



**CPS 2013 RFP
FINAL PROJECT REPORT**

Project Title

Evaluation of pathogen survival in fresh water sediments and potential impact on irrigation water quality sampling programs

Project Period

January 1, 2014 – December 31, 2014

Principal Investigator

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Objectives

Objective 1: Determine the distribution and survival of FIB (E. coli and enterococci), pathogens, and pathogen surrogates in sediments of aquatic mesocosms exposed to seasonal variations.

Objective 2: Investigate the resuspension and subsequent partitioning of FIB and pathogen surrogates from surface sediments using an indoor fluvium to simulate high flow events in surface water sources.

Funding for this project provided by the Center for Produce Safety through:

CPS Campaign for Research

FINAL REPORT

Abstract

Potential contamination of fresh produce with human pathogens via irrigation water has long been recognized. One area of uncertainty involves the transport of pathogens in fresh water sediments to the water column. This study aims to evaluate the relationship between pathogens and fecal indicator bacteria (FIB) in fresh water sources over time and the role sediments have in harboring and distributing pathogens in water resources. To complete this objective, aquatic mesocosms were used to evaluate the survival and behavior of 14 different pathogens including *Listeria monocytogenes*, *Salmonella*, and Shiga toxin-producing *Escherichia coli*, 5 surrogates—*L. innocua*, *L. seeligeri*, PRD1, murine norovirus, and *Salmonella* Typhimurium—and 2 FIB including *Enterococcus faecalis* and *E. coli*. Mesocosms were designed to simulate the natural environment and diurnal variations including UV radiation and ambient temperature. In addition, 2 different fresh water systems – one lake and one river – were used as sources of water and sediments for constructing the mesocosms (7 L of water with 3-5 cm of sediment per mesocosm). To evaluate the attenuation and release of FIB and surrogate microorganisms in fluvial systems, an indoor fluvium was used at both base (1 L/s) and high (2 L/s) flow rates. Microorganisms (MO) were inoculated at 10^5 per ml for each mesocosm and flume set up. For the mesocosms, water and sediment samples were collected for up to 28 days to determine pathogen, surrogate, and FIB concentrations in the water column and sediments. For the indoor flumes, water samples were collected over the first 24 h of the input phase (recirculation of MO inoculated water) followed by collection of sediment samples after 24 h to determine MO attenuation. To determine release or resuspension of MO from sediment to the water column, the flume sediments were subjected to a flushing phase (recirculation of water without MO) and water samples were collected over a 4 h period. Spring, summer, fall, and winter mesocosm experiments have been completed, and base flow and high flow fluvium trials have been performed. Results from the mesocosm experiments suggest that all microorganisms steadily declined in the water column regardless of the season; however, the winter mesocosm resulted in a less rapid decline—most MO detectable out to 14 days—overall when compared to other seasons when the majority of MO declined by day 7 or less. Attenuation and persistence of MO in the sediments tended to be greater in the cooler seasons—fall and winter. Finally, *Salmonella enterica* serovars were more persistent in both water and sediment samples when compared to other MO, and generic *E. coli* often followed a similar pattern as *Salmonella* whereas enterococci tended to trend with the *Listeria monocytogenes* strains. This may indicate the need for two FIB during microbial water quality analysis though generic *E. coli* would clearly be the more conservative choice. For the fluvium experiments, MO were detected in water at consistent levels for the first 4 h of the input phase with a 1 to 3- \log_{10} decrease after 24 h depending on the microorganism. Overall, *E. coli* persisted at greater concentrations in water for both the base and high flow input phase of the fluvium experiments. Analysis of MO in sediment at 24 h showed a significant difference ($p = 0.0117$) between base and high flow attenuation of MO. During the flushing phase, more MO were resuspended in the water during base flow as opposed to high flow after 4 h. Moreover, *E. coli* was resuspended into the water column at higher concentrations followed by *Salmonella* Typhimurium (ATCC 53647) and PRD1 bacteriophage. Upon completion of analysis, these data will inform the design of risk-based sampling programs and the formation of pathogen fate and transport models that better predict potential health risk related to irrigation water quality and fresh produce.

Background

For the last 4 decades, fecal indicator bacteria (FIB) densities have been relied upon to determine recreational water quality. Both generic *Escherichia coli* and enterococci have been reported as the best indicators of potential health risks from recreational water contact (USEPA, 2012). Based on the FIB density exceedance criteria established by the USEPA for recreational water, in 2010 the lettuce and leafy green sector of the fresh produce industry (i.e. Leafy Green Marketing Agreement [LGMA]) adopted similar microbial limits for irrigation water quality standards (LGMA, 2010). Currently there are no national irrigation water quality standards; however, the Food Safety Modernization Act (FSMA) Proposed Rule for Produce would require the same standards as adopted by the LGMA (Table 1).

As outlined in numerous reviews, the potential contamination of fresh produce with pathogens of human health concern via irrigation water has long been recognized (Gerba and Choi, 2006; Hanning et al., 2009; Pachepsky et al., 2011; Steele et al., 2004). The variety of irrigation water sources—groundwater wells, ponds, rivers, streams, municipal water, and reclaimed (treated wastewater) water—combined with the diversity of potential waste stream inputs have resulted in a complex issue when trying to understand and address the fate and transport of both FIB and pathogens in agricultural water resources. Even in light of this complexity, the selection of a single indicator organism—in this instance, *E. coli*—for all potential pathogens present in irrigation water has been the status quo. The basic criteria for selection of indicator organisms are that they must be 1) rapidly measurable, 2) representative of the pathogens of concern, and 3) display similar fate and transport characteristics (Brookes et al. 2005). Previous research has demonstrated that generic *E. coli* only consistently meets one of these criteria—rapid measurement—arguably an important criteria, but in the end does this really meet the intended purpose of protecting public health?

Another FIB, enterococci, has been recommended throughout the years as well and is preferentially used/recommended for saltwater and marine beaches. In addition, recent research suggests that enterococci may better represent the complexities of microbial survival within both the water column and sediment (Wanjugi and Harwood, 2013)—important when considering the impacts of microbial redistribution during turbulent flow (e.g., heavy rain events) or mechanical disruptions within aquatic systems (Suslow, 2010). Even though selection of enterococci as the FIB of choice would be just another version of the status quo, enterococci may be a better, more conservative FIB than *E. coli*.

Though research on indicator organisms for pathogens in water has been ongoing for decades, there are still some critical knowledge gaps that have not been addressed. First, the use of bacterial indicators as predictors of health risk is based on the presumption that the indicator bacteria have survival properties similar to the pathogens that cause disease. However, to date, the majority of studies have focused on the indicator organisms, with less quantification of actual human pathogens (Hipsey et al., 2008; Irvine and Pettibone, 1993). This is primarily due to the need to analyze large sample volumes because of low pathogen concentrations and difficulties in detection methodology. Some studies have reported simplified survival experiments comparing generic *E. coli* or enterococci to one other pathogen such as *E. coli* O157:H7 (Jenkins et al., 2011) or just looked at pathogen survival without including FIB (Avery et al., 2008). However, this simplification does not take into account the differences between multiple strains or even wild-type strains of pathogens and their correlation with FIB.

Second, although it has been known for some time that significant populations of FIB are harbored in fresh water bottom sediments, very little is known about pathogen persistence in

these sediments as few studies have addressed this (Pachepsky and Shelton, 2011). A related knowledge gap is that it is also not well known how much the increased concentrations of FIB during precipitation events is due to runoff vs. resuspension and whether these increases in FIB correlate with increases in pathogen loads (Pachepsky, 2006). A recent study by Yakievich et al. (2013) reported the prolonged release of *E. coli* after artificial high-water flow events even when water levels returned to base flow indicating continued detachment from sediments. Unfortunately, this study did not measure levels of pathogens or other FIB such as enterococci. For some perspective on the potential contributions of the sediments to microbial load, a review by Pachepsky and Shelton (2011) described sediment densities of *E. coli* ranging from 1 to 500,000 CFU or MPN per gram of dry weight sediment from an analysis of over 20 published studies. In the end, microbial settling and resuspension are really the essential processes controlling pathogen and indicator bacteria in fresh water including water sources used for irrigation purposes.

Overall, studies investigating the relationship between pathogens and FIB are limited for both fresh water and their sediments. Therefore, the purpose of this proof of concept study is to provide supporting evidence 1) for the relationship between pathogens and FIB in fresh water sediments and 2) for enterococci as a valid and perhaps more conservative predictor of health risk related to the presence of human pathogens in irrigation water sources. As stated by Droppo et al. (2008), "*the lack of understanding around pathogen/sediment associations may lead to an inaccurate estimate of public health risk, and, as such, possible modification of sampling strategies to reflect this association may be warranted.*" Through completion of this study, these data could better inform the implementation of risk-based sampling programs and the formation of pathogen fate and transport models that better predict potential health risk within the context of irrigation water quality.

Based on this, we hypothesize that fresh water sediments act as significant reservoirs for pathogens in irrigation water sources and that selection of indicator organisms should be weighted for settling, attachment, survival, and resuspension properties in sediments as opposed to solely properties in the water column. In addition, pathogens are hypothesized to behave more similarly to the FIB enterococci as opposed to generic *E. coli* under certain conditions. This is based on 1) persistence in sediments, 2) more resistant to predation, and 3) stronger correlation with human health risks and outcomes. Upon completion, we may determine that selection of different indicator organisms may be appropriate for different watershed and seasonal variations.

Research Methods

Preparation of microorganisms.

In this study, FIB, bacterial surrogates and pathogens as well as viral surrogates were used. Table 1 lists all microorganisms used and justification for selection. Prior to each set of mesocosm or fluvium experiments, bacteria inocula were prepared using the corresponding culture medium (Table 2). Briefly, cultures of each bacteria were prepared at 37°C overnight with shaking. The cells were harvested by centrifugation at 4,000 × g for 5 min at 4°C and resuspended in 5 mL of 0.1% buffered peptone water (BPW). The centrifugation and washing steps were repeated three times. The final concentration of the cell suspension were determined by spread plate method on the respective agar medium and corresponding incubation temperature (Table 2).

Murine norovirus (Type 1) was prepared and assayed as described previously (Gibson and Schwab, 2011c). Briefly, MNV stocks will be propagated in RAW 264.7 cells and stock titers will be determined by plaque assay in 6-well plates. PRD1 bacteriophage was prepared and assayed as described previously (Gibson and Schwab, 2011b). Briefly, PRD1 stocks were propagated in *S. Typhimurium* LT2 (ATCC 19585) and stock titers were determined by double agar overlay assay.

Collection and preparation of water and sediments.

Approximately one to two weeks prior to mesocosm experiment set-up, 60 L water samples were collected from the White River and Beaver Lake in Northwest Arkansas. Sediment samples were collected from Wedington Lake and the Illinois River up to one month prior to experiment set-up. The water was filter sterilized using an 80 kDa molecular weight cut off ultrafilter, and the sediments were sterilized by autoclaving twice and then thoroughly dried on a tarp in the greenhouse. To verify sterilization procedures, samples were analyzed for the presence of *E. coli*.

Assembly of fresh water mesocosms.

Each mesocosm was assembled in 20 L cylindrical, polypropylene tanks as follows. Initially, 3 cm of prepared sediment were added to each tank (i.e. 6 tanks each for river and lake mesocosm set-ups) followed by 7 L of ultrafiltered lake or river water. The mesocosms were left to equilibrate for 24 hours to allow the sediments to settle out of the water column. Prior to inoculation with microorganisms, a Shiruba Aqua Power aquarium head pump (max. capacity of 40 gal/h) was placed in each mesocosm to allow for aeration. For river water mesocosms, pumps were placed on the lowest setting and operated continuously. However, for lake water, pumps were placed on a timer and operated 1 min for every hour to simulate the longer residence time observed in lakes, ponds, and reservoirs. In addition, HOBO Dataloggers were placed in each mesocosm to record water temperature and light intensity. To moderate temperature, the mesocosms were placed in large containers filled with water. Figure 1 shows the basic mesocosm set-up. Following mesocosm set-up, each mesocosm was inoculated with microorganisms as follows with all mesocosms containing enterococci and generic *E. coli* except M7 (mesocosm = M, set = #):

M1: *S. Javiana*, *L. innocua*, *L. monocytogenes*, MNV, PRD1

M2: *S. Montevideo*, *L. seeligeri*, *L. monocytogenes*

M3: *E. coli* O111, *S. Newport*

M4: *E. coli* O145, *S. Thompson*

M5: *E. coli* O157, *S. Typhimurium*

M6: *E. coli* O26, *S. Typhimurium* (ATCC 53647)

MC: CONTROL with water and sediment ONLY

Mesocosm – sample collection and analysis.

Microorganisms were inoculated at 10^8 CFU or PFU per 100 ml or 10^6 PFU per 100 ml (MNV only). Mesocosms were gently stirred after addition of microorganisms and allowed to settle and equilibrate for 30 min. After the 30 min period, a sample will be collected for Day 0. The experiments were completed over a 3 to 4-week period during each calendar season (i.e. Spring, Summer, Fall, Winter). During the 4-week period, samples were collected on a daily basis at the same time each day for the first 7 days then on a weekly basis for the remaining 2 to 3-week period. Water samples (50 ml) were collected first followed by collection of the

sediment samples using a sterile, PVC pipe constructed with a perforated elbow piece to allow water to drain from the sediment (Figure 2). This was done to avoid resuspending the sediment and falsely elevating the concentration of microbes in the water column.

For detection of bacterial pathogens, surrogates, and FIB in water, samples were serially diluted in BPW and select dilutions (50 to 1000 μ l) were plated on the corresponding agar for each pathogen and incubated at the appropriate temperature and duration (Table 2). PRD1 bacteriophage were detected in water and sediments by first serially diluting the samples in BPW as described for bacteria.

For detection of bacterial pathogens, surrogates, and FIB in sediments, 5 g (wet weight) of sediment samples were added to 30 ml of BPW. The samples were then mixed by hand for 2 min over a 10 cm arc to separate microorganisms from sediment. The sample was then allowed to settle for 30 s and the eluent was decanted into a sterile container. An additional 20 ml of BPW was added to the same sediment sample and gently swirled for 10 s, settled for 30 s, and decanted into the same container with the first rinse. Samples were then serially diluted in BPW and analyzed as described for water.

Flume – assembly, sample collection and analysis.

Based on McDowell and Sharpley (2003), a fluvium was utilized to simulate a flowing fluvial system to study the behavior of microbial surrogates in base and high flow conditions. Each flume measures 10-m-long by 20-cm-wide by 20-cm-deep with a variable slope angle of 0 to 15%. A 300-L capacity reservoir is associated with each flume (Figure 3). The fluvium is designed with plumbing and a pump associated with each flume to allow water to be recirculated over the sediment from the “upstream” end with flow rates varying from 1 to 20 L per second. Similar to McDowell and Sharpley (2003), experiments were performed in two phases (i.e. input phase and flushing phase) as well as at two flow rates—base flow (1 L/s) and high flow (2 L/s). All experiments were performed using sterilized river sediment, dechlorinated tap water (DTW), and the following FIB and surrogates: *E. coli* (ATCC 35218), *E. faecalis* (ATCC 29212), *L. innocua*, *L. seeligeri*, *Salmonella* Typhimurium (ATCC 53647), and PRD1 bacteriophage. Samples were analyzed as described for the freshwater mesocosms. Each flow rate experiment was performed twice in duplicate.

For the input phase (i.e., surrogates inoculated in DTW were introduced to flow over the sediment), sediment was placed in two flumes of the fluvium to a depth of approximately 3 cm, and the flumes were set at approximately 1 to 2% grade to achieve the appropriate flow rate. Each reservoir was filled with 180 L of DTW and pumped over the sediment at 1 to 2 L/s for 30 min to allow for equilibration. Following the initial equilibration, surrogate microorganisms and FIB were introduced into the flume reservoirs. Microorganisms were seeded at 10^8 CFU or PFU per 100 ml. Immediately after the introduction of microorganisms, a sample was taken from each flume and additional samples taken after 30 min and then every hour for 4 h and then a final sample after 24 h. At the culmination of the input phase, a composite sample was taken of the sediment at the top, middle, and end of each flume to determine the concentration of microbes attenuated in the sediments.

During the second phase, or flushing phase, the reservoirs were drained and cleaned with 70% ethanol followed by flushing with fresh tap water. Flow was initiated again at 1 or 2 L/s, and water samples were collected at the 10 and 30 min time points and then every hour after the start of flow for 4 h. The flushing phase allowed for quantification of microbial resuspension from the sediment to the water column over time.

Results

Statistical analyses of the data are ongoing as the last samples from the winter mesocosm experiment were collected on December 22, 2014. In addition, due to the number of variables to consider along with the 14 microorganisms analyzed, I plan to consult with a statistician about the most appropriate statistical tests to apply to these data. However, trends in the data by sample type (sediment versus water), microorganism type (FIB, pathogen, or surrogate), and season have been qualitatively analyzed. Figure 4 shows the level of each microorganism over time (1 to 28 days) by season and sample type. Surrogates and FIB have been indicated with dotted lines and corresponding color palettes for each group of microorganisms. For instance, *Listeria* spp. are indicated with a maroon to pink color palette while *Salmonellae* and *E. coli* are indicated with green and blue color palettes, respectively. Because of the amount of data and in an effort to limit the number of figures, it was decided that this was the best way to show trends and differences in microorganisms.

Overall, based on Figure 4, there are several observations that can be made. First, all microorganisms steadily declined in the water column regardless of the season; however, it is important to note that the winter mesocosm resulted in a less rapid decline overall when compared to other seasons, especially the spring. This may be related to the difference in temperature and diurnal variations though data obtained from the HOBO dataloggers are still being compiled and integrated. Second, attenuation and persistence of microorganisms in the sediments tended to be greater in the cooler mesocosm experiments conducted in the fall and winter. Finally, *Salmonella enterica* serovars tended to be more persistent in both water and sediment samples as indicated by the green trend lines on Figure 4. Moreover, generic *E. coli* often followed a similar pattern as *Salmonella* whereas enterococci tended to trend with the *Listeria monocytogenes* strains. This may indicate the need for two FIB during microbial water quality analysis though generic *E. coli* would clearly be the more conservative choice. Figures 5 and 6 have been provided to demonstrate the persistence of microorganisms in water and sediment, respectively, by type of mesocosm—lake or river. Again, application of statistical tests will allow for further validation of these observations.

Results from fluvium experiments are shown in Figures 7-9. Figure 7 shows the persistence of FIB and surrogate organisms in either base or high flow water over a 24 h period. In general, microorganisms were detected in water at consistent levels for the first 4 hours with a 1 to 3- \log_{10} decrease after 24 h depending on the microorganism. Overall, *E. coli* persisted at greater concentrations in water for both the base and high flow input phase of the fluvium experiments. Initial one-way ANOVA of the average concentrations of microorganisms in water samples at 24 h, regardless of flow rate, indicates a significant difference ($p < 0.05$) between *E. coli* ($5.25 \log_{10}$) and *L. innocua* ($3.95 \log_{10}$), *L. seeligeri* ($2.72 \log_{10}$), and enterococci ($3.15 \log_{10}$), but not *S. Typhimurium* (53647) and PRD1 bacteriophage. Although there are slight variations in the initial concentrations of each microorganism, this does seem to be a factor in the concentrations of microbes in water after 24 h.

To determine the attenuation of FIB and surrogates in the fluvial sediments, samples were collected from the top, middle, and end of the flumes after the water was drained from the reservoir. The results of microbial attenuation in these sediments by flow are shown in Figure 8. One-way ANOVA indicates a significant difference ($p = 0.0117$) between base and high flow attenuation of microorganisms in sediment at 24 h. Base flow sediment had $4.5 \log_{10}$ CFU or PFU/ml whereas high flow sediment contained $3.9 \log_{10}$ CFU or PFU/ml. This is expected since

a higher flow rate would likely aid microbial retention in the water column as opposed to attenuation in the sediment.

The final aspect of the fluvium experiments was to evaluate the resuspension of FIB and surrogates into the water column from the sediments during a flushing phase. The results of microbial resuspension in the water column by flow rate are shown in Figure 9. Overall, more microorganisms are resuspended in the water during base flow as opposed to high flow after 4 h. Moreover, *E. coli* was resuspended into the water column at higher concentrations followed by *Salmonella* Typhimurium (ATCC 53647) and PRD1 bacteriophage. This difference is probably related to the fact that less microorganisms are attenuated in high flow sediments when compared to base flow sediments (Figure 8) though statistical analyses will be completed to confirm.

Outcomes and Accomplishments

The original objectives of this project were as follows. Objective 1 aimed to determine the distribution and survival of FIB (*E. coli* and enterococci), pathogens, and pathogen surrogates in sediments of aquatic mesocosms exposed to seasonal variations. Objective 2 investigated the resuspension and subsequent partitioning of FIB and pathogen surrogates from surface sediments using an indoor fluvium to simulate high flow events in surface water sources. Based on the data presented in the results section, we were able to complete both of these objectives within the proposed project timeline while making a few adjustments.

First, we originally proposed to use five *Listeria monocytogenes* strains, but instead we used only four since we eliminated strain J1-101 because this isolate was no longer available in our culture collection. Additionally, we proposed to evaluate MNV (Type 1) as a surrogate for human norovirus, but we encountered difficulties with cytotoxicity of the samples and low recovery efficiencies from sediments during method optimization. However, all experiments included MNV except for the fluvium experiments, and therefore, all samples have been archived for future analysis by either cell culture if the method can be optimized or by reverse transcription, real-time PCR for relative quantification. Another adjustment to the protocol was the exclusion of duplicate mesocosm set-ups which was based on the initial results of two separate trial runs prior to the spring mesocosm experiment. An additional aspect of this project that was challenging related to the logistics of mesocosm set-up and daily sampling as well as processing the samples and plating for each of the different microorganisms. During the initial week of each mesocosm experiment, my whole research group was involved in sample processing making this truly a team effort.

Finally, an unexpected outcome of this project was the lack of persistence of enterococci and rejection of our hypothesis. As we continue to analyze these data and refer back to the peer-reviewed literature, we may be able to determine the reason for the relative instability of enterococci in these mesocosms and fluvial systems. A potential reason could be the propensity for enterococci to enter into the viable but not culturable (VBNC) state when stressed combined with the use of an ATCC *E. faecalis* strain as opposed to an environmental isolate.

Summary of Findings and Recommendations

As indicated previously, there is still a tremendous amount of data analysis to perform, and thus, any summary of findings should be considered preliminary. Overall, the data suggests that *Salmonella* is more persistent than *Listeria* spp. (more specifically, *L. monocytogenes*) and

STECs in both the water and sediments of the mesocosms, though there seems to be some strain dependent factors at play. In addition, the FIB *E. coli* seems to be a fairly conservative indicator for predicting microbial water quality and pathogen behavior although enterococci trended with *L. monocytogenes* which may be useful in future studies of this pathogen in the environment. With respect to pathogen surrogates, *Salmonella* Typhimurium (ATCC 53647) seemed to follow the same survival/persistence patterns as the pathogenic *Salmonella* serovars indicating its potential usefulness as a surrogate for studying pathogenic *Salmonella* survival. Surrogates for *L. monocytogenes*—*L. innocua* and *L. seeligeri*—were overall more stable than the *L. monocytogenes* strains used in the present study. Based on these preliminary data and observations, my recommendation would be that *E. coli* is suitable FIB for predicting microbial water quality. In addition, I would suggest that future studies focus on evaluation of microorganisms within real world freshwater systems using novel passive sampling techniques for detection of FIB and pathogens over time.

APPENDICES

Publications and Presentations (required)

There are no publications or presentations to report. However, an abstract has been submitted to the 2015 International Association for Food Protection (IAFP) Meeting for consideration. In addition, we anticipate preparing possibly two manuscripts for peer-review based on this project.

Budget Summary (required)

Category	Amount
Salary/Wages	\$20,991.21
Supplies	\$24,467.49
Travel	\$1,787.36*
Indirect	\$807.43
TOTAL	\$48,053.49

*\$1,104 remaining for travel to 2015 CPS Research Symposium in Atlanta, GA. Therefore, I will need additional support from CPS if I cannot pull from other areas of the budget once the final amounts have been confirmed.

Tables and Figures

Table 1. Agricultural Water Quality Standards from FSMA Proposed Rule for Produce¹

Type of Irrigation	Upper Limit of <i>E. coli</i> MPN or CFU / 100 mL	
	Single Sample	5-Sample Geometric Mean
Contact (overhead)	126	235
No contact (furrow, drip)	126	576

¹ <http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334552.htm#E>

Table 2. Bacteria and virus strains proposed to be utilized in this study.

Microorganism	Strain	Role	Justification
<i>E. faecalis</i>	ATCC 29212	FIB	Commonly used control strain for enterococci (Sapkota et al., 2007)
<i>E. coli</i>	ATCC 35218	FIB	Reported to closely mimicking <i>E. coli</i> O157:H7 inactivation kinetics (Gurtler et al., 2010)
<i>E. coli</i>	O157, O26, O111, O145	Pathogen	O157:H7 has been associated with fresh produce outbreaks in the past (Beuchat, 1996); other STECs - O145, O111, O26 - also implicated in outbreaks
<i>L. innocua</i>	FSL C2-008	Surrogate	C2-008 has been used previously in fresh produce studies (Oliveira et al. 2011) and <i>L. innocua</i> has been used as a suitable surrogate for LM (Monfort et al., 2000)
<i>L. monocytogenes</i>	R2-574, N1-017, F6-154, R2-499	Pathogen	Strains isolated from outbreaks (Cornell Food Safety Lab Designation)
<i>L. seeligeri</i>	ATCC 35967	Surrogate	Contains virulence factors similar to <i>L. monocytogenes</i> and isolated from the environment (Ly and Müller, 1990)
Murine norovirus	Type 1	Surrogate	Surrogate used for human noroviruses (Richards, 2011)
PRD1		Surrogate	Bacteriophage used as surrogate for human enteric viruses (Gibson and Schwab, 2011)
<i>S. Javiana</i>	ATCC BAA-1593	Pathogen	
<i>S. Montevideo</i>	G4639	Pathogen	<i>Salmonella</i> serovars previously implicated in fresh produce outbreaks (Bhagat et al., 2010; Chang et al., 2012; Xia et al., 2012)
<i>S. Newport</i>	ATCC 6962	Pathogen	
<i>S. Thompson</i>	RM1987	Pathogen	
<i>S. Typhimurium</i>	ATCC 14028	Pathogen	
<i>S. Typhimurium</i>	ATCC 53647	Surrogate	Commonly used avirulent strain of <i>Salmonella</i> (Grasso et al., 2010)

Table 3. Parameters for stock production and detection of bacteria in mesocosm subsamples.

Microorganism	Broth medium	Agar medium	Incubation Temp / Time
Enterococci	TSB	m-Enterococcus	35°C / 24 - 48 h
Generic <i>E. coli</i>	TSB	3M Petrifilm™ <i>E. coli</i> /Coliform	37°C / 18 - 24 h
STECs	TSB	CHROMagar™ STEC	37°C / 18 - 24 h
<i>Listeria</i> spp.	TSB	MOX, RAPID' <i>L.mono</i>	30°C / 24 - 48 h
<i>Salmonella</i> spp.	TSB	XLT4	37°C / 18 - 24 h

TSB = tryptic soy broth; LB = luria broth; TSB-YE = TSB yeast extract; MOX = Modified Oxford; XLT-4 = xylose-lysine-tergitol 4

Figure 1. Mesocosm set-up.

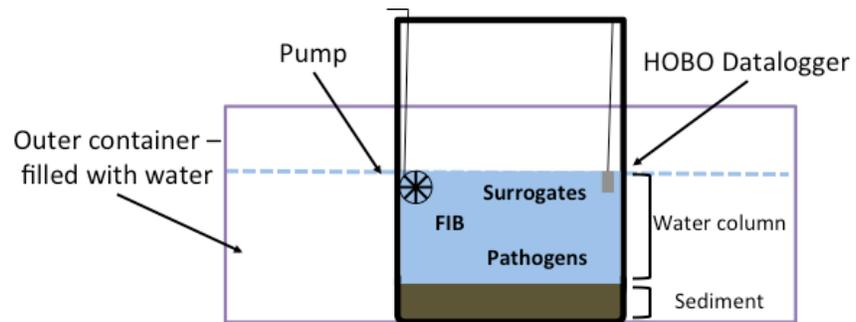


Figure 2. Sediment sampling tool.



Figure 3. Schematic of fluvarium set up from McDowell and Sharpley (2003).

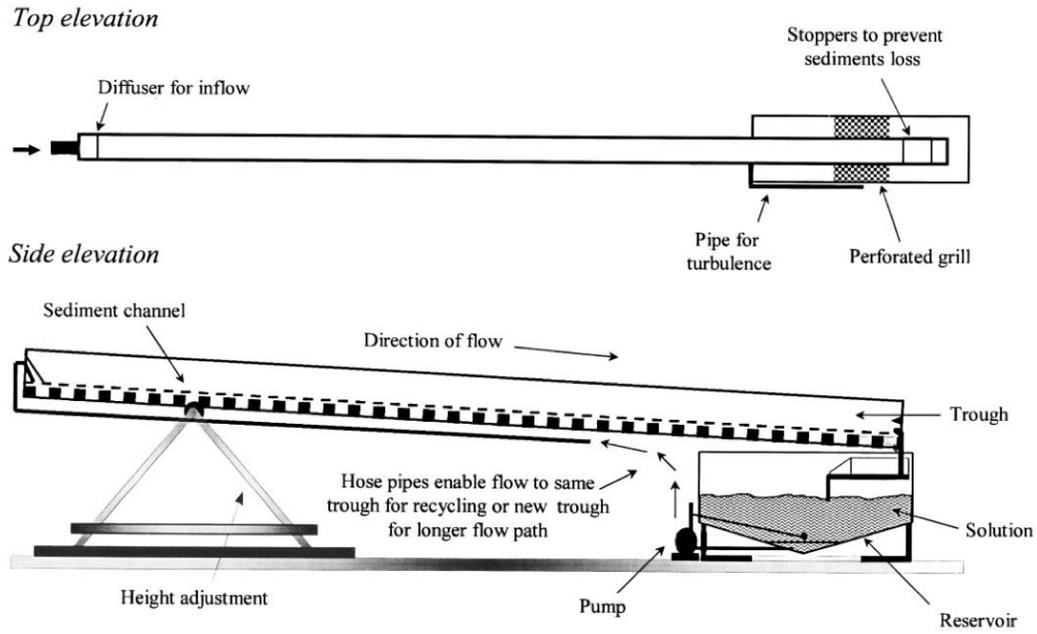


Figure 4. Survival of microorganisms over time by season and sample type.

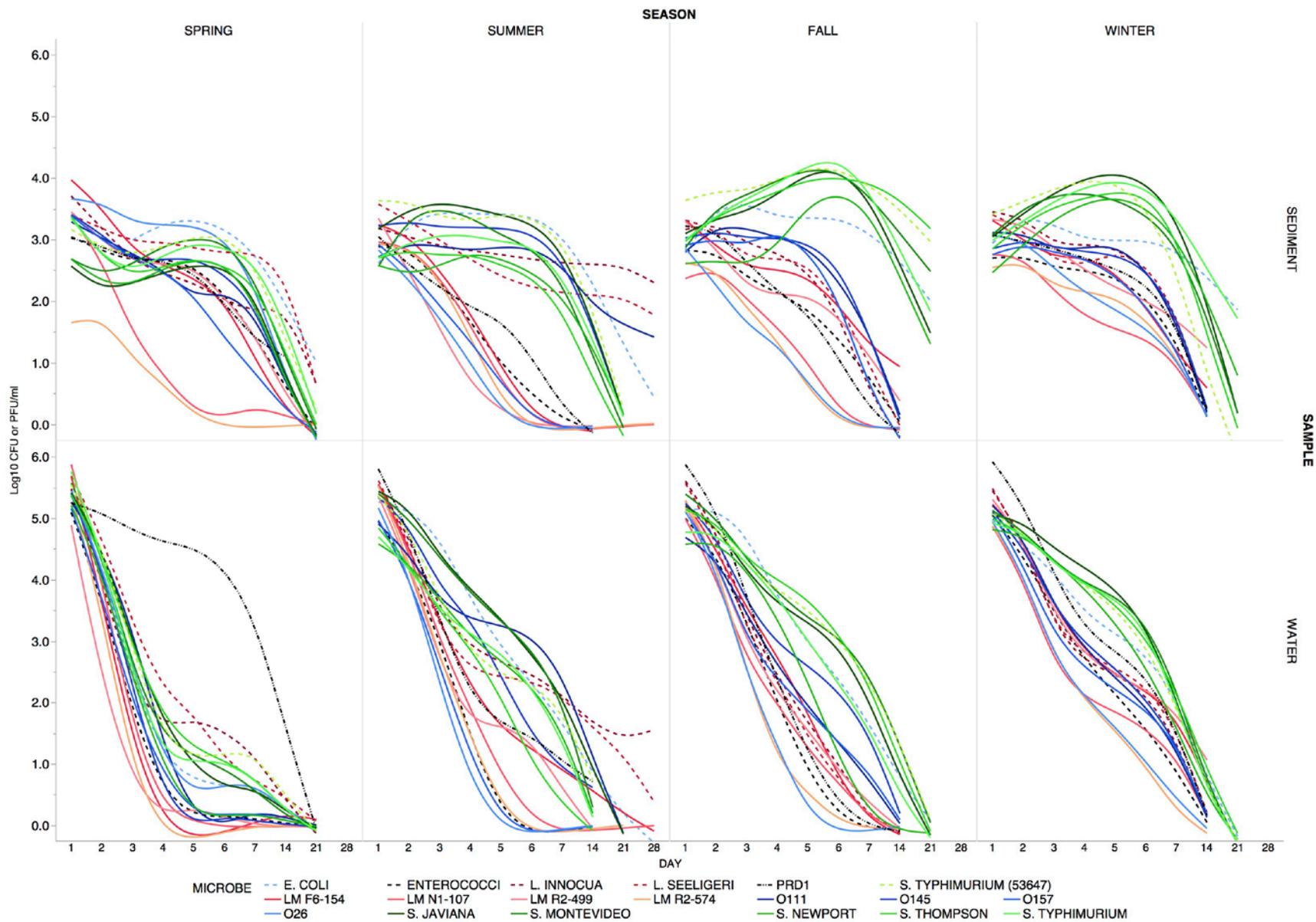


Figure 5. Survival of microorganisms in water over time by season and water type.

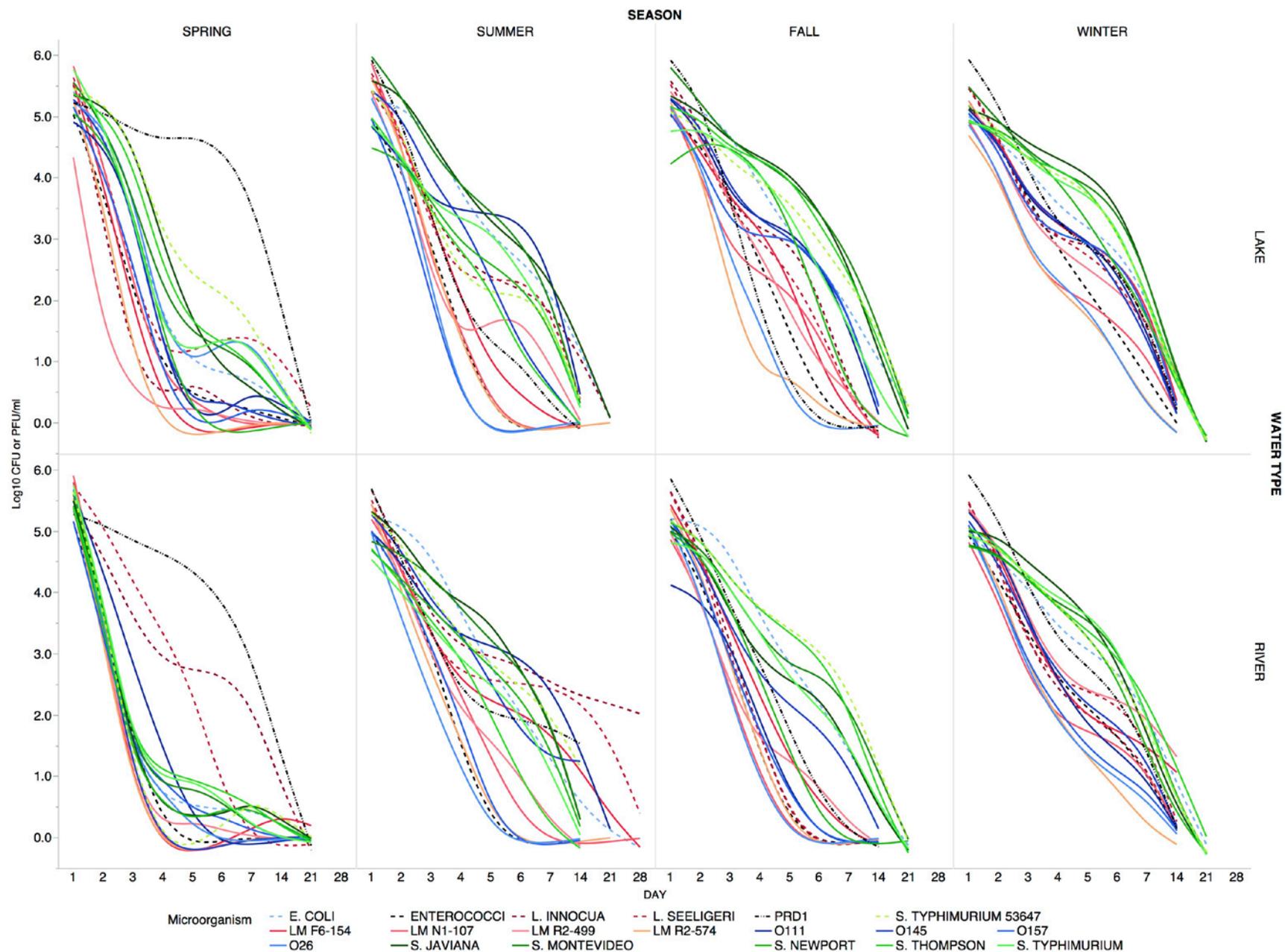


Figure 6. Survival of microorganisms in sediment over time by season and sediment type.

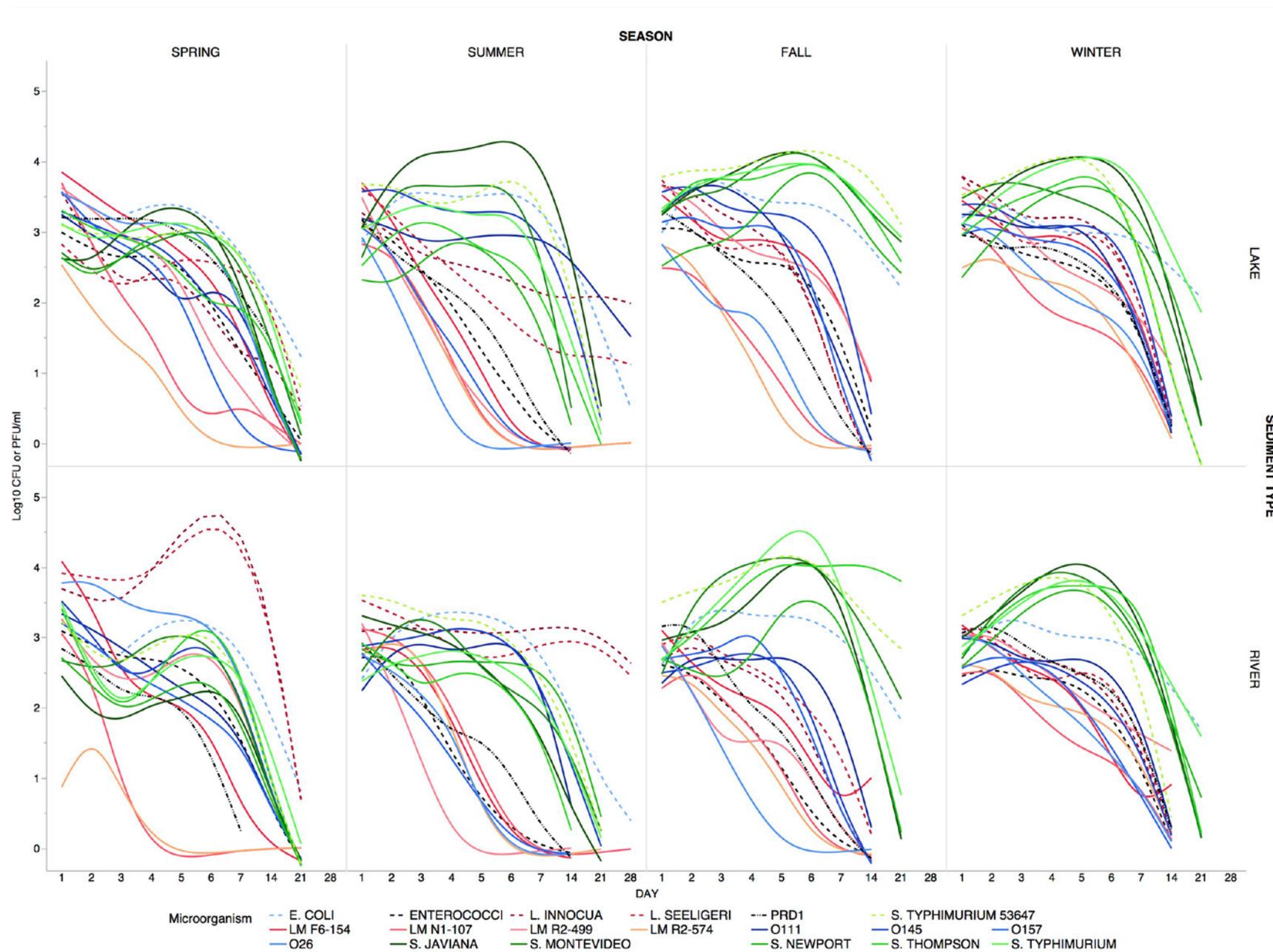


Figure 7. Persistence of microorganisms in the water column of a fluvial system by flow rate—base and high.

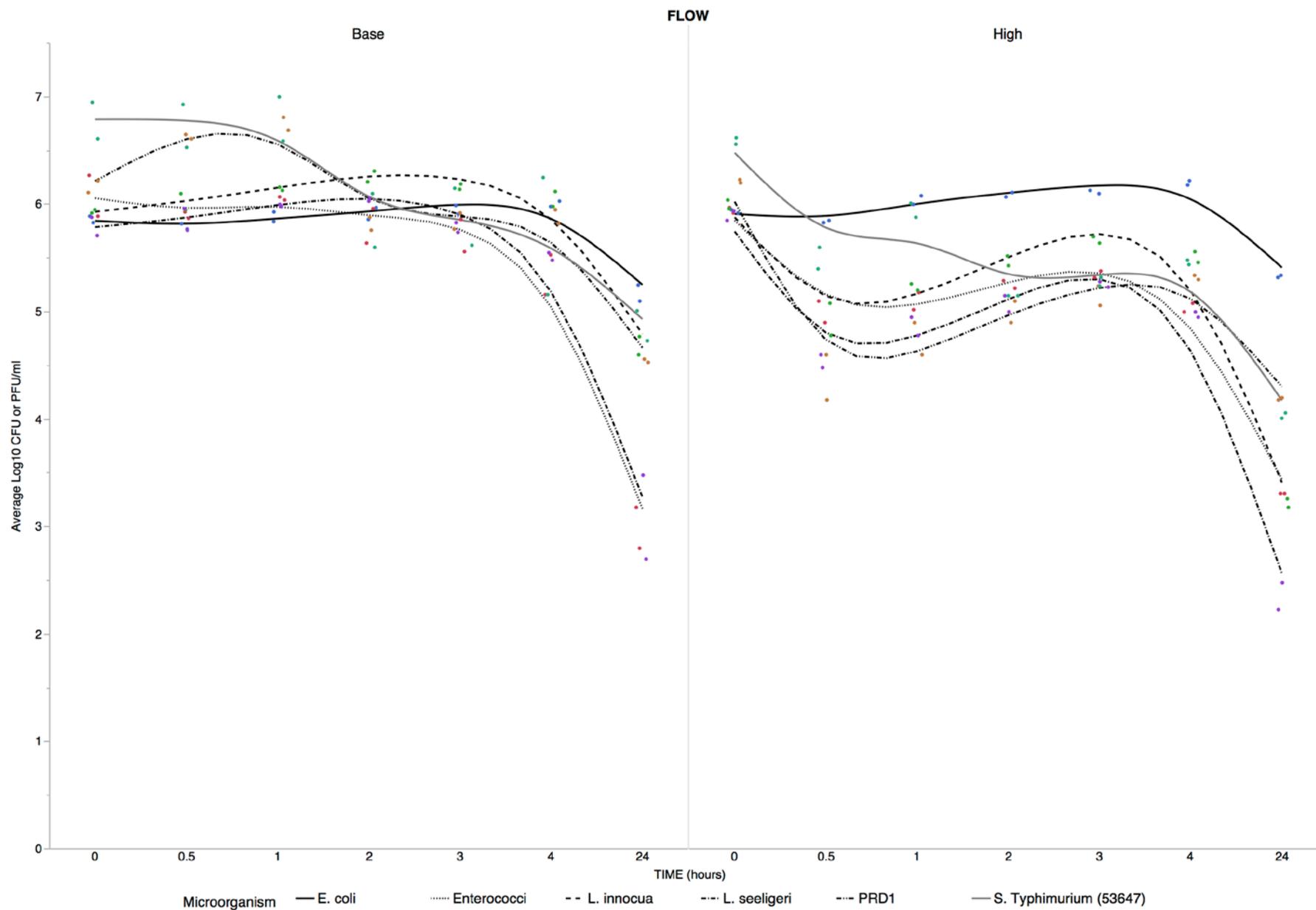


Figure 8. Attenuation of microorganisms in sediments at 24 hours post inoculation in flume water by flow rate—base and high.

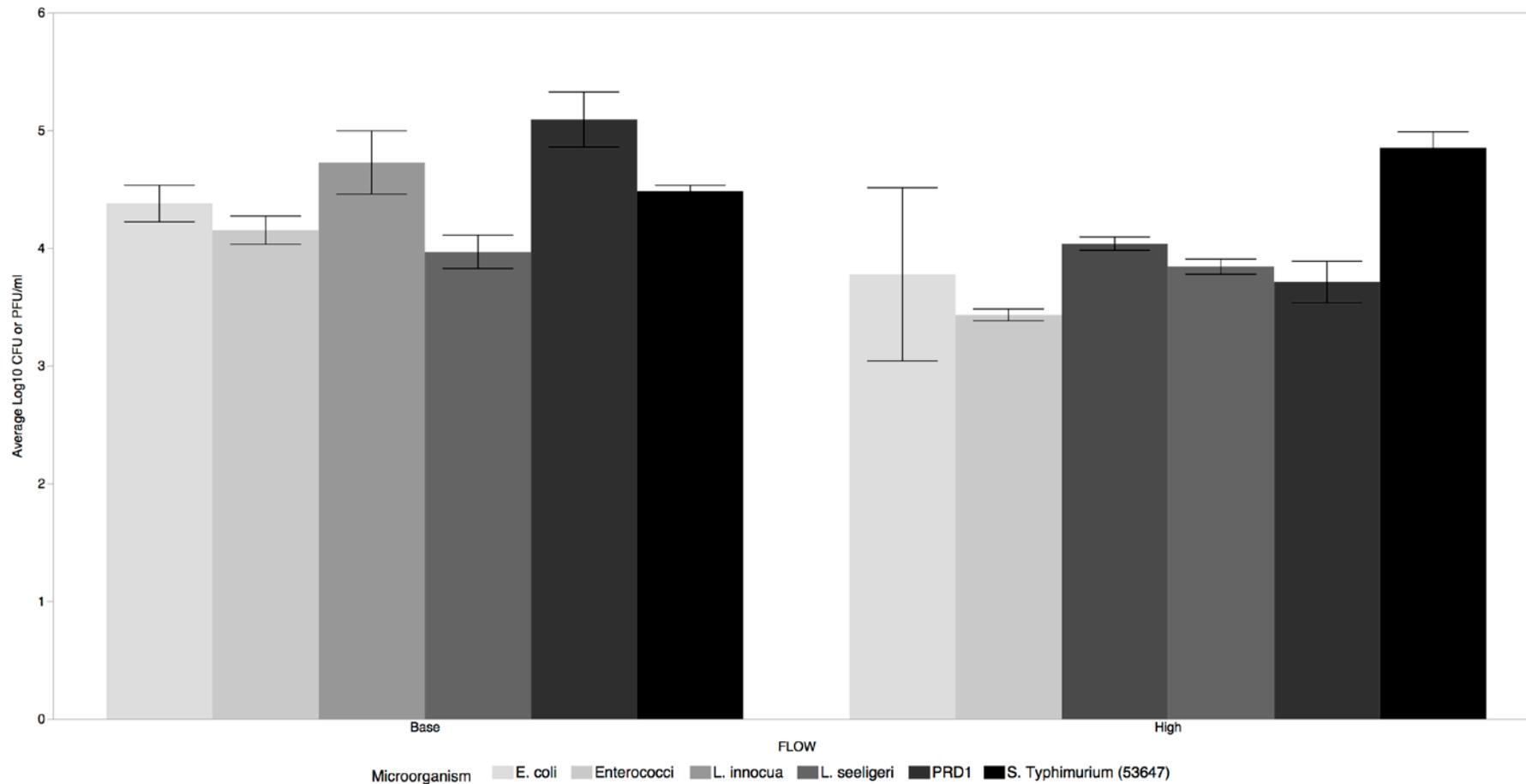


Figure 9. Resuspension of microorganisms from sediment to the water column during the flushing phase of the fluvium experiments by flow rate—base and high.

