Introduction

Escherichia coli and *Enterobacteriaceae* bacteria

The state of Michigan contains over 11,000 freshwater lakes and has extensive shorelines on Lake Michigan, Lake Huron, and Lake Superior. These bodies of water are at risk for contamination by fecal coliforms, specifically the gram-negative bacterium, *Escherichia coli* (*E. coli*) (Department of Natural Resources 2007). *E. coli* is a bacilli-shaped, commensal bacterium existing as about 700 strains or forms usually harmlessly residing in the gastrointestinal tracts of humans, warm-blooded animals, and birds (Department for Health and Human Services 2008, Alabama Water Watch Program 2004). However, some strains, such as the O157:H7 *E. coli* strain found in undercooked meat, are highly pathogenic. Other harmful strains can cause severe diarrhea in healthy adults, lysis of red blood cells and kidney failure (hemolytic-uremic syndrome) in children, and even urinary tract infections and meningitis among women and newborns (The Merck Manuals: Online Medical Library 2003).

Both pathogenic and non-pathogenic bacteria are commonly found in the rod-shaped, anaerobic family *Enterobacteriaceae* (or "Entero family" in Fig. 1), and subdivided into coliform and non-coliform species. In Figure 1, the tree branch indicating "coliforms" include "fecal coliforms" that are bacteria from the gastrointestinal tracts of warm-blooded animals, such as *E. coli*, that can grow at 44.5˚C, and "non-fecal coliforms" that are free-living, benign microorganisms found in soil and water. Together, these "total coliforms" are used as a testing indicator for drinking water (Alabama Water Watch Program 2004, Ely 2006). Other genera in

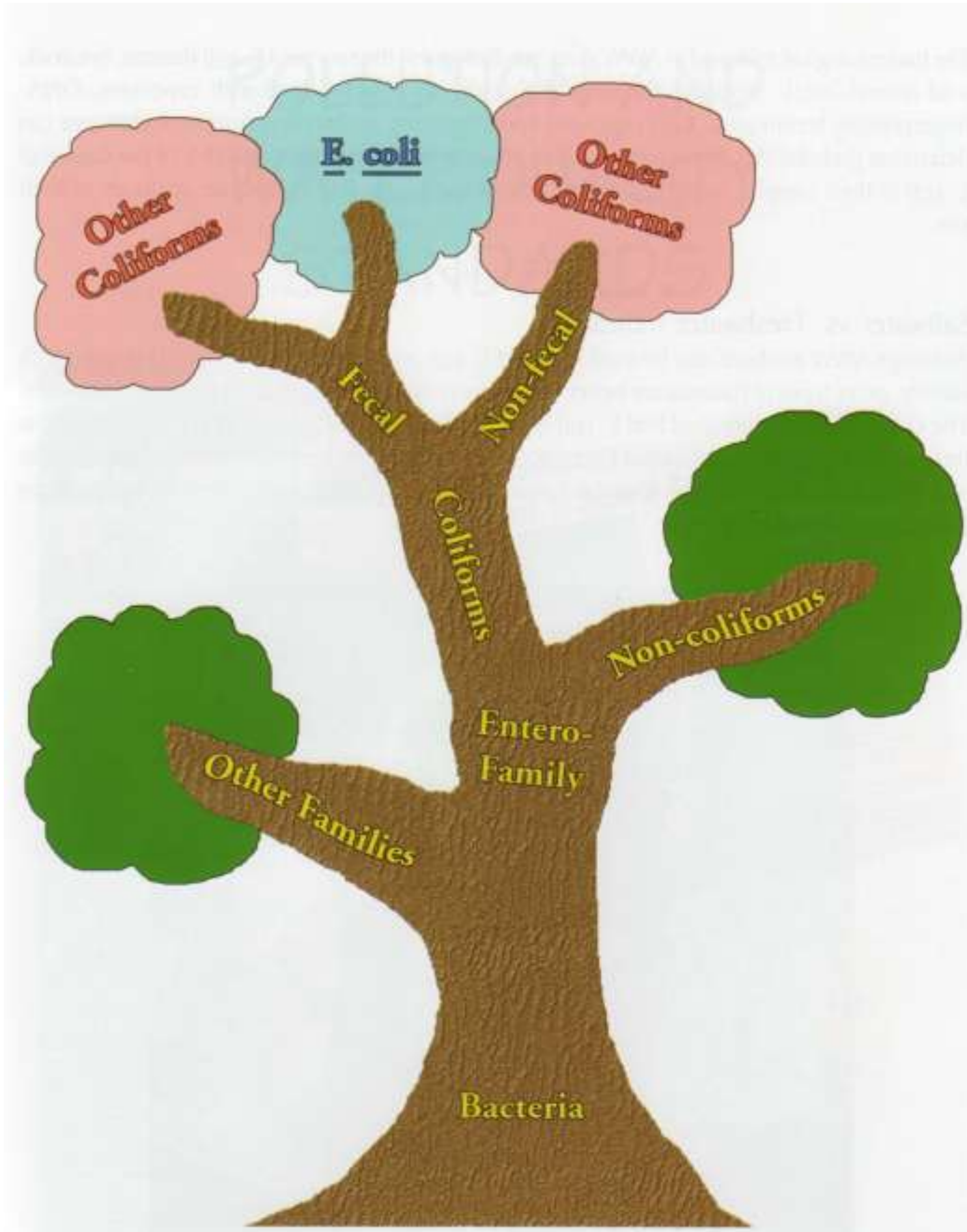

Figure 1. ***Enterobacteriaceae*** **Family Bacteria Tree (Alabama Water Watch Program 2004)**

the family which have more strains that are pathogenic, yet occur less commonly, than *E. coli*, are *Enterobacter*, *Klebsiella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, and *Shigella*, causing diseases such cholera, typhoid fever, shigellosis, salmonellosis, and gastroenteritis (Brock et al. 1986). Additional, more serious, waterborne pathogens include the hepatitis virus (*Cryptosporidium*) and protozoan parasites like *Giardia* (Ely 2006). Since it is impractical to test for each of these pathogens in drinking and recreational waters, the U.S. Environmental Protection Agency (U.S. EPA) determined *E. coli* as the best fecal coliform indicator for freshwaters since it has a strong correlation to other pathogenic bacteria that might also be present in the waters. As humans excrete an average of 20-200 billion *E. coli* each day, high counts in drinking and recreational waters could indicate possible contamination by other pathogenic bacteria that pose a health risk to humans (Alabama Water Watch Program 2004, U.S. EPA 2000).

Furthermore, *E. coli* can be rapidly washed from soils, groundwater, and septic systems and into rivers, streams, and lakes after large amounts of precipitation. Therefore, water sources can be contaminated by *E. coli* which can cause diseases, such as gastroenteritis, in individuals in direct body contact with the polluted water (U.S. EPA 2006).

E. coli water quality standards

The U.S. EPA has administered regulations for sampling, monitoring, and reporting an *E. coli* sample above the standard of 235 CFU (Colony Forming Units) per 100 mL water sample for recreational, ambient waters. Based on an average of at least five samples collected over a 30-day period, advisory signs are posted on beaches if the *E. coli* counts exceed 126 CPU per 100 mL. These standards were set based on the number (8) of approximate cases of gastroenteritis per 1000 recreational water users (Wisconsin Department of Natural Resources

2008). Furthermore, the U.S. Congress in 2000 passed the Beaches Environmental Assessment and Coastal Health Act (BEACH Act), requiring each U.S. state to set bacterial contamination monitoring standards on beaches (U.S. EPA 2002). For beaches in the Great Lakes region, these standards include *E. coli* counts of 0 to 234 CPU/ 100 mL to classify water as “Good” for swimming and allow beaches to remain open to the public. Counts of 235 to 999/ 100 mL result in an advisory of “Caution,” meaning increased risk for encountering pathogens with “full body submersion.” Counts higher than 1000 CPU/ 100 mL require beach closure to avoid potential illness resulting from swimming (Wisconsin DNR 2001, Kleinheinz et al. 2006). The BEACH Act was an addition to the Clean Water Act of 1972 that required all 30 coastal and Great Lake states to implement U.S. EPA’s recommendations for routine water monitoring and sampling (Ely 2006).

E. coli testing methods

There are several methods used to test for *E. coli*, the standard indicator of fecal contamination in ambient freshwaters. General methods include membrane filtration, pour plates, and multiple-well methods. The membrane filtration method involves a water sample to be drawn through a membrane filter that captures the bacteria and incubates in a Petri plate. Most useful for small concentrations of *E. coli* in water samples, three *E. coli* membrane filtration methods created and approved by the U.S. EPA are mTEC (“TEC” is “thermotolerant *Escherichia coli*”) (EPA Method 1103.1, Standard Methods 9213D), modified mTEC (EPA Method 1603), and the MI method (EPA Method 1604). Other membrane filtration methods include m-ColiBlue24 from Hach Company (also approved by the U.S. EPA), and Coliscan MF from Micrology Labs. Two methods especially useful for concerned and responsible citizens wanting to test lake waters involve the simple pour-plate methods, including Micrology Lab’s

Coliscan® Easygels® and the 3M Petrifilm *E. coli*/Coliform Count Plate, especially created for the food industry. However, these methods are not approved by the U.S. EPA. Finally, multiple-well methods include IDEXX Colilert Quanti-Tray (approved by the EPA) and IDEXX Colisure Quanti-Tray (not approved by the EPA), both of which are based on tube fermentation methods, are simple compared to more work-intensive methods, but are more expensive (Ely 2006).

In 2004 and 2005, a comparative study was done by volunteering monitoring programs in Iowa, Indiana, Michigan, Minnesota, Ohio, and Wisconsin of several testing methods for *E. coli*. Those who participated in the research utilized both "simpler" testing techniques like Coliscan® Easygels® incubated at 35°C and at room temperature, the 3M Petrifilm *E. coli*/Coliform Count Plate, and the "more technical" methods including Coliscan-MF and IDEXX Colisure and Colilert with Quanti-Tray/2000. Comparing their *E. coli* counts with results from the U.S. EPA's laboratory, the volunteers concluded that the most accurate and methodologically convenient testing techniques were IDEXX Colisure and Colilert, Coliscan® Easygels® incubated at 35°C, and 3M Petrifilm (O'Brien 2006).

The medium used to identify *E. coli* fecal coliforms in this research, Coliscan® Easygels®, is a chromogenic-containing medium with two sugar substrates, "Red Gal" (6-chloro-3-indolyl-ß-D-galactosidase) and "X-gluc" (5-bromo-4-chloro-3-indolyl-ß-D-glucuronide) (Ely 2006). These sugar substrates are linked to dyes used to detect fecal coliforms in water samples. The enzyme ß-galactosidase, found in all coliforms including *E. coli*, acts on the "Red Gal" substrate which causes the coliform colony to turn pink. The second substrate, "X-gluc," dyes bacterial colonies containing the enzyme ß-glucuronidase a blue-green color, such as in non-coliforms like *Salmonella* spp. or *Shigella* spp. Since the *E. coli* bacterium produces both

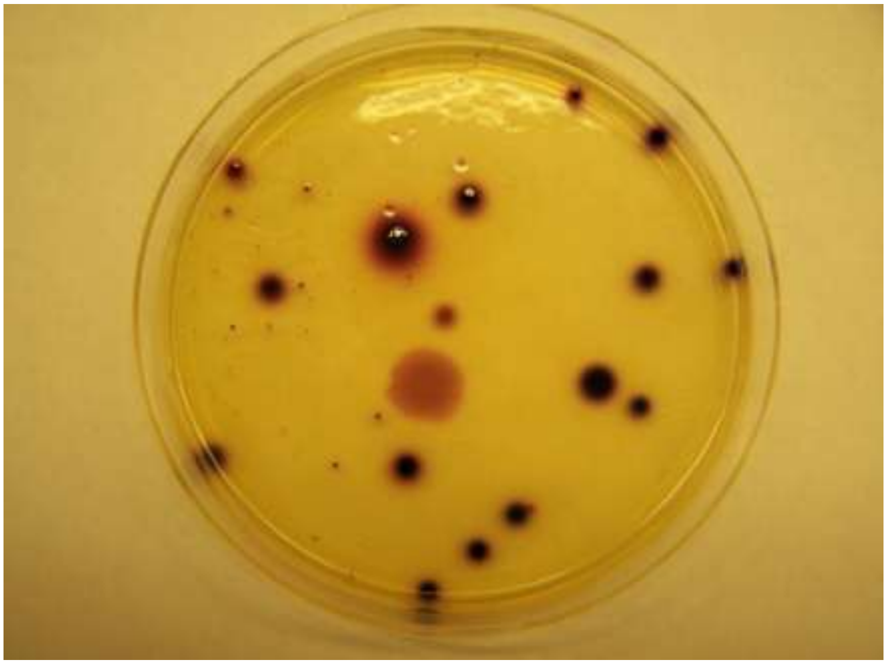

Figure 2. Sample Petri Plate of Coliscan® Easygel® with coliforms

enzymes ß-galactosidase and ß-glucuronidase (pink + blue), the Easygel® medium is able to differentiate *E. coli* colonies, which turn purple (Fig. 2), from other coliforms and non-coliforms, which turn pink or blue-green (Micrology Laboratories 2005, 2007). Coliscan® Easygel® is the only medium that detects both enzymes in *E. coli* and is more convenient and applicable to this study of several weeks in Northern Michigan.

E. coli contamination and sources

Studies have shown that *E. coli* counts are influenced by many sources including waterfowl and livestock waste, septic system effluent, and stormwater runoff (Meyer et al. 2005). This bacterium must be monitored during the summer swimming months and especially after rainfall when counts are usually high (Ishii et al. 2006, Van Donsel et al. 1967). According to previous research in temperate and Great Lakes regions, *E. coli* counts in lakes are at their peak during the summer months and are lowest during the winter (Ishii et al. 2006, Ksoll et al. 2007). Other research has found that fecal coliform counts can vary within a season and tend to be decreased on sunny days as opposed to cloudy days (Whitman et al. 2004). This may be verified by other studies noting the relation of rainfall with increased fecal coliform counts in lake waters, nearshore waters, sediment, and foreshore sand samples (Fong et al. 2007, Francy et al. 2006, Jeng et al. 2005, Kinzelman et al. 2004, Marsalek and Rochfort 2004, Meyer et al. 2005, Nevers and Whitman 2005, Scopel et al. 2006, Standridge et al. 1979, Van Donsel et al. 1967, Whitman and Nevers 2003). The reasons for increased *E. coli* counts influenced by rain runoff into the lakes are many. In areas especially heavily populated by waterfowl, it has been documented that rain runoff washes the bird feces (bird *E. coli* identified by PCR DNA fingerprinting) into nearby waters (Francy et al. 2006, Ksoll et al. 2007, Meyer et al. 2005, Standridge et al. 1979). Although some research argues that the commensal *E. coli*, unlike the other less common fecal coliform *Salmonella* spp., is not likely to live in nonhost environments outside of warm-blooded animals (Winfield and Groisman 2003), more current research notes the survivability and reproduction ability of *E. coli* strains in foreshore sands in the tropical, subtropical, and even temperate regions such as the Great Lakes areas (Hartz et al. 2008, Ishii et al. 2006, Kenzelman et al. 2004, Kon et al. 2007, U.S. EPA 2005, Whitman and Nevers 2003, Whitman et al. 2006). *E. coli* counts are a threat to water quality in the Great Lakes region and are influenced by stormwater runoff during summer storms and other factors like wind, wave height, and groundwater infiltrating sewer and septic systems (Fong et al. 2007, Kinzelman et al. 2004, Nevers and Whitman 2005).

During the summer of 2008, two related objectives were researched in Big Twin Lake, Oxbow Lake, and Starvation Lake: (1) baseline testing of *E. coli* counts on the lakes, and (2) a testing the impact of rain runoff on *E. coli* counts in the lakes. It was hypothesized that *E. coli* counts would be higher after rainfall when compared to the *E. coli* baseline data for each lake. The null hypothesis was that the *E. coli* counts in the lakes will not rise after rainfall.

Materials and Methods

Study Sites

Samples were collected from three lakes in the region of Mancelona, Michigan: Big Twin Lake, Oxbow Lake, and Starvation Lake (Fig. 3). From June 16, 2008 to July 30, 2008 there were eleven samplings completed at either two or three sites on each lake. At Starvation Lake (Fig. 3, 4) samples were collected at a resident's (Bob VanVynckt) dock at the south end (Site 1, S1, N44˚50.428', W084˚57.237') and at the public access on the lake's north end (Site 2, S2, N44˚50.986', W084˚56.579'). At Oxbow Lake (Fig. 3, 4), samples were taken from the East side of the lake (Site 1, O1, N44˚49.723', W084˚58.568') and the other off the dock in the lake's second basin (Site 2, O2, N44˚49.646', W084˚58.693'). At Big Twin Lake (Fig. 3, 5), the sample sites were off the public access dock (Site 1, BTL1, N44˚49.663', W084˚58.63'), the "causeway" beach near the Party Store and Little Twin Lake (Site 2, BTL2, N44˚49.191', W084˚58.204'), and off the Au Sable Institute (ASI) beach dock (Site 3, BTL3, N44˚49.390', W084˚57.458').

Starvation Lake (Fig. 4) is an oblong-shaped lake about 125 acres (50.6 hectares) in area with about 120 resident homes (J. Ross, personal communication, 2008). It ranges from approximately 5 to 47 m in depth (Michigan Interactive 1995). According to annual student reports conducted in the summer Limnology class at the ASI, Starvation Lake is classified as an oligotrophic to oligotrophic-mesotrophic lake, as defined by its low nutrient content, density of fish and plants, and deep depth (Fowler and Maple 2003). Students also have reported abundant Canadian geese on Starvation Lake, which are understood to contribute to several instances of high *E. coli* counts at certain sites. The State of Michigan's

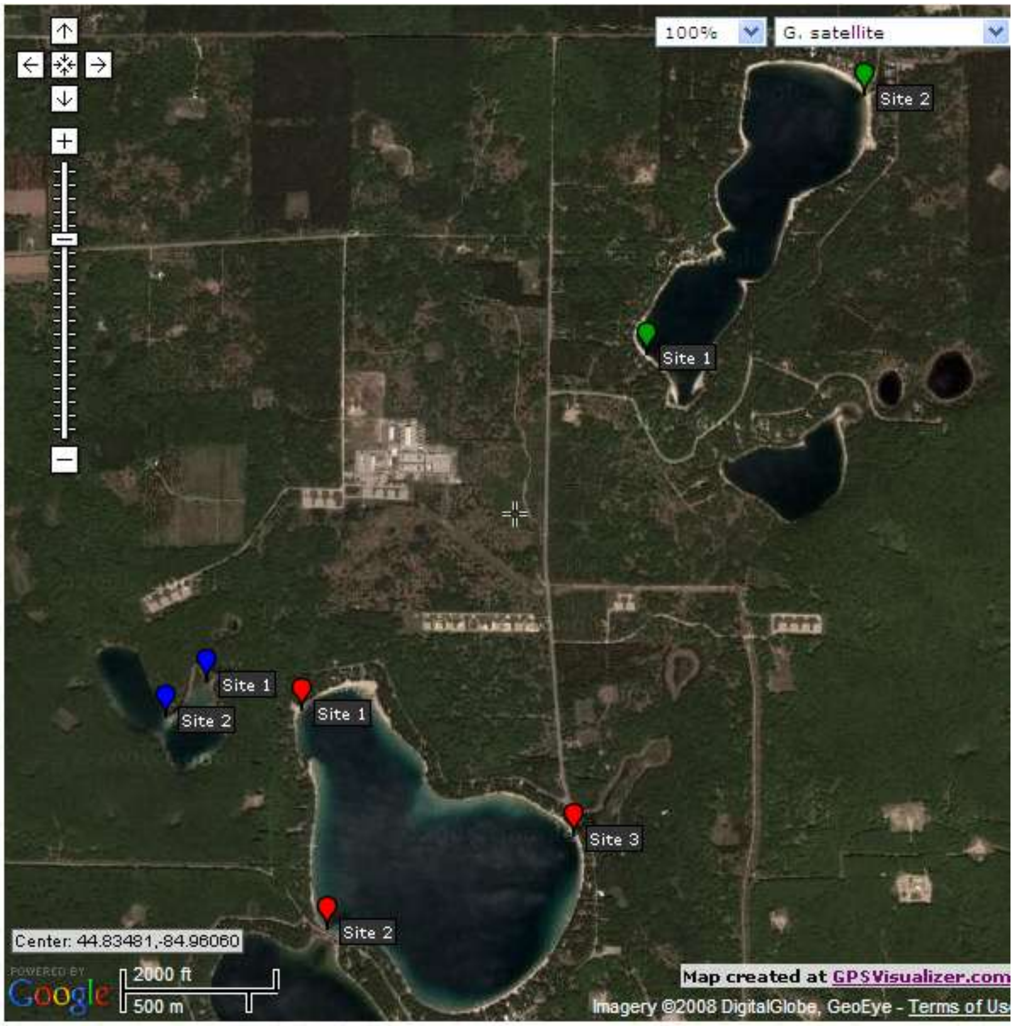

Figure 3. Sampling Sites at Lake Starvation, Oxbow and Big Twin (http://www.gpsvisualizer.com/map?output_google)

Figure 3 Legend:

Starvation Lake (green markers)

Site 1: residents' (VanVynckt family) dock (left side)

Site 2: public access (left side of dock)

Oxbow Lake (blue markers)

Site 1: East side

Site 2: Residents' (Southwell family) dock (left side)

Big Twin Lake (red markers)

Site 1: public access (left side of dock)

Site 2: Causeway (between Big Twin and Little Twin Lakes)

Site 3: ASI beach dock (right side)

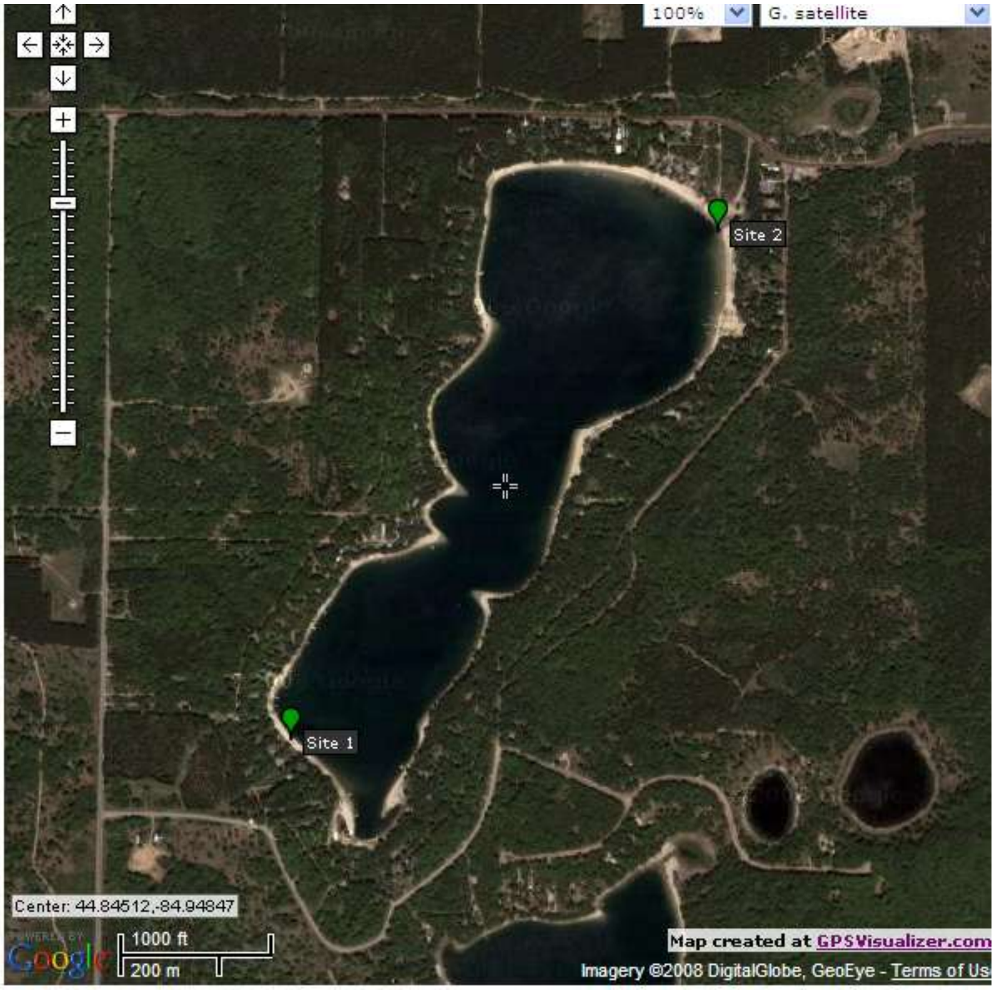

Figure 4. Sampling Sites at Starvation Lake
(http://www.gpsvisualizer.com/map?output_google)

Figure 4 Legend:
Starvation Lake
Site 1 (S1): residents' (VanVynckt family) dock (left side)
Site 2 (S2): public access (left side of dock)

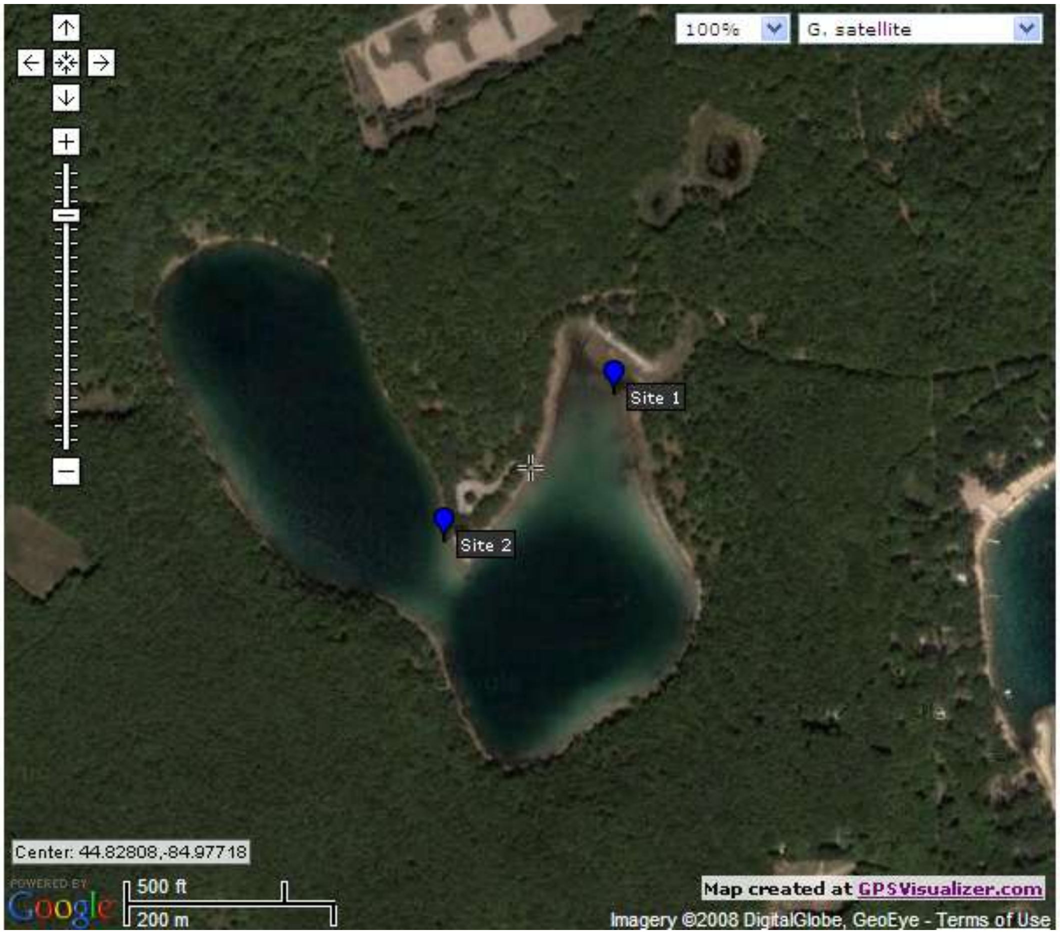

Figure 5. Sampling Sites at Oxbow Lake
(http://www.gpsvisualizer.com/map?output_google)

Figure 5 Legend:
Oxbow Lake
Site 1 (O1): East side
Site 2 (O2): Residents' (Southwell family) dock (left side)

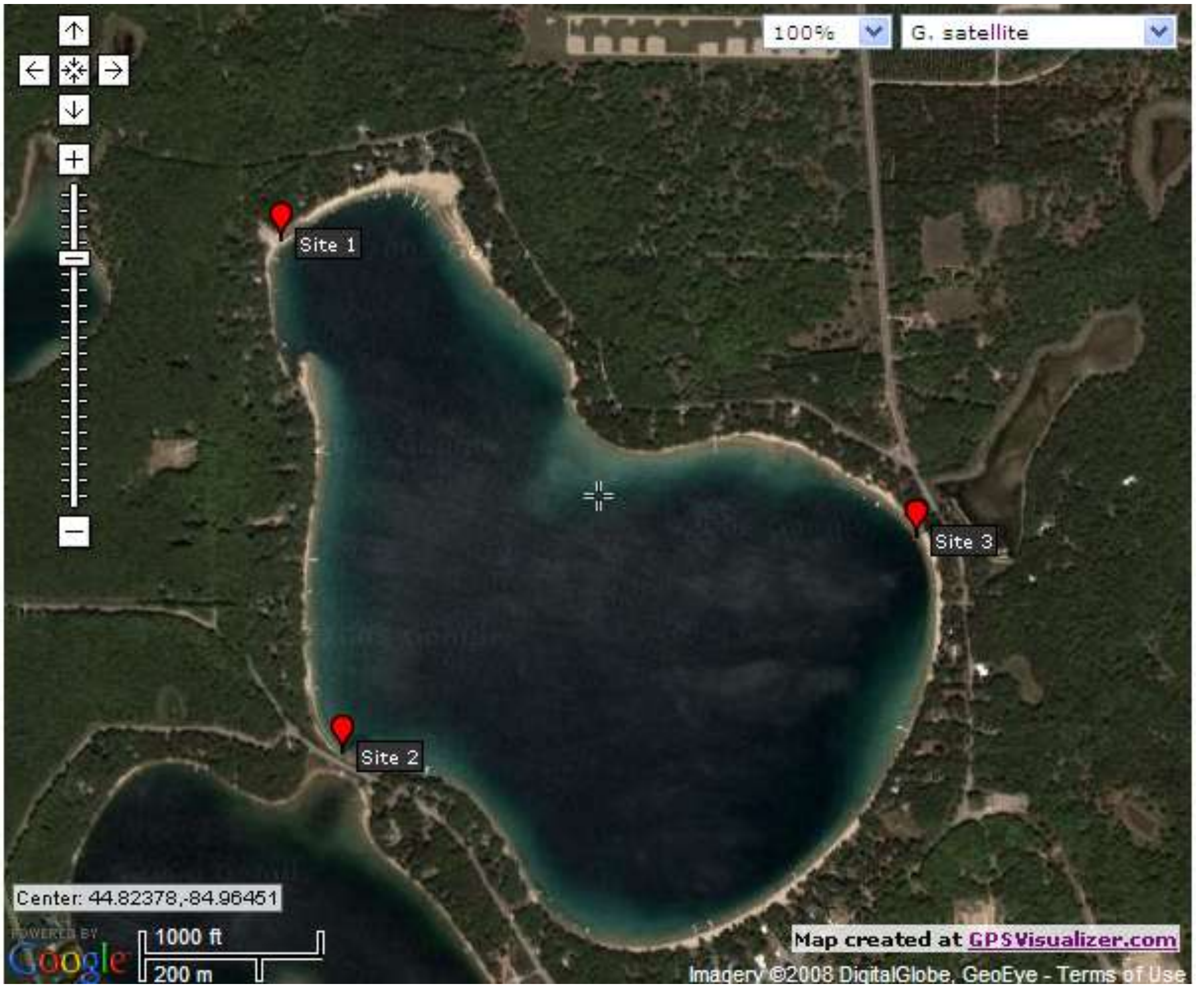

Figure 6. Sampling Sites at Big Twin Lake
(http://www.gpsvisualizer.com/map?output_google)

Figure 6 Legend:
Big Twin Lake
Site 1 (BTL1): public access (left side of dock)
Site 2 (BTL2): Causeway (between Big Twin and Little Twin Lakes)
Site 3 (BTL3): ASI beach dock (right side)

Department of Natural Resources has also noted Michigan's problem of nuisance geese that have ranged from numbers of about 9,000 geese in 1970 to 300,000 geese today. Nesting during March and April, Canadian geese are attracted to cultured landscapes, such as residents' homes on lake fronts, that provide grass shoots and aquatic vegetation (Department of Natural Resources 2007).

Oxbow Lake (Fig. 3, 5) is a small private lake with only one house. According to former student reports, Oxbow lake has been classified as primarily an oligotrophic to oligotrophic-mesotrophic lake due to its shallower depth (up to 10 m in one point) and greater concentration of algae, vegetation, and animals. The former lake owners (the Whelan family), former ASI students in 1998 and 1999, and researchers this year note that there is an increase of wildlife and waterfowl on and near the lake waters, as especially confirmed by waterfowl fecal matter on the walkways leading the to residents' dock noted this summer (Fig. 5, Site 2). Whereas *E. coli* counts were above the U.S. EPA standard of 235 CFU/ 100 mL in 1997 at Site 2 (Fig. 5), the counts were well below that standard at both Sites 1 and 2 the following year (Berkey et. al 1997, Barnett and Stutzman 1998). It has been suggested that the differences in counts between the years result from differing wildlife (geese, beaver, deer, etc) influence on the lakes, rather than the human influence, especially since the Whelan's had moved their septic field to a distant hill where septic effluent would have a minimum impact on the lake.

Understanding the patterns of animal life on Oxbow Lake is important in interpreting *E. coli* results, especially since erosion is an increasing problem on the lake, leading to more seepage runoff from contaminated soils into the lake. According to Barnett and Stutzman (1998), there are thirteen eroded areas on the west side of Oxbow Lake where nutrients wash into the lake from surrounding soils and watersheds. This increase of sediment and runoff into the lake might have been influenced by a 1923 fire that swept Oxbow's watersheds. Found to cause long-term effects, the fire burning piles of dry brush may have produced a heat intense enough to sterilize the soils from recovering its previous vegetation. In addition, the slopes next to the lake were primarily planted with young red oak trees that do not stabilize the sandy soils very well, as foraging of deer and beaver also contribute to the soil and nutrients washing into the lake after heavy rains (B. Barr, personal communication, 1998). The Southwell family now owns the Oxbow Lake property and treat it by the stipulations of the Grand Traverse Regional Land Conservancy to preserve the natural wildlife and lake conditions.

Big Twin Lake (Fig. 3, 6) is chemically and physically dissimilar from the isolated Oxbow Lake. It has a surface area of 215 acres (87 hectares), ranges from 10 to 80 m in depth, and is heavily populated with summer residents. Like most of the lakes in the Northern Michigan area, the lake is surrounded by nutrient-poor sand and has no outlet or inlet, so groundwater directly influences water quality. Also classified as an oligotrophic-mesotrophic lake for its deep waters and relatively low amounts of biota and nutrients, there have not been significant increases in *E. coli* at all three sites during the July month when annual student sampling was conducted over the years since 1998 (Girod and Stowell 2004).

Methods

Collection of Water Samples:

Three replicate water samples to establish a baseline were collected from each of the seven sites on the three lakes in 2008 for six weeks (Fig. 3) for a total of ten sampling days. Six days of weekly baseline sampling were conducted: June 20, 24, July 5, 10, 15, and 25. In order to test impact of runoff, four after-rainfall sampling days were chosen: June 16, July 3, 18, and 23 based on amount of rainfall (at least 0.3 inches) and time of the rainfall event (within 24 hours of the rainfall event). If a rainfall event was sampled for *E. coli*, then the baseline sampling for that week was conducted before the rainfall event had occurred (as predicted by Mancelona, MI weather on www.weather.com), or at least two days after the rainfall sampling. Previous research has shown that high concentrations of *E. coli* after rainfall do not begin to subside until three to seven days after the rainfall event (Jeng et al. 2005). Also, it was important to conduct the after rainfall sampling after the "first flush" or immediately after the first event of rainfall following a dry period (Alabama Water Watch Program 2004).

At each site, physical, chemical, and weather (wind and solar) conditions were obtained to compare to optimal growing conditions for *E. coli* bacteria. As recent research has shown *E. coli* surviving in interstitial sands and waters, the surrounding water pH, temperature, and nutrients can influence *E. coli* to conquer bacterial competition by living in environmentally and nutritionally optimal environments (Kon et al. 2007). Optimal growing conditions for *E. coli* include a pH of 6.0-7.0, a temperature of 37˚C, lower D.O. (dissolved oxygen) in the water, and little to no influence of UV rays (such as within sands) (Whitman et al. 2004, O'Neal and Hollrah 2007, Todar 2007). The level of pH was measured with a pH meter at each sampling site June 16, 20, and 24; however, lack of adequate equipment hindered further pH testing for the remainder of the study. Temperature (˚C) was also taken before collecting samples on each sampling date with a temperature electrode along with D.O. (Dissolved Oxygen). Wind speed at each of the sites was measured with a hand-held anenometer during the 4^{th}, 6^{th}-10^{th} sampling dates. As most collection times were conducted between 7 am to 2 pm (except for July 15 on Big Twin Lake), air temperature, amount of precipitation, predicted winds, and general weather conditions were recorded based on reports for Mancelona, MI weather on www.weather.com. Nine of the ten sampling times were completed before noon, as previous research has shown a decline in *E. coli* counts from samples collected in the afternoon or evening as opposed to the morning (Whitman et al. 2001). All sampling notes can be referred to in Appendix A.

Sampling containers with attached handles were thoroughly washed with bleach and rinsed with tap water before collection. In order to collect the samples at each of the sites, a sampling container was first washed two or three times with the water surrounding that site several feet out from nearshore in order to clear out the water residue from other sites. This same rinsing procedure also occured between each of the three replicates at each site. With a single pipet designated for use at each site, each 5-mL sample was collected from the nearshore water obtained with the sampling container and deposited directly into labeled and thawed Coliscan® Easygel® bottles. Each site's designated pipet was rinsed three times between replicate samples with the same rinsing waters as the sampling container. Upon collection, the Coliscan® Easygel® bottles were immediately placed on ice (4-10˚C) until being processed in the laboratory (Micrology Laboratories 2007).

Isolation and Colony Counts:

The 21 water samples (for each sampling time) in the Coliscan® Easygel® bottles were processed either immediately or within 1-2 hours, according to protocol by Micrology

Laboratories (2005) that recommends processing samples within 6 hours of collection. Upon arrival to ASI, the Coliscan® Easygel® bottles with sample mixtures were shaken, added to labeled Petri dishes, gently swirled, covered, and left at room temperature for approximately 30-60 minutes until the gel solidified. The solidified Petri dishes (Fig. 2) were then inverted and incubated at 35-38˚C for 32-48 hours (Hobson 2007, Micrology Laboratories 2005).

After incubation, the plates were inspected for coliform (pink or red, no smaller than a dot approximately 1 mm in diameter), fecal coliform (blue, purple, or blue-green, indicating *E. coli*), and total coliform (coliform + fecal coliform) colonies, as suggested by Micrology Labs protocol, Alabama Water Watch Program, and personal communication with Jonathan Roth, creator and patent holder of Coliscan® Easygel® (Micrology 2005, 2007, Alabama Water Watch Program 2004, J. Roth, personal communication, 2008). Since beginning at the 4th sampling date some fecal coliform colonies appeared teal or blue-green, personal contact with Jonathan Roth assured the classification of these colonies as *E. coli*. All these colony values were multiplied by 20 to determine the amount of coliforms per 100 mL. If the number(s) exceeded 200, the bacteria was considered as too numerous to count (TNTC) in statistical studies (Hobson 2007).

For further research to be conducted at ORU, selected fecal coliform isolates were preserved on a master plate and refrigerated (4-10˚C). Five *E. coli* colonies from each of the collection sites (Lakes Oxbow, Big Twin, and Starvation) were chosen and streaked (onto pre-poured Easygel® plates to verify the identification of the purple *E. coli* colonies and for further experimentation. These plates were incubated at about 35-37˚C to grow the colonies for further tests.

Data Analysis:

Average, standard deviations, and standard errors of the mean per 100 mL were calculated for all replicate samples, and graphs displaying counts per 100 mL and standard error of the mean error bars were created. With R Commander statistical software (Fox 2008), *E. coli*, general coliform, and total coliform counts per 5 ml sample were analyzed with the parametric One-Way and Two-Way Analysis of Variance (ANOVA) statistical test for the impact of rain on the counts. The ANOVA One-Way test was used to test the significance or impact of rainfall on *E. coli*, general coliforms, and total coliforms. The ANOVA Two-Way test was used to test for the significance of rainfall, the lakes themselves, and rainfall and the lakes together on *E. coli*, general coliform, and total coliform counts per 5 mL sample. The raw data (CFU/ 5 mL) were used in the statistical tests since there was less deviation among counts than with the counts at 100 mL. For both ANOVA tests, a 95% confidence level was used.

Results

Starvation Lake

Table 2 shows that coliform counts varied among the sites and lakes. At Bob VanVynckt's dock at Starvation Lake (Site S1), *E. coli* counts varied from 0 to 480 CPU/ 100 mL, general (pink) coliforms ranged from 0 to 2220 CFU/ 100 mL, and total coliforms from 0 to 2220 CFU/ 100 mL. At the second site on Starvation Lake at the public access (Site S2), counts for *E. coli* ranged from 0 to 100 CFU/ 100 mL, general coliforms from 20 to 1960 CFU/ 100 mL, and total coliform count was also 20 to 1960 CFU/ 100 mL (Table 1). Figure 7 displays the total coliform and *E. coli* counts, and Figure 9 shows total coliform data for both sites, indicating after rainfall sampling dates (6/24, 7/3, 7/18, 7/23). The highest *E. coli* counts that exceeded the 235 CPU/ 100 mL standard was only at Site 1 on an after-rainfall sampling date (Fig. 10).

Statistically, the One-Way ANOVA results in Table 3 show impact of rainfall specifically for raw (CPU/ 5 mL) *E. coli*, general coliform, and total coliform counts. The p-value for *E. coli* on Starvation Lake was 0.0256, verifying that rainfall significantly affects *E. coli* counts.

Table 2. Coliforms counts/100 mL grouped by sampling date and site.

Sample Date		Site S1			Site S2			Site O1			Site O2		
		E. coli	GC	TC	*E. coli*	GC	TC	*E. coli*	GC	TC	*E. coli*	GC	TC
6/16/2008	R1	0	100	100	0	20	20	20	140	160	20	40	60
	R2	0	120	120	20	120	140	20	660	640	0	100	100
	R3	0	0	0	0	40	40	20	420	440	0	80	80
	Average	0.00	73.33	73.33	6.67	60.00	66.67	20.00	406.67	413.33	6.67	73.33	80.00
	STD	0.00	64.29	64.29	11.55	52.92	64.29	0.00	260.26	241.11	11.55	30.55	20.00
	SEM	0.00	37.12	37.12	6.67	30.55	37.12	0.00	150.26	139.20	6.67	17.64	11.55
6/20/2008	R1	0	1900	1900	40	480	520	260	200	460	0	20	20
	R2	0	2220	2220	20	480	500	20	340	360	0	0	0
	R3	0	1840	1840	60	540	600	0	380	380	0	60	60
	Average	0.00	1986.67	1986.67	40.00	500.00	540.00	93.33	306.67	400.00	0.00	26.67	26.67
	STD	0.00	204.29	204.29	20.00	34.64	52.92	144.68	94.52	52.92	0.00	30.55	30.55
	SEM	0.00	117.95	117.95	11.55	20.00	30.55	83.53	54.57	230.94	0.00	17.64	17.64
6/24/2008	R1	0	880	880	0	200	200	0	720	720	0	340	340
	R2	0	1300	1300	0	1960	1960	20	340	720	0	60	60
	R3	20	1240	1260	20	1840	1840	80	500	560	0	80	80
	Average	6.67	1140.00	1146.67	6.67	1333.33	1333.33	33.33	520.00	666.67	0.00	160.00	160.00
	STD	11.55	227.16	231.80	11.55	983.33	983.33	41.63	190.79	92.38	0.00	156.20	156.20
	SEM	6.67	131.15	133.83	6.67	567.72	567.72	24.04	110.15	53.33	0.00	90.18	90.18
7/3/2008	R1	480	740	1220	40	1280	1320	60	660	720	1880	480	2260
	R2	220	400	620	20	760	780	40	220	260	1540	560	2100
	R3	360	900	1260	100	520	620	40	440	480	1200	240	1440
	Average	353.33	680.00	1033.33	53.33	853.33	906.67	46.67	440.00	486.67	1540.00	426.67	1933.33
	STD	130.13	255.34	358.52	41.63	388.50	366.79	11.55	220.00	230.07	340.00	166.53	434.66
	SEM	75.13	147.42	206.99	24.04	224.30	211.77	6.67	127.02	132.83	196.30	96.15	250.95
7/5/2008	R1	0	940	940	0	300	300	80	1920	2000	0	340	340
	R2	0	1200	1200	0	1000	1000	120	1420	1540	0	400	400
	R3	20	740	760	0	680	680	160	1060	1220	20	540	560
	Average	6.67	960.00	966.67	0.00	660.00	660.00	120.00	1466.67	1586.67	6.67	426.67	433.33
	STD	11.55	230.65	221.21	0.00	350.43	350.43	40.00	431.90	392.09	11.55	102.63	113.72
	SEM	6.67	133.17	127.71	0.00	202.32	202.32	23.09	249.35	226.37	6.67	59.25	65.66
7/10/2008	R1	0	480	480	0	560	560	180	920	1100	180	600	780
	R2	0	200	200	20	820	840	300	1260	1560	40	230	2340
	R3	0	440	440	20	980	1000	100	860	960	60	190	1960
	Average	0.00	373.33	373.33	13.33	786.67	800.00	193.33	1013.33	1206.67	93.33	340.00	1693.33
	STD	0.00	151.44	151.44	11.55	211.97	222.71	100.66	215.72	313.90	75.72	226.05	813.47
	SEM	0.00	87.43	87.43	6.67	122.38	128.58	58.12	124.54	181.23	43.72	130.51	469.66
7/15/2008	R1	0	140	180	0	800	800	120	220	340	140	120	260
	R2	40	180	220	20	540	560	140	420	560	80	180	260
	R3	0	180	180	0	1100	1100	20	400	420	80	360	440
	Average	26.67	166.67	193.33	6.67	813.33	820.00	93.33	346.67	440.00	100.00	220.00	320.00
	STD	23.09	23.09	23.09	11.55	280.24	270.55	64.29	110.15	111.36	34.64	124.90	103.92
	SEM	13.33	13.33	13.33	6.67	161.80	156.20	37.12	63.60	64.29	20.00	72.11	60.00
7/18/2008	R1	0	80	80	20	340	360	2340	820	3160	3160	3380	3480
	R2	0	120	120	20	680	700	2680	780	3460	3460	2060	2100
	R3	0	100	100	20	320	340	2200	800	3000	3000	1820	1860
	Average	0.00	100.00	100.00	20.00	446.67	466.67	2406.67	800.00	3206.67	3206.67	2420.00	2480.00
	STD	0.00	20.00	20.00	0.00	202.32	202.32	246.85	20.00	233.52	233.52	840.00	874.30
	SEM	0.00	11.55	11.55	0.00	116.81	116.81	142.52	11.55	134.82	134.82	484.97	504.78
7/23/2008	R1	0	220	220	20	360	380	20	220	240	0	180	180
	R2	0	180	180	20	320	340	100	420	520	20	60	80
	R3	0	140	140	20	320	340	140	240	380	0	140	140
	Average	0.00	180.00	180.00	20.00	333.33	353.33	86.67	293.33	380.00	6.67	126.67	133.33
	STD	0.00	40.00	40.00	0.00	23.09	23.09	61.10	110.15	140.00	11.55	61.10	50.33
	SEM	0.00	23.09	23.09	0.00	13.33	13.33	35.28	63.60	80.83	6.67	35.28	29.06
7/25/2008	R1	0	80	80	40	1680	1720	100	840	940	0	100	100
	R2	0	120	120	0	1440	1440	120	1320	1440	0	220	220
	R3	0	20	20	20	1740	1760	60	560	620	0	160	160
	Average	0.00	73.33	73.33	20.00	1620.00	1640.00	93.33	906.67	1000.00	0.00	160.00	160.00
	STD	0.00	50.33	50.33	20.00	158.75	174.36	30.55	384.36	413.28	0.00	60.00	60.00
	SEM	0.00	29.06	29.06	11.55	91.65	100.66	17.64	221.91	238.61	0.00	34.64	34.64

Table 2. (cont.)

Sample Date		Site BTL1			Site BTL2			Site BTL3		
		E. coli	GC	TC	*E. coli*	GC	TC	*E. coli*	GC	TC
<u>6/16/2008</u>	R1	20	20	40	40	160	200	440	240	680
	R2	20	20	40	20	0	20	280	100	380
	R3	20	20	40	0	20	20	300	140	440
	Average	20.00	20.00	40.00	20.00	60.00	80.00	340.00	160.00	500.00
	STD	0.00	0.00	0.00	20.00	87.18	103.92	87.18	72.11	158.75
	SEM	0.00	0.00	0.00	11.55	50.33	60.00	50.33	41.63	91.65
6/20/2008	R1	300	280	580	40	40	80	440	460	900
	R2	220	360	580	20	40	60	520	660	1180
	R3	200	700	900	80	0	80	540	600	1140
	Average	240.00	446.67	686.67	46.67	26.67	73.33	500.00	573.33	1073.33
	STD	52.92	223.01	184.75	30.55	23.09	11.55	52.92	102.63	151.44
	SEM	30.55	128.75	106.67	17.64	13.33	6.67	30.55	59.25	87.43
6/24/2008	R1	0	60	60	0	0	0	80	420	500
	R2	20	60	80	0	0	0	80	60	140
	R3	0	60	60	40	80	120	80	180	260
	Average	6.67	60.00	66.67	13.33	26.67	40.00	80.00	220.00	300.00
	STD	11.55	0.00	11.55	23.09	46.19	69.28	0.00	183.30	183.30
	SEM	6.67	0.00	6.67	13.33	0.00	40.00	0.00	105.83	105.83
<u>7/3/2008</u>	R1	20	420	440	560	820	1380	20	600	620
	R2	0	480	480	560	800	1360	20	360	380
	R3	20	380	400	680	760	1440	60	220	280
	Average	13.33	426.67	440.00	600.00	793.33	1393.33	33.33	393.33	426.67
	STD	11.55	50.33	40.00	69.28	30.55	41.63	23.09	192.18	174.74
	SEM	6.67	29.06	23.09	40.00	17.64	24.04	13.33	110.96	100.88
7/5/2008	R1	0	20	20	0	60	60	20	600	620
	R2	20	40	60	40	100	140	20	360	380
	R3	0	60	60	40	200	240	60	220	280
	Average	6.67	40.00	46.67	26.67	120.00	146.67	33.33	393.33	426.67
	STD	11.55	20.00	23.09	23.09	72.11	90.18	23.09	192.18	174.74
	SEM	6.67	11.55	13.33	13.33	41.63	52.07	13.33	110.96	100.88
7/10/2008	R1	0	60	60	0	40	40	0	20	20
	R2	40	60	100	0	20	20	0	40	40
	R3	0	120	120	0	0	0	0	0	0
	Average	13.33	80.00	93.33	0.00	20.00	20.00	0.00	20.00	20.00
	STD	23.09	34.64	30.55	0.00	20.00	20.00	0.00	20.00	20.00
	SEM	13.33	20.00	17.64	0.00	11.55	11.55	0.00	11.55	11.55
7/15/2008	R1	20	240	260	60	320	380	60	560	620
	R2	40	180	220	40	360	400	60	360	420
	R3	40	280	320	0	320	320	20	360	380
	Average	33.33	233.33	266.67	33.33	333.33	366.67	46.67	426.67	473.33
	STD	11.55	50.33	50.33	30.55	23.09	41.63	23.09	115.47	128.58
	SEM	6.67	29.06	29.06	17.64	13.33	24.04	13.33	66.67	74.24
<u>7/18/2008</u>	R1	20	240	260	0	160	160	120	80	360
	R2	20	80	100	0	100	100	100	60	320
	R3	0	160	160	0	120	120	60	40	160
	Average	13.33	160.00	173.33	0.00	126.67	126.67	93.33	60.00	280.00
	STD	11.55	80.00	80.83	0.00	30.55	30.55	30.55	20.00	105.83
	SEM	6.67	46.19	46.67	0.00	17.64	17.64	17.64	11.55	61.10
<u>7/23/2008</u>	R1	0	140	140	0	160	160	20	260	280
	R2	0	80	80	0	120	120	0	340	340
	R3	0	20	20	20	140	160	0	180	180
	Average	0.00	80.00	80.00	6.67	140.00	146.67	6.67	260.00	266.67
	STD	0.00	60.00	60.00	11.55	20.00	23.09	11.55	80.00	80.83
	SEM	0.00	34.64	34.64	6.67	11.55	13.33	6.67	46.19	46.67
7/25/2008	R1	0	280	300	0	40	40	100	160	260
	R2	0	700	700	0	0	0	0	80	80
	R3	20	240	260	0	60	60	0	40	40
	Average	6.67	406.67	420.00	0.00	33.33	33.33	33.33	93.33	126.67
	STD	11.55	254.82	243.31	0.00	30.55	30.55	57.74	61.10	117.19
	SEM	6.67	147.12	140.48	0.00	17.64	17.64	33.33	35.28	67.66

Key:
GC: General coliforms
TC: Total coliforms
R1,2,3: Replicates 1, 2, 3
STD: Standard Deviation
SEM: Standard Error of the Mean

All counts are coliforms/ 100 mL nearshore water sample.

BOLD and <u>UNDERLINED</u> sampling dates are AFTER RAINFALL sampling dates. All other dates are part of the weekly baseline.

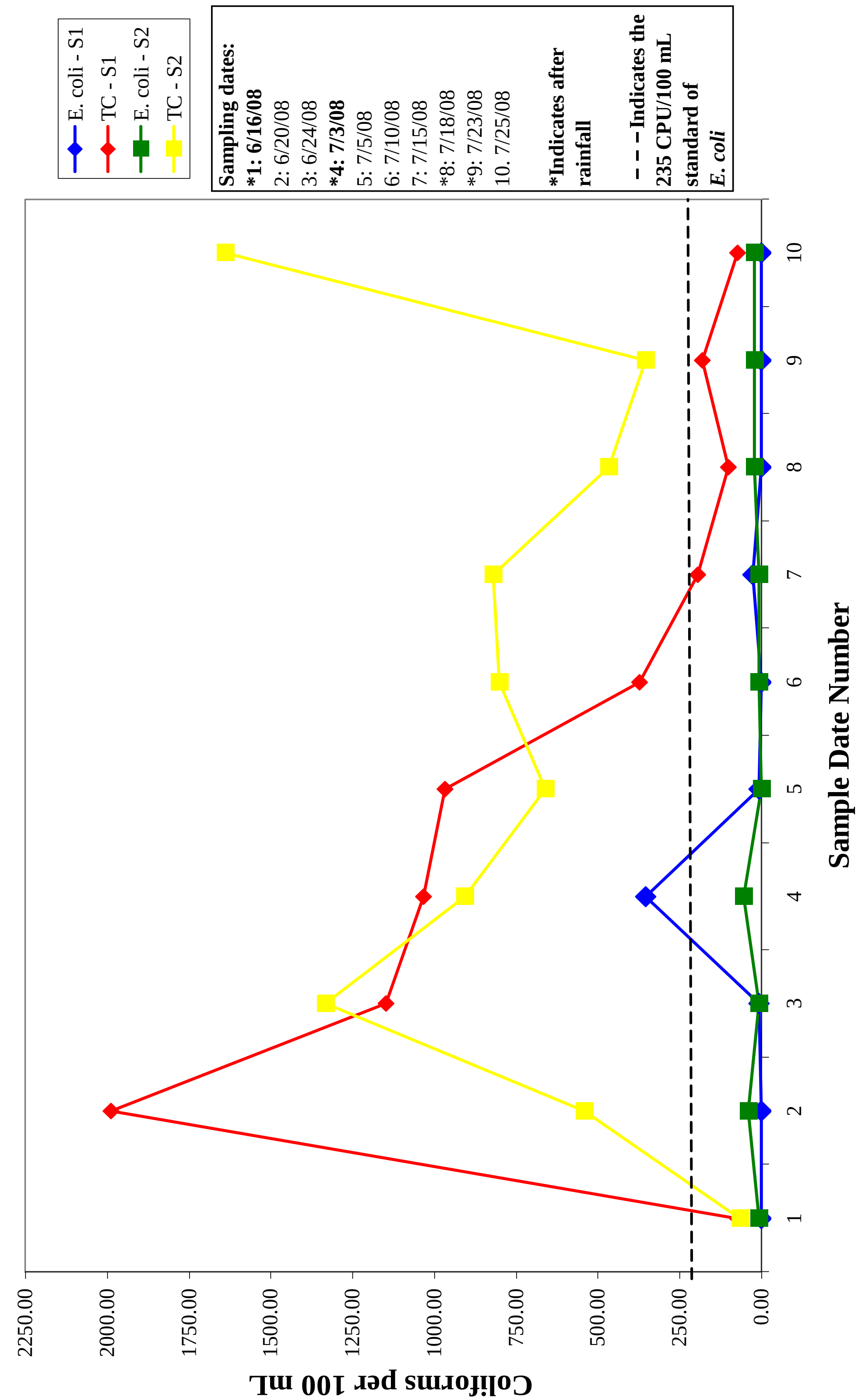

Figure 7. Total coliform and *E. coli* counts in Starvation Lake

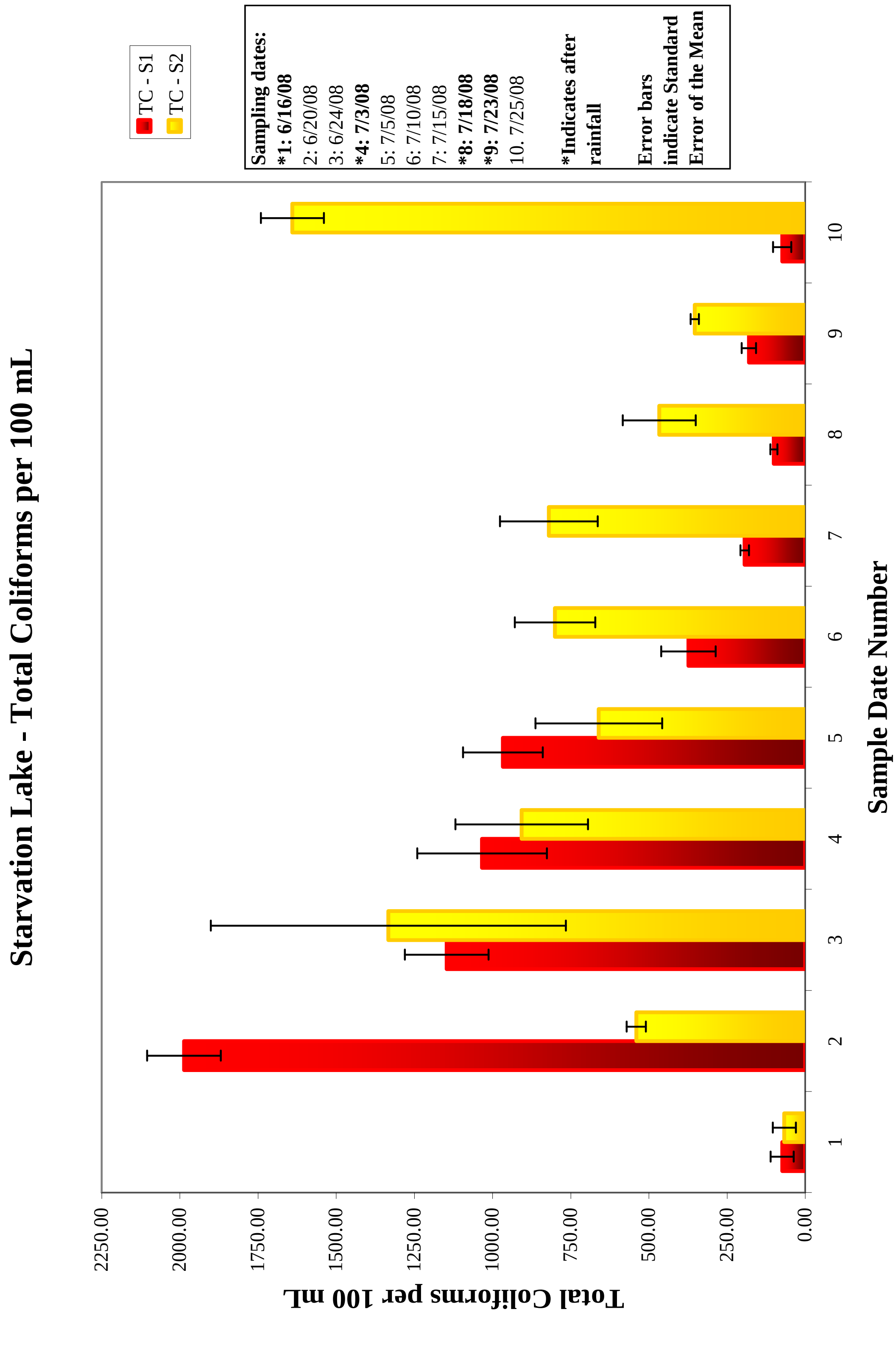

Figure 8. Total coliform counts in Starvation Lake

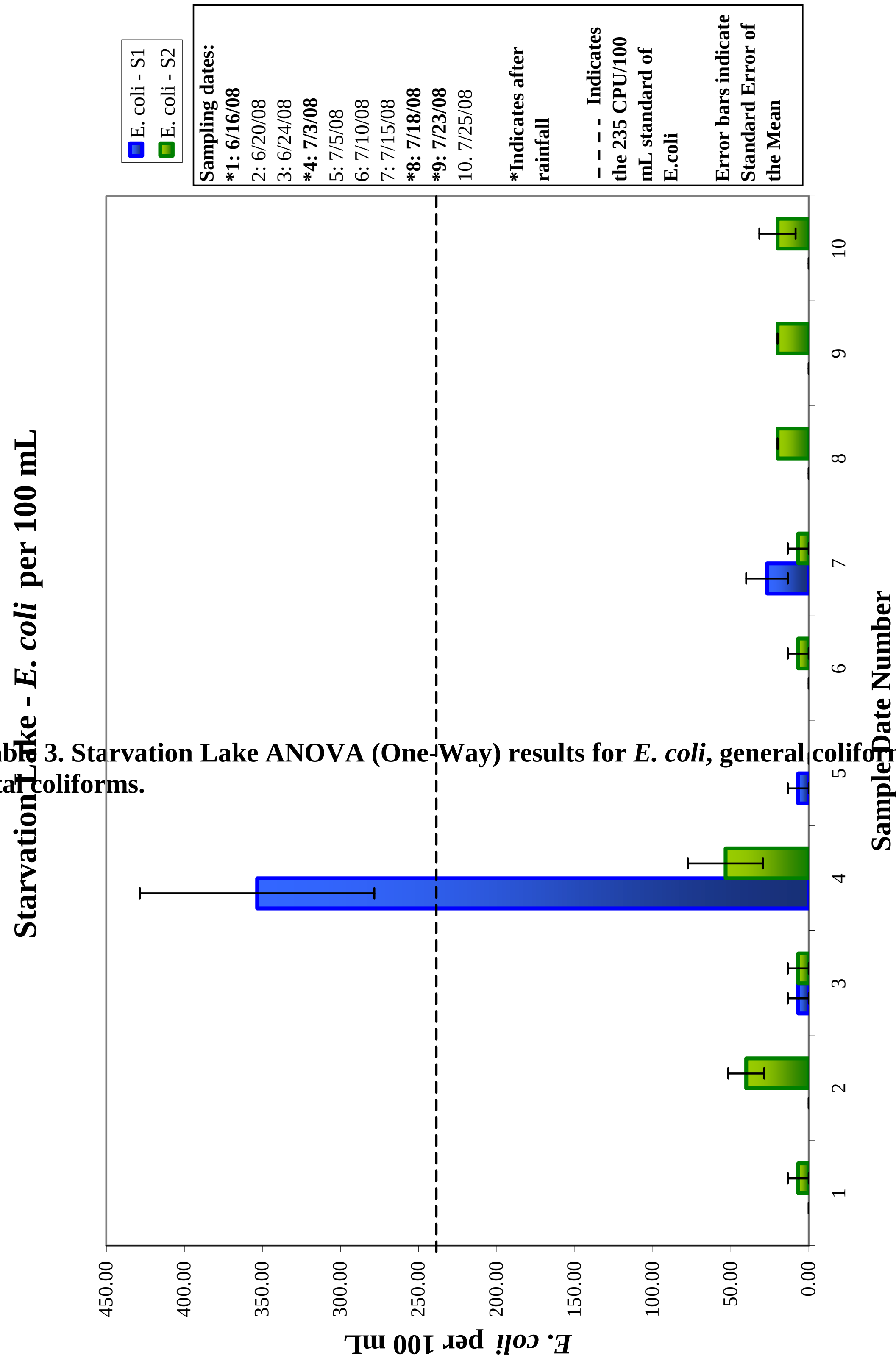

Figure 9. *E. coli* counts in Starvation Lake

Table 3. Starvation Lake ANOVA (One-Way) results for *E. coli*, general coliforms, and total coliforms.

Response: <u>E. coli</u> (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	80.28	80.28	5.2534	0.0256	*
Residuals	58	886.31	15.28			
		Rain			**No Rain**	
Mean		2.8333			0.4722	
Standard Deviation		6.1338			0.7741	
Count		24			36	
Response: General coliforms (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	9996	9996	14.277	0.00037	***
Residuals	58	40610	700			
		Rain			**No Rain**	
Mean		17.0417			43.3889	
Standard Deviation		16.1608			31.4427	
Count		24			36	
Response: Total coliforms (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	8304	8304	11.046	0.001544	**
Residuals	58	43604	752			
		Rain			**No Rain**	
Mean		19.8750			43.8889	
Standard Deviation		20.0311			31.3394	
Count		24			36	

Table 3 Key:	Significance codes:
Df: Difference	"***" = 0
Sum Sq: Sum of Squares	"**" = 0.001
Mean Sq: Means Square	"*" = 0.01
Pr (>F): P value	"^" = 0.1
Signif.: Significance	"#" = 1

Oxbow Lake

At Oxbow Lake, the first site on the East side of the lake (Site O1) had significant variances with *E. coli* ranging from 0 to 2680 CFU/ 100 mL, general coliforms from 140 to 1920 CFU/ 100 mL, and total coliforms from 160 to 3460 CFU/ 100 mL. Oxbow Lake's second site at the Stonewell's dock (Site O2), also demonstrated significant increases with counts varying from 0 to 3460 CFU/ 100 mL for *E. coli*, 0 to 3380 CFU/ 100 mL for general coliforms, and 0 to 3480 CFU/ 100 mL for total coliforms (Figs. 10, 11). It is significant to note that at the 7/18/2008 sampling date when the highest *E. coli* counts were recorded at both sites on Oxbow Lake, the highest counts for the general coliforms were also recorded at O2 (Table 2). Also, the highest *E. coli* counts that exceeded the 235 CPU/ 100 mL limit were sampling days after rainfall (7/3 and 7/18) (Fig. 10).

For the individual One-Way ANOVA for the significance of rainfall on the E. coli, general coliform, and total coliform counts, only the *E. coli* p-value was significant at 0.00014 (Table 4). The general coliform p-value was 0.407, and for total coliforms it was 0.053, showing no impact of rainfall on those counts.

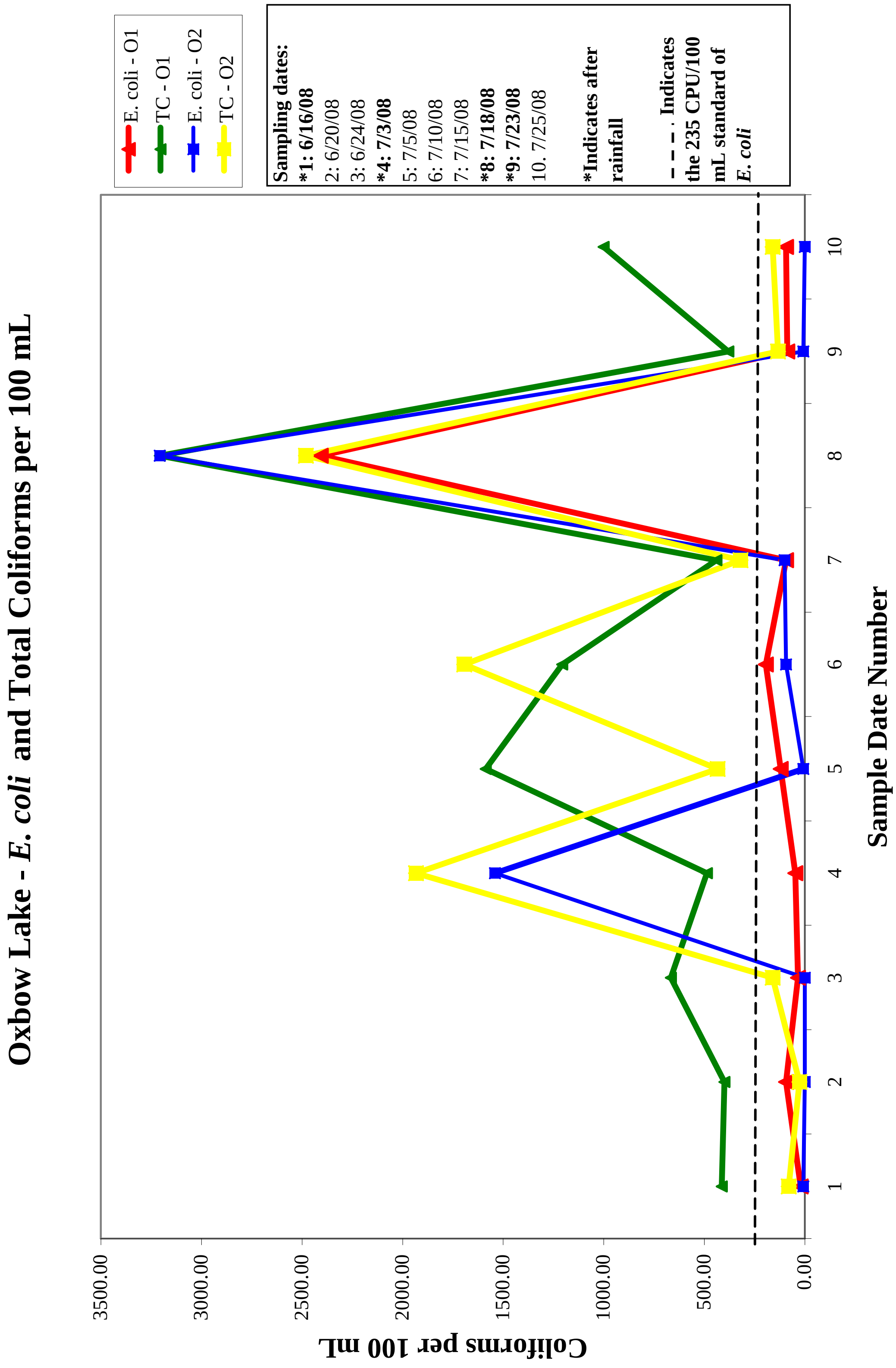

Figure 10. Total coliform and *E. coli* counts in Oxbow Lake

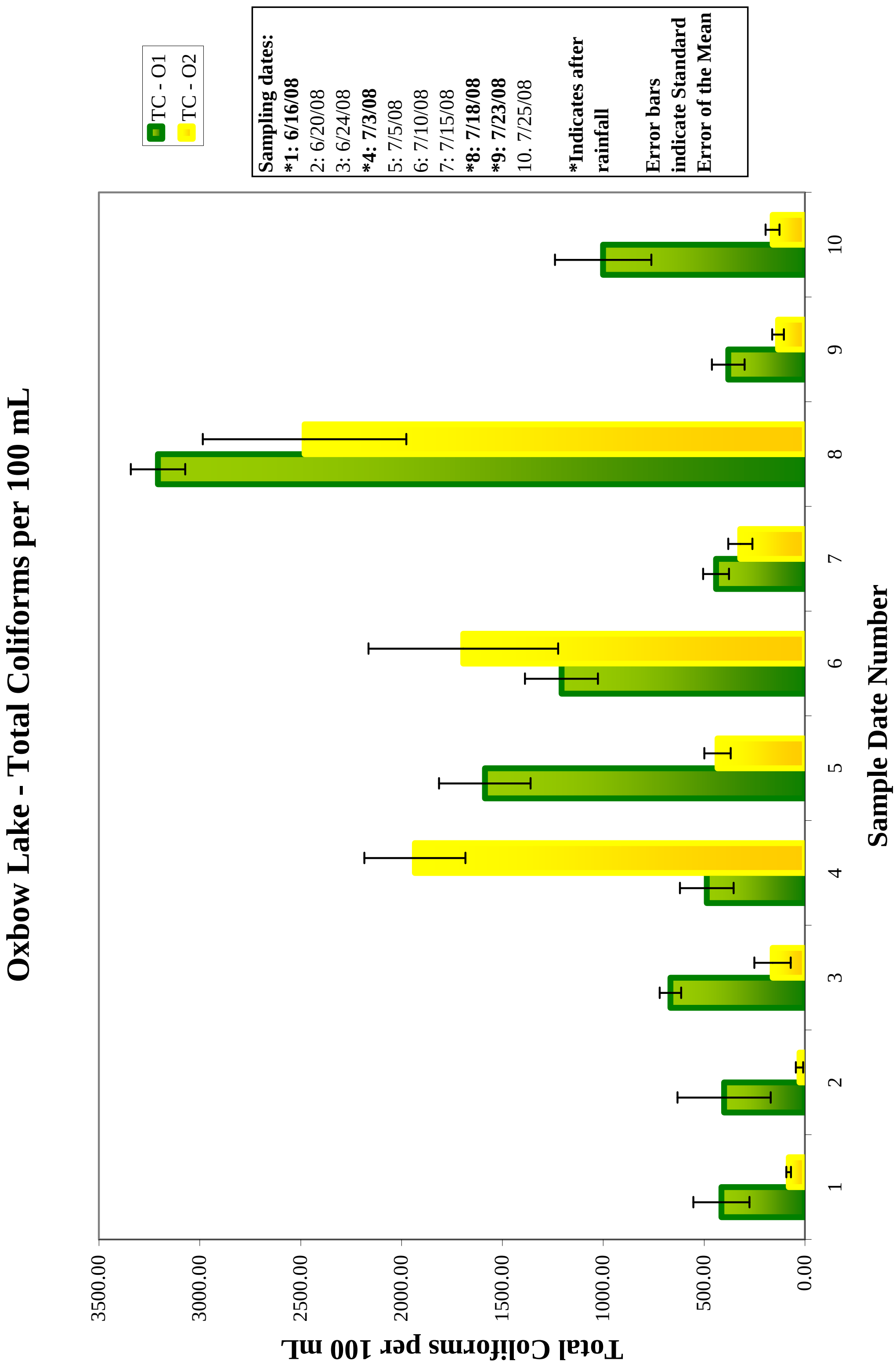

Figure 11. Total coliform counts in Oxbow Lake

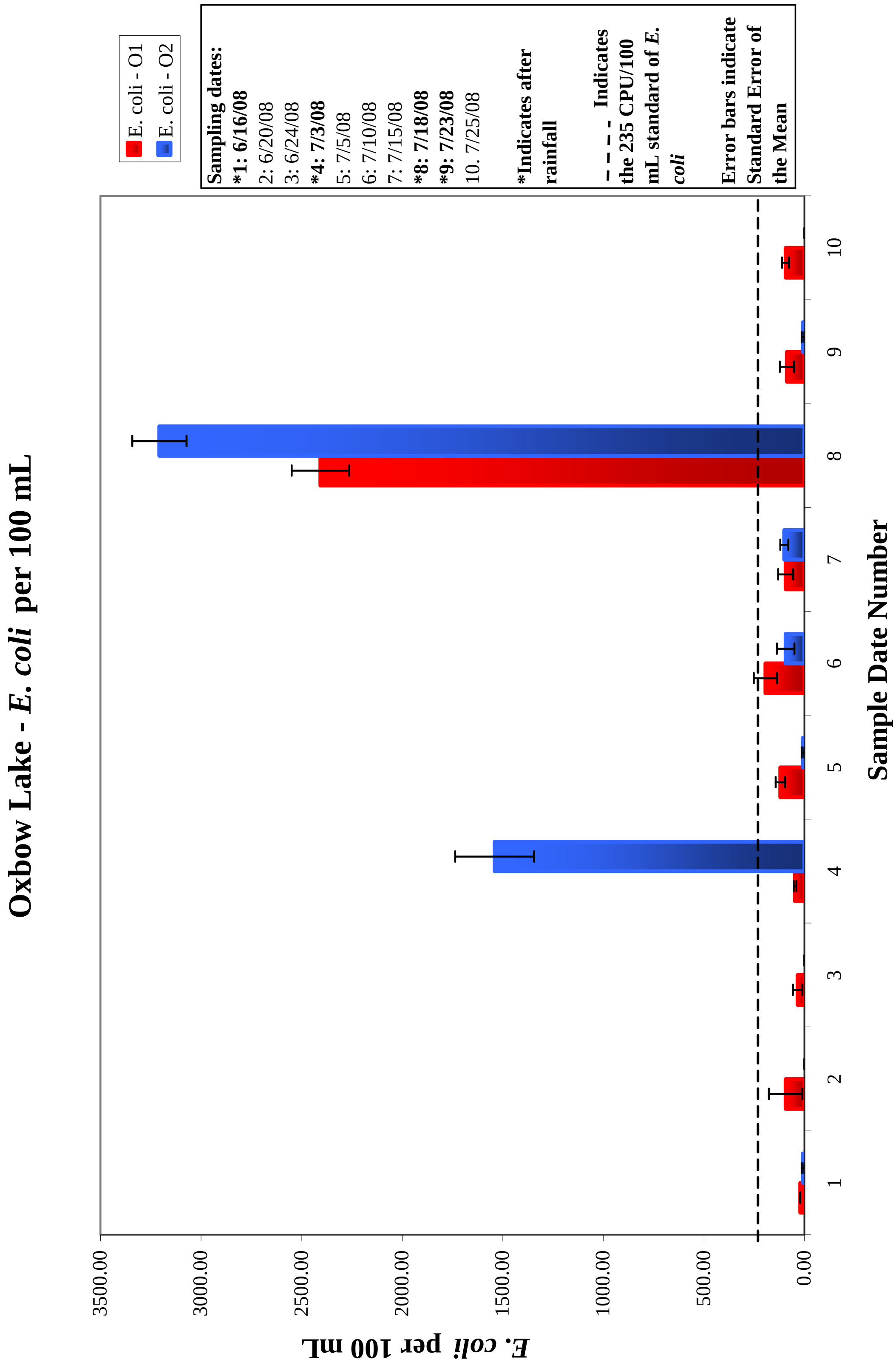

Figure 12. ***E. coli*** **counts in Oxbow Lake**

Table 4. Oxbow Lake ANOVA (One-Way) results for *E. coli*, general coliforms, and total coliforms.

Response: E. coli (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	25773	25773	16.627	0.00014	***
Residuals	58	89905	1550			
		Rain			**No Rain**	
Mean		45.7500			3.4444	
Standard Deviation		62.3338			3.9239	
Count		24			36	
Response: General coliforms (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	629	629	6988	0.4066	
Residuals	58	52242	901			
		Rain			**No Rain**	
Mean		31.1667			24.5556	
Standard Deviation		38.7900			22.4464	
Count		24			36	
Response: Total coliforms (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	7775	7775	3.8984	0.0531	^
Residuals	58	115674	1994			
		Rain			**No Rain**	
Mean		56.9583			33.7222	
Standard Deviation		60.2347			30.3434	
Count		24			36	

Table 4 Key:	Significance codes:
Df: Difference	"***" = 0
Sum Sq: Sum of Squares	"**" = 0.001
Mean Sq: Means Square	"*" = 0.01
Pr (>F): P value	"^" = 0.1
Signif.: Significance	"#" = 1

Big Twin Lake

At Big Twin Lake, the *E. coli* counts at the public access dock (Site BTL1) were 0 to 300 CFU/ 100 mL, general coliforms were 20 to 700 CFU/ 100 mL, and total coliforms were also from 20 to 700 CPU/ 100 mL. At Big Twin Lake's second site at the "causeway" (Site BTL2), *E. coli* counts ranged from 0 to 680 CFU/ 100 mL, general coliforms from 0 to 820 CFU/ 100 mL, and total coliforms from 0 to 1440 CFU/ 100 mL. As it was with Oxbow Lake, it is also significant to note that at the 7/3/2008 sampling date when the *E. coli* counts were high, the general (pink) coliforms were also similarly high (Table 1). Big Twin Lake's third sampling site at the ASI beach dock (BTL3) had *E. coli* counts ranging from 0 to 440 CFU/ 100 mL, general coliforms form 0 to 660 CFU/ 100 mL, and total coliforms from 0 to 1180 CPU/ 100 mL (Figs. 10, 11, 12). At the first and second sampling dates, E. coli counts were above the 235 CFU/ 100 mL standard at the ASI beach, as the first sampling date was after rainfall. The highest E. coli counts for Site 2, the causeway, were at the third sampling date, which was not after rainfall. The public access (Site 1) had the highest E. coli counts of 240 CPU/ 100 mL on the 2nd sampling date, which was also not after a rainfall event. During the remaining rainfall sampling days (7/3 and 7/18), there was no significant increase in E. coli in any of the three sites.

On Big Twin Lake, the *E. coli* p-value for significance was 0.3011 (Table 5), showing that rainfall is not a significant factor with *E. coli* counts. The general coliforms and total coliforms p-values are also not significantly impacted by rainfall.

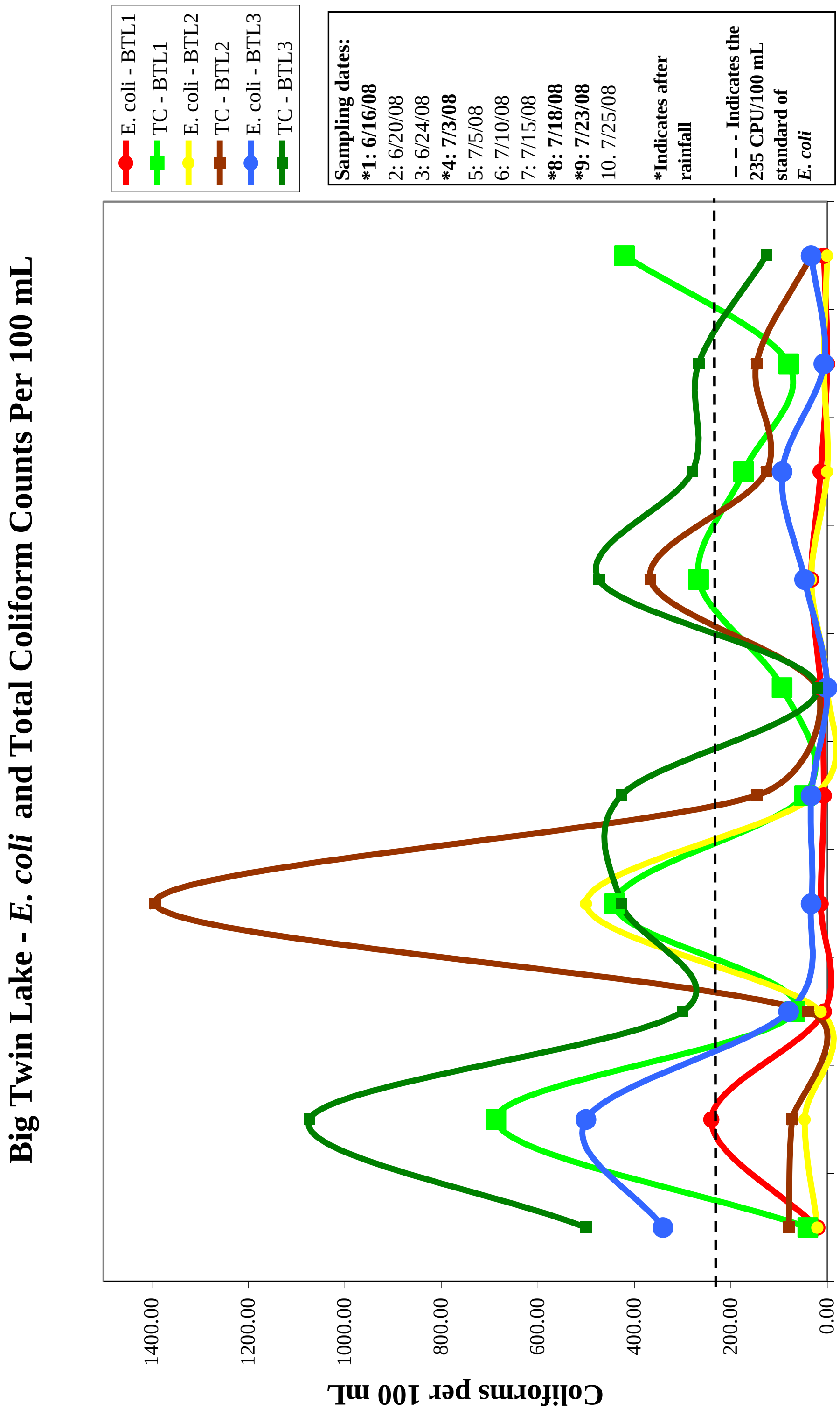

Figure 13. Total coliform and *E. coli* counts in Oxbow Lake

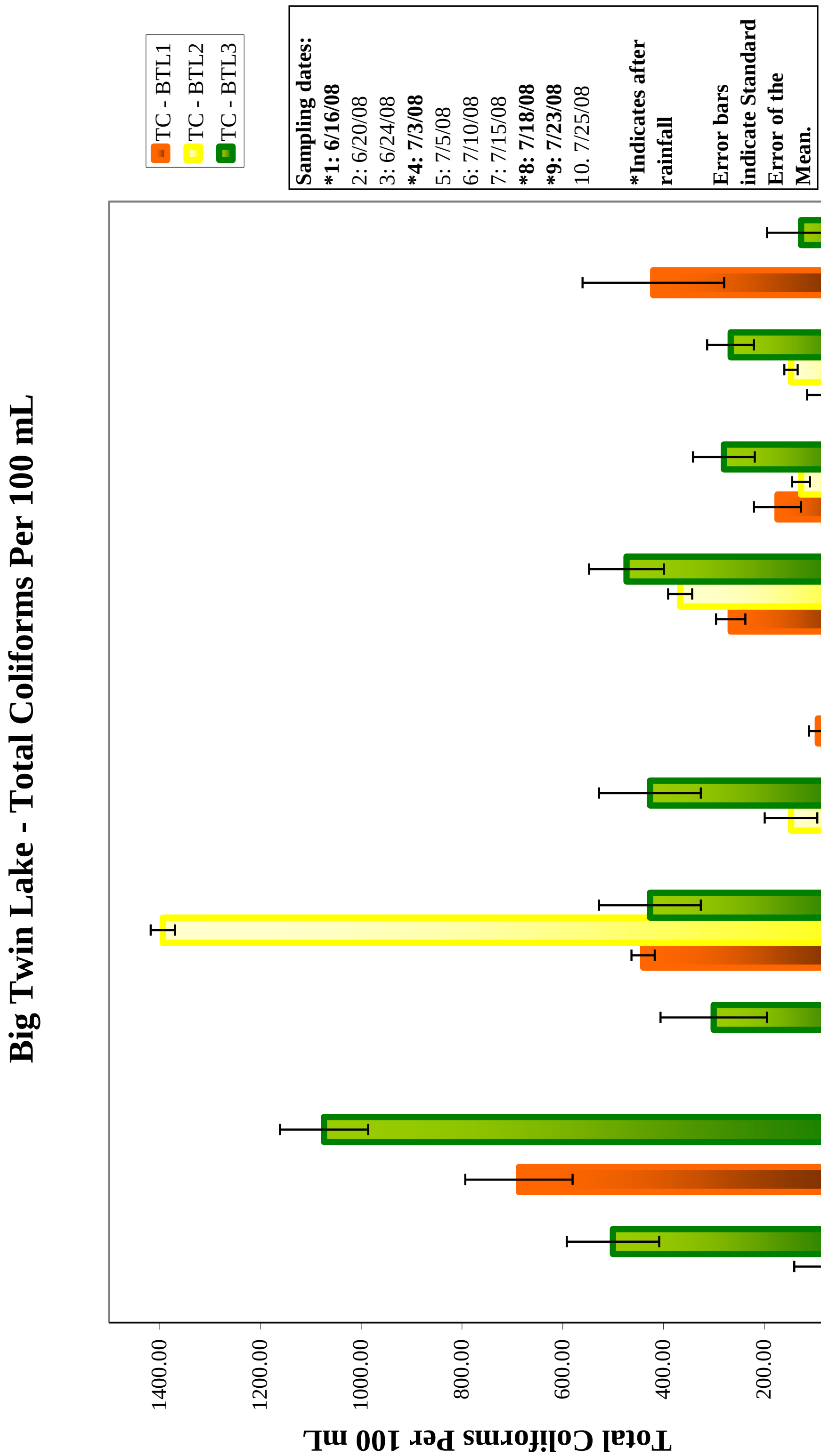

Figure 14. Total coliform counts in Oxbow Lake

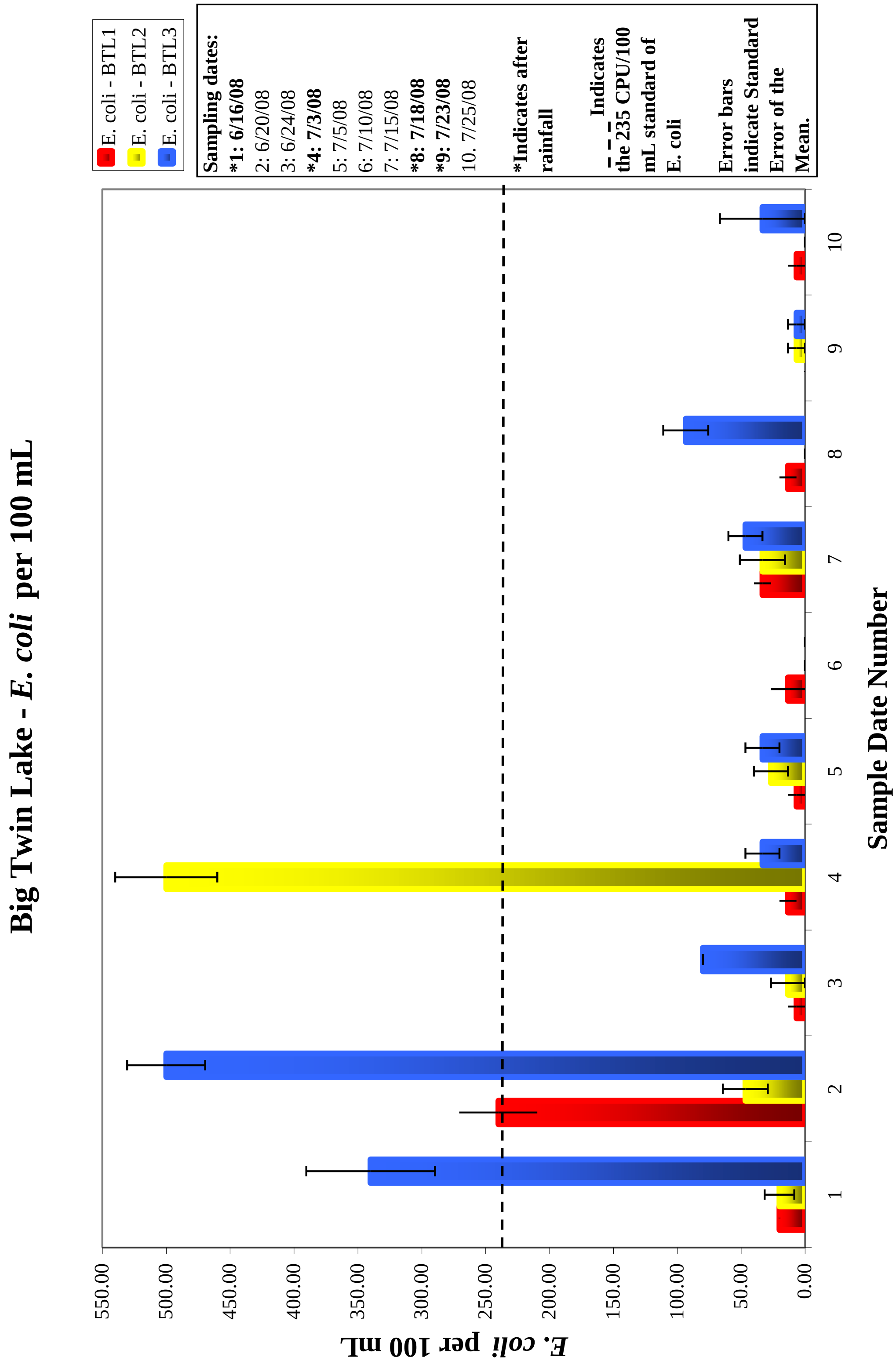

Figure 15. *E. coli* counts in Oxbow Lake

Table 5. Big Twin Lake ANOVA (One-Way) results for *E. coli*, general coliforms, and total coliforms.

Response: E. coli (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	60	60	1.0821	0.3011	#
Residuals	88	4879.6	55.4			
		Rain			**No Rain**	
Mean		4.7777			3.1111	
Standard Deviation		9.1061			6.1080	
Count		36			54	
Response: General coliforms (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	36.3	36.3	0.3236	0.5709	#
Residuals	88	9869.1	112.1			
		Rain			**No Rain**	
Mean		11.1667			9.8704	
Standard Deviation		11.1829			10.1796	
Count		36			54	
Response: Total coliforms (CFU/ 5 mL)						
	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr (>F)**	**Signif.**
Rain or No Rain	1	260.4	260.4	0.9975	0.3206	#
Residuals	88	115674	1994			
		Rain			**No Rain**	
Mean		16.4722			13.0000	
Standard Deviation		18.2967			14.5732	
Count		36			54	

Table 5 Key:	Significance codes:
Df: Difference	"***" = 0
Sum Sq: Sum of Squares	"**" = 0.001
Mean Sq: Means Square	"*" = 0.01
Pr (>F): P value	"^" = 0.1
Signif.: Significance	"#" = 1

Two-Way ANOVA for all lakes

Table 6 shows Two-Way ANOVA results for significance of rainfall on all lakes overall, the impact of the lakes on the counts, and the affect of both rainfall and lake on counts of *E. coli*, general coliforms, and total coliforms. The *E. coli* p-value as impacted by the lake was 1.60E-06, 1.62E-05 for rainfall, and 7.07E-08 for both lake and rainfall.

Table 6. All lakes ANOVA (Multi-Way) results for *E. coli*, general coliforms, and total coliforms

Response: E. coli (CFU/ 5 mL)

	Sum Sq.	Df	F value	Pr (>F)	Signif.
Lake	13376	2	14.261	1.60E-06	***
Rain or No Rain	9153	1	19.517	1.62E-05	***
Lake + Rain/ No Rain	16760	2	17.869	7.07E-08	***
Residuals	95671	204			

	Rain	No Rain
Mean		
Lake Starvation	2.8333	0.4722
Oxbow	45.7500	3.4444
Big Twin	4.7778	3.1111
Standard Deviation		
Lake Starvation	6.1338	0.7741
Oxbow	62.3334	3.9239
Big Twin	9.1061	6.1080
Count		
Lake Starvation	24	36
Oxbow	24	36
Big Twin	36	54

Response: General coliforms (CFU/ 5 mL)

	Sum Sq.	Df	F value	Pr (>F)	Signif.
Lake	20787	2	20.642	6.86E-09	***
Rain or No Rain	1302	1	2.586	1.09E-01	#
Lake + Rain/ No Rain	9359	2	9.294	1.37E-04	***
Residuals	102720	204			

	Rain	No Rain
Mean		
Lake Starvation	17.0417	43.3889
Oxbow	31.1667	24.5556
Big Twin	11.1667	9.8704
Stanadard Deviation		
Lake Starvation	16.1608	31.4427
Oxbow	38.7900	22.4464
Big Twin	11.1829	10.1796
Count		
Lake Starvation	24	36
Oxbow	24	36
Big Twin	36	54

Response: Total coliforms (CFU/ 5 mL)

	Sum Sq.	Df	F value	Pr (>F)	Signif.
Lake	32559	2	18.222	5.23E-08	***
Rain or No Rain	81	1	0.090	7.64E-01	#
Lake + Rain/ No Rain	16258	2	9.099	1.64E-04	***
Residuals	182251	204			

	Rain	No Rain
Mean		
Lake Starvation	19.8750	43.8889
Oxbow	56.9583	33.7222
Big Twin	16.4722	13.0000
Standard Deviation		
Lake Starvation	20.0311	31.3394
Oxbow	60.2347	30.3434
Big Twin	18.2967	14.5732
Count		
Lake Starvation	24	36
Oxbow	24	36
Big Twin	36	54

Table 6 Key:

Sum Sq: Sum of Squares
Df: Difference
Pr (>F): P value
Signif.: Significance

Significance codes:

"***" = 0
"**" = 0.001
"*" = 0.01
"^" = 0.1
"#" = 1

Discussion

As confirmed by low a p-value of 0.0000162 for the significance of rainfall on all the lakes, the hypothesis was accepted that rainfall increases *E. coli* counts in Starvation, Oxbow, and Big Twin Lakes (Table 6). However, individual p-values for Starvation Lake (0.0256), Oxbow Lake (0.00014), and Big Twin Lake (0.3011) show that only Starvation and Oxbow Lakes have high *E. coli* counts impacted by rain. Also, *E. coli* counts vary among the sites and lakes as shown by Figs. 9, 12 and 15 displaying the *E. coli* counts/ 100 mL for each lake and site.

There are several possible factors contributing to the variance of *E. coli* counts among the lakes. With Starvation Lake, the only instance of *E. coli* above the 235 CFU/ 100 mL standard was at Site 1 on the fourth sampling date and after a rainfall of 0.33 inches the night (7/2/2008) before sampling (Fig. 9). This sudden increase may be due to the period of dry weather before the rainfall event. Sampling notes in Table 1 (Appendix A) show that it had only rained 0.03 inches three days (6/29) before the 7/2 rain event, and 0.37 inches on 6/28. This pattern of rainfall was different from the rainfall samplings on 7/18 and 7/23. Before the eighth sampling run (7/18), there was a sudden influx of rain the night previous to sampling with 0.86 inches of precipitation. It had only rained a small amount of 0.19 inches two days (7/15) before that rainfall event, so weather before the eighth-run rainfall was fairly dry. Much research confirms the increase of *E. coli* after rain events due to the bacterium residing in beach sands and sediment, with the highest concentrations of *E. coli* in foreshore sands. Kinzelman et al. (2004) has found the highest *E. coli* counts in foreshore sands as opposed to submerged sands and waters, as Jeng et al. (2005) found high after-rainfall *E. coli* counts that did not decrease until three to seven days after the rainfall event. In addition, one study (Whitman et al. 2006), conducted along Southern Lake Michigan, found *E. coli* compacted in the beach sands even 5 m out from the shore. These conclusions are valid in explaining the influx of water through the beach soils and sands, washing concentrated *E. coli* into the nearshore waters. However, with several days of rain before sampling, it is possible that excess water after the first water "flush" could dilute the site and *E. coli* could die off after a few days since it is possibly more exposed to UV light as opposed to the dark and warm foreshore sands, which is most like a warm-blooded intestinal gut that it usually thrives in. This situation is similar to O'Neal and Hollrah (2007), who found no peaks of *E. coli* during the whole month of August due to frequent rain diluting the sampling sites.

Furthermore, many factors could have also influenced the increase of *E. coli* the fourth sampling date. The Site 1 increase on Starvation Lake could be due to both human and animal influence, since it is affected by waterfowl, beaver, and possibly septic effluent. The site is a few meters in front of the resident's home, which shares the shoreline with other lake residents that also have septic systems. Normally, however, these septic tanks at Starvation Lake are behind the houses away from the shoreline and elevated above the water table to allow adequate percolation of waste water through the soils (D. Riehl, J. Ross, and S. Marcus, personal communication, 2008). Also, research such as Whitman et al. (2001) finds that the primary sources for fecal pollution are less frequently human sources (except for, at times, sewer effluent), but more often waterfowl. However, others, such as Sankararamakrishnan and Guo (2005), show that humans have a greater impact of *E. coli* counts especially after rainfall.

It is more probable that an increase in *E. coli* counts is due to animal influence since geese are often sighted and complained about by Northern Michigan Lake residents (C. Nostwold, personal communication, 2008). Crowe (2007), Ksoll et al. (2007), Meyer et al.

(2005), Standridge et al. (1979), Whitman et al. (2001), and Whitman and Nevers (2003) also have all noted the highest *E. coli* counts in areas of higher waterfowl, duck, or sea gull concentration. It is more likely that this conclusion is accurate since there are fewer animal sources (waterfowl, beaver, deer, raccoon) for fecal contamination washed by the rain, such as in areas also affected by other factors like agricultural and livestock runoff (O'Neal and Hollrah 2007, Crowe 2007).

On Oxbow Lake, there were the highest counts of *E. coli* out of all three lakes, as it also had the lowest p-value (0.0000162) for rain impacting the *E. coli* counts (Fig. 12, Table 4). The three high peaks were all after rainfall events. Both of the highest peaks were at Site 2, the resident's dock, where the counts were well above the 1000 CFU/ 100 mL Great Lakes beach-closure count. Site 1 (East end) also had a high peak on the eighth sampling date (after rainfall) when Site 2 had *E. coli* counts above 3000 CFU/ 100 mL. It has been noted several times as documented in Table 1 (Appendix A) that bird feces were present on the walkways above Site 2. Other animals noted when sampling were heron, bald eagle, beaver, and domestic dog. It is also significant to note that only one resident home is located on the lake above the shoreline, so there are fewer factors from humans (i.e. septic effluent from many homes, human activities with pets, etc.) that contribute to fecal contamination. The residents have also recently re-built their home and replaced their septic tank, so it is even more probable that the high fecal contamination is due to wildlife. Also, since there is only one home that is not frequently occupied during the year, there may be a greater "flush" of *E. coli* from soils, feces, and the septic tank after rainfall compared to areas with frequent human activities on the soils. Oxbow Lake also has a greater littoral zone, especially at Site 1, and shallower depth throughout the lake compared to Starvation and Big Twin, so *E. coli* could more easily concentrate around the shoreline with less water dilution after frequent rain.

Big Twin Lake had the most variance of *E. coli* since one of the three peaks above the 235 CFU/ 100 mL standard occurred on a normal or baseline sampling date (Fig. 15). Since rain does not significantly impact *E. coli* counts on Big Twin Lake according the high p-value of 0.3011 (Table 5), as Whitman et al. (2001) confirms, it is nearly impossible to detect a single factor that influences the high *E. coli* counts. However, the high counts on the ASI beach (Site 3) on the second sampling date (baseline) could be mostly correlated to geese and other animals since residents in the area have made comments of geese gathering on the shoreline during the night. Also, all three of the sites on Big Twin Lake are not directly under homes; and, it is unknown as to how old, where they are located, and where the effluent from septic tanks percolates into the lake water. Big Twin Lake is also a deeper lake, which makes it less common for *E. coli* to concentrate in shallower waters, and making it more susceptible for rain storms to dilute the sampling sites. As Whitman et al. (2001), Kinzelman et al. (2004), Kon et al. (2007), Nevers and Whitman (2005), Standridge et al. (1979), have noted the correlation of wind direction, wind intensity, and water currents with increased *E. coli* counts, there were no correlations with wind speed, wind direction, and currents in this study since some studies have shown correlations of high counts with wind speed only if it is over 20 mph.

It is also possible that the *E. coli* counts were lower at Big Twin Lake since it was usually sampled later on in the day, as opposed to Starvation and Oxbow Lakes. Whitman et al. (2001) completed a study on a Chicago beach, collecting hourly samples from the morning into the evening in both foreshore sands and nearshore waters. They found that the *E. coli* counts significantly decreased as the afternoon progressed. In addition, Whitman et al. (2004) found *E.*

coli count decreased on sunny days compared to cloudy days in Lake Michigan. It is probable that the counts at Big Twin could be variable due to the conclusions discovered by other studies, and it would beneficial for further research to involve a sampling time series over 24 hours to detect solar and temperature effects on *E. coli* counts. It would also be advantageous to conduct this study in human vs. animal-dominated sites.

Other helpful analysis to confirm the sources of the *E. coli* found in the lakes would be to complete PCR DNA Fingerprinting to detect human vs. animal-derived *E. coli* in a molecular laboratory. Detecting the different *E. coli* strains could be difficult, however, as Meyer et al. (2005) has noted the problems of interpreting the results of genotypic testing since strains that match *E. coli* isolates are often not 100% similar.

DNA fingerprinting could also be conducted to test for the survivability of *E. coli* in foreshore sands and sediment. A similar study was conducted by Ishii et al. (2006) who used HFERP (horizontal, fluorophore-enhanced repetitive extragenic palindronmic PCR) to indicate that soil-derived *E. coli* genotypes were present over time and reproducing over winter months in Lake Superior.

In conclusion, the null hypothesis that rainfall will have no impact on *E. coli* counts was rejected in this study. With the example of Big Twin Lake not showing significance of rain on the *E. coli* counts, it is difficult to pin-point exact predictors of high *E. coli* count in the lakes (i.e. precipitation amount) without further research to validate the results. Lake residents, however, should be cautioned if a rain influx follows a period of dry weather, especially in areas of greater animal activity.

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Literature Cited

Alabama Water Watch Program. 2004. Bacteriological Monitoring. Auburn University, AL.

Barnett A, Stutzman BC. 1998. A limnological study of Oxbow Lake. Au Sable Institute of Environmental Studies. Unpublished report.

Barr B. 1998. Personal communication about Oxbow Lake history. As cited by Barnett and Stutzman.

Berkey R, Sorenson M, Tassone F. 1997. A limnological study of Big Twin and Oxbow Lake. Mancelona, MI: Au Sable Institute of Environmental Studies. Unpublished report.

Brock TD, Brock KM, Ward DM. 1986. Basic Microbiology with Applications, 3rd ed. Englewood Cliffs, NJ: Prentice-Hall. 310-313 pp.

Crow G. 2008. Photography of research methods and sites. Mancelona, MI.

Crowe A. 2007. *E. coli*: A Permanent Resident of our Beaches? The Lake Huron Centre for Coastal Conservation. <http://lakehuron.ca/index.php?page=e-coli> Accessed 2008 Jul 25.

Department for Health and Human Services. 2008. *Escherichia coli*. Centers for disease control and prevention. Division of foodborne, bacterial, and mycotic disease. <http://www.cdc.gov/nczved/dfbmd/disease_listing/stec_gi.html#1> Accessed 2008 May 20.

Department of Natural Resources. 2007. "Lake Maps By County." <http://www.michigan.gov/dnr/0,1607,7-153-30301_31431_32340---,00.html> Accessed 2008 Jun 27.

Ely E. 2006. Bacteria methods for recreational waters: a short history and guide. The Volunteer Monitor: The National Newsletter of Volunteer Watershed Monitoring. San Francisco. 18: 8-10.

Fong T-T, Mansfield LS, Wilson DL, Schwab DJ, Molloy SL, Rose JB. 2007. Massive microbiological groundwater contamination associated with a waterborne outbreak in Lake Erie, South Bass Island, Ohio. Environ. Health Persp. 115 (6): 856-864.

Fowler A, Maple J. 2003. Starvation Lake. Au Sable Institute of Environmental Studies. Limnology Lake Report. Unpublished report.

Fox J. 2008. The R Commander: a basic-statistics GUI for R. development version: 1.4-0. < http://socserv.mcmaster.ca/jfox/Misc/Rcmdr/> Accessed 2008 Aug 15.

Francy DS, Bertke EE, Finnegan DP, Kephart CM, Sheets RA, Rhoades J, Stumpe L. 2006. Use of spatial sampling and microbial source-tracking tools for understanding fecal contamination at two Lake Erie beaches. U.S.G.S. Survey Sci. Invest. Rep. 2006: 5298, 29 pp.

Girod A, Stowell J. 2004. Limnological survey of Big Twin Lake. Mancelona, MI: Au Sable Institute of Environmental Studies.

Hartz A, Cuvelier M, Nowosielski K, Bonilla TD. 2008. Survival potential of *Escherichia coli* and *Enterococci* in subtropical beach sand: implications for water quality managers. J. Environ. Qual. 37: 898-905.

Hobson, R. 2007. An analysis of antibiotic resistance in *Escherichia coli* isolated from the Arkansas River and effluent from a wastewater treatment plant. Oral Roberts University. Tulsa, OK. Senior Paper.

Horne AJ, Goldman CR. 1984. Limnology, 2^{nd} ed. New York: McGraw-Hill. 462 p.

Ishii S, Ksoll WB, Hicks RE, Sadowsky MJ. 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. Appl. Environ. Microbiol. 72: 612-621.

Jeng HAC, Englande AJ, Bakeer RM, Bradford HB. 2005. Impact of urban stormwater runoff on estuarine environmental quality. Estuarine, Coastal and Shelf Science 63: 513-526.

Kinzelman J, McLellan SL, Daniels AD, Cashin S, Singh A, Gradus S, Bagley R. 2004. Non-point source pollution: determination of replication versus persistence of *Escherichia coli* in surface water and sediments with correlation of levels to readily measurable environmental parameters. J. Water Health 2: 103-114.

Kleinheinz GT, McDermott CM, Leewis MC, Englebert E. 2006. Influence of sampling depth on *Escherichia coli* concentrations in beach monitoring. Water Res. 40 (20): 3831-7.

Kon T, Weir SC, Howell ET, Lee H, Trevors JT. 2007. Genetic relatedness of *Escherichia coli* isolates in interstitial water from a Lake Huron (Canada) beach. Appl. Environ. Microbiol. 73:1961-1967.

Ksoll WB., Ishii S, Sadowsky MJ., Hicks RE. 2007. Presence and sources of fecal coliform bacteria in epilithic periphyton communities of Lake Superior. Appl. Environ. Microbiol. 73: 3771-3778.

Marcus S. 2008. Personal communication about Starvation Lake. Mancelona, MI.

Marsalek J, Rochfort Q. 2004. Urban wet-weather flows: sources of fecal contamination impacting on recreational waters and threatening drinking-water sources. J. Toxicol. Environ. Health, Part A 67: 1765-77.

The Merck Manuals: Online Medical Library. 2003. *Enterobacteriaceae* Infections. Merck & Co., Inc. <http://www.merck.com/mmhe/sec17/ch190/ch190h.html> Accessed 2008 Jul 30.

Meyer KJ, Appletoft CM, Schwemm AK, Uzoigwe JC, Brown EJ. 2005. Determining the source of fecal contamination in recreational waters. J. Environ. Health 68: 25-30.

Michigan Department of Natural Resources. 2007. Nuisance geese. <http://www.michigan.gov/dnr/0,1607,7-153-10370_12145_25065-59467--,00.html> Accessed 2008 Jul 29.

Michigan Interactive. 1995. Michigan Lake Maps: Starvation Lake. <http://www.fishweb.com/maps/kalkaska/starvation_lake/index.html> Accessed 2008 Jul 29.

Micrology Laboratories. 2005 Aug 15. Detection of waterborne coliforms and *E. coli* with Coliscan® Easygel®. <http://www.micrologylabs.com/Home/Our_Methods/Coliscan_Easygel/Coliscan_Easygel_Instructions> Accessed 2008 Jun 16.

Micrology Laboratories. 2007 Nov 28. FAQ about the use and interpretation of Coliscan® Easygel® and Coliscan® Membrane Filter Methods. <http://www.micrologylabs.com/Home/FAQ> Accessed 2008 Jun 16.

Nevers MB, Whitman RL. 2005. Nowcast modeling of *Escherichia coli* concentrations at multiple urban beaches of southern Lake Michigan. Water Res. 39 (20): 5250-60.

Nostwold C. 2008. Personal communication about Starvation Lake. Mancelona, MI.

O'Brien E. 2006. Volunteer conduct bacteria methods comparison study. The Volunteer Monitor 18: 1, 3-6.

O’Neal SW, Hollrah DN. 2007. Monitoring of total coliform and *Escherichia coli* levels in a second order stream in West-Central Oklahoma. Proc. Okla. Acad. Sci. 87:69-75.

Riehl D. 2008. Personal Communication about Starvation Lake. Mancelona, MI.

Ross J. 2008. Personal communication about Starvation Lake, Mancelona, Michigan.

Roth J. 2008. Personal communication about Coliscan® Easygel® methods. Micrology Laboratories.

Scopel CO, Harris J, McLellan SL. 2006. Influence of nearshore water dynamics and pollution sources on beach monitoring outcomes at two adjacent Lake Michigan beaches. J. Great Lakes Res. 32: 543–552.

Standridge JH, Delfino JJ, Kleppe LB, Butler R. 1979. Effect of waterfowl (*Anas platyrhynchos*) on indicator bacteria populations in a recreational lake in Madison,Wisconsin. Appl. Environ. Microbiol. 38: 547-550.

Todar K. 2007. Todar’s online textbook of bacteriology: nutrition and growth of bacteria. University of Wisconsin-Madison, Department of Bacteriology. <http://www.textbookofbacteriology.net/nutgro.html> Accessed 2008 Jun 30.

U.S. EPA. 2000. Improved enumeration methods for the recreational water quality indicators: *Enterococci* and *Escherichia coli*. Office of Science and Technology, Washington D.C. EPA/821/R-97004. <http://www.epa.gov/waterscience/beaches/files/rvsdman.pdf> Accessed 2008 Jun 27.

U.S. EPA. 2002. National Beach Guidance and Required Performance Criteria for Grants. EPA-823-B-02-004. US Environmental Protection Agency, Washington, DC.

U.S. EPA. 2005. Microbial source tracking guide document. EPA/600/R-05/064. <http://www.epa.gov/nrmrl/pubs/600r05064/600r05064.pdf> Accessed 2008 Aug 15.

U.S. EPA. 2006. “Basic information about *E. coli* 0157:H7 in drinking water.” <http://www.epa.gov/safewater/contaminants/ecoli.html> Accessed 2008 May 20.

Van Donsel DJ, Geldreich EE, Clarke NA. 1967. Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution. Appl. Environ. Microbiol. 15: 1362-1370.

Whitman RL, Horvath TG, Goodrich ML, and Nevers NB. 2001. Characterization of *E. coli* Levels at 63rd Street Beach. US Geological Survey. (The 'Whitman Report' for the City of Chicago).

Whitman RL, Nevers MB. 2003. Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. Appl. Environ. Microbiol. 69: 5555–5562.

Whitman RL, Nevers MB, Byappanahalli MN. 2006. Examination of the watershed-wide distribution of *Escherichia coli* along Southern Lake Michigan: an integrated approach. Appl. Environ. Microbiol. 72: 7301-7310.

Whitman RL, Nevers MB, Korinek GC, Byappanahalli MN. 2004. Solar and temporal effects on *Escherichia coli* concentration at a Lake Michigan swimming beach. Appl. Environ. Microbiol. 70: 4276 – 4285.

Winfield MD, Groisman EA. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli.* Appl. Environ. Microbiol. 69: 3687–3694.

Wisconsin Department of Natural Resources. 2001. Water Quality Standards for Wisconsin Surface Waters. NR/102. Department of Natural Resources. Madison, WI.

Wisconsin Department of Natural Resources. 2008. Beach monitoring program requirements. <http://www.dnr.state.wi.us/org/water/wm/WQS/Beaches/BeachMonitoringRequirements.pdf> Accessed 2008 Jun 20.

Appendix A

TABLE 1: Sampling Observations and Notes									**Starvation Lake, Site 1**					**Starvation Lake, Site 2**					**Oxbow Lake, Site 1**				
Sampl. Date #	**Date**	**Time**	**Time of incub.**	**Incub. temp. (°C)**	**Time of count**	**General Weather**	**Chance of rain (%)**	**Amount Precip. (inches)**	**T (°C)**	**pH**	**DO (mg/L)**	**MPH**	**Observ.**	**T (°C)**	**pH**	**DO (mg/L)**	**MPH**	**Observ.**	**T (°C)**	**pH**	**DO (mg/L)**	**MPH**	**Observ.**
1	**6/16/2008**	9am	3:40pm	38-39	6/18, 8:10am	61°F, overcast, some rain		??	19.6	9.6			rainy, cloudy	19.6	9.6			coudy and cold					partly cloudy, slight breeze
2	6/20/2008	9am	3:30pm	37-38	6/22, 8:15am	60°F, sunny. Will be high of 75°F, winds SW 5-10 mph	30		20.0	9.3			warm, clear & sunny skies	20.9	9.3			warm, clear & sunny skies	23.0	9			sunny skies
3	6/24/2008	9am	2:50pm	37-38	6/26, 9am	some clouds, mostly sunny, 64-70°F, 60-65% humidity	10		20.4	10.2		2	sunny, very slight breeze	20.7	10.5		2.5-8	clear and sunny	22.5	9.7			clear, sunny, very slight breeze
4	**7/3/2008**	11:30am	6:45pm	35-37	7/5, 3pm	60°F, sunny, winds N at 10-15 mph; storm the night before		0.33 (7/2), 0.03(6/29), 0.37 (6/28)	21.7		7.85	9 to 10	sunny, cold air temp.	21.4		8.17	6 to 10	strong currents toward shore	24.0		10.02	5 to 8	
5	7/5/2008	7am	12:15pm	37	7/7, 9am	sunny, cool, no winds, high of 79°F			19.6		8.13		anenometer not working for all sites	18.7		8.86			14.2		9.30		
6	7/10/2008	9:30am	2:20pm	37	7/12, 8am	67°F, partly cloudy, winds SE 3 mph,	30	0.39 (7/7), 0.03 (7/6)	22.4		8.36	2 to 4	sunny, surface pollen/grass on water	22.8		8.28	5 to 9	currents towards shore and white foam	24.5		8.75	6 to 9	small currents toward shore
7	7/15/2008	12:15pm	6:45pm	37	7-17, 11am	sunny, 79°F, SSW of 14 mph, high of 83°F, slight chance of thunderstorm	30		22.7		8.70	2 to 9	sunny, slight winds	24.4		7.52	13 to 14	sunny, strong winds & currents; dog walked through site	27.1		9.10	10 to 14	windy, sunny, clear skies
8	**7/18/2008**	8am	1pm	37-38	7-20, 1:30am	69°F, cloudy, sprinkling, high 78°F, winds WSW 5-10mph, scattered thunderstorms	40	0.86 (7/17), 0.19 (7/15)	22.5		9.23	0	cloudy, no rain	22.5		8.28	3 ot 4	cloudy, beginning to sprinkle	22.6		6.62	4 to 7	slight breeze, cloudy, water yellower than normal
9	**7/23/2008**	9am	12:30pm	37	6:30pm, 7-24	sunny, partly cloudy, 60s°F, high 81°F, winds N 10-15 mph	20	0.69 (7/22), 0.95 (7/19), 0.82 (7/17)	22.4		8.33	0	sunny, no clouds, clear skies	22.8		8.00	0	sunny, clear skies, no wind/current	21.2		8.35	0	sunny, clear skies, slight tint of yellow in water
10	7/25/2008	9:30am	2pm	38	9am, 7-27	high 83°F, winds SW 10-20 mph, sprinkles early, scattered thunderstorms in afternoon	40		22.6		8.46	2 to 5	sunny, few clouds, no rain	22.0		8.15	6 to 8	partly cloud, wind, abundant white foam at shore, current, sediment in site - sand eroded	23.9		8.40	6 to 10	partly cloudy

KEY:	T: Temperature
	DO: Dissolved Oxygen
	MPH: Miles Per Hour
	Observ.: Observations on field
	BOLD and UNDERLINED are AFTER RAINFALL sampling dates
	All other dates represent the weekly baseline.

Table 1 (cont.)		**Oxbow Lake, Site 2**					**Big Twin Lake, Site 1**					**Big Twin Lake, Site 2**					**Big Twin Lake, Site 3**				
Sampl. Date #	**Date**	**T (˚C)**	**pH**	**DO (mg/L)**	**MPH**	**Observ.**	**T (˚C)**	**pH**	**DO (mg/L)**	**MPH**	**Observ.**	**T (˚C)**	**pH**	**DO (mg/L)**	**MPH**	**Observ.**	**T (˚C)**	**pH**	**DO (mg/L)**	**MPH**	**Observ.**
1	**6/16/2008**					partly cloudy/ sunny; wind blowing to site										current/ wind toward site					strong wind toward site
2	6/20/2008	20.7	8.9			sunny skies	21.5	8.9	21.10	8.8	sunny with some clouds	21.1	8.8			sunny with some clouds	21.4	8.6			sunny with some clouds
3	6/24/2008	20.6	9.9			sunny, very slight breeze; goose droppings on walkway	20.4	9.4		2	sunny, waited for sediment to settle since boat drove through site btwn 2nd and 3rd reps.	21.1	9.4			sunny	22.0	9.2			more breezy, more clouds, white foam at shoreline
4	**7/3/2008**	22.8		8.28	6 to 8		22.6		7.88	~5-7	waited for sediment to settle after boat disruption; strong currents from boats	24.8		7.35	3 to 7	currents from boat	23.8		7.45	~5-6	some currents, sunny & warm air
5	7/5/2008	18.9		10.65		many kids in the water previous night	18.6		9.74			19.6		9.04			19.5		8.56		slight winds towards shore
6	7/10/2008	23.2		8.47	5 to 7	sunny with some clouds, more calmer winds	22.6		7.75-8.15	2 to 5	sunny, no breeze/winds/clouds	23.5		7.90	6 to 8	winds blowing opposite from shore	25.3		7.10-7.11	7 to 9	strong currents & white foam; sunny
7	7/15/2008	24.6		8.80	4 to 8	sunny, slight breeze	23.3		9.22	0	sunny, storm clouds, gentle sprinkle	22.7		9.40	9 to 10	rainy, then sunny and cloudy	25.4		8.37	14 to 15	windy, sunny, tide currents towards shore
8	**7/18/2008**	23.5		8.11	2 to 5	cloudy and muggy	22.6		8.11	0 to 2	cloudy and muggy	22.6		8.32	0 to 2	cloudy and muggy	22.4		7.64	3 to 7	cloudy, muggy, breeze, cool air, currents & white foam towards shore
9	**7/23/2008**	22.6		8.59	5 to 7.5	sunny, clear, slight breeze, abundant bird fecal matter on walkway, waters with surface algae	22.4		8.29	2 to 4	clear skies, sunny, no sediment in water	23.3		8.37	0 to 7	sunny, clear skies, small current & white foam at shore	23.5		8.13	3 to 5	sunny, clear skies, small current & some white foam at shore
10	7/25/2008	23.4		9.10	2 to 10	sunny	24.1		8.15	2 to 5	sunny, slight/ no breeze, small current	23.6		8.14	2 to 7	sunny, small current, winds toward lake from land	22.9		7.82	6 to 10	sunny, strong current towards shore

Appendix B

Photographs of Research

Big Twin Lake

Site 1: Public access dock

Site 2: Causeway

Site 3: Au Sable Institute beach dock

Starvation Lake

Site 1: VanVynckt resident's dock

Site 2: Public access dock

Oxbow Lake

Site 1: East end

Site 2: Stonewell resident's dock (Crow 2008)

Sampling methods on the lakes

Crow (2008)