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Fate and Transport of *E. coli* in Cedar Creek Watershed, Texas

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Abstract. Presence of *E. coli* is recognized as an important indicator of fecal contamination leading to impairment of a water body. Apart from point and non-point sources, in-situ re-growth is believed

to be a considerable source of *E. coli* in many cases. There is a need to identify, characterize, and quantify *E. coli* loads from various sources in a watershed. To understand re-growth phenomenon better, the fate of *E. coli* needs to be monitored under different environmental factors such as temperature, moisture content and pH.

Wildlife and range cattle manure samples have been collected and analyzed for their *E. coli* content using EPA recommended Method 1603. Growth and die-off rates at different temperatures (0°C, 10°C, 25°C, and 50°C), moisture conditions (0%, 5%, 25%, 50%, 75% dry-basis) and pH (acidic, alkaline and basic) are being monitored. The concentrations measured within and among different temperatures over a period of 7 days showed highly variable counts. Potential *E. coli* loads calculated will then be incorporated in a newly developed model called Spatially Explicit Load Enrichment Calculation Tool (SELECT) to predict *E. coli* loads resulting from various identified sources in Cedar creek watershed of south central Texas. Findings of this research will aid in Watershed Protection Plan (WPP) development and Total Maximum Daily Load (TMDL) development to address impairment from point and non-point source pollution of *E. coli* in the State of Texas. The results and findings of our study will be discussed in this paper.

Keywords. E .coli, manure, TMDL, Texas watershed

Introduction

Presence of *Escherichia coli* (*E. coli)* is recognized as an important indicator of fecal contamination leading to impairment of a water body. Apart from point and non-point sources, in situ re-growth is believed to be a considerable source of *E. coli (*Byappanahalli et al. 2003, Muirhead et al. 2004*)*. There is a need to identify, characterize, and quantify *E. coli* loads from wildlife and range cattle in a watershed. To understand re-growth phenomenon better, the fate of *E. coli* needs to be monitored under different environmental factors such as temperature, moisture content, and pH. It is sometimes assumed that once these bacteria are shed and as they enter soil or water, their population declines rapidly. However, studies (Fenlon et al. 2000, Jiang et al. 2002, Islam et al. 2004) suggests that under certain favorable environmental conditions including optimum temperature, moisture, PH, nutrient levels, and competition with other common bacteria, *E. coli* survive for a much longer period of time.

Fecal coliform, particularly *E. coli* are considered a reliable indicator of recreational water quality. Tyrrel et al. (2003) suggest that there is evidence that catchment sources (e.g., septic tanks or sewage sludge) of fecal pollution become the dominant input following rainfall events. Other catchment sources of pollution include animal feces deposited on land by grazing, scavenging and predatory animals and birds. The transport of microorganisms from land into waterways can have detrimental effects on water quality and human health. If pathogenic strains of *E. coli* are ingested then they may cause illness by multiplying and/or producing toxins in the small intestine. A single sample criterion of 235CFU/100ml (Colony Forming Units) for recreational water quality is set for impairment of water resources by the U.S Environmental Protection Agency (Byappanahalli et al 2003). Several water streams across USA are impaired because they do not meet the adequate bacterial quality standards (USEPA, 2008) .
Cedar creek watershed, located partly in Brazos County and partly in Robertson County in East Central Texas is one of the several water bodies deemed impaired because it did not meet bacteria criteria. Cedar creek watershed has little or no urban influence hence the main sources of bacteria is assumed to be of non-point sources such as fecal material of animals added to the water either through direct deposition or indirectly through runoff from land.

A number of environmental factors and management practices affect the fate and transport of *E. coli* in rural and agricultural landscapes. There is a need to identify the leading environmental factors that influence transport, persistence, re-growth, and survival of *E. coli* in landscapes (e.g., temperature, moisture content, waste source, soil type, rainfall, nutrient status, etc). Also

re-growth of *E. coli* in landscapes due to favorable environmental conditions (e.g., rainfall after dry weather conditions) is one of the major phenomena that affect *E. coli* concentration in streams (TSSWCB, 2007). The objective of this study was to identify, characterize and quantify *E. coli* loads from four different wildlife sources and then monitor the survival, growth and re-growth of *E. coli* at four different temperatures over a period of seven days.

Methods and Materials

Cedar creek watershed is located in Brazos County & Robertson County in East central Texas. Fecal samples from different wildlife sources were collected from the study area. The fecal material was collected by trapping the animals. A grid-design was used for 42 traps per ranch in the watershed, each measuring 81 cm x 25 cm x 30 cm. (Raccoon/feral cat Tomahawk Live Trap, Tomahawk, WI). 150-m spacing between traps has been shown to adequately sample animals that are highly attracted to aromatic baits (e.g., raccoons and opossums). Randomly located trap arrays were used in order to capture armadillos, rabbits, and skunks (e.g., species less attracted to bait). The arrays were fabricated out of 61-cm tall chicken fencing with 61-cm long wooden stakes. Each array had 8-12 armadillo/rabbit traps (43 traps total for each ranch; 48 cm x 15 cm x 15 cm; Tomahawk Live Trap, Tomahawk, WI) with variable array setups designed to take advantage of the local vegetative community and topography.

All field samples were refrigerated until analyzed and enumerated for *E. coli* using EPA method 1603. In this method water sample is filtered using membrane filters, which retains the bacteria. A direct count of *E. coli* in water can be obtained in water based on the development of colonies that grow on the on the surface of the membrane filter sitting on a nutrient medium. The nutrient medium for analyses was prepared by adding 45.6 g of dehydrated modified membrane-Thermotolerant *Escherichia coli* (modified mTEC) agar powder to 1L of distilled water and boiling the mixture for 1 minute. The mixture was autoclaved at 121°C for 15 min and poured into 9 x 50mm petri dishes. The mixture was allowed to solidify in the plates at room temperature. To process, the fecal samples were first thawed until they reached room temperature and then 1 gm of substrate was taken from each sample and the bacteria from the substrate were elutriated by centrifuging for 2 minutes with 9.5ml of sterile distilled water. The suspension was serially diluted and filtered using Micropore membrane filters. Initial filtration and plating was done using 10 ml of sterile water and used as a control, to ensure that the experiments were performed under sterile conditions. A sterile membrane filter was placed on the filter base with the gird side up and the funnel was attached to it so that the membrane filter

was held between the funnel and filter base. The dilution to be plated was vortexed for about 10 s to distribute the bacteria uniformly and poured into the funnel. Filtration was done using a vacuum pump until all the water above the membrane filter is sucked. After filtration, the membrane filter with the bacteria retained on it, was removed aseptically from the filter base and placed onto the hardened modified mTEC agar in the petri dish. Modified mTEC agar is a selective and differential medium and is used for chromogenic detection of *E. coli*. The plate was covered and incubated in inverted position for 2 h at 35± 0.5 °C to resuscitate the stressed cells. The sides of the funnel were then rinsed with methanol followed by distilled water to sterilize for the next use. The process was repeated for all the dilutions to be plated. After 2 hours of incubation the plates were transferred into a Whirl-Pak bag. The bag was sealed and kept in a test tube rack with the plates inverted. The rack was immersed in a 44.5 ± 0.2 °C water bath for 22-24 h and then the plates were removed from the water bath and the number of red/magenta colonies were counted and recorded. If growth was observed on a control sample than that test was rejected. Only the plates having colonies between 30 and 300 were used to report *E. coli* concentrations as colony forming units (CFUs) per g of fecal material.

Along with enumeration of *E. coli* the moisture content of all the samples was also determined by drying 1 g of the wet sample for 24 h and recording the dry weight. Moisture content was calculated on dry basis [(Wet wt. – Dry wt) × 100 ÷ Dry Wt.]

Once colonies were obtained on mTEC agar, one randomly selected colony from each sample was isolated by streaking on Luria-Bertani LB Agar and incubated at 35°C for 24 h. This was conducted as a positive control to confirm that the experiments actually did produce *E. coli* and not some other organism. After the colonies were obtained on LB Agar, one randomly selected colony was again streaked on MacConkey agar. If colonies were obtained on both media then it positively confirmed that the bacteria isolated were *E. coli*. LB agar and MacConkey agar were used for this procedure since they are selective for *E. coli*.

Subsequently, selected *E. coli* samples were tested for survival by subjecting them to different temperatures. Three samples were randomly selected from two species (Cattle and Raccoon). One g of each sub-sample was mixed with 100 ml of distilled water. After inoculation, the mixture was replicated three times and 1 ml of the solution from each replicate was added to 9.0 ml of distilled water and thus was serially diluted. The dilutions were analyzed and enumerated for *E. coli* content using Method 1603 described above to get the initial concentration of the bacteria in the solution. Thus Day 0 counts were obtained. Each sub-sample from the solution (used to obtain Day 0 counts) was then divided into four equal volumes and stored at 0°C,

10°C, 25°C and 50°C, respectively. Similarly the parts were serially diluted and plated after 24h (Day 1), 72h (Day 3), 120h (Day 5) and 168h (Day 7). The *E. coli* numbers were reported as CFU per 10ml.

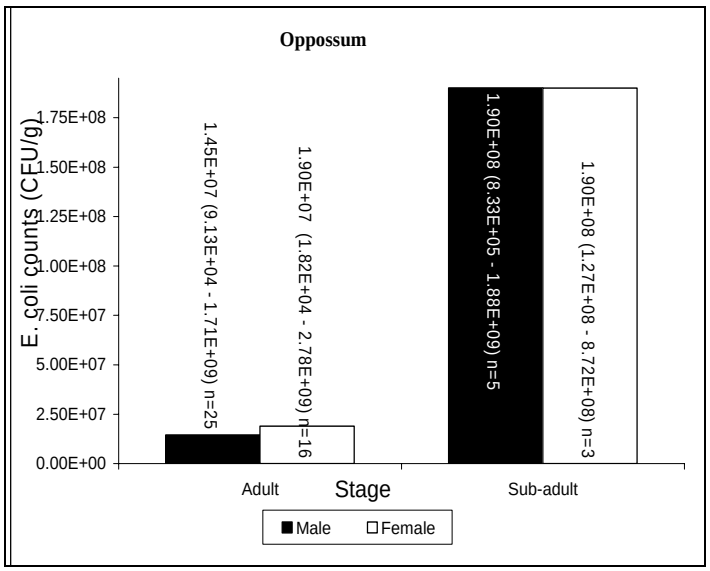
RESULTS

The *E. coli* concentrations from cattle and wildlife feces samples collected from the Cedar creek watershed were recorded in CFU per g of fecal material. Table 1 presents the background concentration of *E. coli* in different species which were analyzed during summer in the watershed. Out of the four species analyzed, median *E. coli* concentrations from opossum (1.60×10^7 cfu/g) and raccoon (1.59×10^7cfu/g) feces were higher than cattle (1.09×10^4 cfu/g) and armadillo (1.01×10^7 cfu/g). The *E. coli* count from cattle feces was found to be lower than all other species.

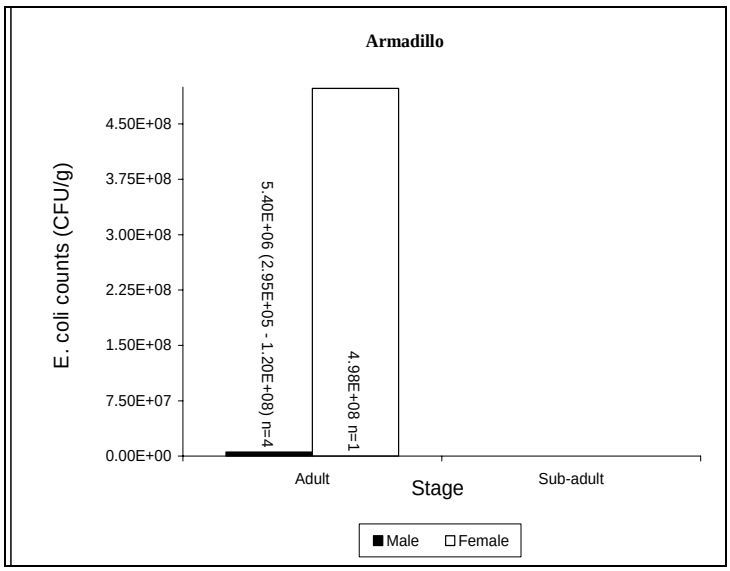
Table 1. E. coli concentration in feces of different species

Species	**Total No. of Samples Processed**	**Total No. of Samples Used**	**CFU/ g (wet wt.)**	
			Median	Range
Armadillo	7	5	1.01×10^7	2.95×10^5 - 4.98×10^8
Raccoon	86	43	1.59×10^7	1.88×10^5 - 3.16×10^9
Opossum	76	57	1.60×10^7	1.82×10^4 - 2.78×10^9
Cattle	23	17	1.09×10^4	9.42×10^1 - 1.92×10^6

The omnivorous behavior of armadillo, opossum and raccoons could be attributed to higher *E. coli* counts than herbivorous cattle. Additionally, feces samples from these animal types showed that median *E. coli* concentrations varied with age and gender (Fig. 1). The *E. coli* analysis of Opossum showed sub-adults shed more bacteria than adults but no large difference was observed between the *E. coli* concentrations of males and females (Fig. 1a). Similarly, adult cattle feces had lower *E. coli* concentrations than calves (Fig. 1b). Conversely, raccoons showed a higher concentration of *E. coli* in feces from adults (Fig. 1c). Adult male raccoons shed up to two orders of magnitude more *E. coli* than female raccoons, whereas, adult female armadillo feces contained more *E. coli* than that of adult males (Fig. 1d).



1a



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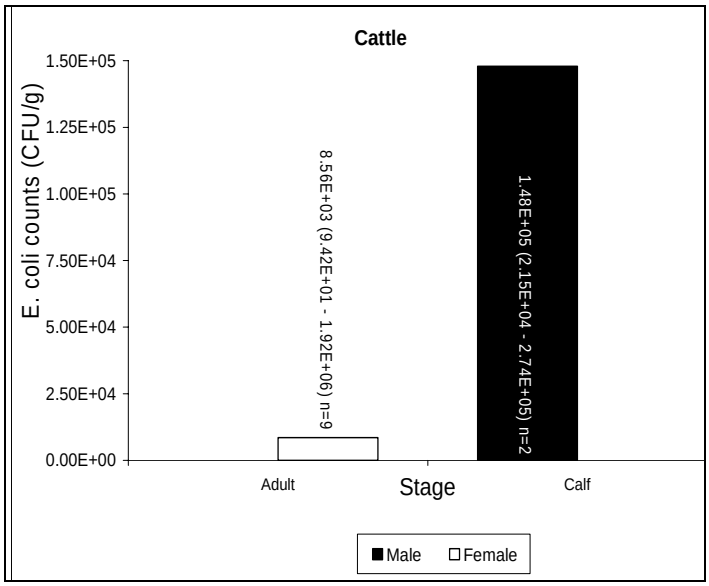
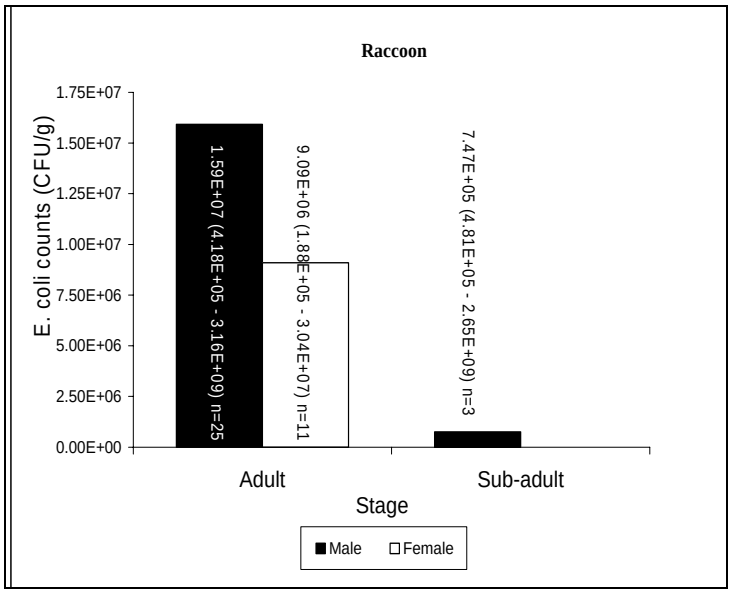


1b



1c

Figure 1. *E. coli concentrations in feces of male and female opossum (1a), cattle (1b), raccoons (1c) and armadillo (1d) at different growth stages.*

The survival and growth of *E. coli* were determined in feces of raccoons and cattle only. The concentrations measured within and among different temperatures over a period of 7 days showed highly variable counts (Fig. 2-3). Day 0 counts in Figs. 2a and 3a show the median background *E. coli* concentrations from cattle and raccoon feces samples at room temperature. Background counts are presented for comparison with the bacterial counts from subsequent

days. The background counts for cattle feces for 0° C are different from 10° C, 20° C and 50° C because the experiments were conducted on different days. The 0° C samples were plated first and 10° C, 20° C and 50° C were plated on the next day. The Day 0 *E. coli* concentrations for 10° C, 20° C and 50° C were less than 0° C (Fig 2b) because the samples went through a freeze and thaw cycle. Kibbey et al. (1978) have reported that freezing and thawing of soils reduces bacterial population.

The results of concentration of *E. coli* from cattle feces at different temperatures over seven days are shown in Fig. 2b. A gradual growth of *E. coli* concentrations was observed at 0° C up to Day 5 followed by a drop on Day 7. At 10° C the bacteria growth was higher for Day 0 to Day 1 which decreased on Day 3. This decline could be due to the depletion of nutrients over time and increased competition for food among bacteria. The *E. coli* concentrations then increased slightly on Day 5 at this temperature, indicating a re-growth. The nutrition for the re-growth could have possibly been due to food supply from the organic matter of the dead bacteria. The *E. coli* levels again decreased on Day 7.

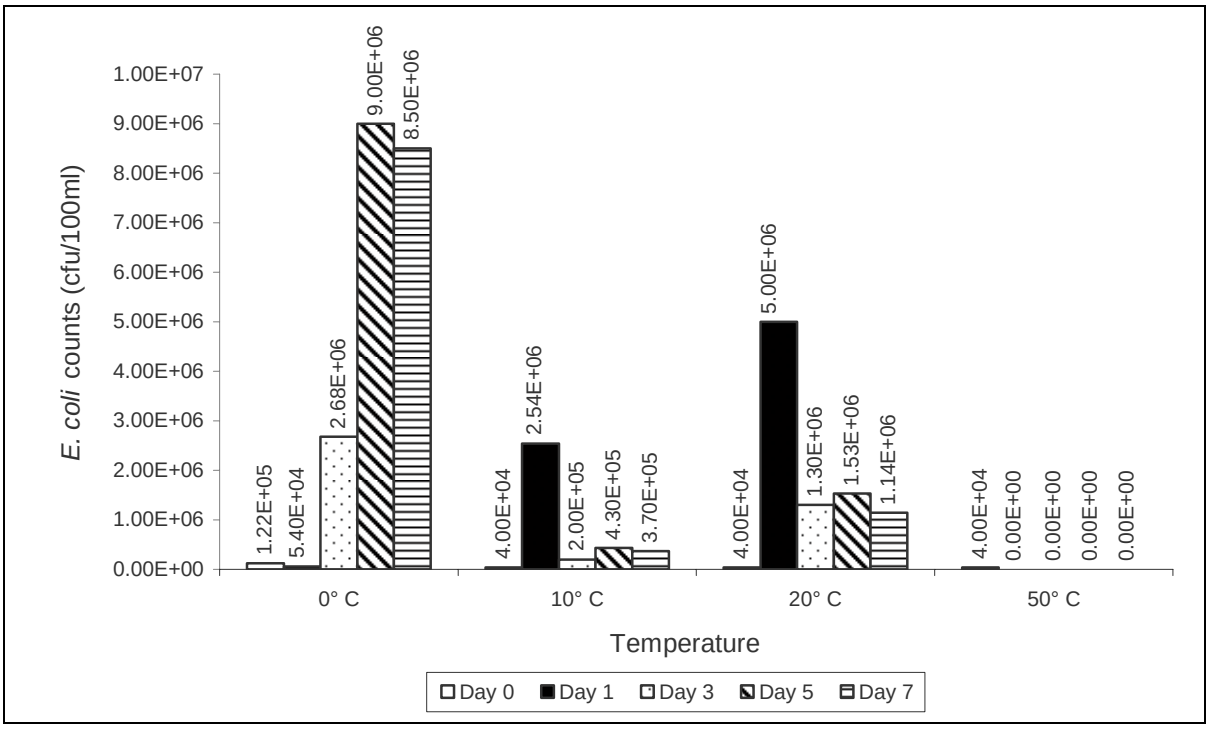


Figure 2a. Survival of E. coli from cattle feces at different temperatures in seven days

At 20° C the growth of *E. coli* showed a similar trend as 10° C but no bacterial growth was observed in cattle feces on any day at the temperature of 50° C. At 0° C the *E. coli* concentrations continued to increase until Day 5, when the highest growth was recorded for cattle feces samples (Fig 2a). Feces samples were frozen while in storage and before being subjected to higher temperatures. Therefore, at 0° C, the bacteria took a longer time to revive themselves whereas increase in counts at 10 and 20° C was observed within one day (Day 1). Also, Fig. 2b shows that at 20° C, the *E. coli* growth was the highest on Day 1. The highest *E. coli* concentration in cattle feces samples was recorded at 0° C on Day 5. By Day 7, *E. coli* concentrations dropped at all temperatures as compared to the previous day (Day 5).

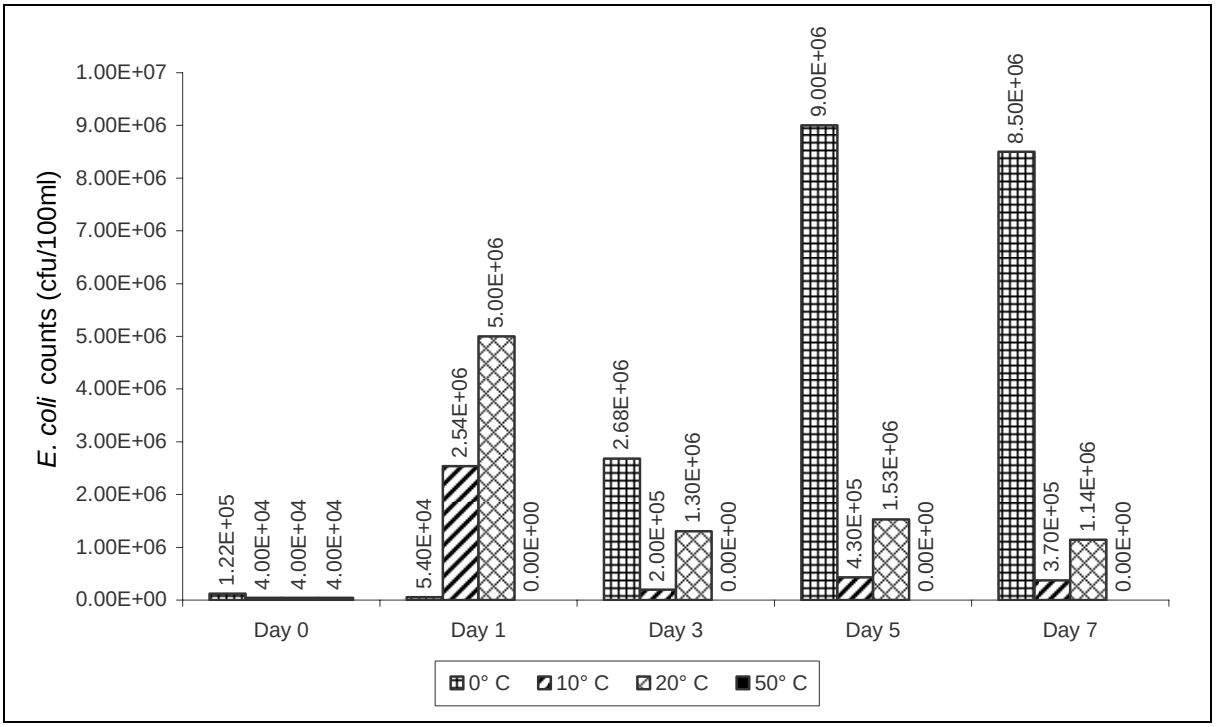


Figure 2b. E. coli concentration from cattle feces on different days

E. coli concentrations from feces samples of raccoons were not similar to concentration from cattle feces (Fig. 3a and Fig 3b). The highest counts for each temperature were observed on Day 0. There was no growth at 50° C on any day after Day 0. All the other temperatures showed a drop in *E. coli* concentrations on Day 1 and an increase on Day 3. On Day 5, the concentrations rose for 10° C and 20° C. The *E. coli* levels on Day 7 rose at 0° C and 20° C but declined for 10° C. The concentrations of *E. coli* from raccoon samples for all temperatures for Day 7 were lower than Day 0. The highest *E. coli* growth was found on Day 7 at 20° C.

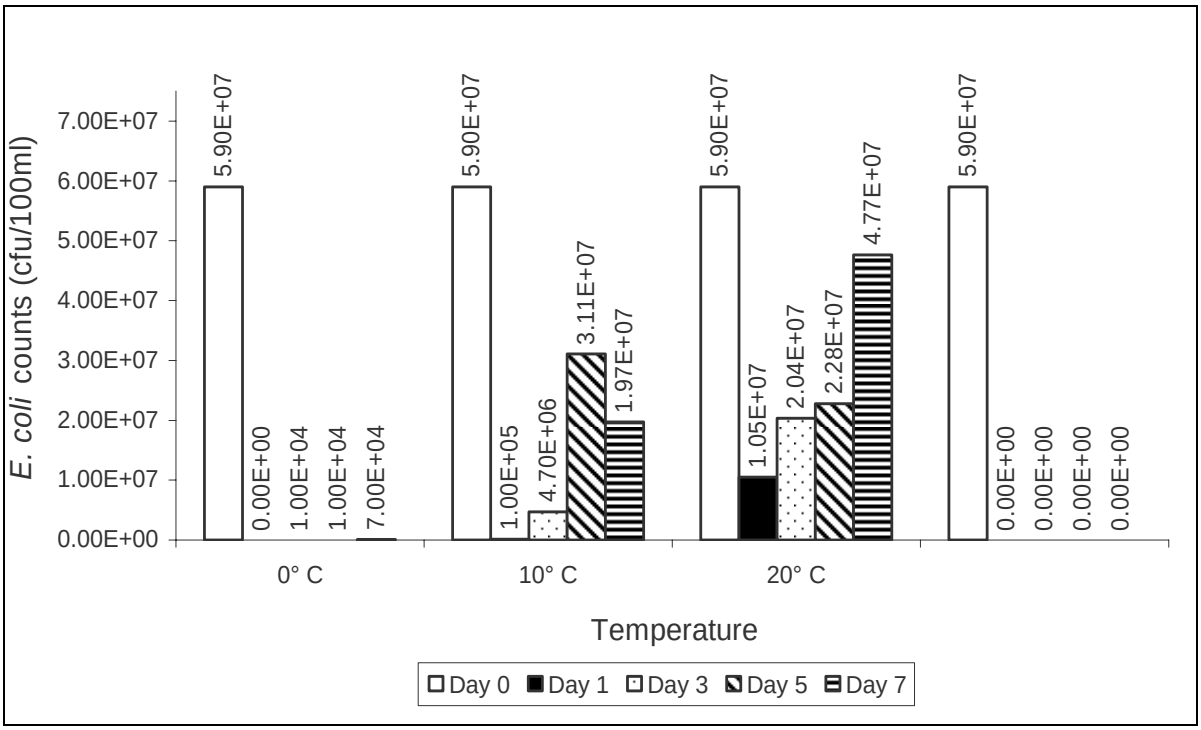


Figure 3a. Survival of E. coli from raccoon feces at different temperatures in seven days

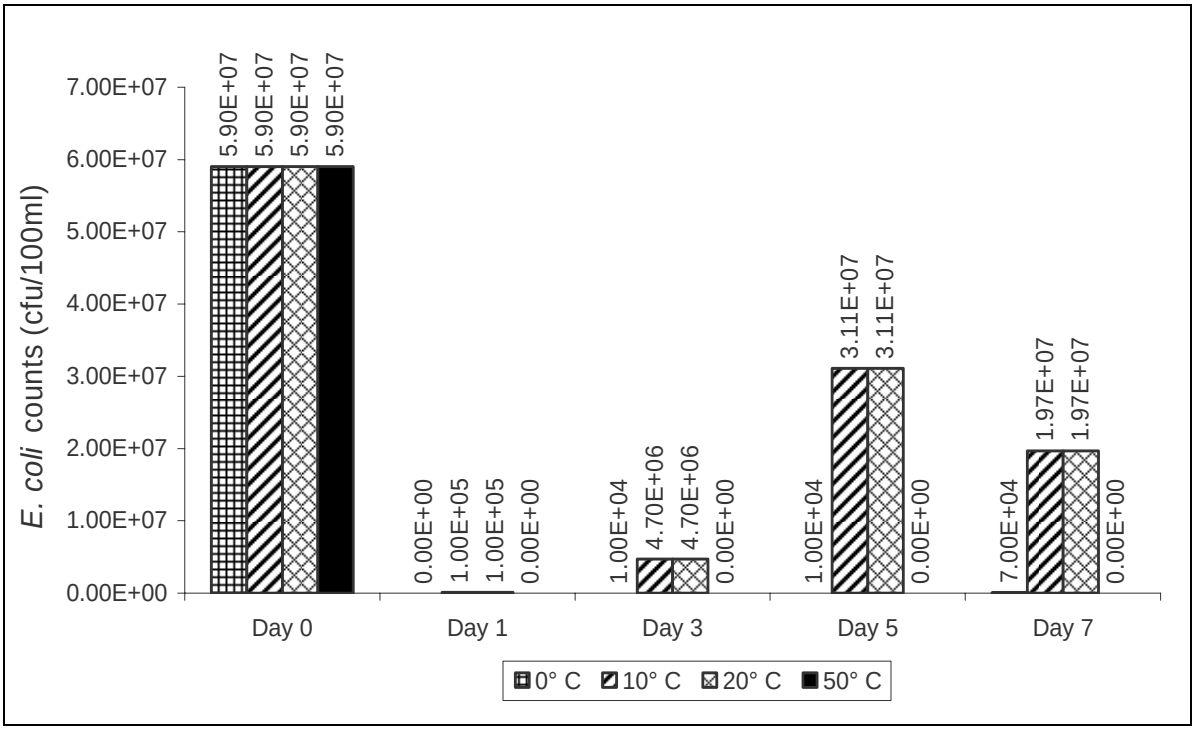


Figure 3b. E. coli concentration from raccoon feces on different days

The results of this study are similar to those by Gerba et al. (1975) and Reddy et al. (1981) who observed that an inverse relationship appears to exist between temperature and bacterial mortality. Survival time of *E. coli* bacteria decreases with higher temperatures. Habteselassie et al. (2007) have also concluded that the *E. coli* survive better at colder temperatures.

Conclusion

E. coli concentrations from feces of different animals was different due to their feed types and the growth and survival of *E. coli* subjected to different temperature conditions showed high variability results over time. Freeze thaw cycles of feces also showed to affect the survivability of the bacteria. This study of *E. coli* bacteria in the feces subjected to different temperature conditions was conducted using the fecal material added to water. The study may have produced different results if conducted using the isolates of the feces since the *E. coli* would not have competed with other bacteria contained in it. Also, the organic matter availability as food for bacteria would have been different under such conditions. Future work will include analysis of the survival and growth of *E. coli* at different moisture conditions.

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