Decentralized Wastewater Influent as a Potential Tool for Community-Level Surveillance of Viral Pathogens and Antimicrobial Resistance

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Introduction

- Wastewater-based surveillance (WBS) has shown promise as an early warning tool for community public health, particularly for viral pathogen trends and antimicrobial resistance (AMR) burden[1,2]
- WBS can act as a surrogate for or supplement to clinical data, especially in healthcare-challenged areas[3]
- AMR a global health threat, exacerbated by overprescription of antibiotics (WV ranks highest in USA[4]) and proliferation in sanitary wastewater
- Only **47%** of WV structures are served by public sewer utilities, leaving the majority on decentralized systems and not directly represented in current WBS measures[5]

Proof of Concept

Learn more!

Onsite monitoring of SARS-CoV-2 and Norovirus in septic tank effluent gravity (STEG) system at 2023 National Boy Scout Jamboree, held at Summit Bechtel Reserve, WV

Objectives

1) Assess feasibility of sampling in decentralized and onsite contexts with various system configuration
2) Investigate the effects of community dynamics (transient, residential, clustered, etc.) and contributing population size on the utility of WBS data from decentralized and onsite systems
3) Compare data obtained from decentralized systems to existing WBS currently ongoing within centralized, large-scale municipal facilities

Methodology

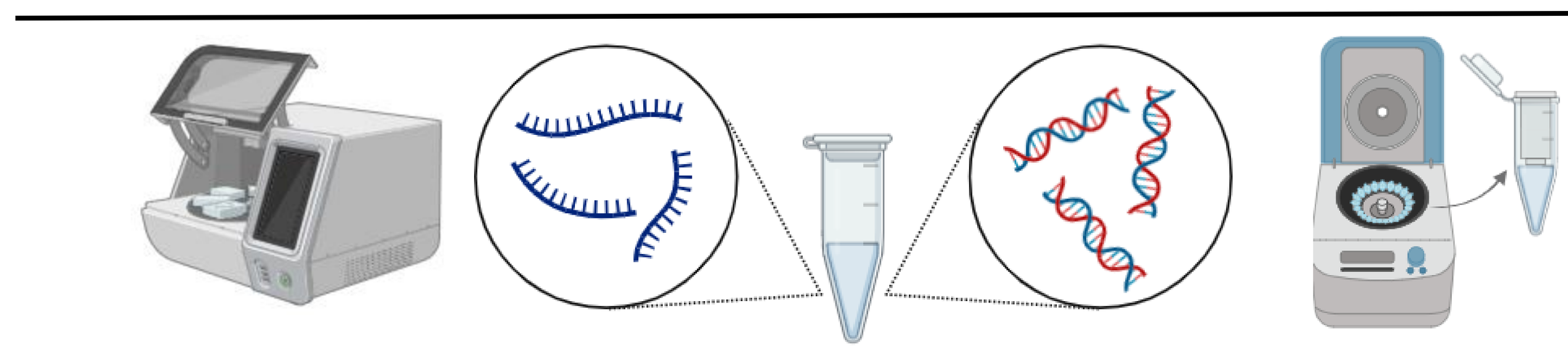
Sampling Regime

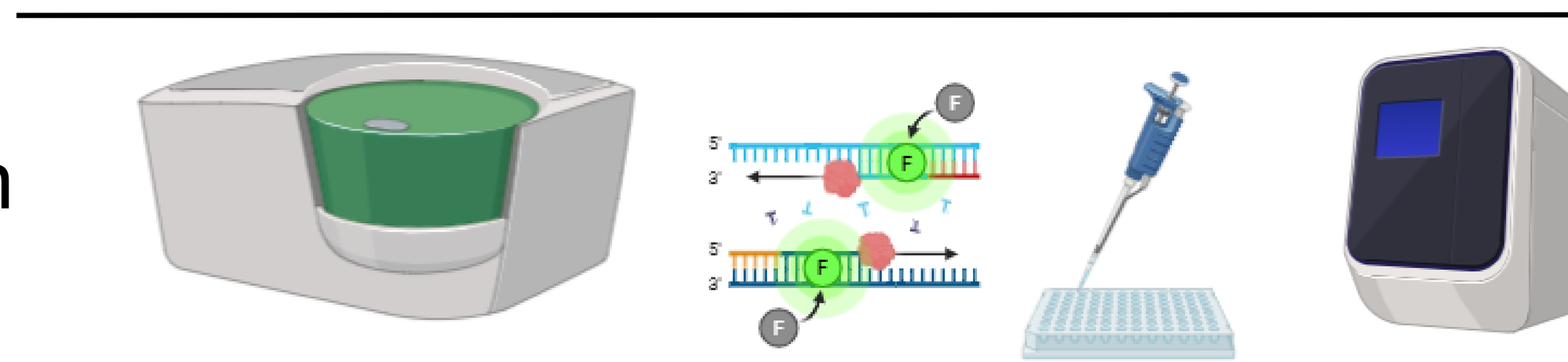
Raw or primary influent (grab samples) collected across multiple dates from:

- Residential Septic & Aerobic Treatment Units/Home Aeration Units
- Clustered Systems & Package Plants of Subdivision Communities
- Transient Communities (such as campgrounds) with Alternative Systems
- Sewered Communities (<2000 persons) with Treatment Lagoons

Laboratory Workflow

Constituent	Viral (RNA)	AMR (DNA)
Genetic Targets	• SARS-CoV-2 • RSV • Influenza A/B • Norovirus	• *bla*TEM • *bla*OXA-48 • *bla*CTX-M • KPC (β-lactam resistance genes)
Nucleic Acid Extraction	Ceres Nanotrap & Applied Bios. MagMAX Wastewater Kit	Filter Concentration & MP Biomed. FastDNA Spin Kit for Soil
Quantification Method	RT-ddPCR	qPCR

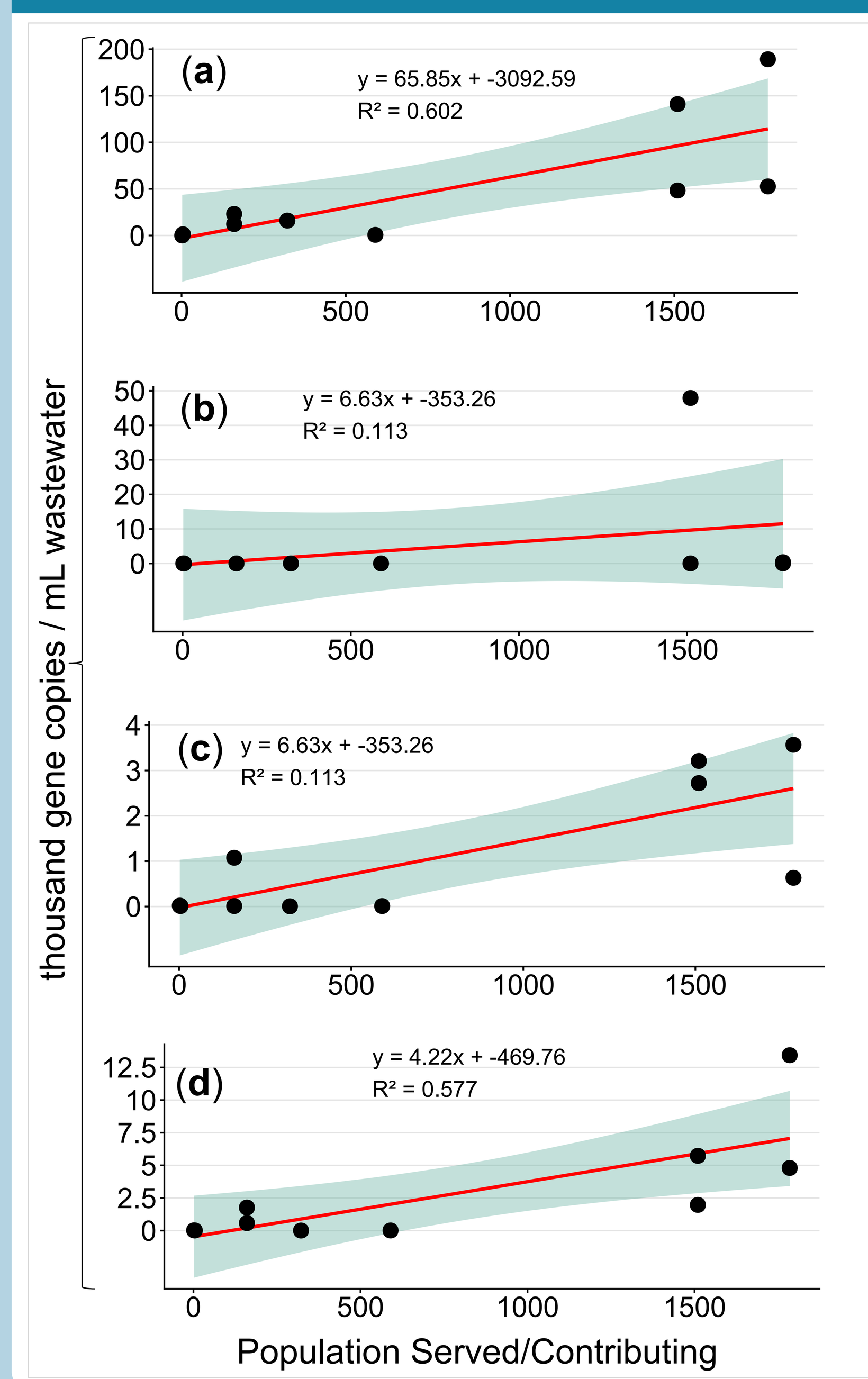




Supplementary Data & Information Collected

Physiochemistry: pH, Temperature, COD, NH_3, TSS, Turbidity
Site Specific: Total Contributing Population, Infrastructure O&M

Preliminary Results & Findings



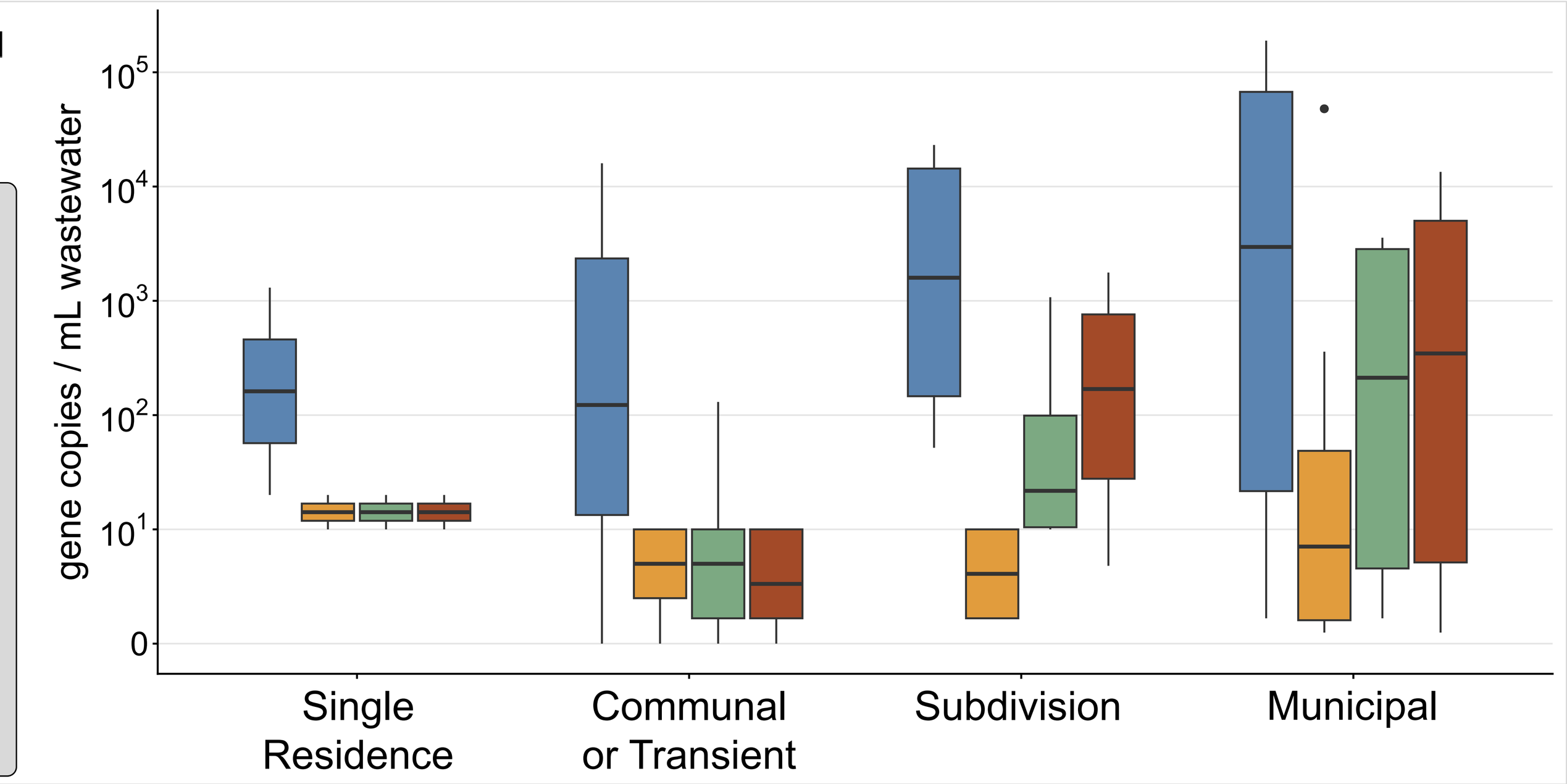
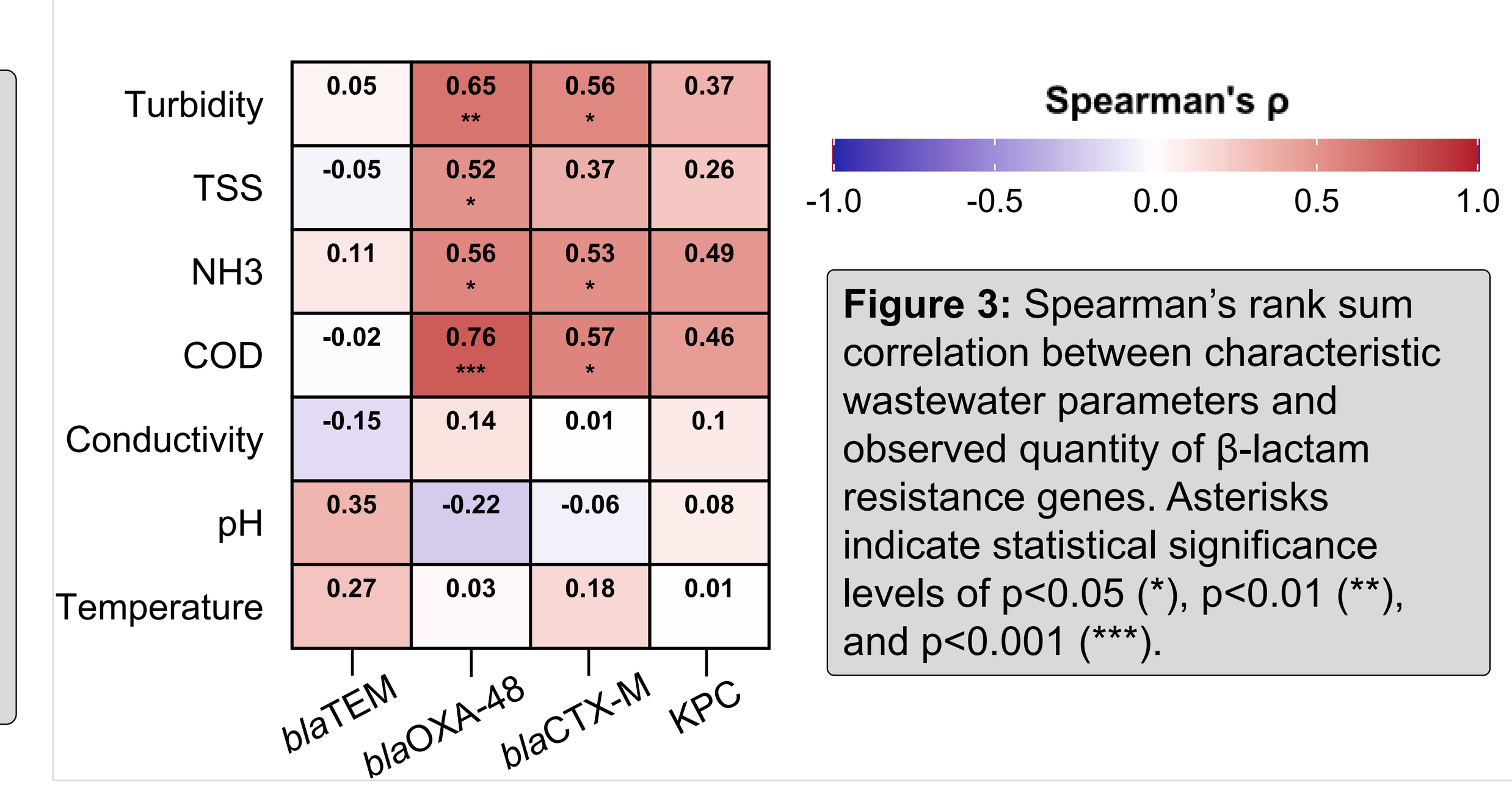


Figure 1 (right): Observed quantities of β-lactam resistance genes, grouped by contributing population type, generally increasing in population size from left to right. Note – communal or transient communities refer to systems where localized gatherings occur; at present, this includes schools and campgrounds.

Figure 2 (a-d, left): Contributing population size versus observed quantity of (**a**) *bla*TEM, (**b**) *bla*OXA-48, (**c**) *bla*CTX-M, and (**d**) KPC β-lactam resistance genes for systems sampled to date, fitted to a linear regression (n = 10).

Figures exclude transient communities (namely, campgrounds) with hypervariable populations.



	*bla*TEM	*bla*OXA-48	*bla*CTX-M	KPC
Turbidity	0.05	0.65 **	0.56 *	0.37
TSS	-0.05	0.52 *	0.37	0.26
NH3	0.11	0.56 *	0.53 *	0.49
COD	-0.02	0.76 ***	0.57 *	0.46
Conductivity	-0.15	0.14	0.01	0.1
pH	0.35	-0.22	-0.06	0.08
Temperature	0.27	0.03	0.18	0.01

Figure 3: Spearman's rank sum correlation between characteristic wastewater parameters and observed quantity of β-lactam resistance genes. Asterisks indicate statistical significance levels of $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***).

Practical Monitoring Challenges

Site access restraints

- Topography/terrain, systems below ground
- Private property and ethical considerations

Representative & reproducible sampling

- Variable sludge age in septic systems

Methodological consistency & efficiency

- Nucleic acid extraction from samples with hypervariable solids content
- Deteriorating systems with limited oversight or operational challenges

Current Key Takeaways

Fairly clear linear trends developing between population served and β-lactam resistance genes

- Might suggest population thresholds exist for meaningful data, *or*
- Normalization by population is warranted

***bla*TEM abundant, regardless of community type**

- Encodes resistance to penicillins and early generation cephalosporins

Higher strength wastewater influent generally corresponds to higher β-lactam concentration

- May be skewed due to rainfall events for systems open to environment or with sewer/conveyance inflow and infiltration

Next Steps

- Continued and repeat sampling; adding more small and mid-size populations to augment the distribution of systems
- Lab method and assay refinement to quantify viral targets as described in methods

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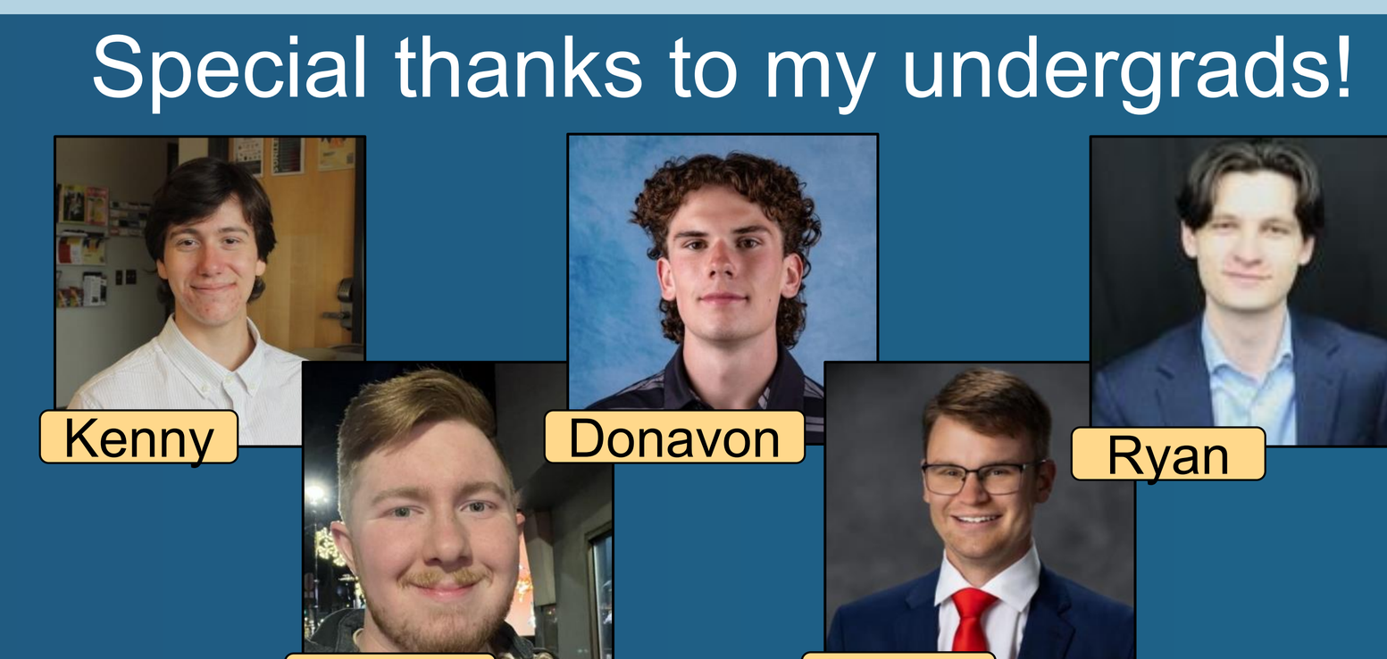
Special thanks to my undergrads!



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