Differential Growth Dynamics Among *Salmonella* Serovars in Surface and Reclaimed Waters Affect Transfer Potential onto Tomatoes

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INTRODUCTION

Salmonella enterica has historically been a food safety concern for agriculture on the Eastern Shore of Maryland [1,2], with surface water and sediments identified as a possible reservoir for multiple strains including *S.* Newport and *S.* Typhimurium [3,4]. For successful plant colonization from water reservoirs, *Salmonella* must survive in water, attach to plant tissue, mitigate stressors on the plant surface, and compete with resident microbiota. It is unclear how habitat history affects the probability of contamination and persistence on fresh produce, or if this ability is serovar specific. **Using multiple *S. enterica* strains of various serovars, this study evaluated 1) *S. enterica* persistence in Maryland surface water microcosms, 2) attachment potential to an abiotic surface, and 3) differential transferability onto tomato fruit in relation to habitat history.** Evaluating these parameters can better estimate the true agricultural risk of *Salmonella* presence in Maryland surface waters.

METHODS

Table 1: Maryland surface water types used in this study.

Water Code	Water Type
MD03	Non-Tidal Fresh
MD04	Tidal Brackish
MD05	Non-Tidal Fresh
MD06	Reclaimed
MD10	Pond

Table 2: Strains used in this study.

Salmonella Strain	Source	Antimicrobial Resistance
Javiana (Jav)	Pond water	Rifampicin (lab-adapted)
Heidelberg (Hed)	Poultry house	Rifampicin (lab-adapted)
4, [5], 12:i:–	Maryland river water	no
Newport (New-)	Maryland river water	no
Newport (New+)	Maryland river water	Multidrug resistant
Typhimurium (Typ-)	Maryland river water	no
Typhimurium (Typ+)	Maryland river water	Multidrug resistant

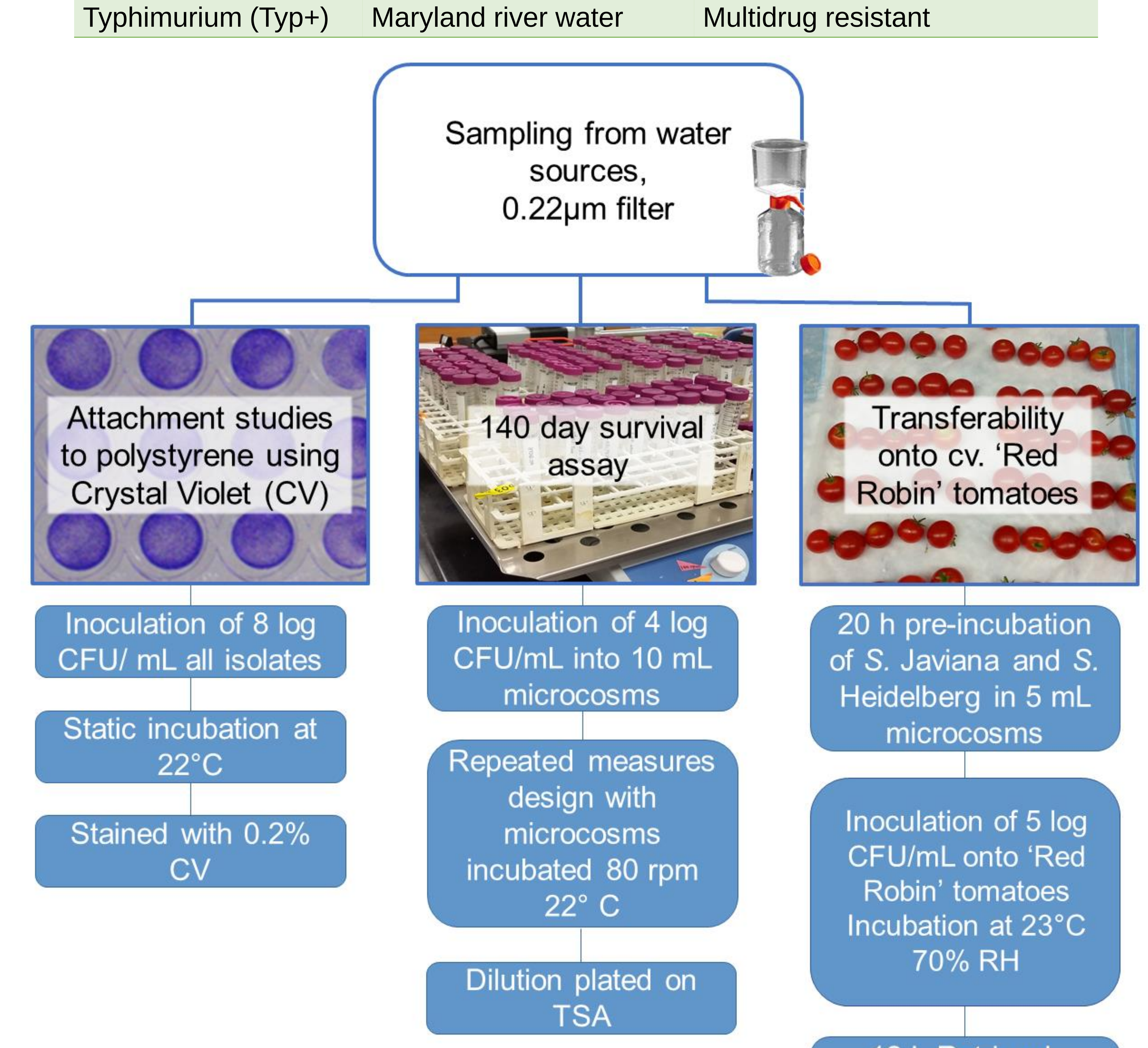

Figure 1: Experimentation workflow. Water for experimentation was collected October 30, 2017.

RESULTS

Persistence in water is significantly influenced by water type, with reclaimed water showing lowest decay rate among all serovars tested.

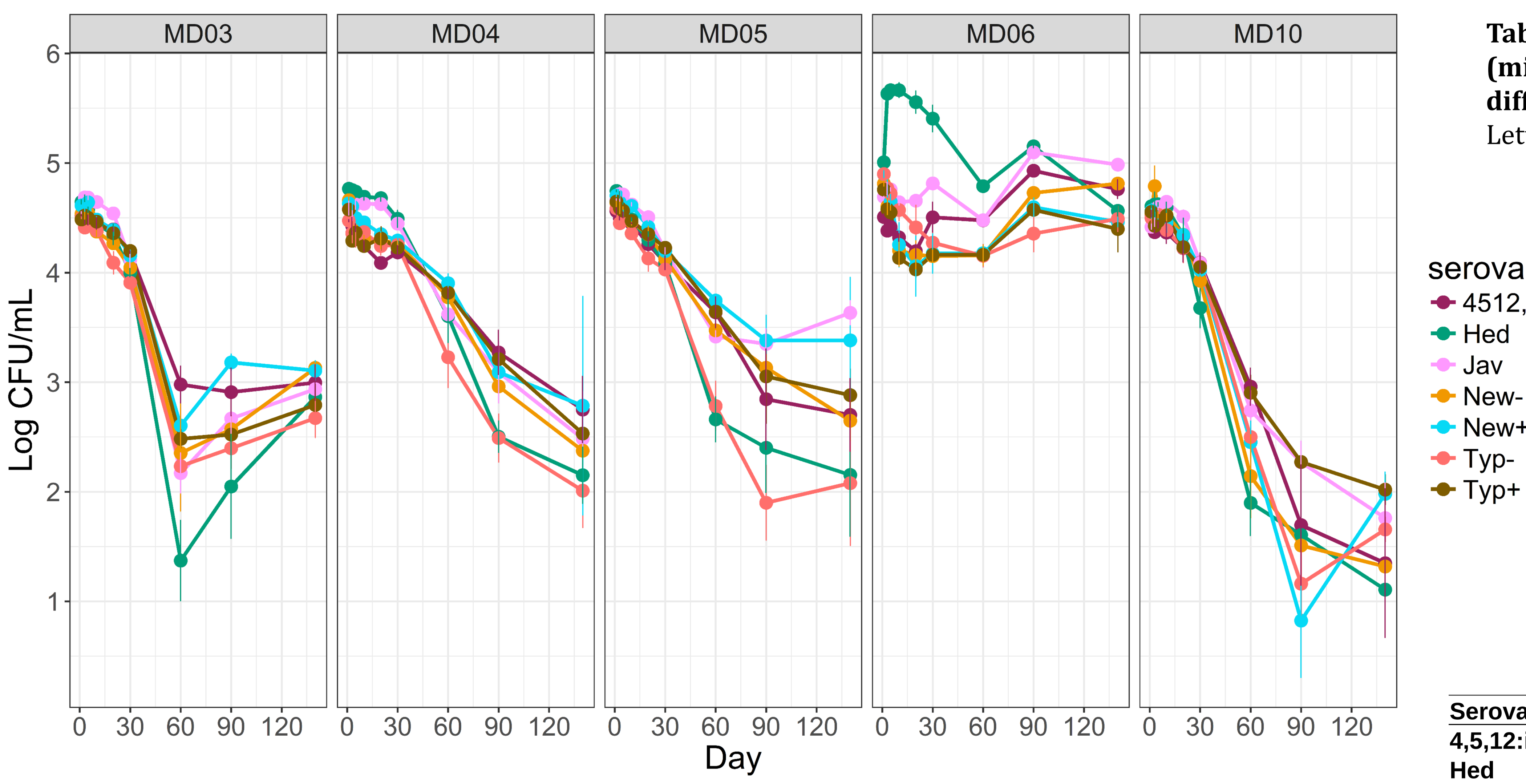

Figure 2: Decay curves over 140 day (d) sampling period of 7 *S. enterica* strains in 0.22µm filtered microcosms. Microcosms were aseptically sampled on D=1,3,5,10,20,30,60,90,140. N=3, error bars represent the standard error of the mean (SEM). While water type is clearly a driver of bacterial persistence, serovar specific dynamics are evident within water types (see table 3, bottom).

Table 3: Decay curve parameters of main effects water type (top) and serovar (middle), as well as decay rate of water x serovar illustrating serovar specific differences (bottom). Curves were fit to Baranyi model using DMFit 3.5 from 1-90 days. Letters denote significant differences within parameter via Tukey HSD (α=0.05)

Water type	Decay rate log CFU/day	Lag	rate*lag	R^2	SE(fit)
MD03	-0.240 ± 0.111 [b]	25.571 ± 1.024 [b]	-6.614	0.935	0.227
MD04	-0.023 ± 0.003 [a]	26.680 ± 2.525 [b]	-0.714	0.897	0.173
MD05	-0.023 ± 0.003 [a]	13.900 [b]	-0.44	0.902	0.202
MD06	-0.015 ±0.017 [a]	69.106 ± 12.478 [a]	2.727	0.507	0.216
MD10	-0.053 ± 0.004 [ab]	20.355 ± 1.137 [b]	-1.061	0.944	0.258

Serovar	Decay rate log CFU/day	Lag	rate * lag	R^2	SE(fit)
4,5,12:i-	-0.021 ± 0.012	35.279 ± 10.679	-0.425	0.825	0.211
Hed	-0.192 ± 0.158	23.863 ± 3.986	-8.514	0.793	0.284
Jav	-0.030 ± 0.026	32.265 ± 12.454	-0.001	0.867	0.161
New-	-0.042 ± 0.018	26.339 ± 3.844	-1.577	0.926	0.235
New+	-0.125 ± 0.085	25.290 ± 3.011	-7.160	0.828	0.218
Typ-	-0.040 ± 0.008	21.052 ± 0.764	-1.011	0.860	0.232
Typ+	-0.044 ±0.012	25.161 ± 3.869	-1.128	0.844	0.165

Serovar	MD03	MD04	MD05	MD06	MD10
4,5,12:i-	-0.053 ± 0.001	-0.012 ± 0.002	-0.021 ± 0.003 [ab]	0.009 ± 0.004 [a]	-0.045 ± 0.011 [ab]
Hed	-0.084 ± 0.044	-0.042 ± 0.005	-0.040 ± 0.007 [b]	-0.006 ± 3.0E-4 [ab]	-0.082 ± 0.016 [b]
Jav	-0.123 ± 0.073	-0.033 ± 0.010	-0.028 ± 0.004 [ab]	0.019 ± 0.017 [a]	-0.044 ± 0.012 [ab]
New-	-0.072 ± 0.032	-0.016 ± 0.001	-0.018 ± 0.001 [ab]	-4.0E-4 ± 8.5E-4 [a]	-0.050 ± 0.005 [ab]
New+	-0.033 ±0.014	-0.016 ± 0.002	-0.017 ± 0.001 [a]	-0.074 ± 0.021 [b]	-0.075 ± 0.004 [ab]
Typ-	-0.053 ± 0.010	-0.037 ± 0.013	-0.036 ± 0.008 [ab]	-0.024 ± 0.018 [ab]	-0.062 ± 0.004 [ab]
Typ+	-0.293 ± 0.132	-0.013 ±0.001	-0.022 ± 0.004 [ab]	-0.043 ± 0.021 [ab]	-0.036 ± 0.006 [a]

Attachment to polystyrene is significantly affected by water type and serovar with *S.* Javiana, Heidelberg significantly lower in attachment capacity over three experimental replications.

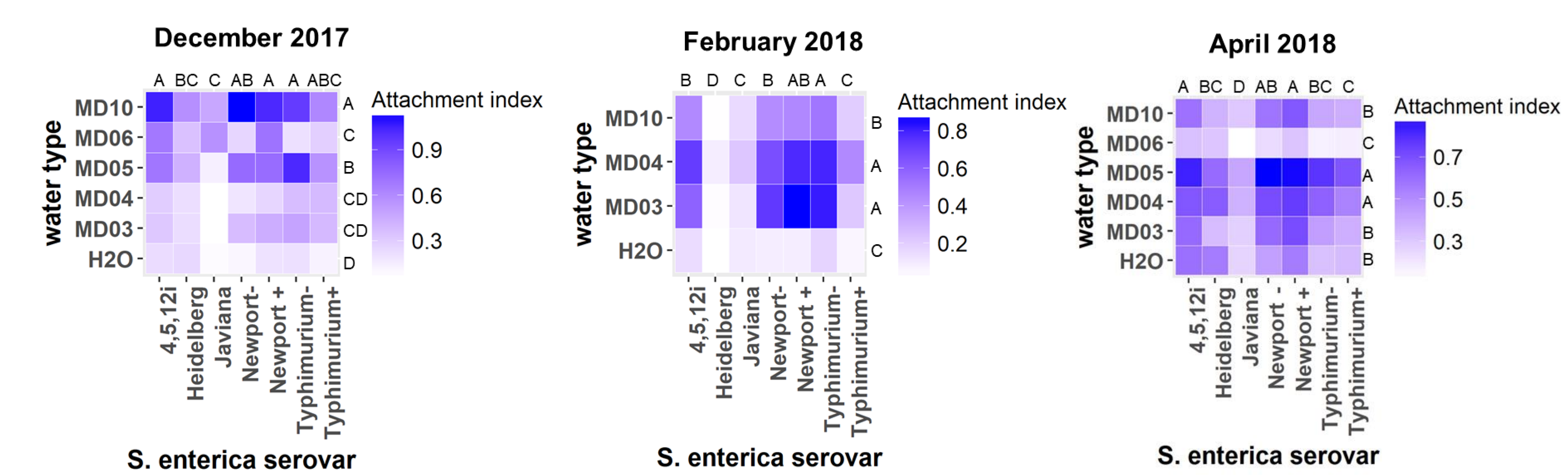

Figure 3: Serovar specific attachment to 96 well plate during static incubation in various water types. N=6 for each experiment. Serovar x water type interaction was significant in December, February experiment but not in April 2018 (*p*=0.0004, *p*=0.0001, *p*=0.5039) via ANOVA(α=0.05). Letters denote significant influence in attachment capacity, either among serovars (across top) or water types (along right side) via Tukey HSD (α=0.05).

S. Javiana transfers to tomatoes at a significantly higher rate than *S.* Heidelberg in all water types tested.

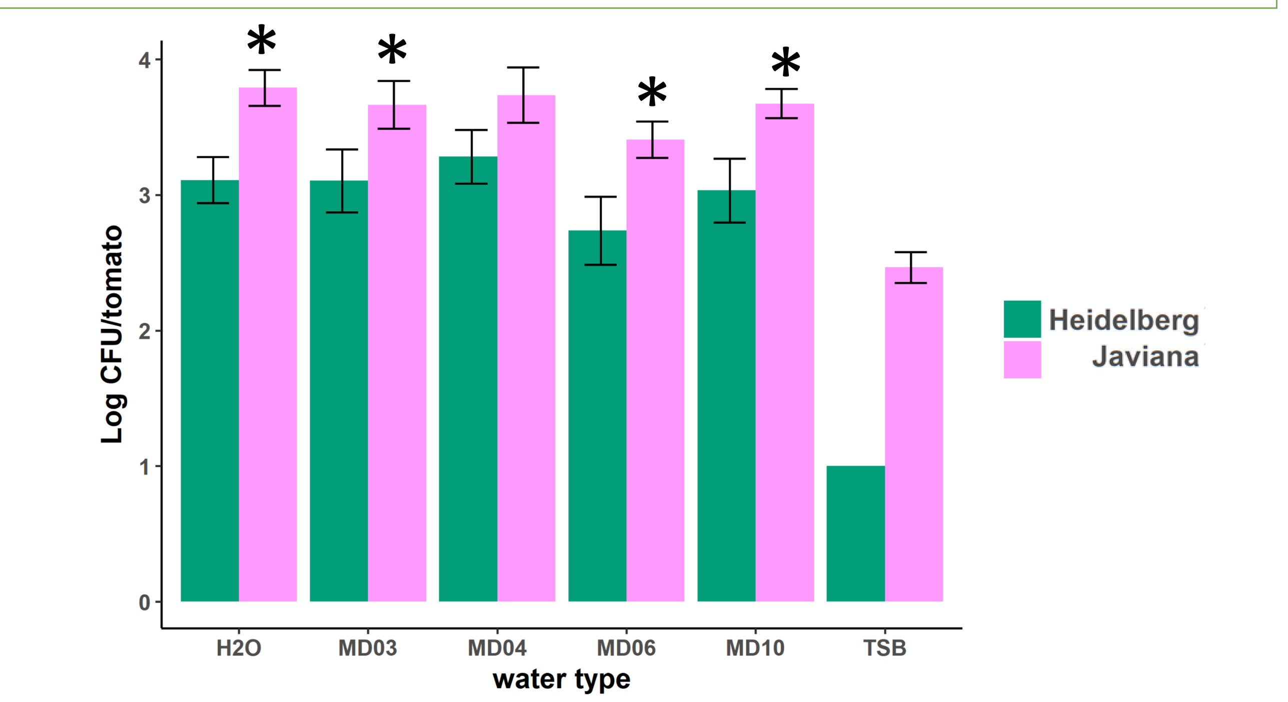

Figure 4: Tomato transferability of *S.* Javiana and *S.* Heidelberg from 24 h water microcosms to cv. "Red Robin" tomatoes. N=5 tomatoes for each treatment combination, experimental design = CRD, error bars = SEM. Asterisks denote *S.* Javiana significantly higher transfer than *S.* Heidelberg via *a priori* contrasts (α=0.05).

CONCLUSIONS

- **S. enterica *decay over time*** was significantly impacted by water type when fit to Baranyi primary decay model (*p*=0.0171). Furthermore, in non-tidal fresh, reclaimed, and pond water (MD05, MD06, MD10) serovar specific decay rates were observed with *S.* Heidelberg exhibiting significantly higher rates of decay than other serovars in MD05 and MD10.
- ***In polystyrene attachment experiments,*** water type and serovar were significant driving factors of attachment capacity (*p*=0.0001*)*. Reclaimed water (MD06) harbored significantly lower attachment than pond and non-tidal freshwater (MD10 and MD05) in December and April. *S.* Newport MDR negative and *S.* 4,5,12:i- attached significantly higher than *S.* Javiana and *S.* Heidelberg in all experimental replicates. No reproducible MDR effect on attachment ability was observed.
- **In S. enterica *tomato transfer,*** Water type did not significantly influence transfer capacity (*p*=0.1859), while serovar was a significant driving factor (*p*=0.0001) in all water types tested except for tidal brackish water (MD04).
- ***Future work includes*** repeating decay experiment with summer water samplings, including culture independent methods to assess viability, and performing transferability experiments with all serovars.
- ***Significance:*** Better understanding risk of *Salmonella* in water sources will lead to more economical and sustainable irrigation management without compromising food safety.

REFERENCES

1. Bennett SD et. al. Epidemiology & Infection. 2015;143(07):1352-9.
2. Greene SK et. al. Epidemiology and Infection. 2008;136(2):157-65. doi: 10.1017/s095026880700859x.
3. Bell RL et. al. Frontiers in Microbiology. 2015;6:415. doi: 10.3389/fmicb.2015.00415.
4. Micallef SA et. al.Environmental Research, Volume 114, 2012,Pages 31-39, ISSN 001- 9351 ,https://doi.org/10.1016/j.envres.2012.02.005.

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