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THE EFFECT OF TEMPERATURE AND DISCHARGE RATES ON THE CONCENTRATION OF *E.coli* IN THE BUFFALO RIVER WATERSHED

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ABSTRACT: Escherichia coli *has the potential to cause human health problems as well as serving as an indicator of water quality. Temperature and flow rates of streams and rivers potentially impact the levels of* E. coli *present. The majority of research on* E.coli *levels has been conducted in warm weather months when the waterways are often used for recreational purposes. Bacterial input to stream sediments may occur during cold weather months and the sediment may act as a bacteria pool that can be resuspended by snowmelt or rainstorm events. The objective of this research was to determine the impact that temperature and discharge rates had on the levels of* E.coli *during cold weather months.* E.coli *levels, temperature, and discharge rates were measured once daily at three sites in the Buffalo River Watershed during early spring 2005. Enumeration of* E.coli *colonies was done using the Coliscan Easygel method. Results showed that levels of E. coli were lower (0-500 cfu/100ml and geometric mean of 32.19 cfu/100ml) than traditionally observed in the Buffalo River Watershed (up to 38,000 cfu/100ml during storm events) during warmer months. No correlation was found between the levels of* E.coli *present and temperature or discharge rates. Since these results are in contradiction to previous findings for the watershed, this study indicates that the water temperature, which averaged 5.5^{o}C, was low enough to suppress levels of* E. coli *and offsets the effects of higher discharge rates.*

INTRODUCTION

The lower part of the Buffalo River (approximately 9km) was designated an Area of Concern (AOC) by the International Joint Commission. The United States Environmental Protection Agency (EPA) defines an AOC as a “geographic area that fails to meet the general or specific objectives of the [Great Lakes Water Quality] agreement where such failure has caused or is likely to cause impairment of beneficial use of the area's ability to support aquatic life”(EPA, 2006). The bacteria enter this AOC from upstream sources as well as 38 combined sewer overflows within the AOC. Although there are no beaches in this area and therefore no beneficial use impairment, the river is often used for recreational purposes, which allows humans to be in close contact with pathogenic bacteria. This study was undertaken in order to determine the impact that water temperature and discharge rates have on the levels of *E.coli* during cold weather months.

LITERATURE REVIEW

The presence of indicator organisms in water suggests fecal contamination from an outside source. The coliform group of bacteria is the most commonly used indicator of contamination. These microorganisms include all aerobic and facultatively aerobic, gram-negative, non-spore forming, rod-shaped bacteria. They also ferment lactose within 48 hours at 35^{o}C. Fecal coliforms are a subset of the total coliform group and include only those species that are present in the intestinal tracts of warm-blooded animals. The fecal coliform group includes *E.coli* and *Klebsiella pneumoniae.* Since it is possible that *K.pneumoniae* can come from other sources besides fecal waste (such as textile waste), *E.coli* is most often used to indicate fecal contamination (Madigan et al., 2000).

E.coli is ever-present in the normal flora of all warm-blooded animals, including humans. Fecal excrement from these animals will always contain this bacterium and is the major source of *E.coli* present in streams and rivers. Two distinct categories exist as sources for bacteria: point and non-point.

Point sources include any source that expels untreated waste directly into a waterway. In most instances, this is due to direct and purposeful introduction of human sewage, sewer equipment malfunction, planned dry weather bypasses, and discharge of storm runoff combined with untreated wastewater as combined sewer overflows (CSO) (O’Shea and Field, 1992). Potential discharge into the Buffalo River AOC could come from any or all of the 38 combined sewer overflows located in the area. The CSO is usually the most significant source of bacteria but there are also smaller point sources present. These include storm sewers, discharges from boats on the waterway, and faulty septic tanks (Brosnan and O'Shea, 1996). Many of the small towns in the upper reaches of the Buffalo River watershed do not have a public sewer system and are dependent on septic tanks for human sewage disposal.

Non-point sources of pollution result from land runoff, precipitation, atmospheric deposition, drainage, seepage, or hydrologic modification. It has been estimated that one-third of the pollutants entering waterways are from non-point sources (Khaleel et al., 1980). The upper reaches of the Buffalo River watershed are located in agricultural and forested areas. Water runoff from these agricultural lands could potentially introduce fecal coliforms into the waterway.

Water runoff is a major contributor to bacterial loading of streams. Land used for agricultural purposes has a strong impact on bacterial loading. A study in Derbyshire England showed a correlation between bacterial concentrations in streams and densities of sheep on adjacent land (Hunter et al., 1999). The results showed that lowest levels of fecal bacteria were present in the winter months and the researchers attributed this to the lack of sheep stocking on adjacent land. Grazing animals on lands adjacent to streams will deposit fecal material onto the land and potentially into the stream itself. The use of manure to enrich cropland introduces bacteria into the water system and different grazing strategies will also affect the water quality (Tiedemann et al., 1988).

It has been shown that bacteria can move through the soil profile during saturated conditions (Rahe et al., 1978; Francy et al., 2000). In periods where there is heavy rainfall, the bacteria will leach from the surface layer of the soil into the lower levels. Once incorporated into the deeper levels, the bacteria will continue to move down slope eventually ending up in the groundwater and streams.

Even in environments without pastureland, bacteria can be introduced into the waterway. This occurs due to the presence of wildlife. Waterfowl can migrate through an area and stop at a water source. When this occurs, they leave behind large amounts of fecal excrement (Hussong et al., 1979). This shows that it is nearly impossible to obtain an environment without the presence of fecal bacteria.

In addition to waterfowl, other wildlife can contribute to bacterial loading of streams. An analysis of Northern Virginia's Four Mile Run Watershed (Simmons et al., 2001) indicated that 45% of the bacteria in the waterway came from avian sources while 55% came from nonhuman mammalian sources. Of these mammalian sources, 33% came from raccoons and another 33% came from deer. The remainder of the sources included canine, rat, feline, opossum, beaver, and muskrat. These results were determined by analyzing the DNA that was extracted from fecal samples collected in the watershed and comparing it to known sources in several DNA libraries.

High *Escherichia coli* levels in streams and rivers potentially can have an impact on human health. *E.coli* is capable of causing health problems in humans including bloody diarrhea and kidney failure (Madigan et al., 2000). If waterways are contaminated by *E.coli* and are used for recreational purposes (such as boating, swimming, or fishing) there is the potential risk for infection. The bacterium enters the human through mucosal membranes as well as skin wounds. The quantity of bacteria in the waterway may be directly related to the potential health impacts of the humans using the water system.

Harrington et al. (1993) performed a study to determine if there was a correlation between the number of fecal coliforms and the health of surfers in Sydney. Approximately 2000 people were involved in this study and they recorded their general health after their exposure to the water. Fecal coliform levels were also monitored during the study. A correlation between incidences of illness and elevated bacterial levels was shown.

Not many studies have attempted to correlate exact water temperature with the number of *E.coli* or fecal coliforms present. The usual method was to assume that in winter, the temperature of the waterway would be lower than that in the summer. In sampling between these seasons, several reports have indicated that the quantity of bacteria present in the winter were higher than in the summer (Goyal et al., 1977). Goyal et al. (1977) assumed that this was due to the elevated die off rate of fecal coliforms with increased temperature. This study was performed in canal communities along the Texas coast. The waters in this area ranged in temperature from $9.0^{o}C$ to $31.0^{o}C$ during this study. Due to this range in temperature, the bacteria were never subjected to extreme cold, which would also affect the die off rate. Therefore, even though the water way is used more in the warm weather months, the levels of bacteria are lower at that time based on the location of the water.

A study of the Buffalo River watershed was conducted to determine the levels of indicator bacteria present. During this study, which ran from the summer of 1992 to the winter of 1993; temperatures ranged from $0^{o}C$ in the winter to $23.9^{o}C$ in the summer. While levels peaked in the summer

Table 1. Bacterial Sampling Locations

Site No.	Latitude	Longitude	Waterway	Description	Municipality
1	42^o51'42.4"N	78^o52'2.3"W	Buffalo River	Bridge on Ohio Street	Buffalo
2	42^o51'49.4"N	78^o49'15.8"W	Upper Buffalo River	Bridge on Seneca Street	Buffalo
3	42^o51'34"N	78^o49'20.3"W	Cazenovia Creek	Bridge on Southside Pkwy.	Buffalo

months, this study showed that the mean level of fecal coliforms remained relatively steady throughout the year (Irvine and Pettibone, 1996).

The quantities of fecal bacteria in the sediments of a stream have been shown to be higher than in the overlying water layer (Loutit and Lewis, 1985; Burton et al., 1987; Sherer et al., 1992). An increase in the water flow rate will also increase the turbidity of the stream. The bottom sediments are stirred which releases bacteria into the water. The sediments in the Buffalo River are comprised mostly of silt and clay (65-99%) with the remainder being sand (US Army Corps of Engineers District (USACE) 1993). Due to the small size of silt and clay, they are easily resuspended into the water column. The river is also subjected to yearly dredging by the USACE, which allows for the resuspension of bacteria from the sediments. This river is often used by ships and their passage though the river has been shown to increase the levels of suspended sediment and fecal coliforms in the waterway (Pettibone et al., 1996).

Previous studies of the river have shown that mean fecal coliform levels were frequently above the state guidelines. Between the summer of 1992 and the spring of 1993, 277 samples were collected from the watershed (Irvine and Pettibone, 1996). The results of this study showed that 69% of the samples exceeded the state guidelines for fecal coliforms. New York State has a guideline for primary contact water not to exceed 200 colony-forming units $100ml^{-1}$. Strictly speaking, this guideline does not apply to the Buffalo River watershed because there are no designated beaches within the area. This guideline will be used for this research (although technically incorrect), because residents have been known to use the waterways for recreation.

METHODS

The Buffalo River Watershed, in Western New York, drains an area of 1155 km^2 (Figure 1). This watershed extends over two counties (Erie and Wyoming) and has a variety of land uses including several small communities and agricultural land. Three major streams feed into this watershed: Cayuga, Buffalo, and Cazenovia creeks. Flow rates of these streams are continuously monitored by the United States Geological Survey (USGS).

Water samples were collected at three sites within the Buffalo River watershed (Figure 1). Table 1 summarizes the sites sampled. Sites 1and 2 were located on the Buffalo River (lower and upper respectfully). Sample site 3 was located on Cazenovia Creek. Sites 2 and 3 were chosen to determine the amount of bacteria entering the river from non-point sources upstream of the City of Buffalo. Site 1 was chosen to determine the total quantity of bacteria leaving the river and the input from point sources. The USGS gage stations (Figure 1) provided information on flow rates for each of the three sites. Water samples were collected from March 24 2005 through April 10 2005. Samples were collected once daily from each of the three sites. A baler attached to a rope was lowered from the center point of the downstream side of the bridge at each location to collect water. The baler was allowed to drop just below the water surface and then brought back up to the bridge.

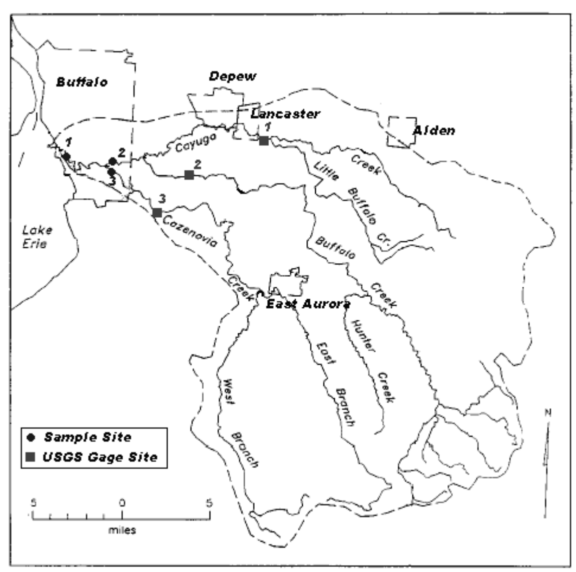

Figure 1. Map of Buffalo River watershed, sample sites, and USGS gage stations

The water in the baler was drained and a second sample was acquired in order to avoid cross contamination between sites. A digital thermometer was used to determine the temperature of the water in the baler. A sterile pipette was used to remove 1ml of water from the baler and the water was then deposited into the Easygel vial. The vials were labeled, placed on ice, and brought back to the lab for analysis.

The water samples were tested for the presence of *E.coli* using the Coliscan Easygel method. The liquid field sample was poured into the treated plates supplied by the Easygel Company. The media was allowed to set for 45-60 minutes and the plates were then inverted. The plates were allowed to incubate at room temperature for 72 hours before being examined. Dark purple colonies were counted and recorded as *E.coli*.

Average daily discharge rates for each site were determined using data provided by the USGS website. Discharge for site 1 was determined by combining the discharge from gages 1, 2, and 3. Discharge for site 2 was determined by combining gage 1 and 2 data (Figure 1). Discharge for site 3 was determined using data provided by gage 3. Since the gage sites are upstream from the sample sites, the discharge rates are approximate. There are additional drainage areas between the gages and the sites so the discharge rates used in this study are underestimated.

RESULTS

During the sampling period, a total of 56 samples (including duplicates) were collected. Table 2 gives a summary of discharge, temperature, and colony counts for each site including the mean and range of values. Mean discharge rates and CFUs were highest at site 1 while mean temperature was highest at site 3. Bacterial counts varied throughout the sampling period based on site and date. Figure 2 shows the number of *E.coli* colonies present per 100ml of water. Figure 3 shows the relationship between colony counts and temperature. Figure 4 shows the relationship between colony counts and discharge. No correlation was determined between temperature ($r = -0.22$) or discharge ($r = 0.036$) at a significance level of 0.05.

DISCUSSION AND CONCLUSION

Abiotic factors, such as temperature and discharge have been shown to affect the number of *E.coli* colonies present in a freshwater system. It has been shown that high water temperatures will decrease the number of fecal coliforms in the water column (Goyal et al., 1977; Nobel et al., 2004). This study was conducted in cold weather where the mean temperature was near 5.5^{o}C. Results of this study indicated that there was no correlation between temperature and the number of colonies present in the water ($r = -0.22$). Temperatures in the watershed ranged from 1.8-11.5^{o}C but there was not a marked change in the number of *E.coli* colonies present.

A lack of correlation was also seen in this study between the discharge rate and the number of *E.coli* colonies present ($r = 0.036$). A snow event occurred on the weekend of April 2nd (6-8 inches of precipitation) which increased the discharge rate at each of the sample sites. The number of *E.coli* colonies showed no response to this increased flow rate. The flow rates for the event of April 2nd would have had the ability to resuspend sediment bound bacteria had the bacteria been viable (Burton et al., 1987). Since there was a range in temperatures and discharge rates, the *E.coli* colonies should have shown a correlation. The explanation for why there was no correlation seems to be that the low water temperatures had a marked effect on the *E.coli*. It appears as though the water temperature never reached a high enough point to allow for the inoculation of the sediment with the bacteria that could later be resuspended.

Table 2. Summary of Discharge, Temperature, and CFU Results

Site No.	**Discharge (m^3s^{-1})**		**Temperature (^{o}C)**		***E.coli* CFU per 100ml**	
	Mean	**Range**	**Mean**	**Range**	**Geometric Mean**	**Range**
1	66.59	17.89 - 177.38	5.3	1.6 - 8.7	112.02	0 - 300
2	41.69	12.09 - 100.13	5.5	1.5 - 8.8	30.63	0 - 500
3	21.82	4.94 - 60.76	5.9	2.5 - 11.5	9.09	0 - 300

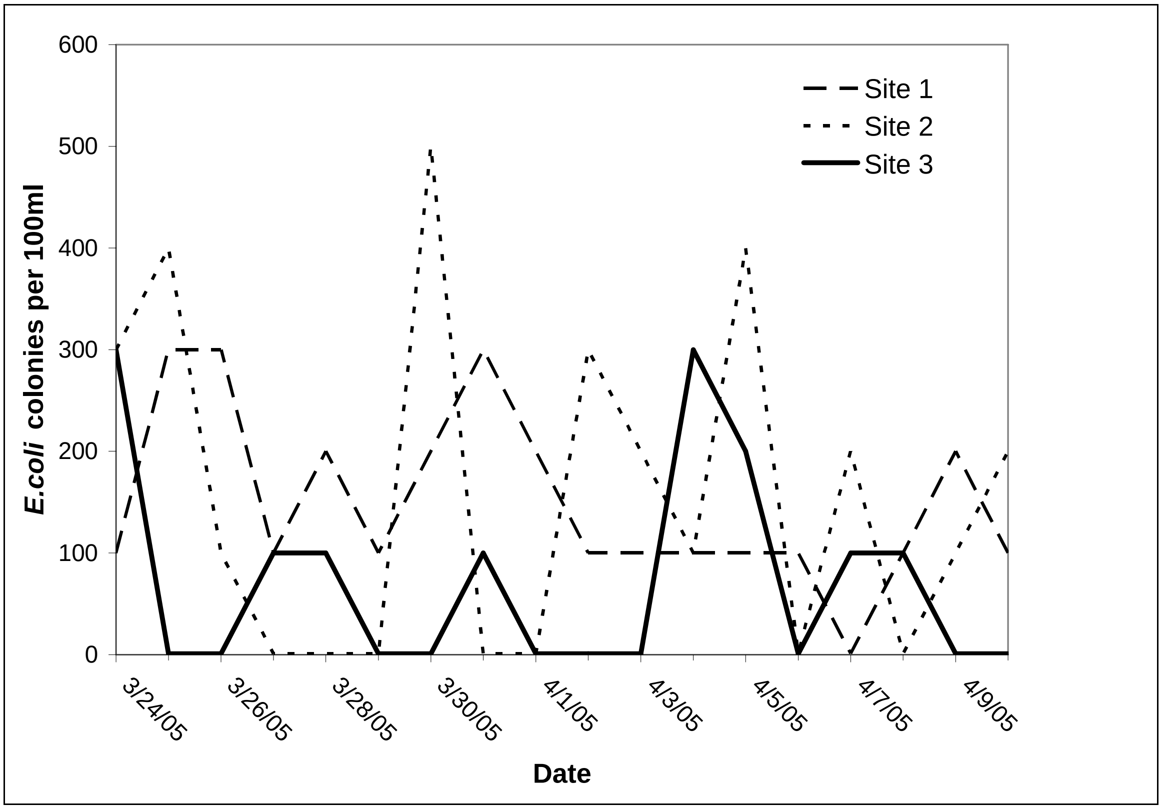

Figure 2. Time Series of colonies present by site

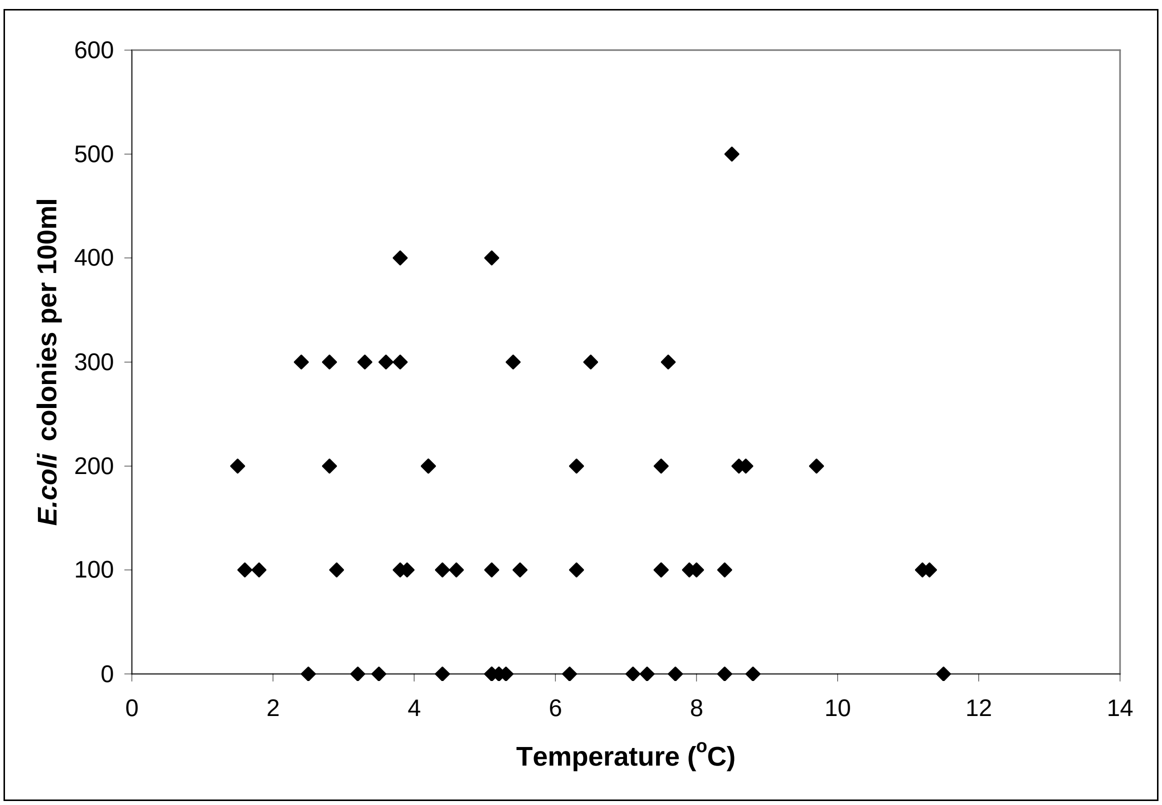

Figure 3. Number of *E.coli* colonies based on temperature (r = -0.22)

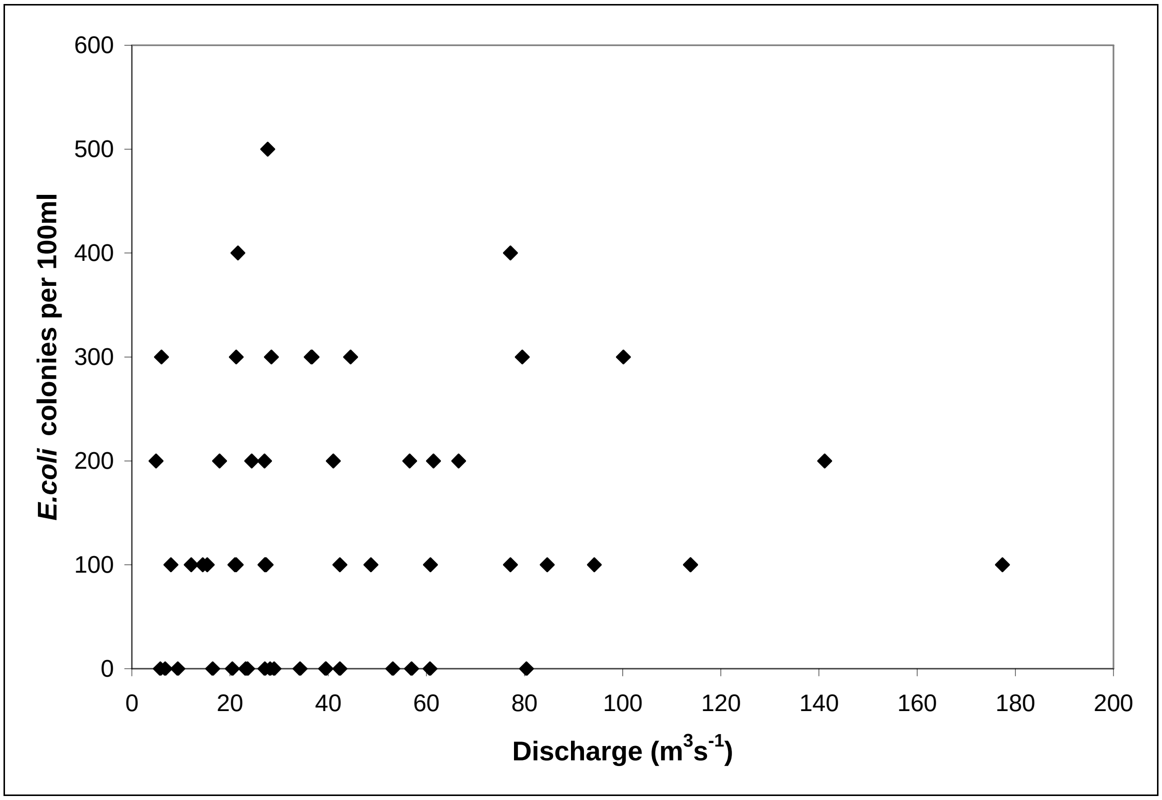

Figure 4. Number of *E.coli* colonies based on discharge (r = 0.036)

Previous studies of this watershed have shown that with warm temperatures, fecal colonies often exceeded state guidelines at the three sites. In an unpublished study, colony counts reached as high at 38,000 per 100ml during storm events. That study was conducted in the late summer and temperatures averaged around 20^{o}C.

Storm events were monitored within this watershed to determine the levels of fecal colonies present in 2000 (Irvine et al., 2005). Sample sites for that study included all three of the sites sampled in this study. The results showed that fecal coliforms ranged from 20 to 450cfu/100ml during times of low discharge. During summer storm events the levels increased and reached over 50,000cfu/100ml.

In this study, the geometric mean of *E.coli* colonies at each site varied based on location in the watershed. Sites 2 and 3, the two upstream sites, had geometric means of 30.63cfu/100ml and 9.09cfu/100ml respectively. The downstream site, Site 1, had a geometric mean of 112.02cfu/100ml. This is in contrast to a previous study of the watershed that indicated that fecal coliform levels decreased in the downstream direction (Irvine et al., 2005). That study was done during warm weather and storm events, which could account for the difference.

While not the usual method for *E.coli* enumeration, the Coliscan Easygel Method proved to be consistent in this study. Three duplicate samples were collected during this study and the results showed no marked difference. Of the duplicates, two showed a difference of 100cfu/100ml and one had zero difference.

Bacterial loading of stream sediments during cold weather conditions may be an important source of free bacteria during warm weather. Bacteria added to the system during cold weather could be stored in the sediments and then resuspended during times of high discharge. The storage and survival appears to be dependent upon water temperature. This study indicates that water temperatures near 5^{o}C are low enough to suppress the levels of *E.coli* and would not allow for storage and resuspension of the bacteria.

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