Water Quality Assessment Of Five Southern Maine Beaches: Comparing ?Escherichia Coli? Levels

Fecal contamination of water is often determined by the amount of ?Escherichia coli (?E. coli?) cells present within a water sample. In this study ?E. coli? was used as the indicator bacteria. Water samples from five different lakes, each with 0, 1, or 2 beach closings respectively, were tested for their ?E. coli? levels to determine if they were truly safe for swimming. It was hypothesized that if the beach had the highest number of closings than it would have the highest current ?E. coli ?count. However, the results showed that the number of closings did not have an impact on the levels of ?E. coli? with Highland Lake having the most closings and only 0. 115 ?E. coli ?cells/mL compared to Sebago Lake with no closings and 0. 447 ?E. coli ?cells/mL. Despite having four samples out of the five test positive for ?E. coli,? the ?E. coli? cells/mL amounts were all well within the standard of 126 ?E. coli? cells/100ml for safe levels of bacteria in freshwater lakes (EPA 2018).

Introduction: Summers in Maine are short, so people take advantage of the warm weather and utilize the lakes for recreational activities. With this increase in the number of people swimming, it is crucial to perform weekly water tests to prevent the spread of enteric pathogens from contaminated water (MD 2018). This is especially important since in recent years recreational water associated outbreaks of gastrointestinal diseases have been high (Yoder ?et al ?2008). To help reduce the public health risks associated with contaminated water, the EPA set water quality standards specifically for freshwater recreational areas in 1989. The standards were based on the studies performed by Cabelli and Dufour which demonstrated that Escherichia coli?, a fecal coliform, was found to be the best indicator for contamination levels in freshwater sources (Cabelli? et al? 1979). More recent findings reaffirmed this claim due to how easily ?E. coli? are grown and their positive correlation with fecal pathogens (Wade ?et al? 2003). Coliforms are small gram negative bacteria that do not produce spores and are able to ferment lactose.

These small bacteria are found in both the environment and in the feces of all warm-blooded animals (Cabelli ?et al? 1982). For this reason, coliforms are used to indicate general contamination of water sources. However, since coliforms are found in multiple environments, a more specific indicator like ?E. coli ?is often used to isolate the cause of contamination. ?E. coli ?is a fecal coliform, meaning it is a type of coliform found in high quantities in the intestines of warm-blooded animals (Cabelli ?et al? 1982). Thus the presence of ?E. coli? in a sample points to fecal contamination. However, most strains of ?E. coli ?are not harmful, but its presence indicates that other fecal pathogens may be present (Wade ?et al? 2003). In this experiment,

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five samples of lake water from public beaches around Southern Maine were tested for fecal contamination. The methods used were a COLISURE kit from IDEXX and the Most Probable Number equation to determine the total number of ?E. coli? cells. It is hypothesized that if the lake was closed most frequently during the summer for failed water tests, then it will currently have the highest level of ?E. coli?.

Methods and Materials:Fifty milliliter (mL) samples of lake water were collected from five different public beaches in Southern Maine. Samples were taken from Highland Lake Beach, Woods Pond Beach, Mousam Lake Beach, Crystal Lake Main Beach, and Raymond Beach on Sebago Lake. Each sterile 50 mL test tube used for collecting samples contained 0. 05 mL of 10% sodium thiosulfate to prevent chlorine from killing the microbes (Lab Manual 2018). Half of a IDEXX COLISURE package was added to each sample respectively and then shaken vigorously. Using half of a 96 well microplate, 44 wells were each filled with 200 microliters (uL) of one sample. Each sample had its own positive controls (2 wells containing 200ul of sterile water inoculated with? E. coli ?culture) and negative controls (2 wells containing 200ul of sterile water). In total, 48 wells were used for each sample. This procedure was repeated for all five samples using a total of three 96 well microplates. All samples were incubated at 37 degree celsius for 4 days. The wells containing the sample were compared to the positive and negative controls to determine the presence of coliforms and ?E. coli?. When the nutrient-indicator chlorophenol- red-?B?-D-galactopyranoside (CPRG) is metabolized by coliforms it releases a magenta product called chlorophenol red. Thus the total number of wells containing a color change, from yellow to magenta, were counted and recorded. This was repeated for all samples. A UV light was then used to observe the presence of ?E. coli ?in the sample. ?E. coli? has a unique enzyme that allows it to metabolize 4-methylumbelliferyl-B-D-glucuronide (MUG) releasing methylumbelliferone which is a fluorescent product. The total number of fluorescent wells were then counted and recorded. The 50ml tube containing the original sample was also observed under the UV light for fluorescence. This was repeated for all samples. The Most Probable Number (MPN) equation was used to calculate the number of ?E. coli cells present in each sample. The MPN equation is: # cells per ml = (1/volume)x In(total # of wells/ # negative wells). A bar graph was then used to compare the total number of ?E. coli? found in each lake and the number of times each beach was closed this past summer.

Results:The overall goal of this experiment was to measure the level of ?E. coli? present in five different lake samples and determine if previous closings impacted the current ?E. coli? levels. The five samples were all collected from public beach accesses within a one hour radius of Portland. The lakes were deliberately selected based on the number of reported closings. To compare the effect of closings on the level of ?E. coli?, samples were collected from two lakes that were never closed, two lakes that were closed only once, and one lake that was closed twice. The figure below visually represents the experimental findings. A bar graph depicting the levels of ?E. coli? found in each of the five lake samples compared to number of closings.Figure

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1. shows the overall level of ?E. coli? found in each sample. The red number above each lake indicates the total number of times the lake was closed this past summer. This highlights the correlation between ?E. coli? levels and number of closings. Sebago Lake had the highest ?E. coli? levels of 0. 447cells/mL despite never being closed. In contrast, Highland Lake, which was closed twice, had equal levels of ?E. coli? to Mousam Lake. Mousam Lake with only 0. 115 cells/mL was never closed. Crystal Lake had the second highest ?E. coli? level of 0. 233 cells/mL and was closed only once. Woods Pond was closed one time but currently did not test positive for any ?E. coli? within the parent sample and the individual wells.

Discussion:After calculating the total number of ?E. coli? cells/mL for each sample, it was observed that the highest ?E. coli? levels were found at a beach that was never closed during the summer. In contrast, the beach containing the lowest levels of ?E. coli? was closed once due to a failed water test. Figure 1. does not show any discernible trend. It was assumed that ?E. coli? levels would remain elevated in frequently closed beaches while still being within safe ?E. coli? levels. In general, the data does not support the hypothesis that ?E. coli ?levels would be higher in lakes that were closed more frequently during the summer. Thus, according to this experiment, there is no direct correlation between the number of times a beach was closed and the level of ?E. coli currently present. This is surprising because frequent closings are often associated with high pollution levels (IJC 1991).

There are many factors that can influence ?E. coli? levels such as water temperature, low water levels, human traffic, stormwater runoff, marinas, leaky sewer lines, and presence of waterfowl (Bacteria 2018). Some of these factors can be used to explain the results found in this experiment. For example, Sebago Lake had the highest ?E. coli ?levels of 0. 447 cells/mL despite having no known closings. The high levels may have been influenced by location. Raymond Beach is next to Route 302 which could account for stormwater runoff containing ?E. coli? from animal waste (Xue ?et al? 2018). In addition, it's a large lake frequented by more people and has high boating traffic. Perhaps Sebago Lake should have been closed this summer, but was not monitored properly. The results of 0 cells/mL for Woods Pond can also be explained. Woods Pond was closed this summer; however it was closed due to a norovirus (M. D. 2018). This coincides with the experimental results of no ?E. coli? cells present. With more prior research, Woods Pond could have been excluded from the lakes labeled as closed. As for Highland Lake, despite being closed twice, the ?E. coli ?levels were found to be equivalent to Mousam Lake which never failed any ?E. coli? testing. Using Mousam's ?E. coli? level as a standard, it could be argued that Highland Lake's ?E. coli? levels recovered due to a decrease in temperature in recent weeks (Shergill2004). In addition to the multiple environmental factors, another source of error could be the lack of representative samples.

For instance, a representative sample may not have been captured within the wells resulting in a low number of overall positive wells. An example of this was Highland Lake which only had

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two positive wells despite having a completely fluorescent parent tube. It indicates that very few ?E. coli? cells were captured while pipetting the sample. The second problem surrounding a representative sample is the lack of multiple samples from each lake. Without repeated results, the experiment lacks accuracy for drawing any significant conclusions. While the results of this experiment did not lead to any major conclusions, the practice of water testing remains important for public health. Even this experiment was able to show that all five lakes currently test within the safe levels of ?E. coli? set by the EPA. However, the EPA does not specify where samples should be taken from which may impact the reading. A future experiment could test the ?E. coli? levels found at different depths and distances from the shore. Perhaps ?E. coli? is best collected right along the shoreline or maybe the more accurate readings come from deep samples further out in the lake. Overall, the better we are at detecting the presence of ?E. coli?, the more we can prevent the spread of enteric diseases and perhaps even determine the source.

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