CONSERVE
A Center of Excellence at the Nexus of Sustainable Water Reuse, Food, and Health

USDA United States Department of Agriculture
National Institute of Food and Agriculture

University of Maryland SCHOOL OF PUBLIC HEALTH

Refining Sampling and Analysis Approaches to Advance Understanding of the Microbiological Risks of Agricultural Water Reuse



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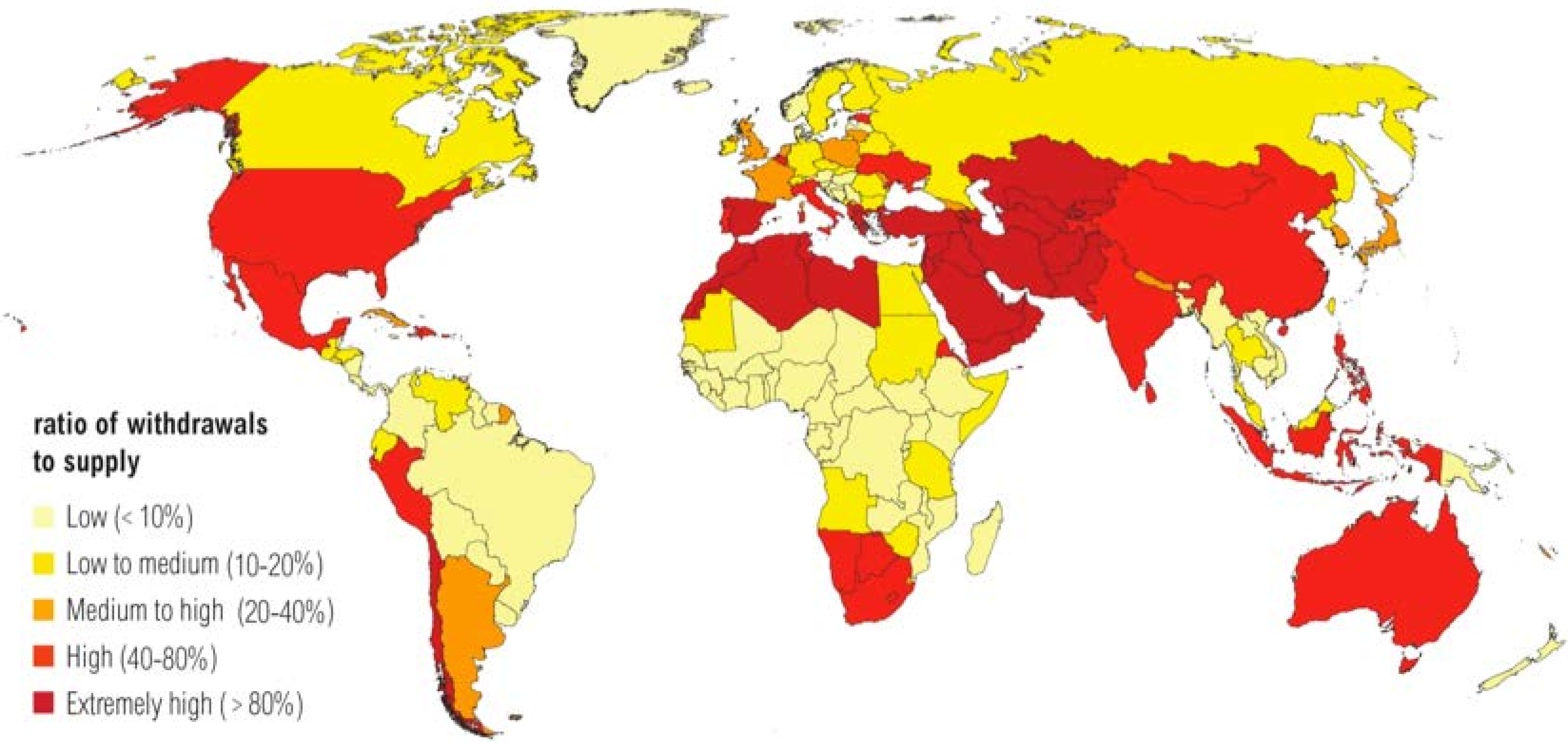
Presentation outline

- Global water scarcity and use of recycled water on food crops
- Mission and activities of CONSERVE Center of Excellence
- Refining sampling and analysis approaches for recycled water
  - Comparing sampling frequency and sample volumes
  - Employing whole genome sequencing
  - Coupling DNA-labeling and sequencing approaches

Increasing water shortages threaten irrigation water resources

Water Stress by Country: 2040



NOTE: Projections are based on a business-as-usual scenario using SSP2 and RCP8.5.

For more: ow.ly/RiWop

WORLD RESOURCES INSTITUTE

Recycled Water: An Important Part of the Solution

- Recycled water = <u>advanced treated</u> wastewater or greywater = reclaimed water = water reuse

- "One Water Approach" = All water has value and should be managed in a sustainable, inclusive, integrated way

- "There is no bad water"

Photo: ClimateTechWiki

Source: Carollo.com

US EPA. 2012. Guidelines for Water Reuse. https://nepis.epa.gov/Adobe/PDF/P100FS7K.pdf

Orchards being irrigated with untreated wastewater in Riverside, CA, 1890-1900.
California Historical Society Collection, 1860-1960

AGRICULTURAL IRRIGATION ACCOUNTS FOR ~30% OF RECYCLED WATER USE IN THE U.S.

Table 3-3. Nationwide reuse summaries of reclaimed water use in agricultural irrigation (adapted from Bryk et al., 2011)

State	Annual Agricultural Reuse Volume	
	mgd	1000 ac-ft/yr
Arizona	23	26
California	270	303
Colorado	2.97	3
Florida	256	287
Idaho	0.27	0.3
North Carolina	1.0	1
Nevada	13.4	15
Texas	19.4	22
Utah	0.81	1
Washington	0.02	0.03
Wyoming	0.89	1

- Number of US States with rules, regulations or guidelines addressing water reuse on:
  - Food crops: 27 states
  - Processed food and non-food crops: 43 states
- Rules, regulations and guidelines dictate **water quality requirements**

US EPA 2012 Guidelines for Water Reuse. https://nepis.epa.gov/Adobe/PDF/P100FS7K.pdf

Food Safety Modernization Act (FSMA)

- FSMA Produce Safety Rule
  - Strict water quality criteria for irrigation water "that is directly applied to growing produce…"
  - *E. coli* standard:
    - Baseline microbial water quality profile for untreated surface water: ≥ 20 samples over 2-4 years
    - Geometric mean (GM) < 126 CFUs of generic *E. coli* per 100 mL
    - Statistical threshold value (STV) < 410 CFUs of generic *E. coli* per 100 mL

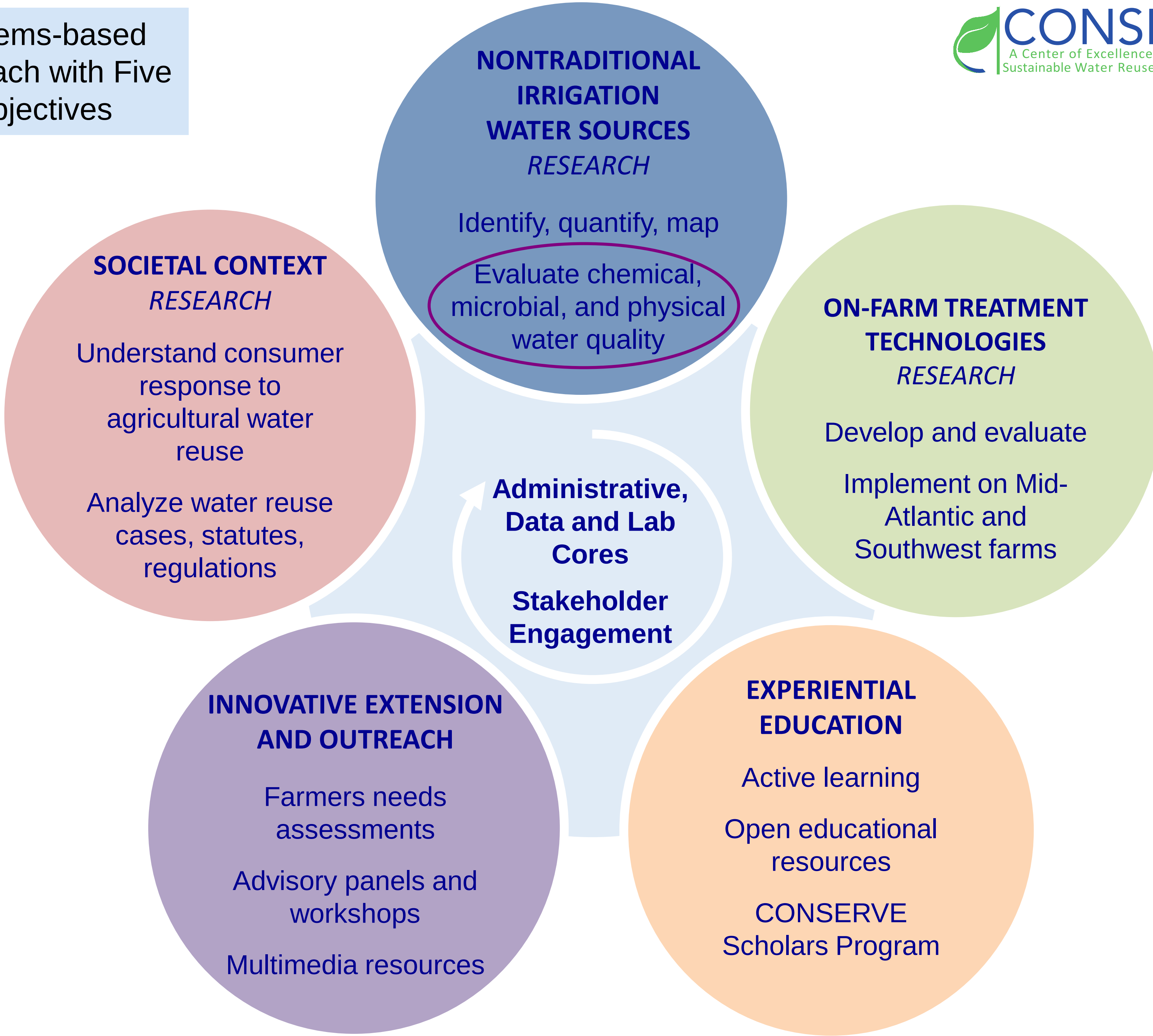
Sustainable on-farm water treatment solutions are needed to enable growers to use recycled (or nontraditional) water sources for food crop irrigation.

CONSERVE

A Center of Excellence at the Nexus of Sustainable Water Reuse, Food, and Health

Our Mission: *To facilitate the adoption of transformative on-farm water treatment solutions that enable the safe use of nontraditional irrigation water on food crops.*

Systems-based Approach with Five Objectives



Nontraditional Irrigation Water Sources *(Research)*

Nontraditional Irrigation Water Quality

(Co-PIs: Sapkota, Kniel, Sharma, Micallef, Hashem, Gerba, Ravishankar, Rock, Parveen, May, Sapkota, Mongodin, Colwell, Pop)

2 Year, Bi-weekly Field Sampling Effort Completed

22 field sites sampled in the Mid-Atlantic and Southwest

>5,000 water samples collected, processed, analyzed for bacterial indicators, pathogens, antibiotic-resistant bacteria, pharmaceuticals, and sent for 16S rRNA sequencing and metagenomic shotgun sequencing

>500 *Salmonella* isolates Whole Genome Sequenced by FDA GenomeTrakr Program

ARIZONA · UNIVERSITY SYSTEM OF MARYLAND · COSMOSID · USDA ARS · UNIVERSITY OF DELAWARE

E. coli Findings: Mid-Atlantic Sites
October 2016-October 2018

SARAH ALLARD

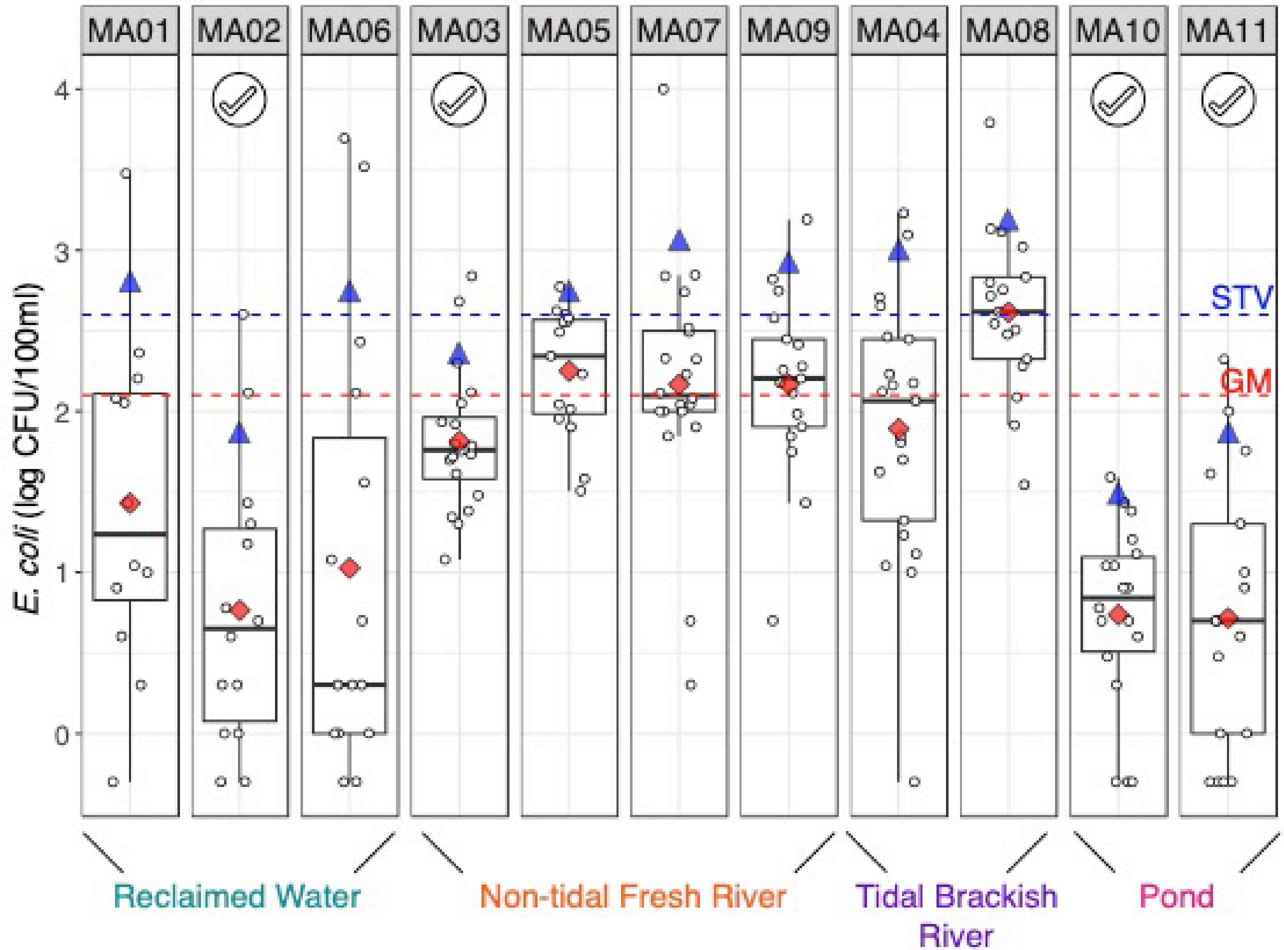
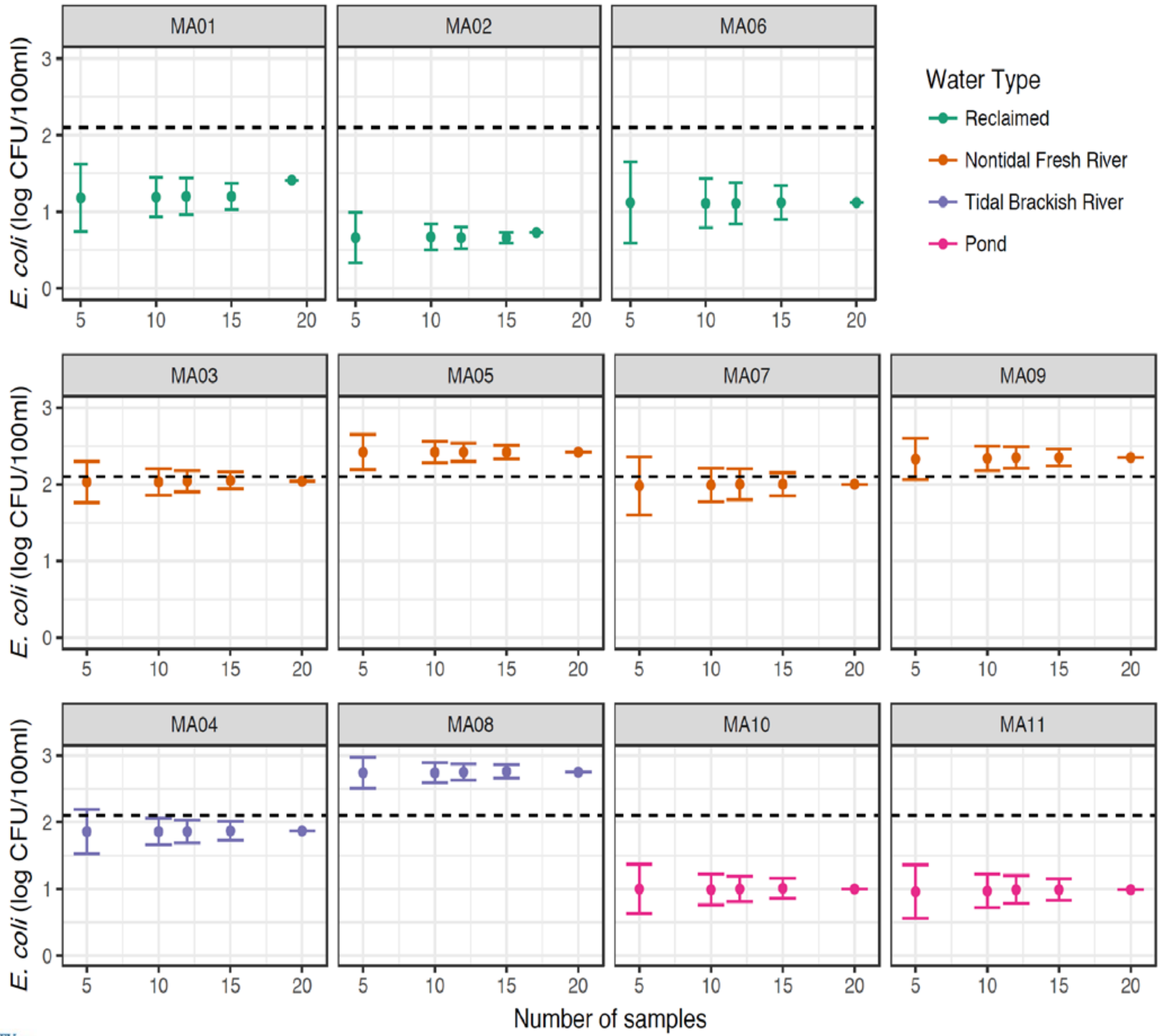


Fig 1: *E. coli* geometric mean (GM, red diamond) and statistical threshold values (STVs, blue triangle) in irrigation water samples collected during the growing season (on sampling days with <0.1 cm precipitation in the previous 24 h) in comparison to standards of the Food Safety Modernization Act, Produce Safety Rule.

Impact of Sampling Frequency on *E. coli* Geometric Means



Created by: Sarah Allard, PhD

Impact of Sample Volume on Pathogen Detection

Table 3: Number (percentage) of total sampling events at each site where each water volume filtered contained *Salmonella* spp. or *L. monocytogenes*.

Site	Water type	# Sampling event	***Salmonella spp.***			***L. monocytogenes***		
			0.1 L	**1 L**	**10 L**	**0.1 L**	**1 L**	**10 L**
MA04	River	34	17 (50%)	16 (47.1%)	27 (79.4%)	9 (26.5%)	6 (17.6%)	14 (41.2%)
MA05	River	32	8 (25%)	15 (46.9%)	25 (78.1%)	25 (78.1%)	29 (90.6%)	29 (90.6%)
MA06	Reclaimed	25	2 (8%)	5 (20%)	8 (32%)	2 (8%)	2 (8%)	2 (8%)
MA10	Pond	35	1 (2.9%)	2 (5.7%)	7 (20%)	2 (5.7%)	2 (5.7%)	3 (8.6%)
MA11	Pond	34	2 (5.9%)	4 (11.8%)	10 (29.4%)	1 (2.9%)	2 (5.9%)	3 (8.8%)
MA12	Produce wash	10	5 (50%)	4 (40%)	6 (60%)	1(10%)	1 (10%)	1 (10%)

Sharma et al. 2019. [Under Review].

Recovering 10L Water Samples Significantly Improved the Likelihood of Detection for Both *Salmonella* spp. and *Listeria monocytogenes*

Pathogen	Volume Comparison	Increase in Likelihood of Recovery	*p*-value
Listeria monocytogenes	1L vs 0.1L	1.2	0.894
	10L vs 0.1L	4.8	0.012
	10L vs 1L	3.9	0.037
Salmonella spp.	1L vs 0.1L	1.7	0.194
	10L vs 0.1L	43.5	<0.0001
	10L vs 1L	25.5	<0.0001

Sharma et al. 2019. [Under Review].

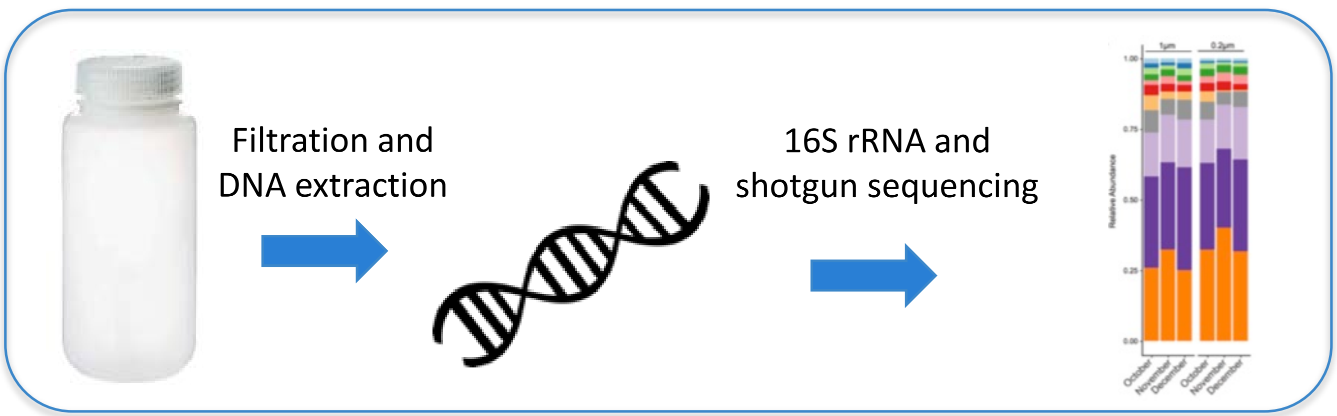
Whole Genome Sequencing of *Salmonella* Isolates Revealed the Presence of 21 Serovars

MA 04 (**River**)	MA 05 (**River**)	MA 06 (**Recycled**)	MA 10 (**Pond**)	MA 11 (**Pond**)	MA 12 (**Processing**)
Newport	**Newport**	**Newport**		**Newport**	**Newport**
Bareilly	**Bareilly**		**Bareilly**	**Bareilly**	
Javiana	**Javiana**				**Javiana**
Typhimurium				**Typhimurium**	**Typhimurium**
4:i:-	**4:i:-**				
Infantis					**Infantis**
	Norwich				**Norwich**
Thompson				**Thompson**	
Anatum	Bovismorbificans	Montevideo		Berta	Liverpool
Enteritidis	Give				Mbandaka
IV44:z36,[z38]:-	Oyonnax				
Johannesburg	IIIa 48:g,z51:-				

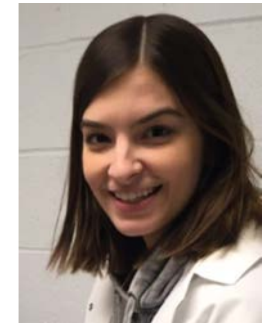
Fig 1: *Salmonella* serovars isolated from recycled and untreated surface waters and whole genome sequenced through the FDA GenomeTrakr Network.

“Who” else is there and what are they doing?

- Important to culture fecal indicator bacteria (e.g. *E. coli)* and pathogens from irrigation water sources
- Bacterial, culture-based work provides only one part of the overall picture of microbial water quality
- DNA-based approaches can improve our understanding of total microbial communities (taxonomy and function) present in water



Bacterial Diversity and Antibiotic Resistance Genes In Recycled and Untreated Surface Waters



JESSICA CHOPYK

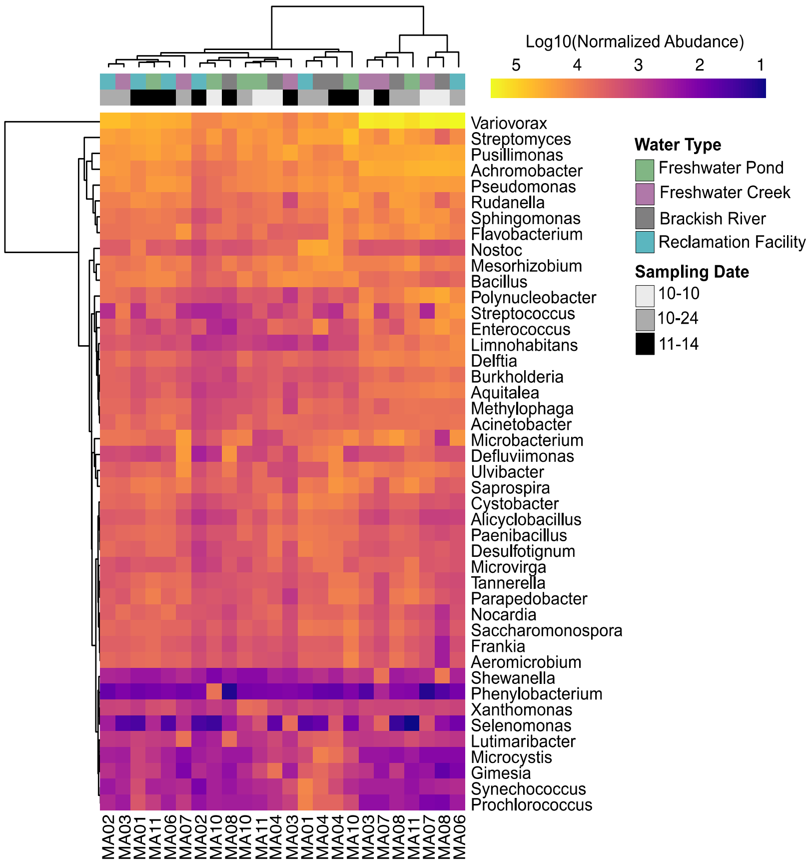


Figure 2: Taxonomic heatmap of the bacterial communities present in recycled and untreated surface water sites by sampling date. Heatmap based on the log-transformed normalized abundance of the most dominant genera (>1% in at least one sample).

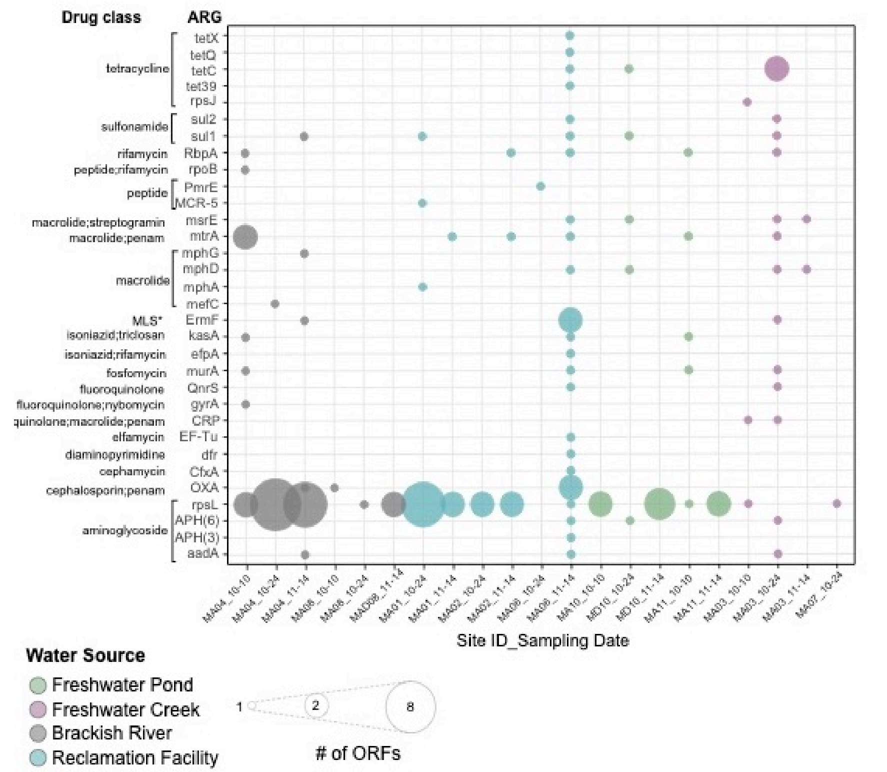


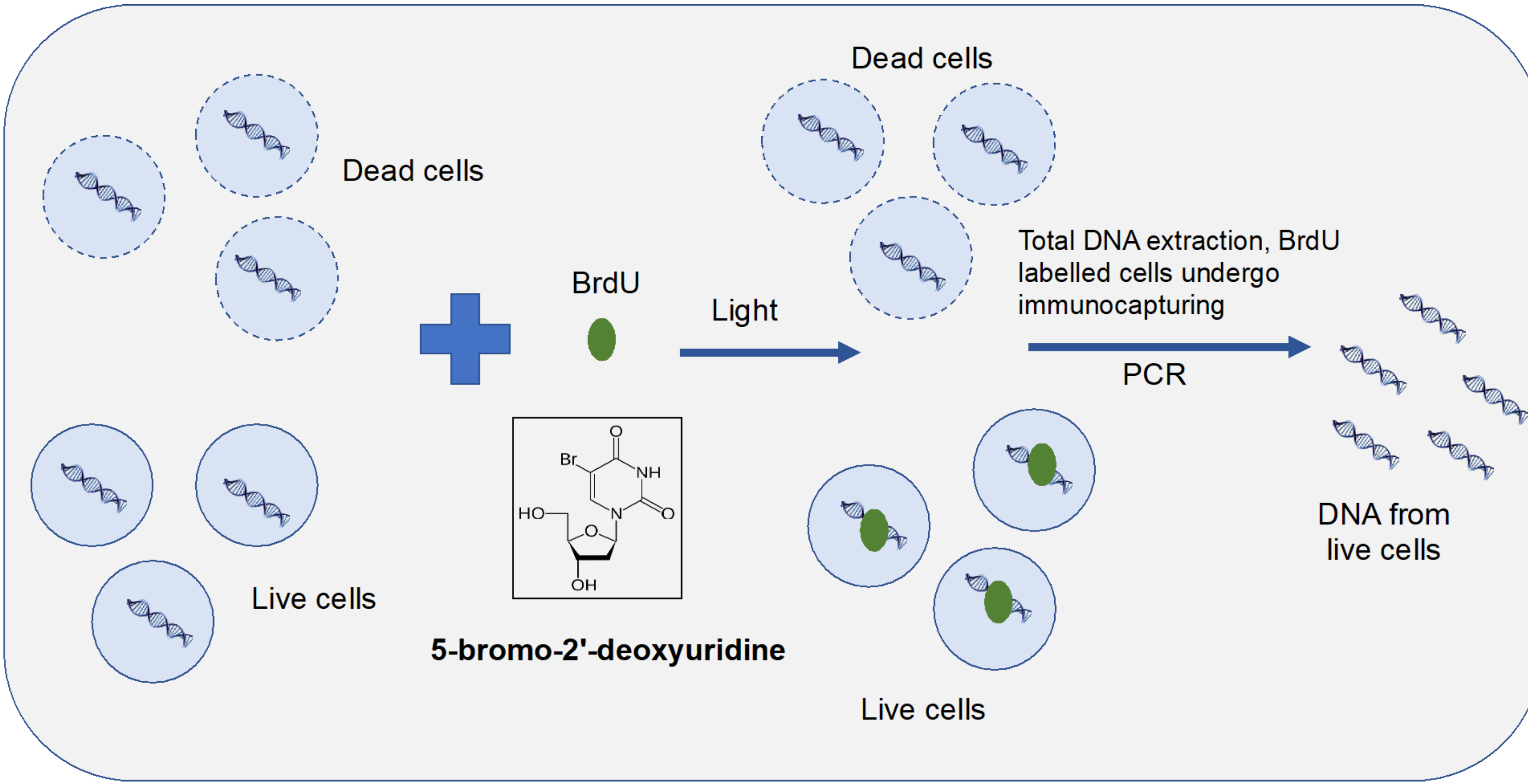
Figure 4: Antibiotic resistance genes (ARGs) predicted in recycled and untreated surface water sites by sampling date. Dotplot showing the ARG-like ORFs present at each water site, with the size of each dot equivalent to the number of translated ORFs with homology to each ARG listed on the y-axis, and the color representative of the water type.

Chopyk et al. 2020. *Water Research*. 169, 115250.

Are they alive (metabolically-active)?

LEENA MALAYIL

Coupling DNA-Labeling and Sequencing Approaches



Malayil et al. 2019. [In preparation].

Identifying Metabolically-active Bacterial Communities in Irrigation Water

Leena Malayil

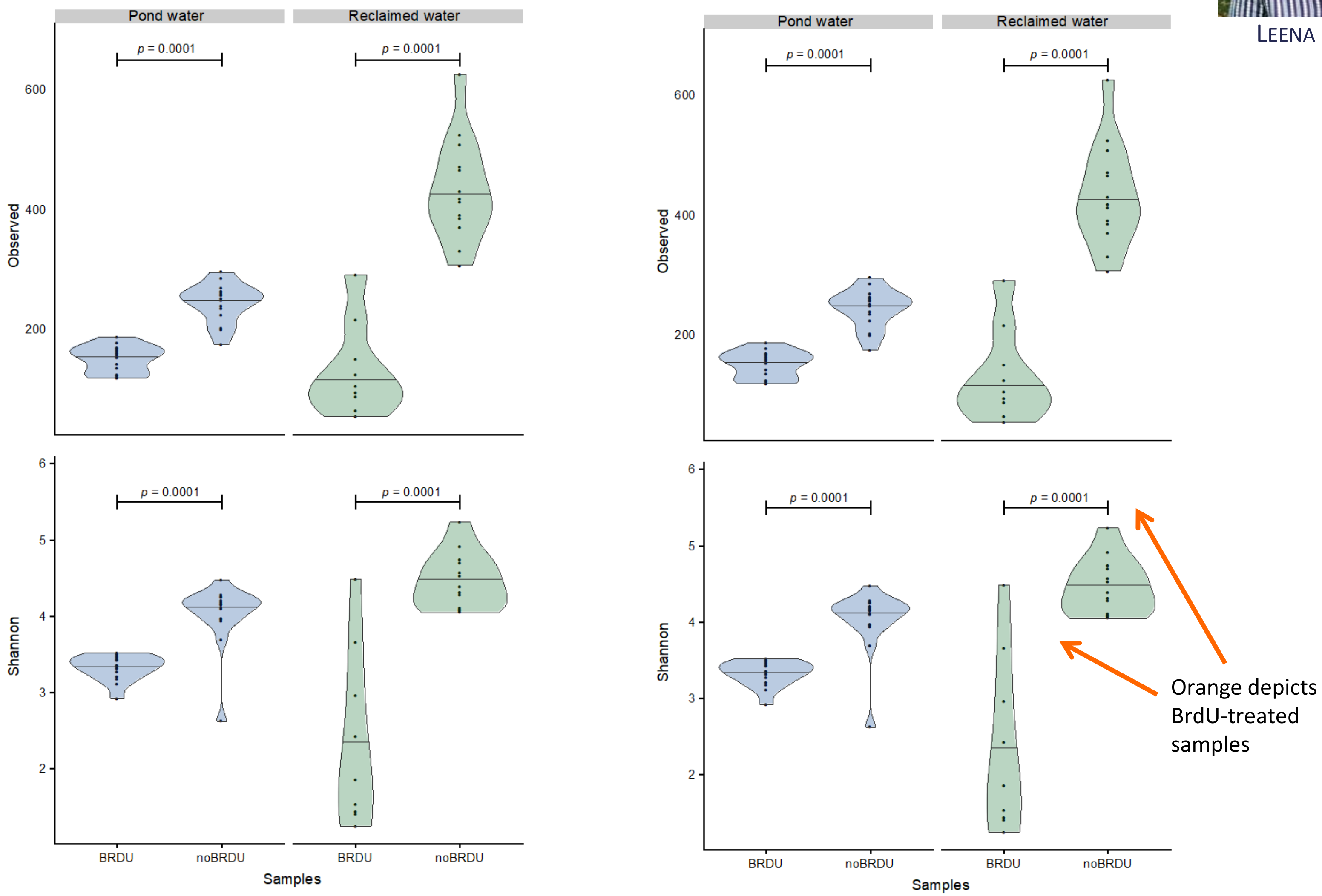


Figure 1: Alpha and beta diversity (rarefied) among BrdU- and non-BrdU-treated reclaimed water (MD06) and pond water (MD10).

Malayil et al. 2019. [In preparation].

Shared and Unique Bacterial Profiles in BrdU and Non-BrdU Treated Recycled Water Samples

LEENA MALAYIL

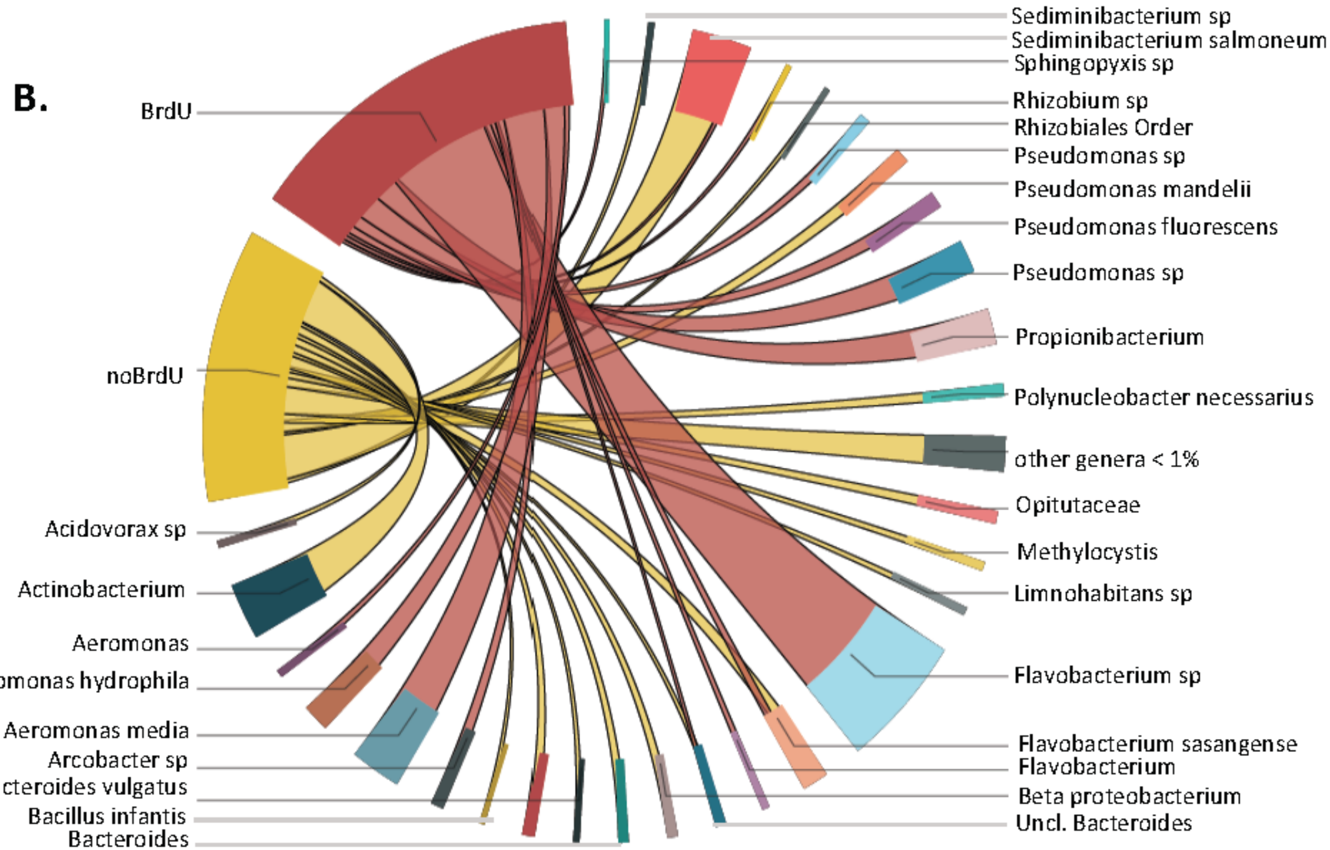


Figure 4, B: Shared and unique bacterial profiles visualized by chord plots between BrdU and non-BrdU-treated reclaimed water samples.

Malayil et al. [In preparation].

Are antibiotic resistance genes present in the metabolically-active fraction of bacterial communities?

Leena Malayil

Reclaimed Water

Figure 5: Relative abundance of antibiotic resistance genes in reclaimed water (A) samples by BrdU-treatment and by sampling month.

Malayil et al. 2019.

TAKE HOME MESSAGES

- Agricultural water reuse is an important part of future water and food security solutions
- Microbiological contaminants can persist in recycled water and untreated surface water
- Larger volume sampling (≥ 10L) is necessary for improved pathogen detection
- Coupled DNA-labeling and sequencing approaches can help improve understanding of the microbiological risks associated with agricultural water reuse

The CONSERVE Team

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