Interim Report

2017 Faecal Loads in South East New Brunswick Waters - Towards Citizen Monitoring
New Brunswick Environmental Trust Fund

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Goals & Introduction

Goal 1: Assemble baseline reference data on the state, patterns and history of faecal indicator organisms in the Northumberland Strait of New Brunswick.

Shellfish surveys dating as far back as 1940 make up a large portion of the data available on water quality for the New Brunswick Northumberland Strait shores. Starting in the early 2000s specific beach testing data started to become more public so more recent data can be sourced back to public access sites.

Our goal is to create a searchable, analyzable overview of the data available, the organisms being tracked and the sites. The dataset can then be made accessible to interested parties, in a format amenable to ongoing extension.

Microbial counts were performed at various labs in New Brunswick, including RPC Moncton, Environment Canada Mobile lab and Caledon.

In the winter of 2017, an environmental microbiology class at Mount Allison University started the assembly, input and analysis of the data. Their research and data culminated in an April 2017 symposium held at the university. The focus for the presentation was to demonstrate the change in bacteria levels to the public in order to engage them with the current issues with the Northumberland Strait water quality. Based on the results from the class, several future directives were proposed, which provided a starting point for the research to be done with the current Environmental Trust Fund grant.

A portion of time spent on this ETF project was dedicated to reformatting the preliminary dataset. The dataset was also checked for errors and missing relevant environmental data including air temperature, water temperature and tide was added. Time was also allocated to creating a sub-dataset for just Parlee Beach, Murray Beach and Aboiteau Beach for analysis of those major tourist beaches.

Goal 2: Evaluate the feasibility of citizen-science or community group monitoring of faecal indicator organisms.

In addition to the data analysis, the class ran a short analysis on three low cost products which could potentially be used to test water quality by “citizen scientists”. Coliscan Easygel plates were used to enumerate the amount of fecal and non-fecal coliforms. The *E. coli* Easygel Cards were to enumerate the amount of *E. coli* in a sample. The *Enterococcus* Easygel Cards were used to enumerate Enterococcus. (Micrology Laboratories). Micrology also produced a card capable of detecting fecal and non-fecal coliforms,an option in the event the other products were not sufficient..

Results

Goal 1: Assemble baseline reference data on the state, patterns and history of faecal indicator organisms in the Northumberland Strait of New Brunswick.

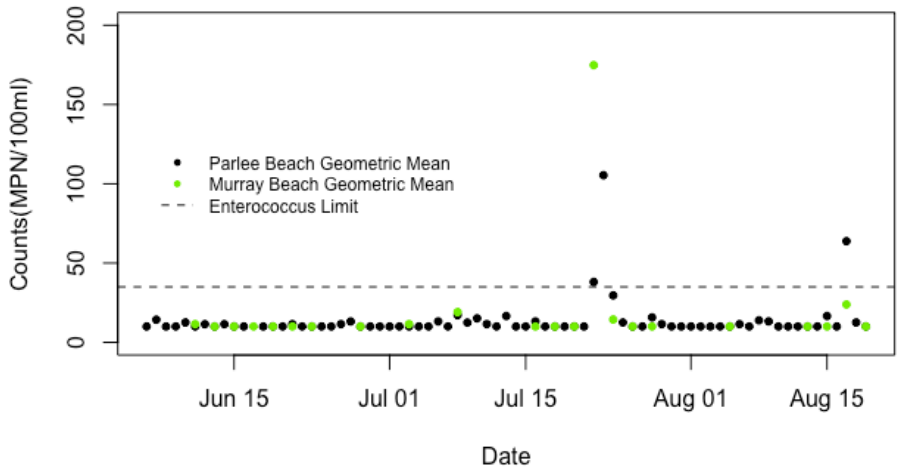

Figure 1. 2017 Levels of *Enterococcus* for Parlee and Murray Beaches, reported as geometric mean. Limit for Geometric mean indicated at 35 MPN/100mL.

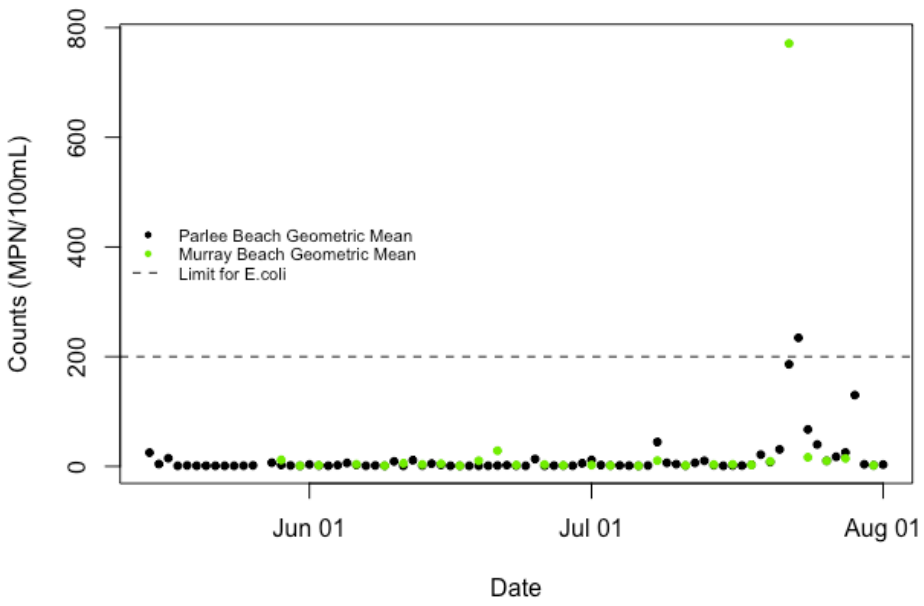

Figure 2. 2017 *E. coli* levels for Parlee and Murray Beaches, reported as a geometric mean of 5 samples. Limit for geometric mean of *E. coli* indicated at 200 MPN/100mL.

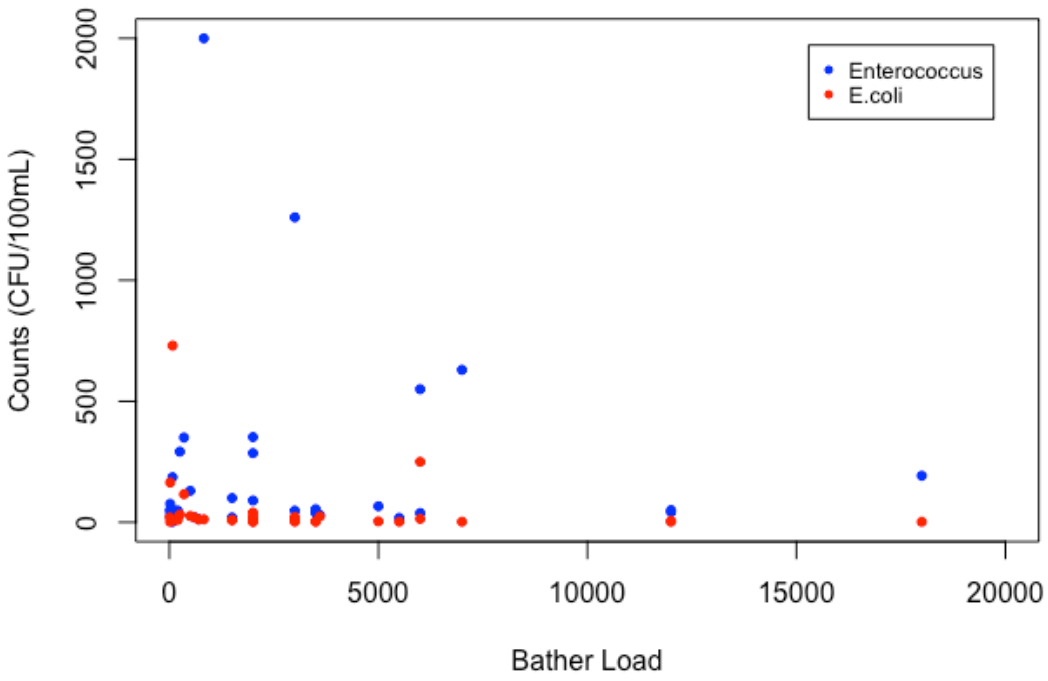

Figure 3. Bacterial Counts vs estimated Bather Load at Parlee Beach for the years 2011,2014,2015 and 2016.

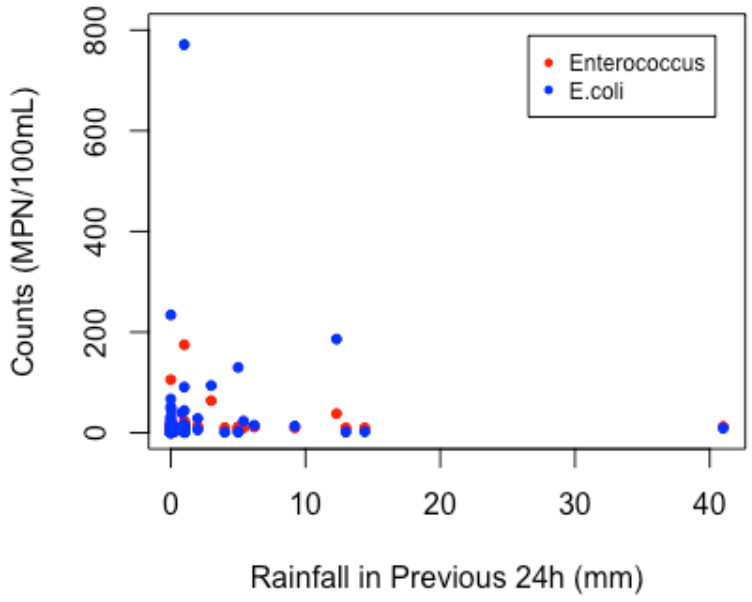

Figure 4. Bacterial Counts vs Rainfall in Previous 24 h at Parlee Beach for the years 2011,2014,2015 and 2016.

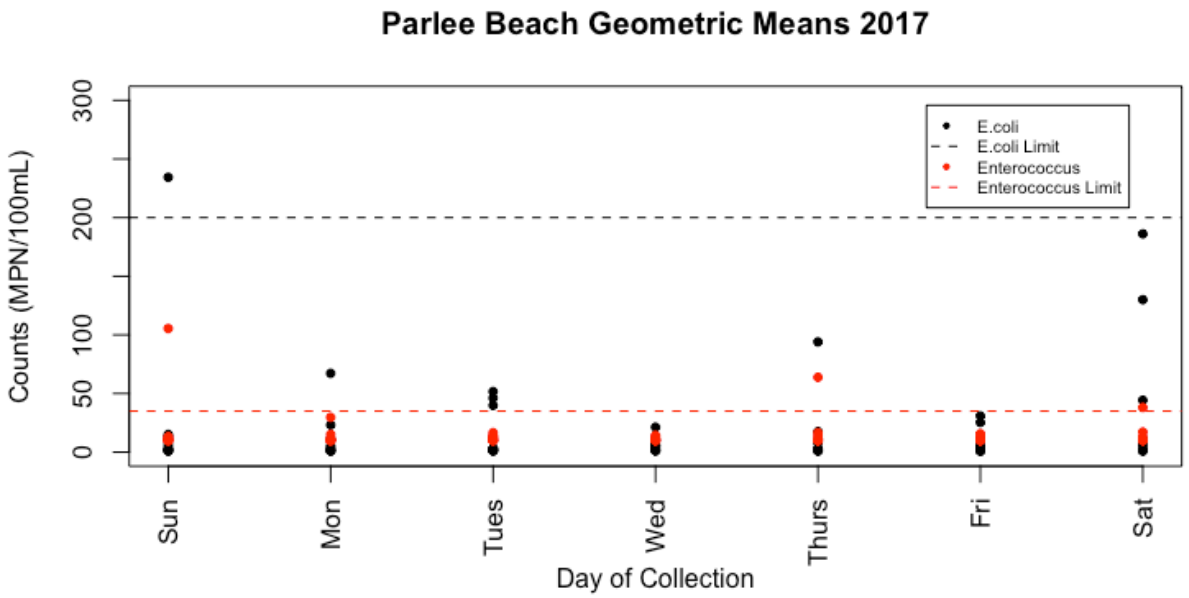

Figure 5.Counts for Parlee Beach Summer 2017 vs. the days of the week the sample was taken.

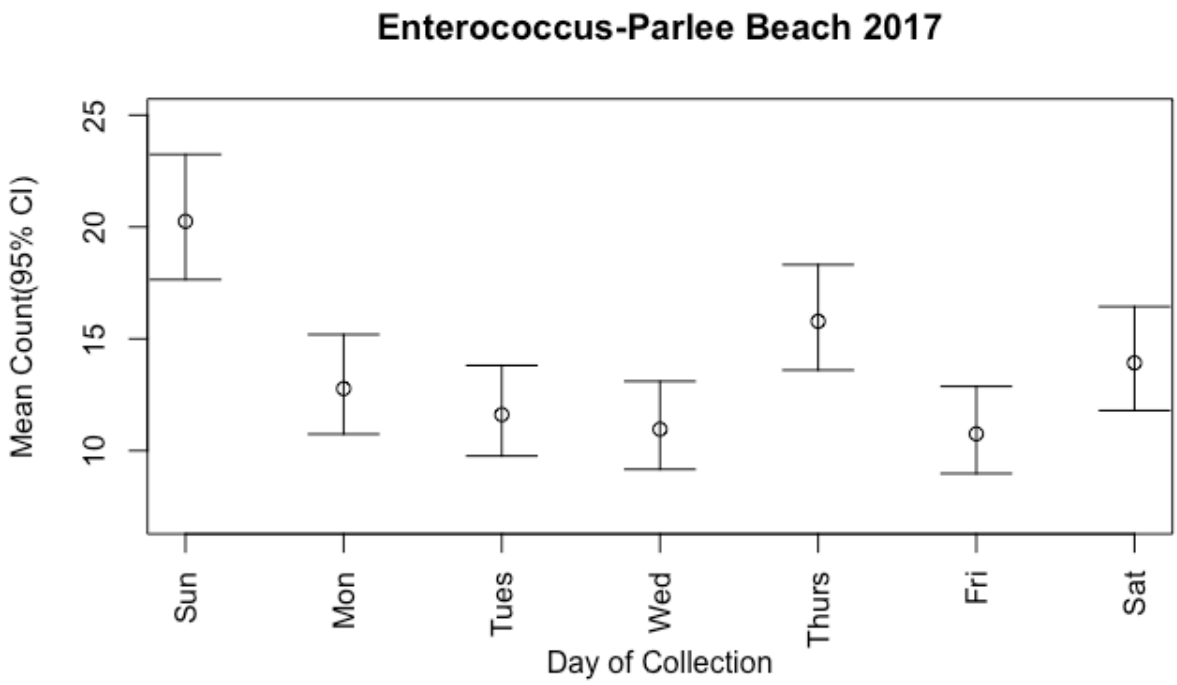

Figure 6.Test of significance of day of the week influence on the *Enterococcus* count recorded for that day. The more the standard error bars overlap, the less significant difference in the overall counts.

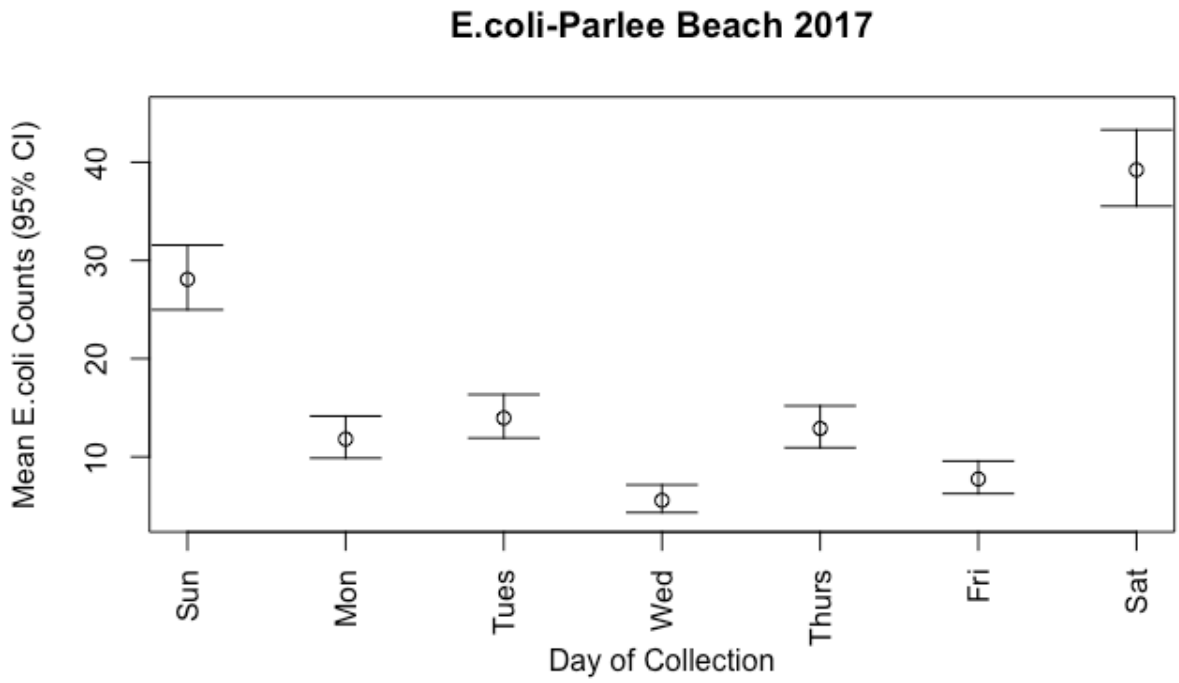

Figure 7.Test of significant of day of the week influence on the *E.coli* count recorded for that day. The more the standard error bars overlap, the less significant difference in the overall counts.

Goal 2: Evaluate the feasibility of citizen-science or community group monitoring of faecal indicator organisms. *Coliscan Easygel Cards*

One of each replicate for *E. coli* can be seen below. Each individual dot was counted as a colony.

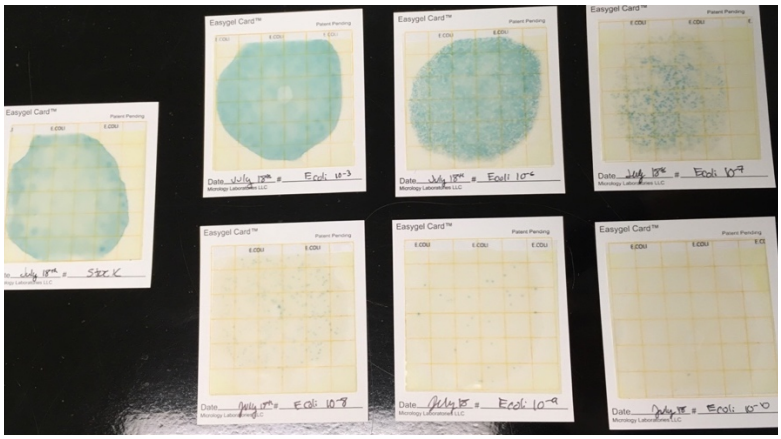

Figure 8. One of each dilution for *E. coli* tested on a Coliscan Easygel Card that was incubated for 24 hours at 37°C. Each card contains 1mL of inoculum, which is an *E. coli* culture diluted in sterile tryptic soy broth at stock, and dilutions of 10^{-3},10^{-6}, 10^{-7},10^{-8},10^{-9},10^{-10}.

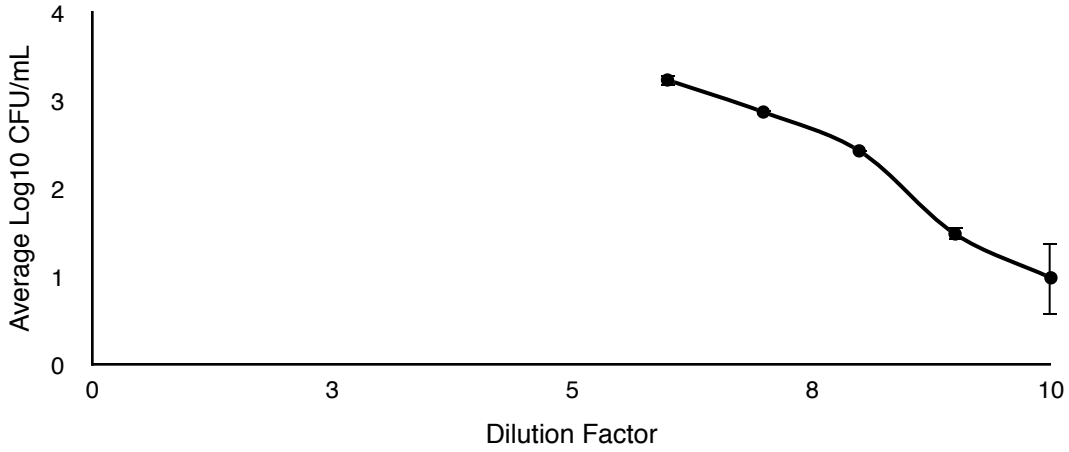

Figure 9. Average Log CFU/mL plotted against log dilution. Error bars indicate standard deviation for each dilution

One of each *EasyCard* replicate for *Enterococcus* can be seen below.

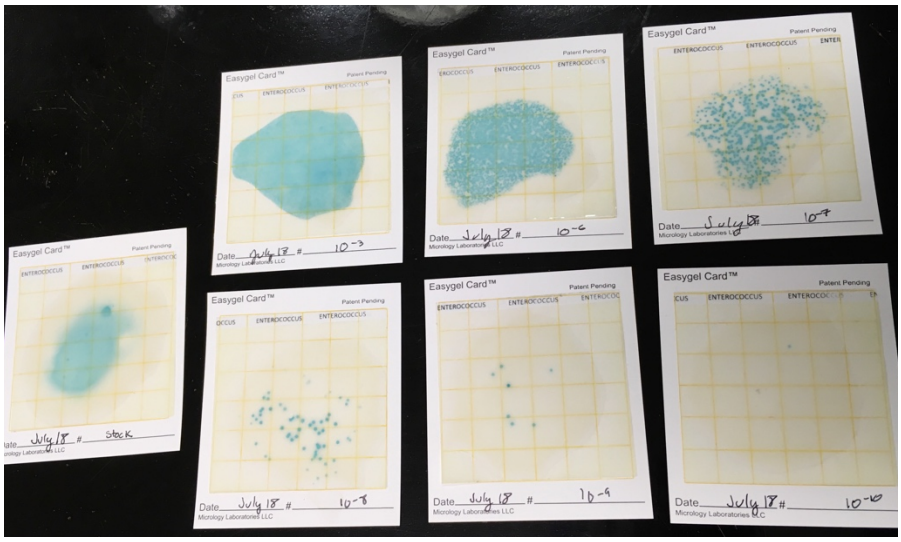

Figure 10. One of each replicate for *Enterococcus* dilutions on EasyCards. Dilutions done in sterile tryptic soy broth at stock and dilutions of 10^{-3},10^{-6}, 10^{-7},10^{-8},10^{-9},10^{-10}.

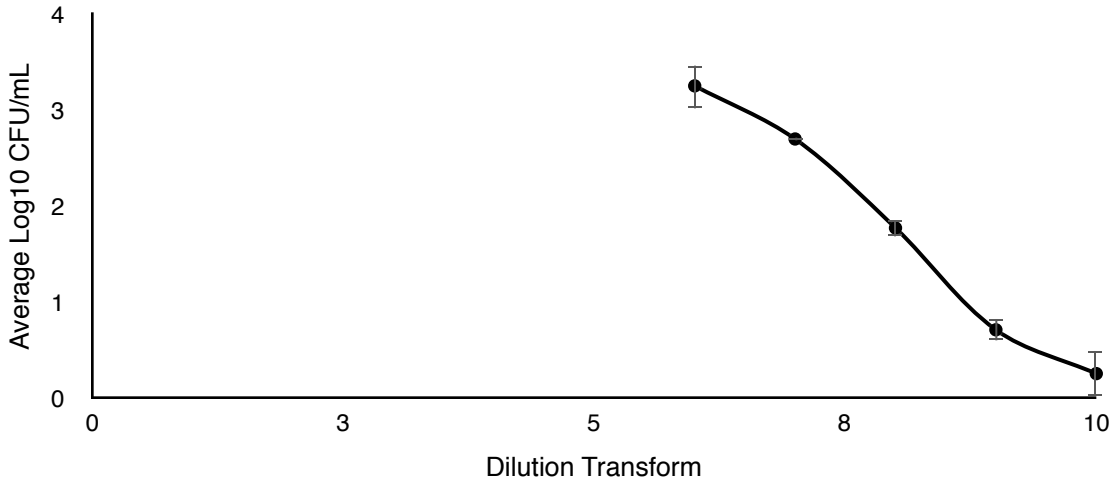

Figure 11. Plot of Log10 CFU/mL versus log dilution for *E. faecalis*. Error bars indicate standard deviation for each dilution.

Coliscan Easygel Pour plates

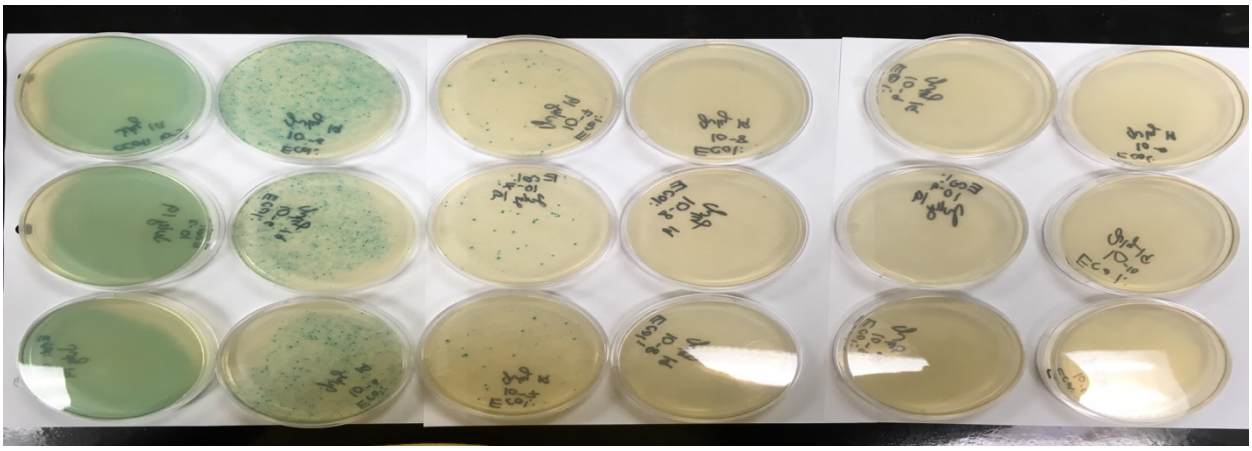

Figure 12. Coliscan Easygel Pour plate test. Left-right dilutions of stock culture to 10^{-3},10^{-6},10^{-7},10^{-8},10^{-10}. All plates were inoculated with 1mL of their specified dilution. Cultures were diluted with sterile tryptic soy broth.

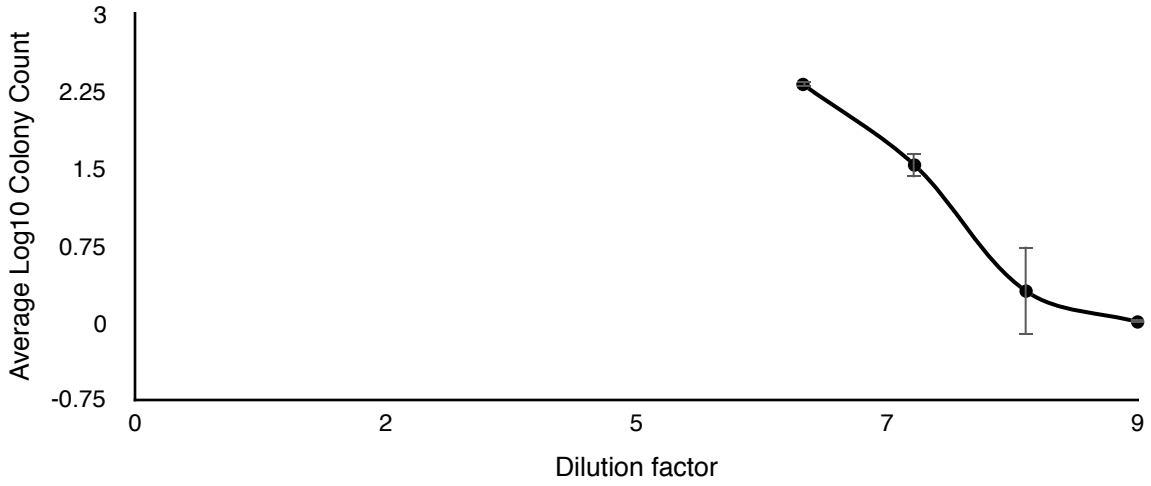

Figure 13. Log of Average CFU/mL plotted against log dilution. Error bars indicate standard error.

Water Samples

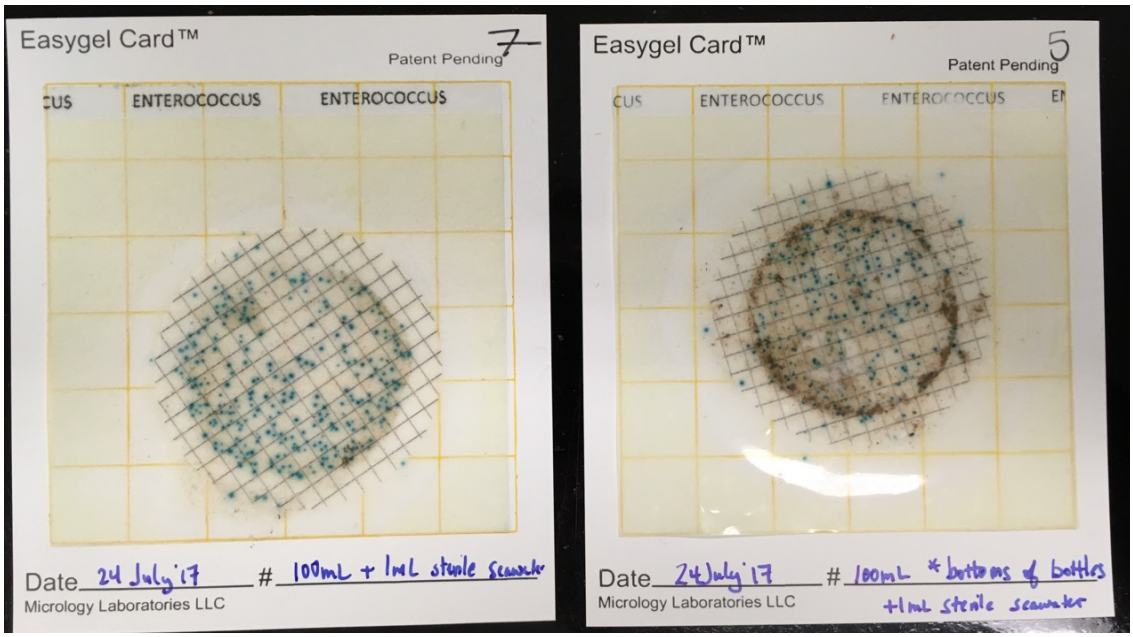

Figure 14. Vacuum filtered water samples from Aboiteau Beach on July 24th, 2017.

Table 1. CFU per specified volume of water of organism of interest.

Organism	Colonies	Volume Filtered
	CFU	mL
E. coli	38	100
E. coli	NA	200
E. faecalis	225	100
E. faecalis	268	100
E. faecalis	NA	200

ECC Card Colour Test

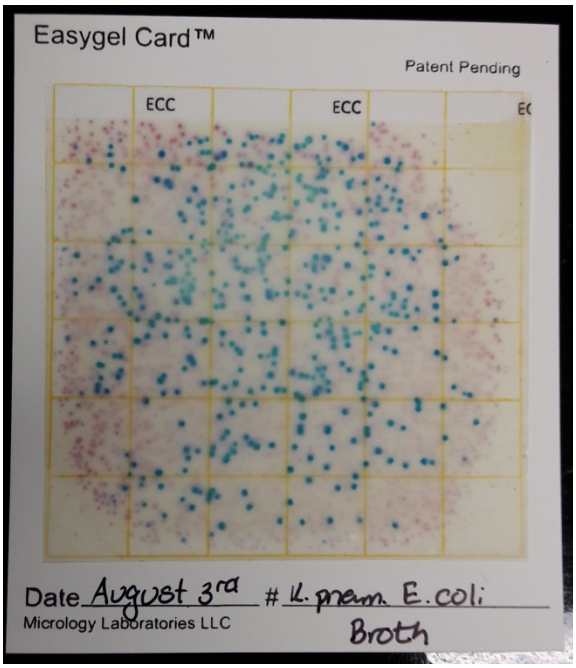

Figure 15. *K. pneumoniae* and *E. coli* dilutions on Coliscan ECC card. Pink colonies are *E. coli* and blue colonies are *K. pneumoniae*. 500μL of a 10^{-7} dilution of each culture was inoculated on the card. Diluent for *K. pneumoniae* was sterile Lab-Lemco broth. Diluent for *E. coli* was sterile tryptic soy broth.

Future Directions

Based on the results of the quantification tests, a high accuracy, low cost non-lab test for marine water seems unlikely. Using cards does not have the same technical issues that can come with the pour plates. However accurate results can only be achieved if the seawater sample is pre-filtered to concentrate the bacteria from seawater, because the reliable quantitation range of the cards is fairly high. There is no cost-effective way to provide an at home alternative for filtration of 100 ml of seawater into a format suitable for placement on the card.

The cards could be used in the field by researchers who have portable filters at their disposal. Both the plates and cards could also be used in a lab setting where all the proper tools and growing environments were available.

For Parlee, analysis of the significance of the day of week on count will be continued. If it seems like the days of the week with the highest counts are changing over time, this could help pinpoint a source and when the issue arose. This might also be a point of consideration for beaches not yet doing everyday testing, using Parlee as a model, one would want to be testing on the days that seem the most significantly different from the normal to best allocate limited testing resources to uncover how much counts fluctuate.

We are currently cross-correlating multiple years of data with rainfall and weather over days preceding the counts.

We have submitted the curated database of microbial counts to the province and continue to add to the growing repository.

Materials and Methods

Control Organisms

E. coli, E. faecalis, P. aeruginosa and *K. pneumoniae* were kept on agar slants. *E. coli, E. faecalis, P. aeruginosa* were on tryptic soy agar while K. pneumoniae were kept on slants made from Lab-Lemco Broth and agar. Tryptic soy agar was made in 150mL batches. Approximately 150mL of distilled water, containing a stir bar, was brought to a rolling boil on a hot plate. For the 2% agar solution, 3g of agar was then added to the water and allowed to stir for 10 minutes. Then 4.5g of the tryptic soy broth powder was added and allowed to stir for 10 minutes. The solution was then removed from heat to allow to cool before pouring into tubes. Tubes were glass with threaded caps, fitted to the slant rack. Tubes had previously been cleaned with bleach, then rinsed according to Campbell Lab protocol. Tubes were not sterilized. Once cool enough to handle, the agar was added to the tubes until tube was approximately 1/3 full. Filled tubes were placed in the autoclave safe slant rack. Cap threads were left loose, and then covered in tin foil before autoclaving on a liquid cycle with a 15-minute exposure time. After autoclaving, tubes were placed at a 20^{o} slant with caps still loose for approximately 30 minutes, then threads were tightened and agar left to solidify.

This same procedure was followed for the Lab-Lemco agar. A 2% agar solution was made, but the amount of broth powder required for 150mL was 1.2g. The Lab-Lemco powder was used as a replacement for nutrient broth since Fisher Scientific was out of nutrient broth powder. Broth was made in the same fashion; however, the agar was not added to solution.

Organisms were sub cultured either every week or every two weeks depending on what experiments were going to be run that week. No biological safety containment hood was available, so a bench work surface was wiped down with ethanol before culturing. Before transferring, threads were loosened on tubes of interest to make for a smoother transfer. The loop was then flame sterilized, the tube containing culture was the opened and flamed, then the loop was inoculated, the top of the original tube re flamed and capped, then the new tube was opened, inoculated and then flamed. The new tube was not flamed before inoculation as it had not been opened since being autoclaved.

24 hours before a test, tubes of broth were inoculated with culture and left to incubate at 37^{o}. After 24 hours, a serial dilution was performed using sterile broth following the schematic below.

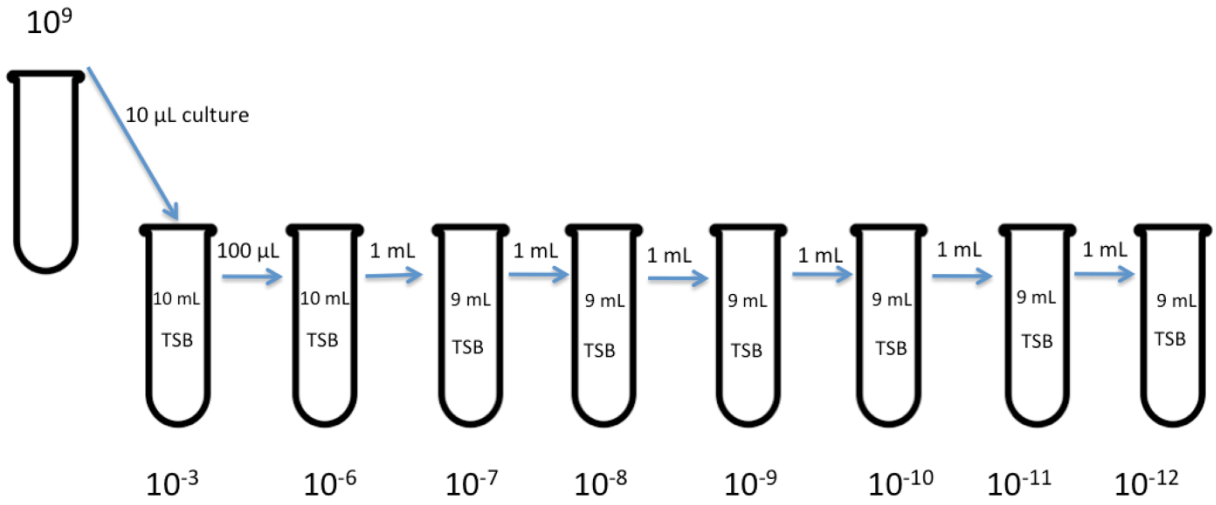

Figure 16.Dilution scheme for Coliscan Easygel Cards. *For K. pneumoniae* sterile Lab-Lemco broth was used as the diluent. For *E. coli*, *E. faecalis* and *P. aeruginosa* sterile tryptic soy broth was used.

Coliscan Easygel Cards

The Coliscan cards and plates contain sugars which are acted upon by beta-galactosidase, an enzyme found in many fecal coliforms. When the sugar is metabolized it releases a pink dye. There is another sugar in the coliscan media which will release a blue dye when metabolized by coliforms using the enzyme beta-glucuronidase. *E. coli* metabolize both of the sugars, resulting in a purple/dark blue colour when inoculated on the Easygel Plates or ECC cards (Which contain the two sugars). The cards intended for only *Enterococcus* and *E. coli* detection contain only the sugar to release the blue dye, hence their teal/blue appearance on a completed test.

A dilution curve was performed with culture standards to determine the detectable range for each product.*Klebsiella pneumoniae* was used as the coliform positive control for ECC cards and *Pseudomonas aeruginosa* was used as the negative control. The Micrology tests are intended for freshwater use, therefore the control organisms specified are more commonly found in freshwater. It was decided to use them for marine samples since it would give an indication of whether the tests were working properly.

In addition to determining the detectable range, there was interest in adapting the laboratory procedures to make the tests more accessible to those without the lab space who were still interested in testing their water. Proposed recipients of the modified test included waterfront cottage owners, watershed management committees like the Shediac Bay Watershed Association, or those interested in plating samples in the field to minimize the time the water had to be transported. Using a filter paper on the Micrology cards allowed a higher volume to be analyzed than the standard Micrology procedure since that procedure only used 1mL of sample water per test, which could not be considered a representative sample at the regulatory limits for *Enterococcus* or *E.*

coli counts. When deciding how much water should be filtered at a time it was decided to test 100mL at a time since number of colonies is typically reported per 100mL of sample water. Methods to modify the filter tower procedure common for most water filtration procedure would have to be considered. Disposable, swinnex-style syringe tips appeared could be an appropriate substitute. The necessity of a pre-filter when filtering sea water samples was also questioned. If the goal was to create an easy to use kit-style procedure, the pre-filter could add more sources for error but may allow for clearer results when it came to counting.

We decided the focus of this research would be on the detection and enumeration of *Enterococcus* since previous research shows it is a better indication of marine water quality. *E. coli* do not survive as long as *Enterococcus* in marine water. Therefore testing *E. coli* could result in a false evaluation of the water quality. *Enterococcus* has a lower limit for recreational water than *E. coli*, 35MPN/100mL compared to 200MPN/100mL. This means tests for *Enterococcus* must be more sensitive in the lower range, which can be difficult to produce when not in the lab environment.

Following determination of detectible range, ideal volumes and organism of interest sea water samples from various locations were collected. Samplers were given the following form to fill out (next page) to make the sampling procedure and data collected as standard as possible. The desired volume to be collected was 500mL, this would allow for analysis of *Enterococcus* to be done in triplicate with the membrane filtration procedure. 100mL per test would be used, allowing the other 200mL to be available for other uses, such as inoculation media for quality control tests.

Each dilution was then tested in triplicate. 1mL of solution was pipetted on to each card using a 1000μL Pipette. Cards were then labelled and left to incubate for 24 hours. When cards were ready to count, they were placed under a dissecting scope. For cards with many (>500) colonies, individual quadrants of the cards were counted and their totals added together. This same procedure was followed for every organism. **

Coliscan Easygel Pour Plates

24 hours before the test, *E. coli* was inoculated in sterile tryptic soy broth 12 hours before the test, the necessary amount of coliscan media bottles were removed from the freezer and placed in the crisper to thaw.

The same dilution scheme was followed for the plates. Each dilution was also done in triplicate. 1mL of inoculum was added to each bottle of media. Following inoculation, the bottles were swirled gently to avoid bubbles while mixing.

Each bottle was then poured into its own Coliscan Easy gel plate. Once poured, they were left on the benchtop for 90 minutes before being turned upside down and incubated for 24 hours at 37^{o}.

Colonies were counted on benchtop without dissecting scope, however, grid paper was placed below the plate to make counting easier.

ECC Card-Colour Test

24 hours before the test a tube of sterile Lab-Lemco Broth was inoculated with *Klebsiella pneumoniae* to use a coliform positive control. A tube of sterile tryptic soy broth was inoculated with E. coli to be the *E. coli* positive control. A serial dilution was performed on both organisms, but only until a concentration of 10^{-7} was obtained. 500μL of the *E. coli* 10^{-7} was added to the card, then 500μL of *Klebsiella pneumoniae* was added. The card was then incubated at 37^{o} for 24 hours.

Water Samples

Water samples were collected as outlined in the form appended Once collected, they were refrigerated until they arrived at the lab (24-36 hours). A sterilized filter tower was set up. Non-sterile 0.2micron filter paper was placed and then clamped in place. 5mL of distilled water was run through the system before the sample was added to ensure that there were no leaks in the system. 100mL of sample water was placed in the tower to be filtered. The cap of the filter tower was left on until the water was about 1cm above the filter paper, then when the water was about 0.5cm above the paper, the suction was turned off. Removing the cap before stopping suctions stops the filter paper from warping when the suction is turned off.

The filter paper is then placed in the center of a coliscan Easygel card (*E. coli* or *Enterococcus*) then 1mL of distilled water is placed on top of the filter paper. It should be added dropwise to ensure the water is distributed evenly. Sea water could also be added. The seawater should be taken up in a 5mL syringe. A syringe filter can then be added and the water filtered through and onto the card. The card was then incubated for 24h at 37^{o}.

Preservation of Cultures

A 20% glycerol solution was made, using 20mL of milliQ water and 5mL of glycerol. This solution was vortexed in 3 short pulses to homogenize. It was then filter sterilized with a syringe filter. 1mL of sterile glycerol solution was placed into sterile 1.5mL micro centrifuge tubes. A flamed loop was then used to transfer the desired culture from the agar slants to the micro centrifuge tube. Tubes were then placed in the -80^{o} freezer.

Sampling Form

Name:_____

Contact Info:_____

Sampling Location (ex. Shediac Bay):_____

_____

Approx. Sample Depth:_____

_____

Date and Time:_____

_____

Air Temperature:_____

Tide:_____

Sampling Method from State of Wisconsin Dept. of Natural Resources
www.wisconsin.gov

(1) **Water should be approximately knee deep, sample should be taken should approximately 6 to 12 inches below the surface of the water.**

(2) Open a sampling bottle and grasp it at the base with one hand and plunge the bottle mouth downward into the water to avoid introducing surface scum.

(3) Position the mouth of the bottle into the current away from your hand. If the water body is static, an artificial current can be created by moving the bottle horizontally with the direction of the bottle pointed away from you.

(4) **Tip the bottle slightly upward to allow air to exit and the bottle to fill**.

(5) Make sure the bottle is completely filled before removing it from the water.

(6) **Tightly close the cap and label the bottle**.

(7) **Store sample in a cooler filled with ice or suitable cold packs immediately. (**Sampling Procedure