# Prevalence of *Escherichia coli* Before and After Storm Events in Big Twin, Oxbow, and Starvation Lakes in Northern Michigan.

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## ABSTRACT

*Escherichia coli* (*E. coli*), a fecal coliform bacteria, primarily resides in the gastrointestinal tracts of warm-blooded animals and birds. *E. coli* is recognized as the indicator of fecal contamination in freshwaters by the U. S. EPA, which sets the water quality standard for recreational waters to 235 *E. coli* per 100 mL. In the Great Lakes region, *E. coli* counts that peaked during summer months have been correlated to stormwater runoff from waterfowl, septic, and agricultural sources. The goal of this study was to detect the impact of rainfall on *E. coli* counts in Northern Michigan lakes.

In June and July 2008, Starvation, Oxbow, and Big Twin Lakes were tested for *E. coli*, general coliforms, and total coliform counts using Coliscan® Easygel® media. Each lake had two or three sites with three sample replicates collected near shore at each site. Ten sampling dates were completed over a period of six weeks, with six weekly baseline collections and four after-rainfall samplings collected within 24 hours of at least 0.8 centimeters (0.3 inches) precipitation.

ANOVA Multi-Way statistical results show that rainfall has significant impact on *E. coli* counts. However, individual statistical analysis for each of the lakes shows that in only Oxbow and Starvation after rainfall *E. coli* counts showed a significant difference from the baseline data. This may be as a result of human versus animal influence on the lakes, which can be further researched using PCR DNA Fingerprinting. Mann-Whitney-U statistical results show that rainfall has significant impact on *E. coli* counts.

## INTRODUCTION

- Michigan is the "land of freshwater lakes" with over 11,000 lakes, including two bordering Great Lakes.
- These lakes are at risk for contamination by fecal coliforms, the most prevalent being *Escherichia coli (E. coli)*, a gram-negative bacterium residing in the gastrointestinal tract of humans, bird, and warm-blooded animals.
- There are over 700 strains of *E. coli*, with a few pathogenic strains such as O157:H7. Other *E. coli* outbreaks over the past 10 years involved meat, vegetables, and recreational waters.
- Both pathogenic and non-pathogenic bacteria are in the family *Enterobacteriaceae* subdivided into coliform and non-coliform species. The coliform bacteria are further subdivided into the fecal (in feces of humans, animals, and birds) and non-fecal coliforms (free-living, benign organisms in water, soil, and vegetation. Total coliform count is used as a drinking water indicator.
- Fecal coliforms not only include *E. coli*, but also other genera that have more pathogenic strains but are less common than *E. coli* such as *Salmonella*, *Shigella*, and *Enterobacter*. These pathogenic strains can cause severe diseases such as cholera, typhoid fever, shigellosis, and salmonellosis. According to the U.S. EPA (U.S. Environmental Protection Agency), fecal coliforms are considered the indicator of fecal pollution in freshwater areas. *E. coli* as the best fecal coliform indicator for freshwaters since it has a strong correlation to other, more pathogenic bacteria that also may be present in the waters.
- An increase of *E. coli* counts is caused by many sources: waterfowl or birds, septic and sewer effluent (rising groundwater due to rains), human bathers, livestock, and stormwater runoff.

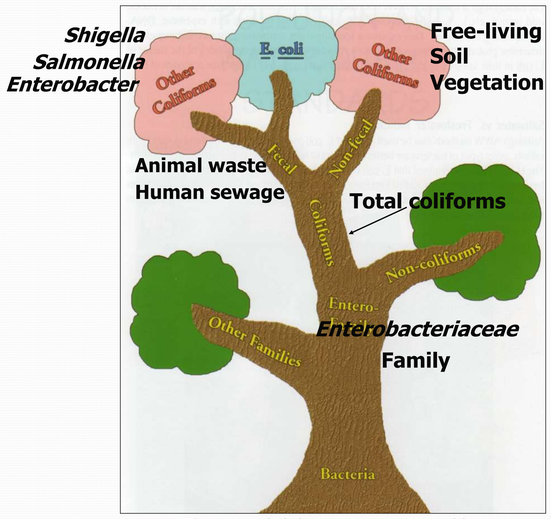



*Figure 1: (Alabama Water Watch)*

## INTRODUCTION

- For the Great Lakes region, the BEACH Act *E. coli* standards are 0 to 234 CFU (Colony Forming Unit)/ 100 mL to classify waters as "good" and beaches are open to the public. Counts of 235 to 999 CFU per 100 mL are "cautionary" and advisory signs are posted on the beach. Counts over 1000 *E. coli* colonies per 100 mL require beaches to be closed.
- There are three methods of testing for *E. coli* in water: membrane filtration, tube fermentation, and the pour-plate method. This research used the pour-plate method, Coliscan® Easygel®, with two sugar substates, Red-Gal and X-gluc, that interact with the enzymes galactosidase and glucuronidase in *E. coli*, producing dark purple, blue, and teal colonies on the plate.
- Hypothesis: *E. coli* counts will be higher after rainfall when compared to the lakes' normal (baseline) *E. coli* counts

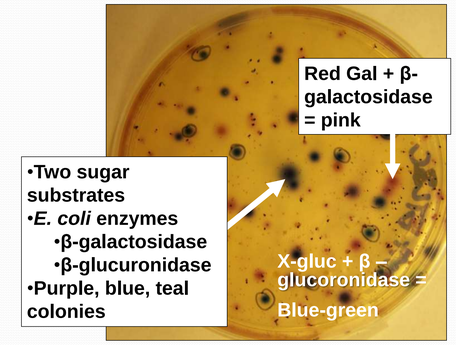



*Figure 2: Coliscan® Easygel®*

## MATERIALS & METHODS

**Sampling**
- Weekly for 6 weeks
  - Baseline sampling every week
  - 4 after-rainfall samplings
    - Within 24 hours of rain event
  - Total of 10 sampling runs
- 3 replicates at each site
  - Total of 21 samples per sampling run


**Observations**
- Water current
- Water transparency
- Animal evidence
- Weather forecast (www.weather.com for Mancelona, MI)
- Amount of precipitation (inches)
- Wind speed & direction

**Measured Dissolved Oxygen (DO) & Temperature**

**Plates Poured; Incubated for 30-45 hours at 35-38°C**

**Colonies Counted & Data Recorded**
- **Purple, blue, teal = *E. coli***
- **Pink = General coliforms**
- ***E. coli* + pink = Total coliforms**

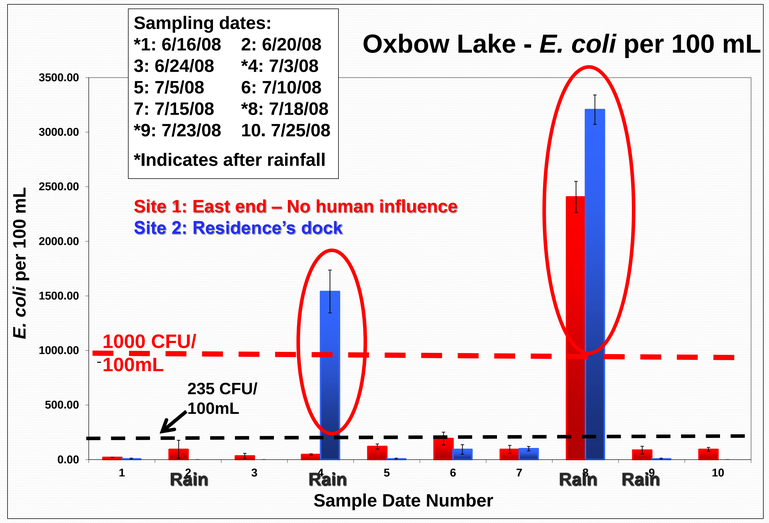
**Statistical Analysis:** R Commander One-Way and Multi-Way ANOVA; Mann-Whitney U


*Figures 3,4,5: Sampling;Testing Sites*

## RESULTS & DISCUSSION

| | RAIN | | NO RAIN | |
|---|---|---|---|---|
| Lake | Mean (raw data) | Standard Deviation | Mean | Standard Deviation |
| Starvation | 2.8333 | 6.1338 | 0.4722 | 0.7741 |
| Oxbow | 45.75 | 62.3334 | 3.4444 | 3.9239 |
| Big Twin | 4.7778 | 9.1061 | 3.1111 | 6.108 |
| Multi-Way ANOVA p-value for Rain/ No Rain variable | | | | 1.62E-05 |

- **Multi-Way ANOVA**: Rain significantly impacts *E. coli* counts for all lakes (p-value: 1.62E-05)
- **One-Way ANOVA**: Variation among sites and lakes
- **Big Twin**: Rainfall does not significantly affect *E. coli* counts-ANOVA p-value = 0.3
  - All three sites not below homes
  - Sampling completed later in the day
  - Excess rains dilute sampling site
  - Deeper lake water
  - No distinct correlation with winds and currents
  - **Many variables**: homes surrounding lake, septic tanks, bird feces
- **Starvation**: Rainfall significantly affects *E. coli* counts – ANOVA p-value = 0.026
  - Highest *E. coli* count after rainfall (0.3 in.)
  - Highest count at resident's dock (Site 2)
  - Possible influence of septic effluent
  - Evidence of bird feces
- **Oxbow**: Rain significantly affects *E. coli* counts – ANOVA p-value = 0.00014
  - Animal influence; bird feces
  - Only one home; greater "flush" after rain
  - Less variables than other lakes
  - Shallower depth
  - Concentrated water site




## CONCLUSIONS

- **Hypothesis accepted**: Rainfall has impact on *E. coli* counts
- No direct correlation of high *E. coli* counts with wind speed, water currents, DO, and temperature
- Caution lake residents of high counts if rain influx following dry weather and in areas of greater animal activity

## FUTURE RESEARCH

- Sampling time series over 24 hours; testing solar and temperature effects at human vs. animal dominated sites
- PCR DNA Fingerprint analysis of human vs. animal *E. coli* at molecular biology lab

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