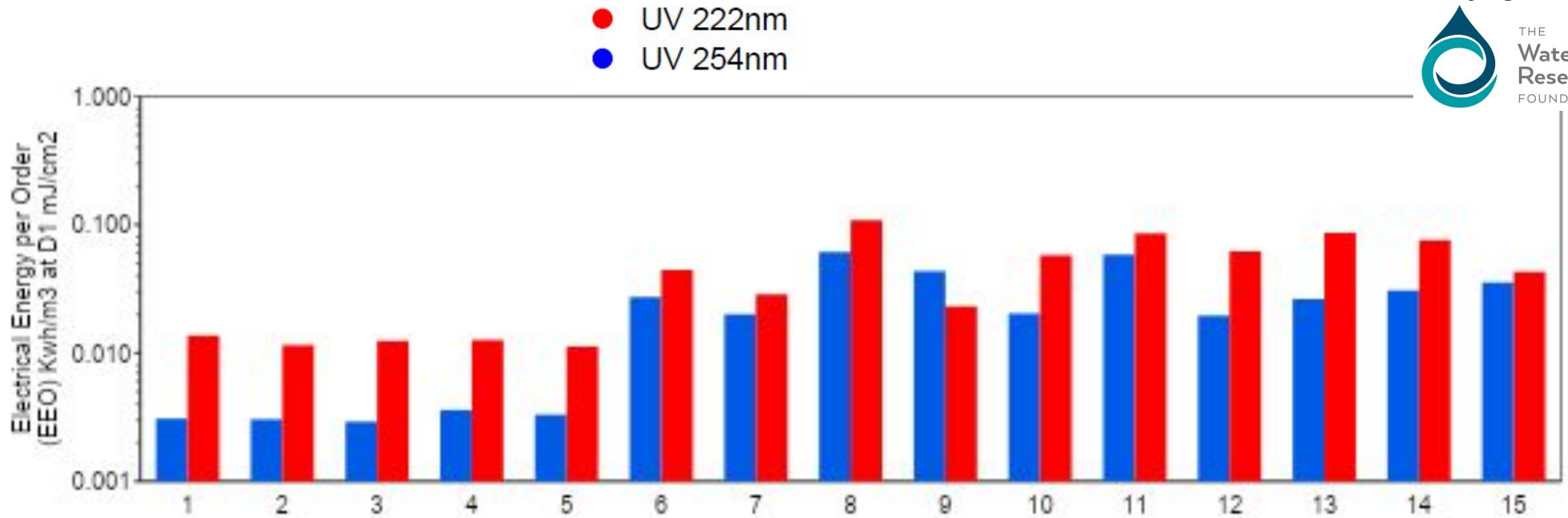
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Mycobacteria virulence factors seem to impact UV disinfection resistance



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Presence and treatment of potential opportunistic *mycobacteria* that can vary across groundwater geology

- Mycobacteria vary across groundwaters
- UV can be part of the solution for combatting OP in water treatment

Other sample site selection considerations

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- Alabama
 - Soil and wastewater management issues

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- Idaho
 - Heavy reliance on GW



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- Nevada
 - *Legionella* in direct-to-distribution wells Atkinson et al 2022
 - Studied chlorine, implementing UV-LED
- Colorado
 - Impacts of UV-LED on biofilms and OPs

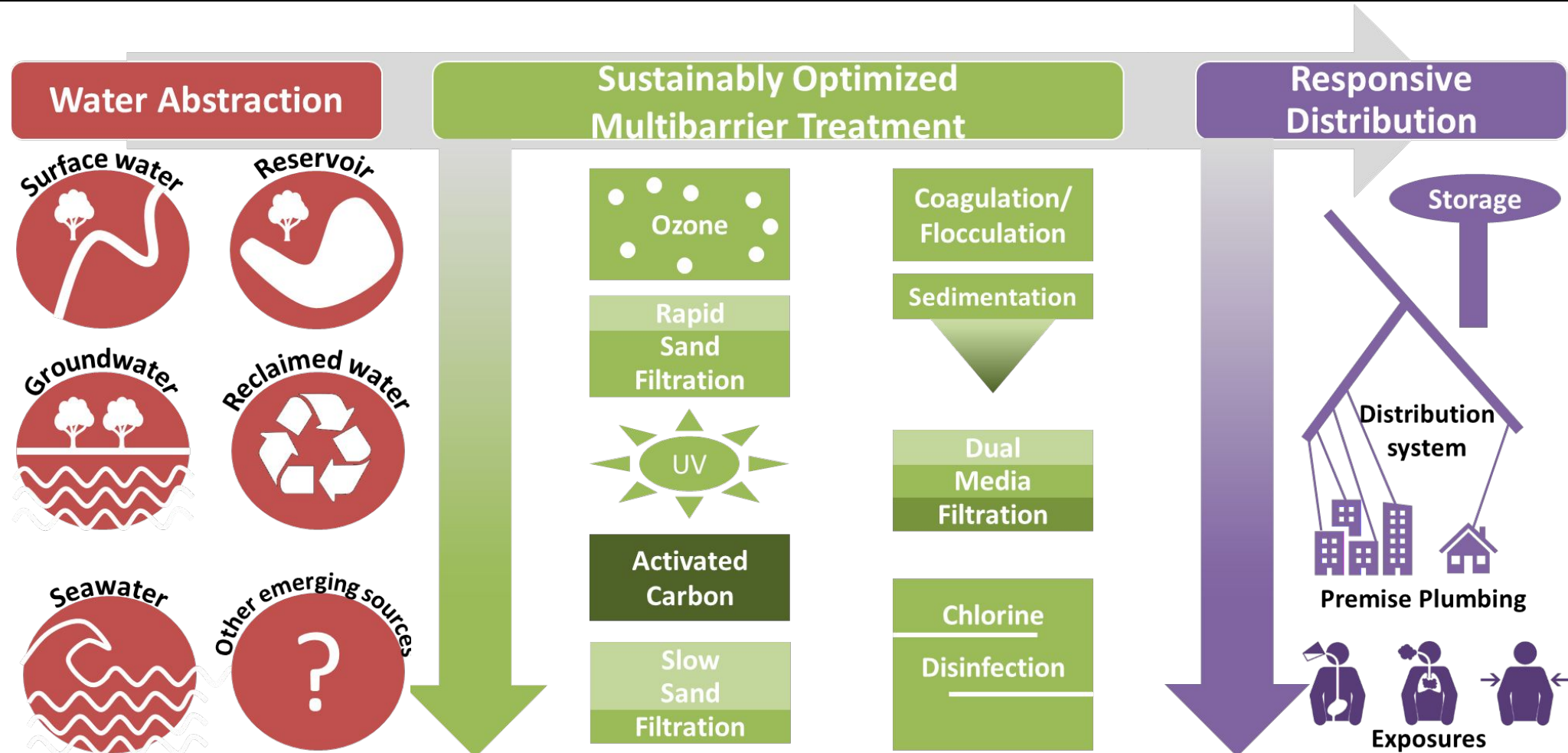


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FOUNDATION

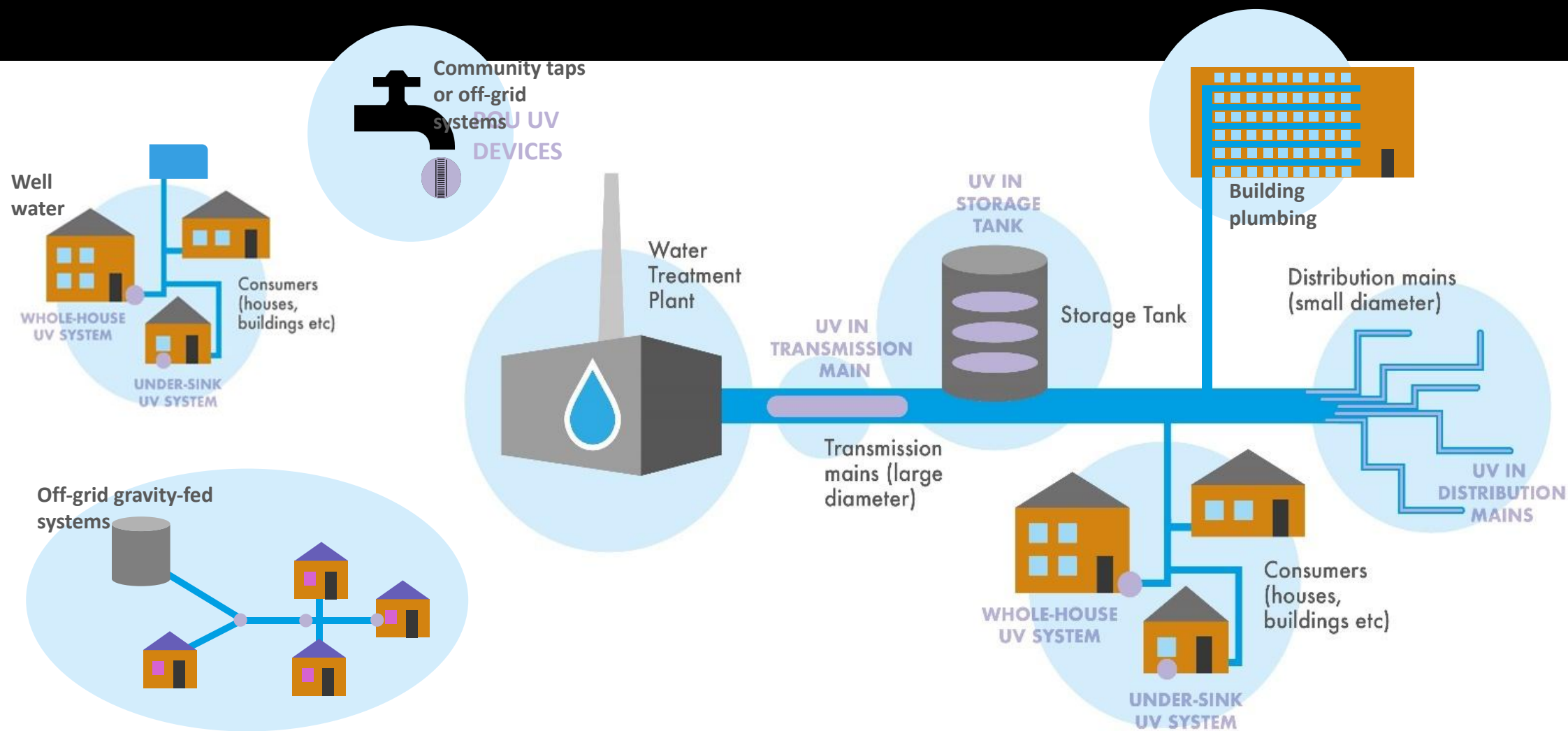
Impact of UV-LED on OP in groundwater microbiomes?

- Nevada
 - Pre and post UV LED
 - Field scale
 - Indigenous microbiomes (includes OP)
- Colorado
 - Pre and post UV LED
 - Bench scale
 - Spiked OP and indigenous microbiomes

Water microbiomes must be optimized for multiple water sources, treatments, and exposure routes



Application of UV doesn't need to be limited to centralized treatment



Presence and treatment of potential opportunistic *mycobacteria* that can vary across groundwater geology

- Mycobacteria vary across groundwaters
- UV can be part of the solution for combatting OP in water treatment

Presence and treatment of potential opportunistic *mycobacteria* that can vary across groundwater geology

- Mycobacteria vary across groundwaters
- UV can be part of the solution for combatting OP in water treatment
and distribution systems

Presence and treatment of potential opportunistic *mycobacteria* that can vary across groundwater geology

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