



Introduction

- Fecal indicator bacteria (FIB) are microorganisms that are used by water resource managers to detect the presence of fecal contamination in water.
- *Enterococcus* and *Escherichia coli* (*E. coli*) reside mainly in the intestines of humans and animals, making them the most reliable fecal indicator bacteria.²
- Standard Methods states “Keep source water, stream pollution, recreational water, and wastewater samples cold but unfrozen ($\leq 10^{\circ}\text{C}$) during transport (≤ 8 hr between collection and lab arrival)”.¹
- After eight hours of storing a sample, the sample becomes “invalid”, which creates obstacles in planning sampling trips and laboratory processing.¹

Research Goal

- The goal of this project was to determine the affect sample holding time has on the final measured concentration of fecal indicator bacteria.



Figure 1: Sample site location on Town Branch Creek.

Methods

Field Collection:

- Water samples were collected from Town Branch Creek in Tahlequah, Oklahoma on October 14 (Trail 1), October 23 (Trial 2), and October 31, 2019 (Trial 3) (Figure 1).
- Three liters of water was collected into a churn splitter. The sample was homogenized and split into 24, 100 mL, sterile sample bottles. Samples were preserved with Sodium Thiosulfate, placed in a cooler, and transported to the GRDA/NSU Research laboratory (Figure 2).

Laboratory Analysis:

- Of the 24 bottles, 12 were used to test for *E. coli* following the IDEXX Colisure Method (SM 9223 A.) and 12 were used to test of *Enterococcus* using the IDEXX Enterolert Method (SM 9230 D.) (Figure 5).
- Samples were processed in triplicate at 2, 8, 24, and 36-hour intervals following collection.
- Samples remained in a refrigerator at 4°C until processing time.

Data Analysis:

- Data were analyzed via Repeated Measures Analysis of Variance (RM-ANOVA) followed by a post hoc analysis with Tukey’s Honestly Significant Difference test (Tukey HSD).

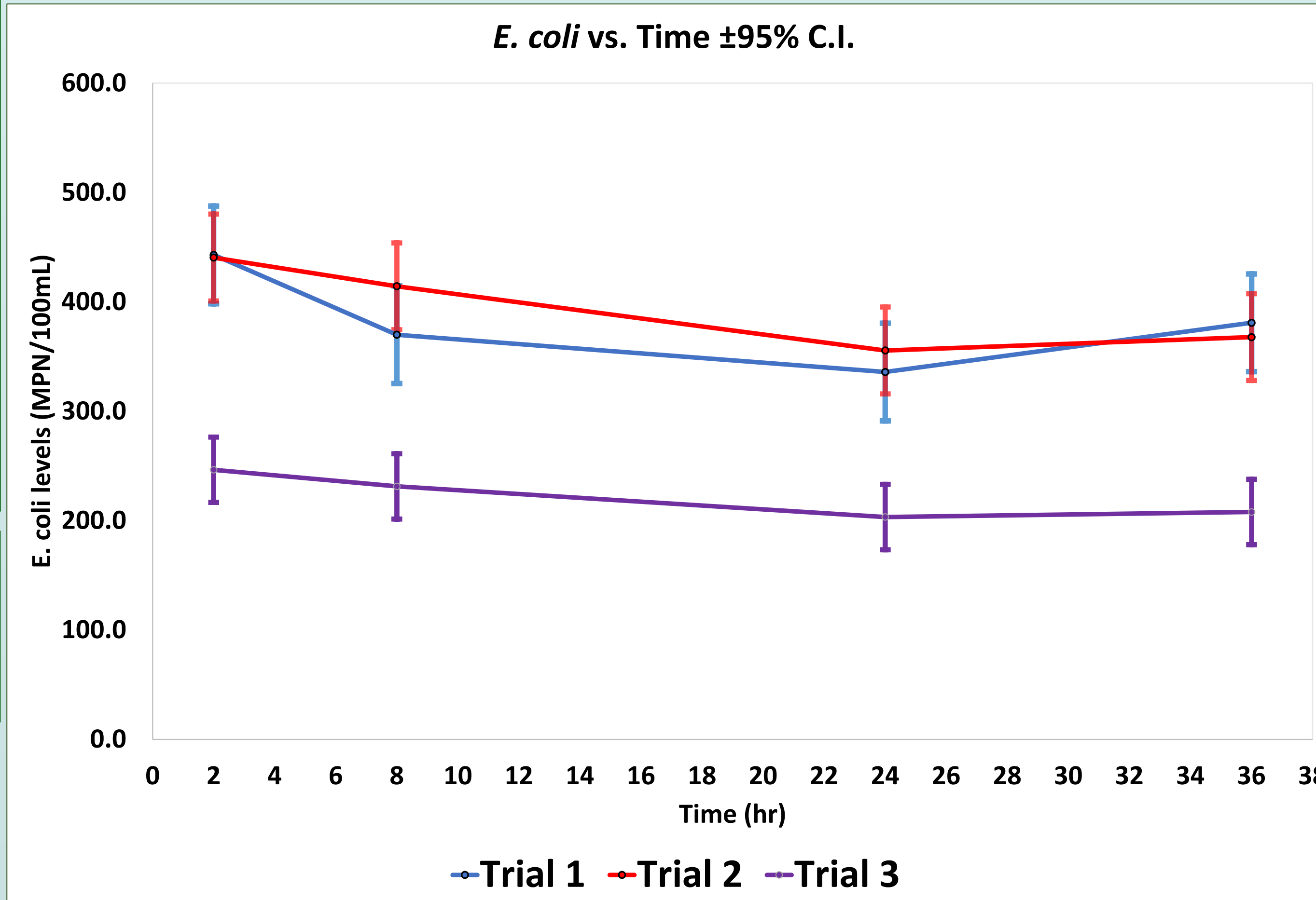


Figure 3: *E. coli* holding time with means separately over all 3 trials.

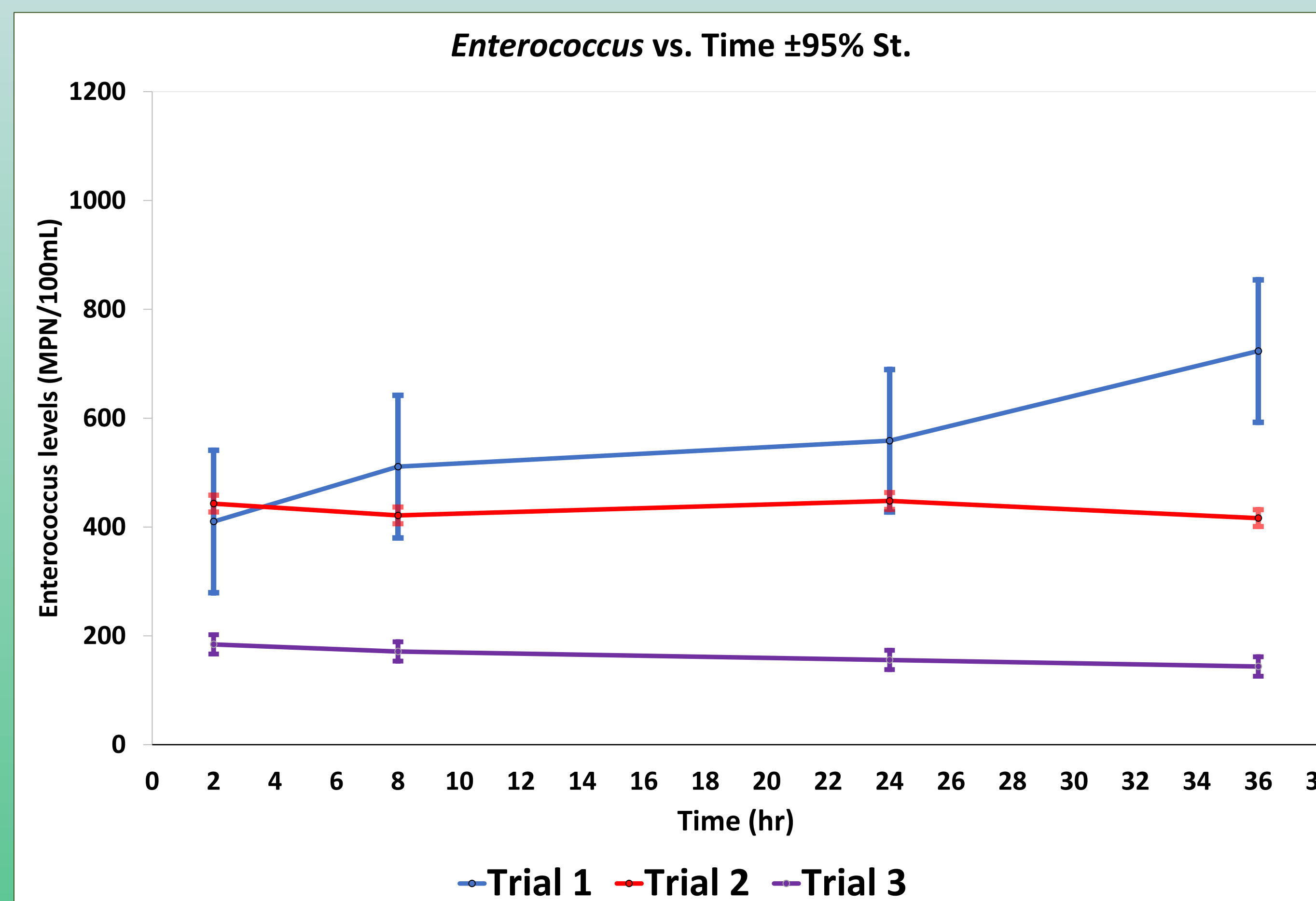


Figure 4: *Enterococcus* holding time with means separately over all 3 trials.



Figure 2: Field and laboratory sampling and processing

Results

- For *E. Coli*, RM-ANOVA main effects found significant differences in average MPN values for holding time ($F=4.411$, $P=0.041$) and trial number ($F=37.151$, $P<0.001$). No significant interaction was found between time and trial ($F=0.323$, $P=0.916$) (Figure 3).
 - Post Hoc Comparisons (Tukey HSD) between holding time within Trials 1, 2, and 3 found no significant differences between average MPN and holding time.
- For *Enterococcus*, RM-ANOVA found a significant interaction between holding time and trial number ($F=8.702$, $P<0.001$) (Figure 4).
 - Post Hoc Comparisons (Tukey HSD) between holding time within trial 1 found significant differences in MPN between 24 and 2 hours, 36 and 2 hours, 36 and 8 hours, and 36 and 24 hours.
 - For trials 2 and 3 no significant differences were found between average MPN and length of holding time.



Figure 5: IDEXX bacteria cards: blank (left) and total coliform (right).

Discussion

- Pope et al. found decreases in *E. coli* bacteria levels over holding time from 4 separate locations.⁴
 - There was a decrease in all 3 trials for *E. coli* levels in this experiment, but findings were not significant.
- Milligan found *Enterococcus* bacteria levels to be significant after 24 hours.³
 - Our overall results differ, as no significance was shown at any time other than in the 1st trial.
- Our data indicates that bacteria results processed outside holding time may still yield informative results for water resource managers.
- Further replication of this study needs to be done to better understand how bacteria reacts as holding time increases.

Literature

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4. Pope, M. L., M. Bussen, M. A. Feige, L. Shadix, S. Gonder, C. Rodgers, Y. Chambers, J. Pulz, K. Miller, K. Connell, and J. Standridge. 2003. Assessment of the Effects of Holding Time and Temperature on *Escherichia coli* Densities in Surface Water Samples. *Applied and Environmental Microbiology* 69:6201–6207.

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