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Seasonal Change in Bacteria Levels and Eluent Comparison for the Enumeration of E. Coli and Enterococci from Recreational Beach Sand

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SEASONAL CHANGE IN BACTERIA LEVELS AND ELUENT COMPARISON FOR THE ENUMERATION OF *E. COLI* AND *ENTEROCOCCI* FROM RECREATIONAL BEACH SAND

by

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ABSTRACT

SEASONAL CHANGE IN BACTERIA LEVELS AND ELUENT COMPARISON FOR THE ENUMERATION OF *E. COLI* AND *ENTEROCOCCI* FROM RECREATIONAL BEACH SAND

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The University of Wisconsin-Milwaukee, 2013
Under the Supervision of Professor Jin Li

Research into the causes of bacterial contamination of recreational beaches has been increasing. Many researchers are now looking to enumerate bacteria from beach sands in order to find the connections between the water and sand quality. As the number of these studies increase, there is a need to determine a standard procedure for enumerating bacteria from sand. The eluent that a researcher chooses could impact the results that they obtain due to ionic strength. The first part

of this paper compares the *E. coli* and *Enterococci* counts of identical sand samples using phosphate buffered saline and distilled water as eluents. The second half of this paper analyzes the role of *Cladophora* in the seasonal change of *E. coli* and *Enterococci* levels in the recreational beach environment.

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Introduction

Millions of people visit over 500 recreational beaches in the Great Lakes region every year (*Liu et al, 2006*). Contamination of these recreational waters is a major environmental concern and public health threat. The primary way that recreational waters and the adjacent beaches are monitored for elevated health risks are through testing for indicator bacteria, *Escherichia coli* and *Enterococci*. *E. coli* are common and natural inhabitants of the intestinal tract of warm-blooded animals, including humans, and greater than 10^6 *E. coli* are generally present in 1 gram of colon material (*Ishii et al, 2007*). *Enterococci* are also natural inhabitants of the intestinal tract of warm-blooded mammals, and they have shown a tolerance in extremes of pH, temperature, salts, and detergents (*Halliday et al,* 2011). Since these bacteria are thought to be unable to survive in the environment, their presence is believed to indicate contamination from human sources such as wastewater treatment plants or runoff water contaminated with fecal bacteria.

In 1986, the United States Environmental Protection Agency produced guidelines recommending *Enterococci* and *E. coli* as appropriate bacterial indicators to monitor recreational waters (*US EPA, 1986*). *Enterococci* are best correlated with health outcomes in marine systems, whereas *E. coli* is best correlated with health outcomes in fresh water systems due to their survivability in different conditions (*Halliday et al, 2011*). The state of Wisconsin uses two different thresholds for its

beach monitoring of *E. coli*. The advisory limit is 235 CFU/100 mL, which prompts the health department to post a yellow “Caution” sign at the beach. The second limit is set at 1000 CFU/100 mL. This is a closure limit and the local health department is responsible for posting a red “Closed” sign in order to notify the public that an elevated health risk is present at the beach. A new set of recreational water quality standards were recommended by the EPA in 2012 (*Table 1*).

Table 1: EPA Recreational Water Quality Standards 2012

<u>**Criteria Elements**</u>	**Illness rate 36/1000**		**Illness rate 32/1000**	
Indicator	*GM (cfu/100mL)*	*STV (cfu/100mL)*	*GM (cfu/100mL)*	*STV (cfu/100mL)*
Enterococci (marine & fresh)	35	130	30	110
E. coli	126	410	100	320

The EPA is no longer recommending the use of a single sample maximum value. They now suggest using a combination of the geometric mean and statistical threshold value in order to determine the magnitude, duration and frequency of bacterial indicator presence. The geometric mean is determined by taking the $\log_{10}$ of the sample values, averaging those values, and then raising the average to the power of 10. The statistical threshold value is derived by estimating the percentile of the expected water quality distribution around the geometric mean criteria value. The new recommendations correspond to an estimated illness rate of 32-36 per 1000 primary contact recreators (*US EPA, 2012*).

Indicator bacteria *E. coli* and *Enterococci* are also used to determine water quality because of the positive correlation of these bacteria to the occurrence of gastrointestinal illnesses in humans (*Wade et al, 2003*). Their densities in coastal waters contaminated with wastewater and urban runoff have been linked quantitatively to swimmer illness in epidemiological studies (*Yamahara et al, 2009*). Sampling efforts for recreational water quality are focused on the near shore waters because this is where the greatest number of swimmers will come into contact with the water, however, this process could be leaving a gap in the data. Many people that visit the beach are not swimming and simply enjoy playing sports or relaxing in the sand. Bacteria counts in the water alone may not be enough to get an accurate picture of beach health. When beach health is tested now they currently use indicator bacteria levels in the water.

The monitoring of beaches has been limited to testing the water for indicator bacteria, however, a number of studies have suggested that beach sand may also be

a significant source for fecal indicator bacteria (*Whitman et al, 2003B*) (*Bonilla et al, 2007*) (*Byappanahalli et al, 2003A*) (*Ishii et al, 2005*) (*Alm et al, 2006*). One study showed that bacteria harbored in sand persisted longer than in water because the bacteria adhered to sediment particles more easily than the free particles in water (*Whitman et al, 2003B*). Indicator bacteria in California beach sands were found to be mobilized by high tides and diffused into the water column (*Yamahara et al,* 2007). More research is needed to determine just how effective rainfall is at releasing bacteria that is adhered to sand and suspend it into the water table. The levels of bacteria in near shore waters could be directly related to the mobilization of bacteria from the sand by either tides or rainfall into the body of water. This is further supported by the discovery of higher levels of fecal indicators in sand rather than in water at most of the locations studied in the Gaza Strip (*Elmanama et al, 2005*). This indicates that the assumption of indicator bacteria only being able to survive in the intestinal tracts of warm-blooded mammals may be incorrect and their levels may not be an indicator to human source pollution. It has also been shown that exposure to sand (i.e. digging) is positively associated with gastrointestinal illness (*Heaney et al, 2009*). This supports the use of indicator bacteria for public health warnings but shows a need for possibly including the testing of beach sand for bacteria when determining the overall health risk of a recreational area.

Several studies have also looked at the transport of microbial pollutants to determine the source of the pollutants (*Boehm, 2003*) (*Whitman et al, 2006*) (*Ge et al, 2010*). The physical and chemical factors influencing the mobility of bacteria in

porous media include solution chemistry, fluid velocity, surface roughness, charge heterogeneity, grain size, and saturation of porous media (*Chen et al, 2012*). A study performed in Milwaukee showed that the bacterial DNA isolates found in beach sand were highly correlated to itself with gull isolates as the next highest percentage (*McLellan 2004*). These results show that the bacteria that attaches to sand not only is maintained there but also grows and adapts to the environment. Another study showed that *E. coli* persisted year round in two freshwater beaches, independent of pollution events (*Byappanahalli et al, 2006*). A study performed in a rain forest in Puerto Rico showed that fecal coliforms may not be derived exclusively from fecal sources and therefore may not indicate fecal contamination (*Rivera et al,* 1988). This shows that *E. coli* may not be an accurate representative of human contamination to recreational waters because of its ability to thrive in the environment.

E. coli that may inhabit a freshwater beach year-round is an important issue. The ability of *E. coli* to inhabit warm, moist sand and survive through the winter can greatly impact current thoughts on sampling for indicator bacteria. This would cause an indigenous population of bacteria to not only survive but also reproduce and grow in number. There are several factors that could allow this population to survive beneath the beach's surface including protection from photo inactivation, nutrient washing from high tides or storm events, warm summer temperatures, presence of nutrient rich algal mats, etc. One study showed that the presence of sand also increased the time that *E. coli* survived regardless of temperature (*Sampson et al, 2006*).

The presence of *Cladophora*, a genus of nuisance green algae, has become an increasingly common occurrence on Great Lakes beaches. This is due to the arrival of an invasive species called zebra mussels. They have increased the water clarity and allow for photosynthetic activity to occur in deeper water than ever before. Another factor that causes growth of *Cladophora* is phosphorous accumulation from storm water runoff (*Englebert et al, 2008*). *Cladophora* is algae that grows in strands or mats on hard substrates in lakes. Most of the *Cladophora* on beaches is due to it unlatching and washing into shallow waters. It is typically found in the near shore water but is also subject to washing up onshore during a high tide or storm event and can become stranded on the beach. *Cladophora* can smell bad when it congregates in the near shore water, which can be a deterrent for people visiting the beach. Some beaches have decided to take care of this problem by raking the algae onto the beach in order to allow it to decompose quicker. This process could lead to increased nutrient transfer into the sand, which allows the bacteria levels to grow even larger (*Whitman et al, 2003A*).

Project Overview

Bacterial adhesion to beach sand is a large and varied field. This project will focus on two different topics in the area of bacterial adhesion to beach sand. The first topic was performed in the lab and focuses on determining a preferred testing eluent for enumerating bacteria from beach sand. The second topic is conducted with field sampling and analyzing the change of bacteria concentration over time at a freshwater beach as well as the impact of *Cladophora* levels.

One of the challenges in determining the amount of bacteria present in sand is to enumerate the particles for testing. A recent study compared various extraction methods that produced the highest amount of bacteria recoveries (*Boehm et al, 2009A*). The research focused on the physical characteristics of the enumeration process including shaking time, shaking mechanism, settling time, number of rinses, eluent removal, and eluent volume to sand weight ratio. The study tested the enumeration of bacteria using five different eluents; phosphate buffered saline (PBS), distilled water, seawater, PBS + tween 80, and DI water + sodium hexametaphosphate $(NaPO_3)_6$. The results of the study showed that the simplest extraction method that produced the highest indicator bacteria recoveries consisted of 2 minutes of hand shaking in either PBS or DI water, a 30 second settling time, one-rinse step, and a 10:1 eluent volume to sand weight ratio (*Boehm et al, 2009A*).

There has been an inconsistency among researchers as to what eluent should be mixed with the sand to enumerate the bacteria. A majority of researchers tend to

use distilled water or PBS while separating the bacteria from the sand for testing (*Table 2*).

Table 2: Comparison of Eluents Used by Researchers

Author	Year	Eluent Used	Shake Time	Type of Bacteria	Medium	Approx. Conc. Range	Technique
Boehm et al.	2009	PBS	1 & 2 min	*E. coli*	Sand	1.2 - 2.2 log MPN/g	Colilert-18, Enterolert, & mEI Agar
				Enterococci		0.7 - 3.6 log MPN/g	
Bonilla et al.	2007		1 min	*Fecal Coliform*	Sand	4x10^4 - 4x10^7 log CFU/g	mFC, mTEC, & mEI Agar
				E. coli		2x10^4 - 2x10^7 log CFU/g	
				Enterococci		4x10^4 - 4x10^6 log CFU/g	
Hartz et al.	2008		1 min	*E. coli*	Sand	0.1 - 10^6 CFU/g	mTEC & mEI Agar
				Enterococci		1 - 10^5 CFU/g	
Boehm et al.	2009	DI Water	1 & 2 min	*E. coli*	Sand	1.7 - 1.9 log MPN/g	Colilert-18, Enterolert, & mEI Agar
				Enterococci		1.3 - 3.5 log MPN/g	
Byappanahalli et al.	2003		2 min	*E. coli*	Sediment	0.5 - 3 log MPN/g	Colilert-18
Sampson et al.	2006		2 min	*E. coli*	Sand	20 - 20000 MPN/g	Colilert-18
Alm et al.	2003		1 min	*E. coli*	Sand	10^4 - 9x10^4 CFU/ g	mTEC & mEI Agar
				Enterococci		1.3x10^4 - 8x10^4 CFU/g	
Boehm et al.	2009	PBS + tween 80	1 & 2 min	*E. coli*	Sand	1.6 - 1.9 log MPN/g	Colilert-18, Enterolert, & mEI Agar
				Enterococci		0.2 - 3.5 log MPN/g	
		DI + 1% (NaPO3)6	30 sec & 2 min	*E. coli*		1.1 - 1.9 log MPN/g	
				Enterococci		1.1 - 3.6 log MPN/g	
Whitman et al.	2003	PBW	5 min	*E. coli*	Sand	2.5x10^5 - 1.1x10^6 CFU/g	mTEC Agar
Whitman et al.	2003		2 min	*E. coli*	*Cladophora*	3 - 6.2 log CFU/g	mTEC & mEI Agar
				Enterococci		0.8 - 6 log CFU/g	

A majority of the studies that used PBS as the eluent chose the EPA's formula which called for the following ratios; 0.32 grams of sodium dihydrogen phosphate, 1.1 grams of sodium monohydrogen phosphate, 8.5 grams of sodium chloride, and 1 liter of distilled water (*US EPA, 2000*). The solution ionic strength influences the extent of bacterial adhesion to a surface (*Chen et al, 2007*). There is a need to determine a preferred eluent type in sand bacteria testing in order to have more comparable results between studies.

Freshwater beaches may have the ability to harbor *E. coli* at detectable levels even during cold weather months. The current system for determining beach health is focused on the fact that indicator bacteria are found in recreational waters due to human source pollution. If levels of bacteria that are naturally growing in the environment due to past pollution events are able to survive in colder months then the current system of testing may be compromised. Further natural events such as rainfall and animal populations could further exacerbate the issue. Animal feces can lead to concentrated areas of bacteria growth. The presence of *Cladophora* has also been shown to harbor high levels of *E. coli* and *Enterococci* (*Byappanahalli et al, 2003B*). One study showed that *E. coli* was detected 63 of 63 samples of *Cladophora* with average levels ranging from 2700 CFU/100 g to 7500 CFU/100 g (*Olapade et al, 2006*) while another study showed that both *E. coli* and *Enterococci* survived over 6 months in sun dried *Cladophora* mats and could regrow upon rehydration (*Whitman et al, 2003A*). These studies show that *Cladophora* can serve as a reservoir for potential pathogens and bacteria (*Ishii et al, 2006*). It can also allow for increased *E. coli* survival and sometimes replication by augmenting beneficial (nutrients,

protection from predation, attachment sites) and reducing detrimental (ultraviolet light) environmental conditions (*Englebert et al, 2008*). Several studies have also shown that the survival of fecal indicator bacteria in ambient environments is strongly influenced by abiotic (salinity, sunlight, temperature, etc.) and biotic (predation and competition) factors (*Whitman et al, 2004*) (*Boehm et al, 2009B*).

Materials and Methods

Sample Locations and Time

Samples of water and beach sand were taken from Bradford Beach in Milwaukee, WI. The beach is located in a highly urbanized area and there is a source of domestic sewage nearby (*Whitman et al,* 2003A). Three different transects were used as sampling locations (*Figure 1*).

Figure 1: Bradford Beach Transect Map

Each transect consisted of three samples; a water sample, a sand sample from the swash zone, and a sand sample from 20 feet inland. The water sample was taken from water at knee depth (approximately 1.5 feet). The swash zone was defined as the area of the beach that is alternately wet and dry due to wave action. Sand

samples from the swash zone were taken from the surface of the beach. The sand samples that were taken 20 feet up shore from the swash zone were extracted from the water table. The depth of the water table varied from 6-21 inches based on topography of the transect and recent rainfall amounts.

Samples were taken from March to May of 2013 once a week. The weekly sampling occurred at the same time each day in order to account for temperature variability of the bacteria levels. An extra sampling day also was conducted the day after the local wastewater treatment plant, operated by the Milwaukee Metropolitan Sewerage District, reported a combined sewer overflow of 595 million gallons. The summer sampling schedule occurred in July and August. Samples were taken three days per week at approximately 9:15 am. There were no samples taken in June because we ran out of supplies and there was a delay in the ordering process.

Water samples were taken in sterilized 500 mL plastic bottles and sand samples were placed in sterile whirl-pak bags. All of the samples were placed into a cooler with ice for transport to the laboratory. The ice was to preserve the bacteria levels that were present in the sample upon initial sampling.

Algae Visual Classification System

A visual classification system was used to determine the levels of algae each sampling day. The rating scale was as follows: 0 for no algae, 1 for low, 2 for moderate, and 3 for high. A "3" rating was when there was no wave action onto the beach due to the thick algal covering in the near shore water, which led to stagnation (*Figure 2)*. A "2" rating had wave action on beach but large amounts of

algae in the water (*Figure 3*). A "1" rating had small amounts of algae visible in the water (*Figure 4*).

Figure 2: Algae Visual Classification "3"

Figure 3: Algae Visual Classification "2"

Figure 4: Algae Visual Classification "1"

The algae classification was done separately for each of the three transects. Due to the arbitrary nature of the classification system, the rating was discussed and agreed upon by the same two researchers over the course of the entire summer to maintain consistency.

Water Sample Laboratory Procedure

All of the samples were processed in the lab within 2 hours of collection. The water samples were first placed onto an Excella E24 Incubator Shaker Platform (New Brunswick Scientific, Enfield, CT) at 200 rpm for 5 minutes. The shaker platform was used to homogenize the samples due to the presence of algae and other suspended particles. It was also used to detach some of the bacteria from the

algae and suspend them in the water. The bottles were then given 5 minutes of settling time. This was done in order for the larger particles to fall to the bottom of the bottle in order for easier pouring purposes. The next step was to pour 100 mL of water into two separate sterile plastic bottles. One of the bottles was used for *E. coli* measuring and the other was used for *Enterococci*. This procedure was repeated for the water samples at all 3 transects.

Sand Sample Laboratory Procedure

First, the samples of sand were mixed thoroughly for homogenization. Next, the sand was weighed and placed into sterile plastic bottles. During the spring months, the sand samples were weighed out to be 50 grams and in the summer months it was reduced to 25 grams. The summer months yielded higher bacteria counts per gram of sand so the amount of sand had to be reduced in order to get bacteria levels within the detection range of the instruments used. During the data analysis portion of the project, all of the bacteria levels were standardized to a unit of MPN/100 grams in order to account for the various sand weights.

Next, 200 mL of eluent was added to each plastic bottle (see “Eluent Comparison Materials and Procedure” section). Eluents are used to detach the bacteria from the beach sand. The bottles were then placed on an Excella E24 Incubator Shaker Platform (New Brunswick Scientific, Enfield, CT) at 200 rpm for 5 minutes. The shaker platform is used to detach the bacteria and suspend them in the eluent. Then the bottles are given 5 minutes of settling time. This is used to allow the sand to settle on the bottom of the bottle. The next step was to pour 100 mL of

eluent into two separate sterile plastic bottles. One of the bottles was used for *E. coli* measuring and the other was used for *Enterococci*. This procedure was repeated for the swash and 20 feet up shore sand samples at all 3 transects.

Bacteria Count Determination

Colilert indicator (IDEXX Laboratories, Westbrook, ME) was used to measure the amounts of *E. coli* in units of "most probable number" (MPN). A packet of Colilert indicator was added to the bottle with 100 mL of water or eluent in it. This was then shaken and then poured into a Quanti-Tray/2000 packet (*Figure 5*).

Figure 5: Quanti-Tray/2000

The packet is divided up into 49 large and 48 small compartments. The Quanti-Tray/2000 packet is then put through the Quanti-Tray Sealer in order to seal the container. These steps were repeated for the *Enterococci* bottles except that Enterolert indicator (IDEXX Laboratories) was used instead of Colilert indicator.

Next, the *E. coli* samples were placed in an incubator at 35°C for 24 hours. The *Enterococci* samples were placed into a separate incubator at 41°C for 24 hours. The next day the samples were removed from the incubators and examined for positive test results. The first step was to take the *Enterococci* samples and count the number of large and small compartments that had a blue fluorescence. The blue fluorescence was determined with a UV lamp. The *E. coli* samples were first analyzed for a yellow color. They were determined positive for total coliforms if they were a yellow color that was equal to or greater than the comparator from IDEXX Laboratories. Next, the UV lamp was used to determine which of the yellow compartments also had fluorescence. If a compartment had both a yellow color and fluorescence then it tested positive for *E. coli*. Based on the number of large and small positive compartments for each sample, an MPN value was obtained from the chart per 100 mL of eluent or water. The MPN value is equivalent to CFU (colony forming units), which is the conventional bacteria measuring value. The MPN/100 mL of eluent for the sand samples was then standardized to be MPN/100 grams of sand for comparison purposes.

Eluent Comparison Materials and Procedure

Eluents are used to enumerate the bacteria that are attached to the beach sand. Most researchers have used either distilled water or phosphate buffered saline. During the spring sampling schedule, distilled water was used as the only eluent. During the summer months, two different eluents were used to compare the effectiveness of bacterial enumeration from beach sand, distilled water and phosphate buffered saline (PBS). The PBS solution was made according to EPA standards; 0.32 grams of sodium dihydrogen phosphate, 1.1 grams of sodium monohydrogen phosphate, 8.5 grams of sodium chloride, and 1 liter of distilled water. The ionic strength of PBS is approximately 1.65 mM while distilled water is approximately 0 mM.

Eluent Comparison

Results

A total of 126 samples were analyzed for *Enterococci* and 116 samples for *E. coli*. Each sample was tested with identical parameters (i.e. sand sample, shaking time, incubation period, etc.) with the only variable being the use of different eluents. A two-tailed paired t-test was performed for both the *E. coli* and *Enterococci* sample sets in order to determine whether PBS or DI water yielded higher amounts of bacteria from the same sample of sand. After conducting the analysis we noticed that there were a few outliers that were skewing the results. This was due to the fact that we occasionally had days of bacteria contamination that was much larger than the normal day and therefore yielded much higher bacteria counts. After eliminating two samples from the *E. coli* statistical analysis and one from the *Enterococci*, a statistically significant relationship was established. The outliers were chosen because they were the only counts that were greater than 2500 MPN/100 grams of sand. We were unable to make a ratio for the effectiveness of each eluent for enumeration. This is most likely due to the limited number of samples that were analyzed. A ratio of effectiveness would be helpful for researchers to have a clearer picture of how accurate their data is based on which eluent they used.

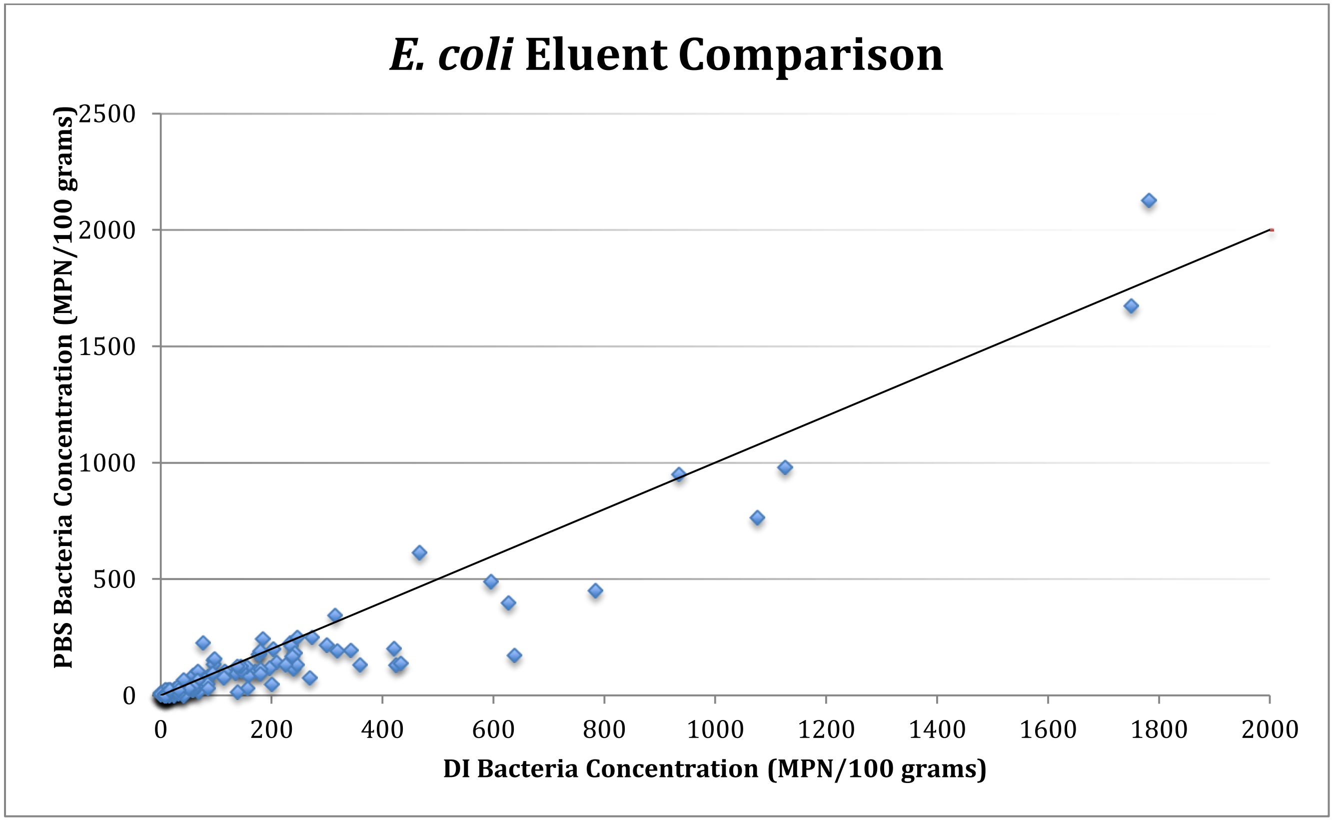
The *E. coli* statistical analysis showed significantly higher bacteria counts using distilled water for enumeration. The average value was 180 MPN/100 grams of sand for distilled water and 144 MPN/100 grams for PBS (*Table 3*).

Table 3: Eluent Comparison *E. coli* Statistical Results

E. coli	**DI**	**PBS**
mean	180.33	144.14
	2 tailed paired t-test	
	P-value	0.0000820
	t	4.0847
	df	115

The p-value was 0.000082, which shows that the data is statistically significant. The mean of DI minus PBS was 36.19 with a 95% confidence interval from 18.6 to 53.7. The results were graphed in order to further illustrate the trend of distilled water yielding higher *E. coli* counts in the sand samples (*Figure 6*).

Figure 6: *E. coli* Statistical Results



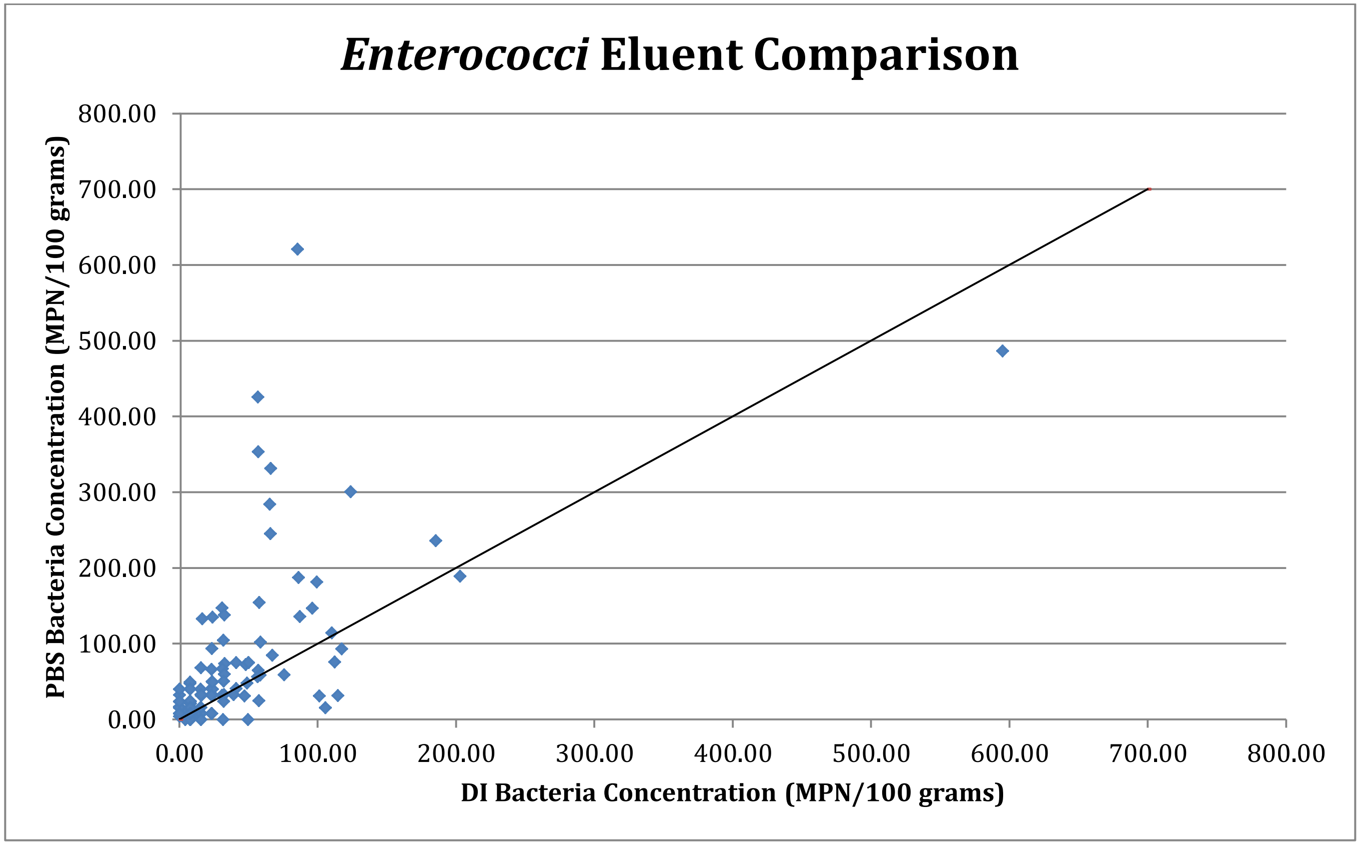
The results for the *Enterococci* data mirrored the *E. coli* data. The *Enterococci* statistical analysis showed significantly higher bacteria counts using PBS for enumeration. The average value was 37 MPN/100 grams of sand for distilled water and 65 MPN/100 grams for PBS (*Table 4*).

Table 4: Eluent Comparison *Enterococci* Statistical Results

Enterococci	**DI**	**PBS**
mean	36.70	64.73
	2 tailed paired t-test	
	P-value	0.000108116
	t	4
	df	124

The p-value was 0.00011, which shows that the data is statistically significant. The mean of PBS minus DI was 28.03 with a 95% confidence interval from 14.2 to 41.9. The results were graphed in order to further illustrate the trend of PBS yielding higher *Enterococci* counts in the sand samples (*Figure 7*).

Figure 7: *Enterococci* Statistical Results



The DI and PBS bacteria counts for *E. coli* and *Enterococci* were compared in order to create a ratio for the eluent used (*Figures 8-9*).

Figure 8: *E. coli* – DI/PBS Ratio

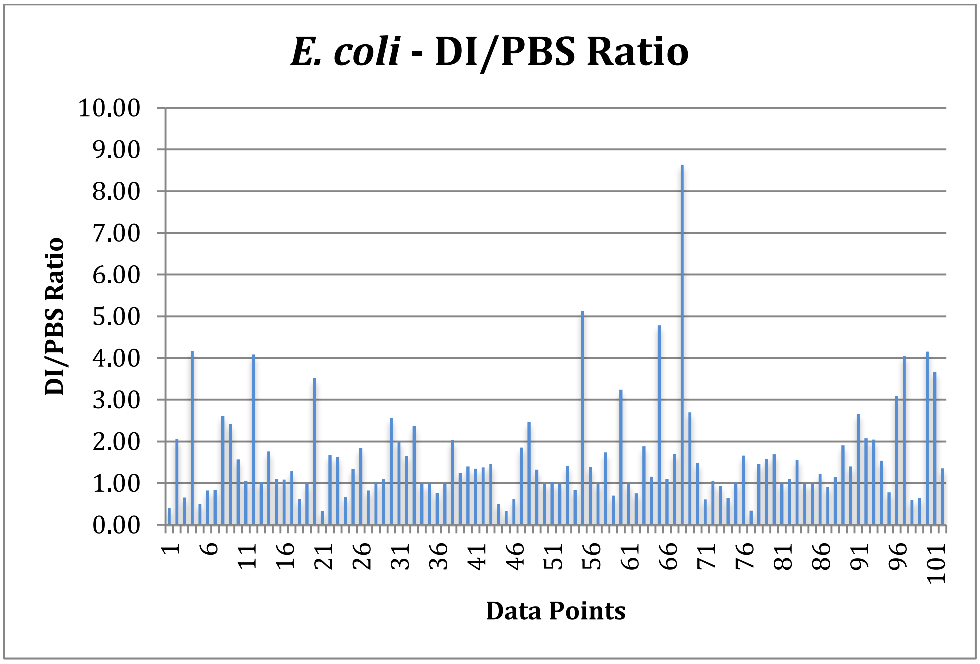
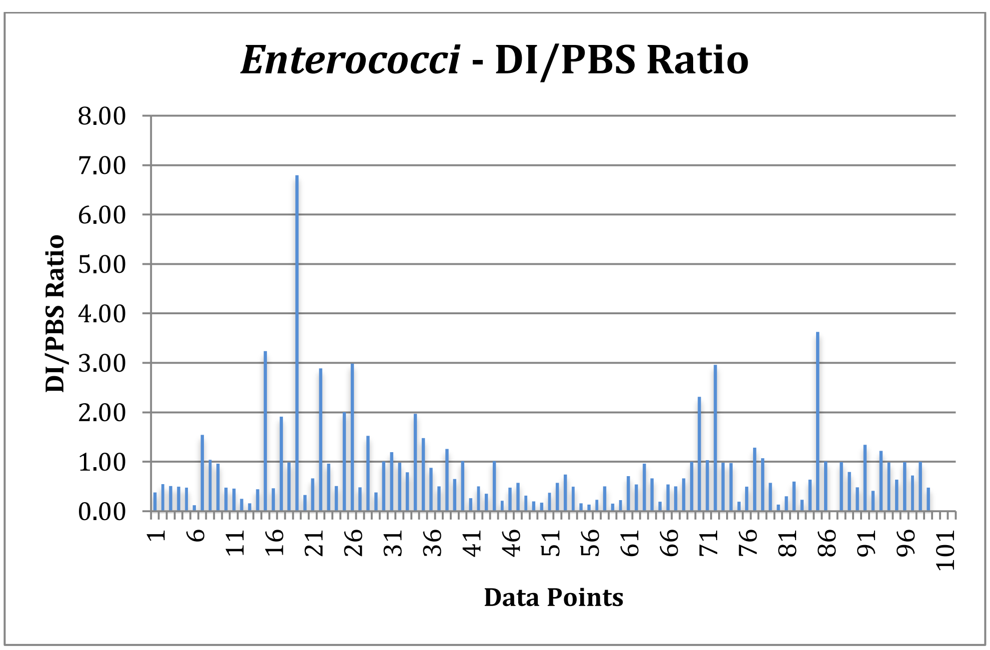


Figure 9: *Enterococci* – DI/PBS Ratio



The ratios were also compared to the bacteria concentrations from the DI eluent (*Figures 10-11*).

Figure 10: DI/PBS Ratio vs. DI *E. coli* Concentration

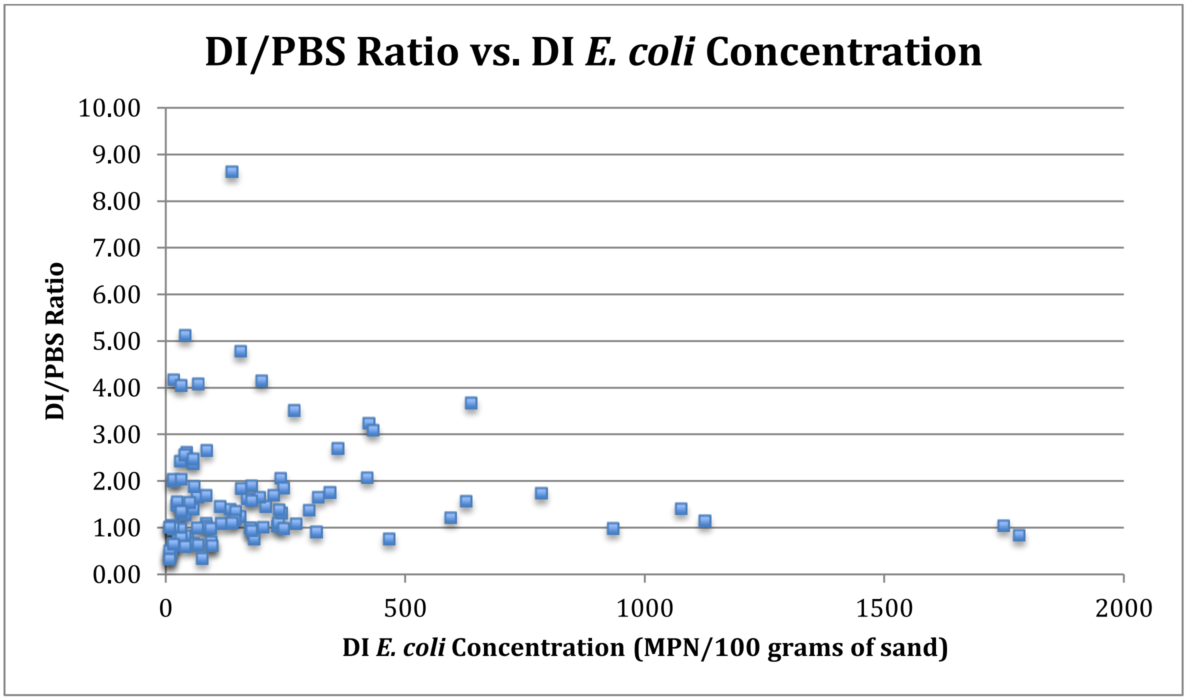
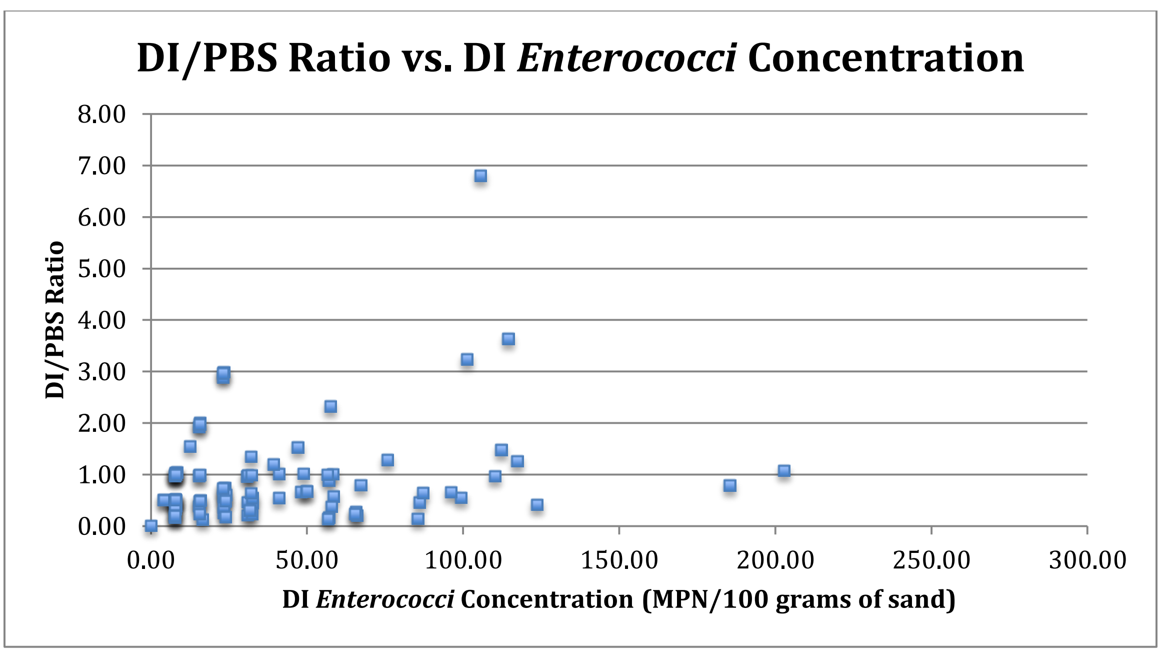


Figure 11: DI/PBS Ratio vs. DI *Enterococci* Concentration



Discussion

These results show that there is a need for more research to adequately choose an eluent. The results show that distilled water is more effective at enumerating *E. coli* from sand samples and PBS is more effective for enumerating *Enterococci*. The ionic strengths of these two eluents could have an impact on the adhesion of bacteria to beach sand*. E. coli* and beach sand are both gram-negative. A study by Walker et al. showed that repulsive electrostatic interactions play a significant role in the adhesion of *E. coli* to a surface. With an increase in the ionic strength of the background solution, the repulsive force between the two gram-negative forces is lessened (*Walker et al, 2005*). This is a factor in the beach sand and *E. coli* interaction because it shows that the PBS solution could be causing greater adhesion forces between the sand and *E. coli* due to its higher ionic strength. The lower ionic strength of distilled water could allow the repulsive forces between the sand and *E. coli* to be increased and therefore aiding in the enumeration of as many *E. coli* cells as possible.

Enumeration levels of *Enterococci* proved to be higher when PBS was used as an eluent. This is in agreement with literature on the role of ionic strength in relation to the sticking efficiency of *Enterococci*. One study showed that as ionic strength increased, the sticking efficiency was greatly decreased for *Enterococci* (*Cail et al, 2005*). *Enterococci* are gram-positive bacterium and therefore, as the ionic strength increases, the repulsion forces increase. The higher ionic strength of PBS could be contributing to the higher *Enterococci* counts by disrupting the adhesion to sand particles.

Osmotic pressure is an important phenomenon to take into account. This occurs when the bacteria cell is unbalanced with the solution around it so that it eventually bursts. Gram-positive bacteria (i.e. *Enterococci*) have been found to be unaffected by osmotic pressure (*Mager et al, 1956*). Distilled water can cause an increase in osmotic pressure on a gram-negative bacteria cell like *E. coli* (*Bayer, 1966*). These results go against research that has been done that shows that osmotic pressure on *E. coli* from distilled water will damage or kill the bacteria. PBS is used to balance the levels of salt between the bacteria cells and the solution so it should enumerate higher levels of bacteria for *E. coli.*

Due to the high variability of the ratios, a definitive ratio conversion from DI to PBS was unable to be developed. Figures 10 and 11 show that the ratios were not dependent on bacteria concentration. As the concentrations increased, the ratios were approximately the same as they were at lower concentrations. This is important to note because if a larger sample set was available, it may be possible to make a DI to PBS ratio conversion. This would allow researchers to compare data from others even if they used different eluents. It would also enable health departments to decide which eluent to use and they could convert their bacteria concentrations into whatever form they may need.

More research is needed to determine why distilled water was able to yield higher bacteria counts than PBS in identical sand samples. As research on bacteria levels in recreational beach sands is done in the future, it will be important to look into the most effective and accurate ways of enumerating bacteria from the sand.

More research is needed in the process of enumerating bacteria from sand before accurate data can be obtained in relation to beach health.

Seasonal Change in Bacteria Levels

Results

A total of 32 sample sets were used to analyze the seasonal change in *E. coli* and *Enterococci* levels. Each sample set was taken on the same date and broken up into 6 categories; *E. coli* swash zone, *E. coli* up shore, *E. coli* water, *Enterococci* swash zone, *Enterococci* up shore, and *Enterococci* water. The date range was from April 3rd to August 21st 2013. The bacteria counts for the swash zone and up shore were recorded as MPN/100 grams of sand and the water samples were recorded as MPN/100 mL.

All of the samples showed increasingly higher bacteria counts as time passed from spring to summer (*Figures 12-17*).

Figure 12: *E. coli* Counts in Swash Zone

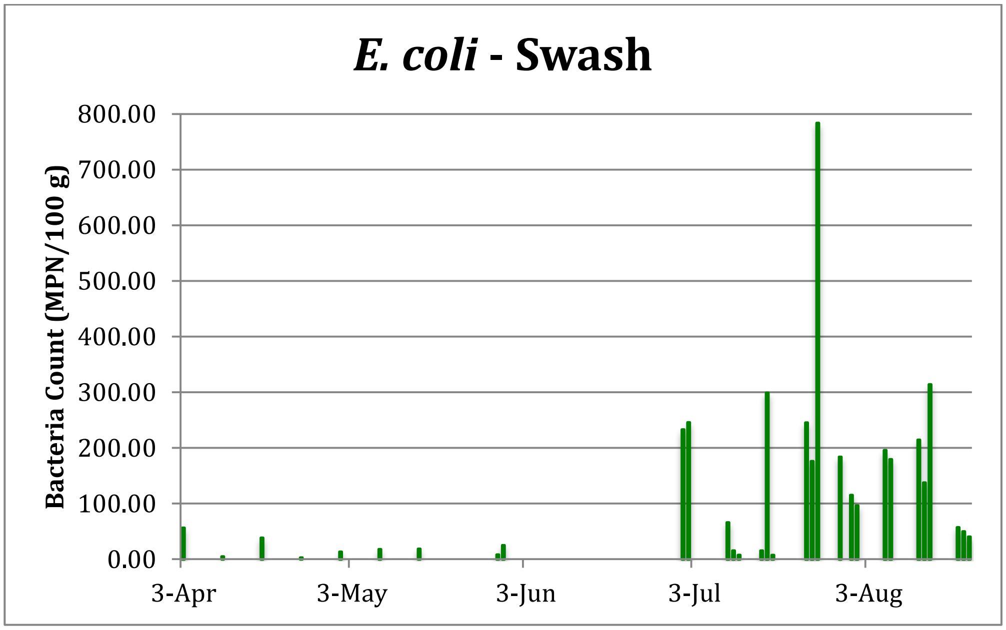


Figure 13: *Enterococci* Counts in Swash Zone

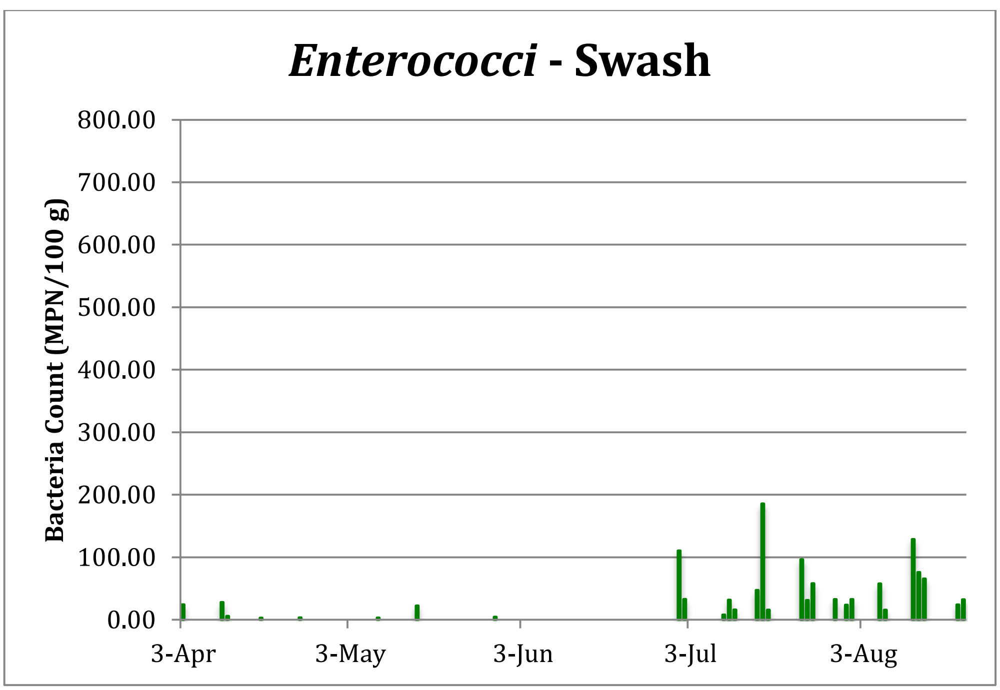


Figure 14: *E. coli* Counts in Up Shore Location

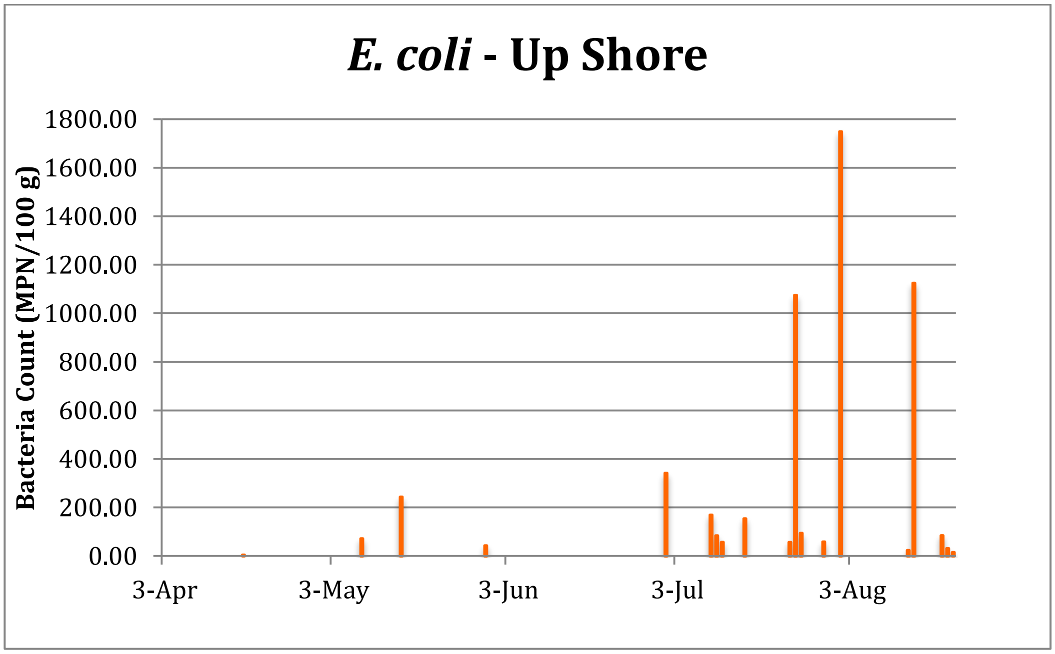


Figure 15: *Enterococci* Counts in Up Shore Location

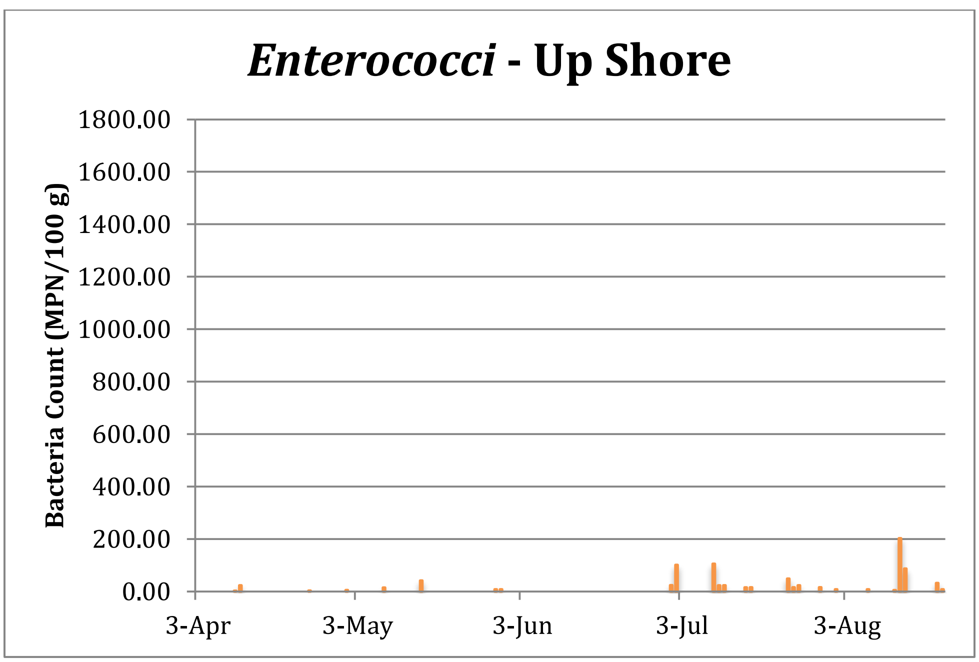


Figure 16: *E. coli* Counts in Water

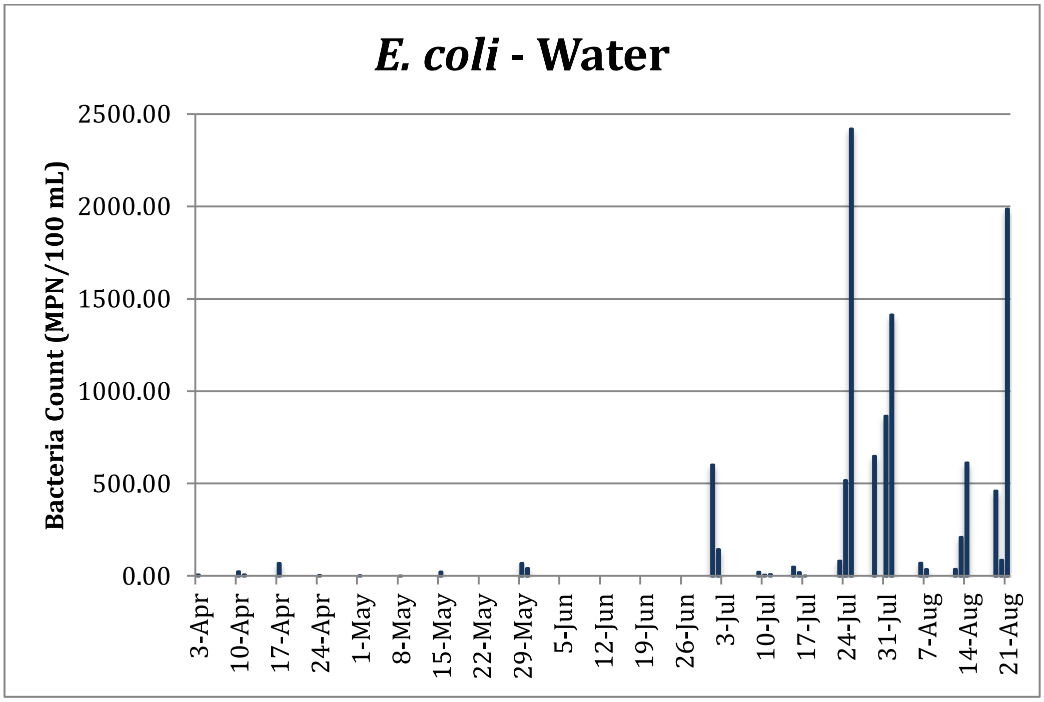
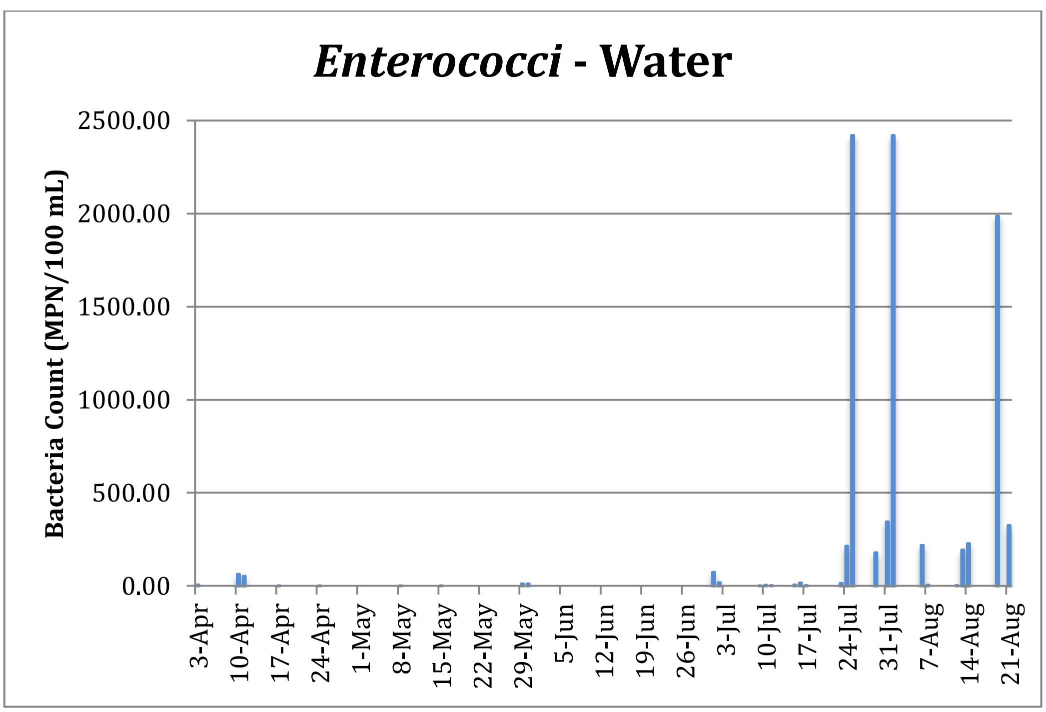


Figure 17: *Enterococci* Counts in Water



The *E. coli* levels in the swash zone and up shore locations increased much more rapidly than the *Enterococci* levels. The water samples showed the greatest amount of change from spring bacteria levels to summer bacteria levels.

The bacteria counts in relation to the algae levels were also analyzed (*Figures 18-23*).

Figure 18: Average *E. coli* Counts in Water and Algae Rating

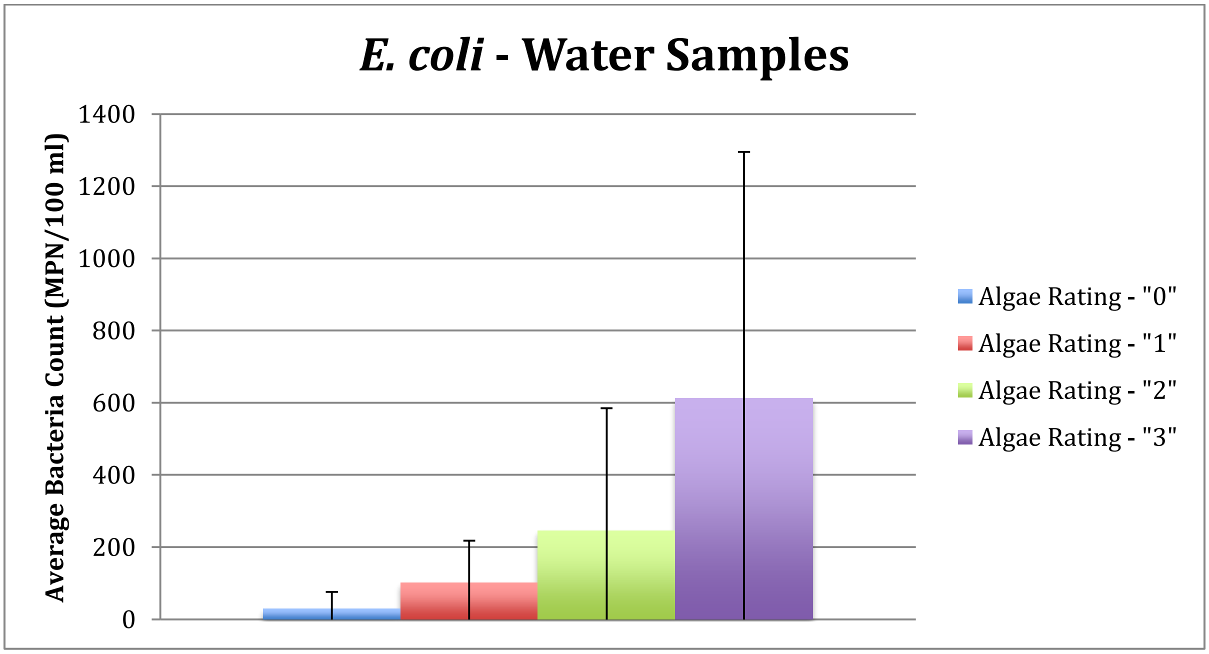


Figure 19: Average *Enterococci* Counts in Water and Algae Rating

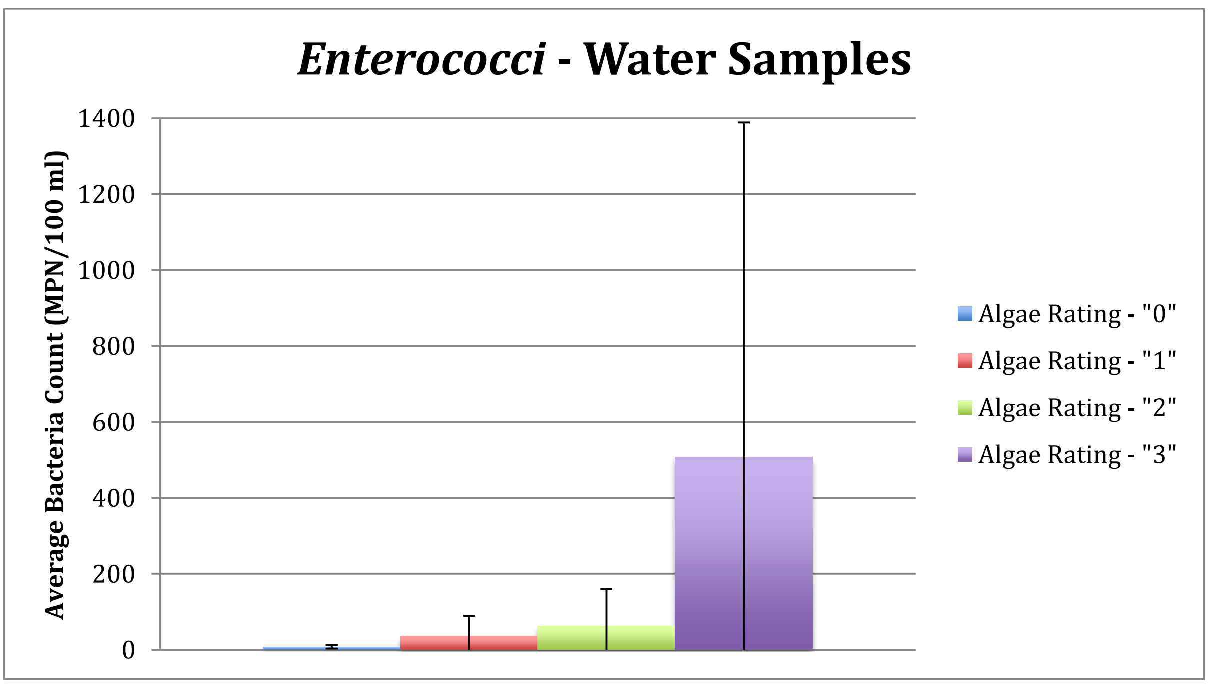


Figure 20: Average *E. coli* Counts in Swash Zone and Algae Rating

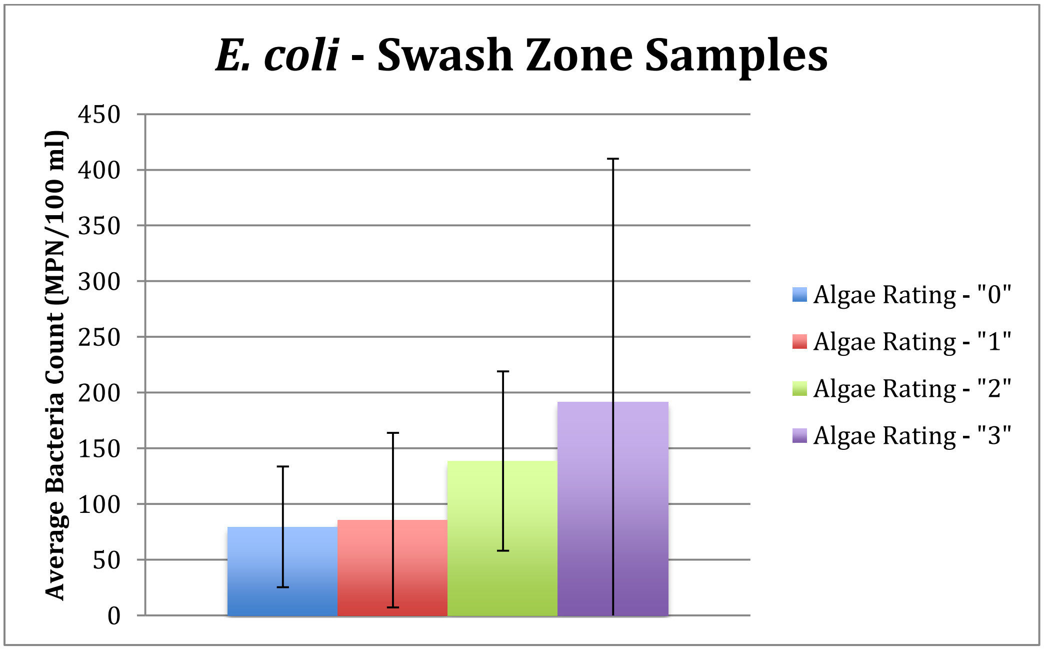


Figure 21: Average *Enterococci* Counts in Swash Zone and Algae Rating

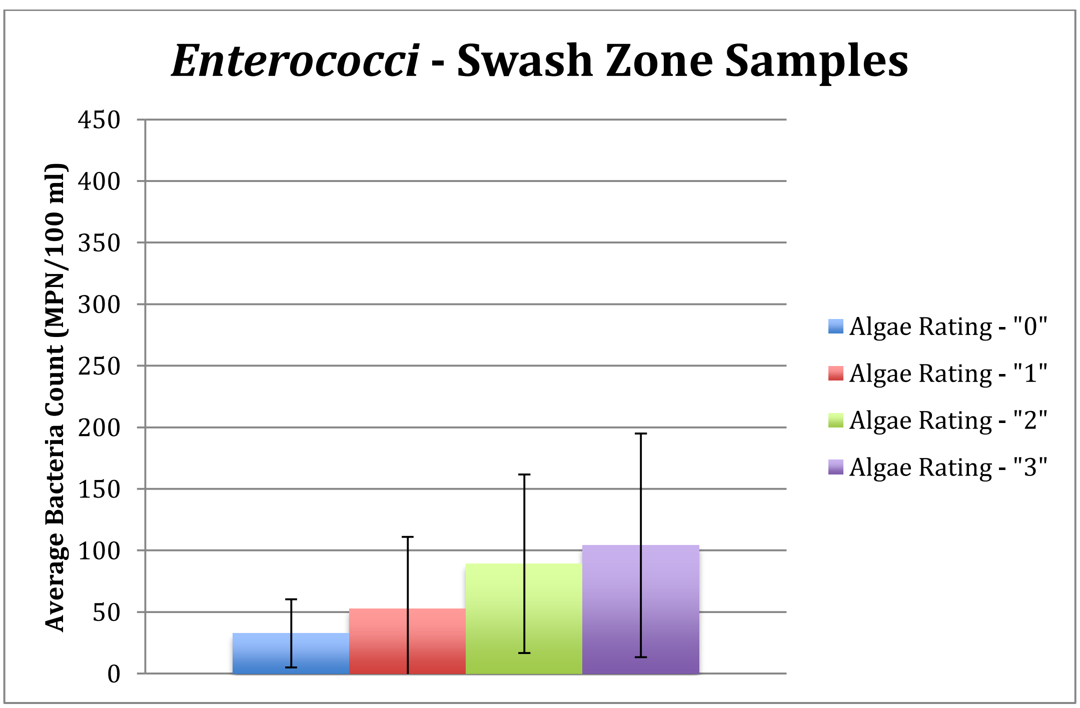


Figure 22: Average *E. coli* Counts in Up Shore and Algae Rating

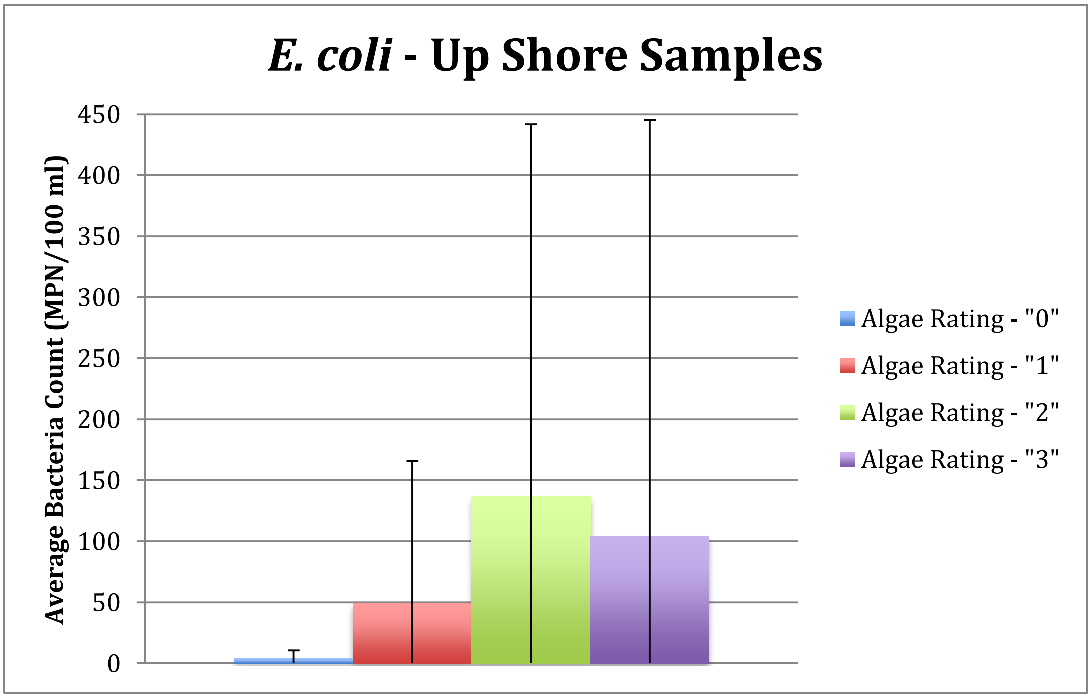
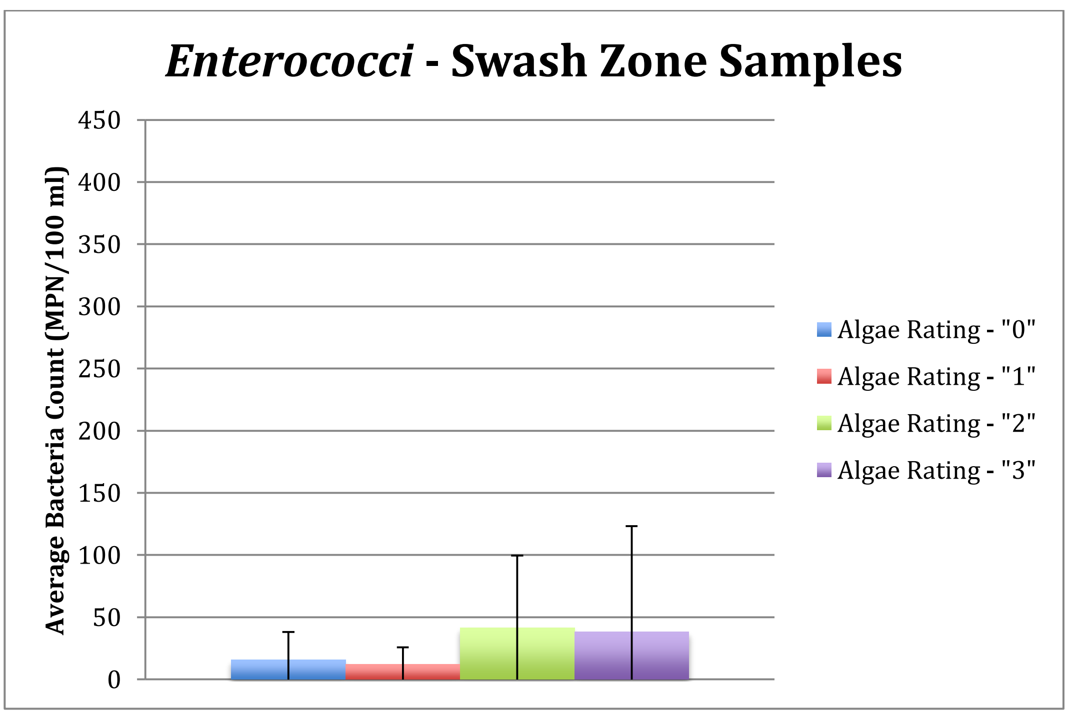


Figure 23: Average *Enterococci* Counts in Up Shore and Algae Rating



As the algae rating increased for the water and swash zone samples, the average bacteria amount also increased. The up shore sand samples did not have a clear relationship between the algae rating and average bacteria levels.

Statistical analysis was performed on each of the six sample sets; *E. coli* swash zone, *E. coli* up shore, *E. coli* water, *Enterococci* swash zone, *Enterococci* up shore, and *Enterococci* water. Two tailed t-tests were used to compare the bacteria counts for each group of algae ratings (*Tables 5-10*).

Table 5: P-Values of Algae Ratings Comparison of *E. coli* in Water

***E. coli* - Water Samples**	
Algae ratings Comparison	*P-value*
0 & 1	0.192961225
1 & 2	0.085157081
2 & 3	0.069718494
0 & 2	0.179337656
0 & 3	0.070860672
1 & 3	0.001978333

Table 6: P-Values of Algae Ratings Comparison of *Enterococci* in Water

***Enterococci* - Water Samples**	
Algae ratings Comparison	*P-value*
0 & 1	0.222317689
1 & 2	0.311403437
2 & 3	0.052729778
0 & 2	0.192995999
0 & 3	0.182638649
1 & 3	0.025346823

Table 7: P-Values of Algae Ratings Comparison of *E. coli* in Swash Zone

***E. coli* - Swash Zone Samples**	
Algae ratings Comparison	*P-value*
0 & 1	0.863794358
1 & 2	0.06007602
2 & 3	0.359568919
0 & 2	0.114342909
0 & 3	0.227552551
1 & 3	0.102262305

Table 8: P-Values of Algae Ratings Comparison of *Enterococci* in Swash Zone

***Enterococci* - Swash Zone Samples**	
Algae ratings Comparison	*P-value*
0 & 1	0.40166341
1 & 2	0.143584559
2 & 3	0.614921592
0 & 2	0.064480749
0 & 3	0.051661664
1 & 3	0.053355905

Table 9: P-Values of Algae Ratings Comparison of *E. coli* in Up Shore

***E. coli* - Up Shore Samples**	
Algae ratings Comparison	*P-value*
0 & 1	0.360085395
1 & 2	0.267902686
2 & 3	0.764228236
0 & 2	0.306292248
0 & 3	0.482941627
1 & 3	0.515851757

Table 10: P-Values of Algae Ratings Comparison of *Enterococci* in Up Shore

***Enterococci* - Up Shore Samples**	
Algae ratings Comparison	*P-value*
0 & 1	0.732834954
1 & 2	0.05258513
2 & 3	0.900426182
0 & 2	0.286672259
0 & 3	0.507937574
1 & 3	0.218481989

Only two of the p-values were statistically significant. Both of them occurred when the algae ratings 1 & 3 were compared in the water samples for both *E. coli* and *Enterococci* (p-values of 0.00198 and 0.0253 respectively). The number of samples for each algae rating were highly variable due to the changing conditions present at the beach on sampling days.

The rainfall amounts and the maximum daily temperatures were graphed from the day before each sampling day for the spring and summer collection periods (*Figures 24-25*).

Figure 24: Rainfall Amounts

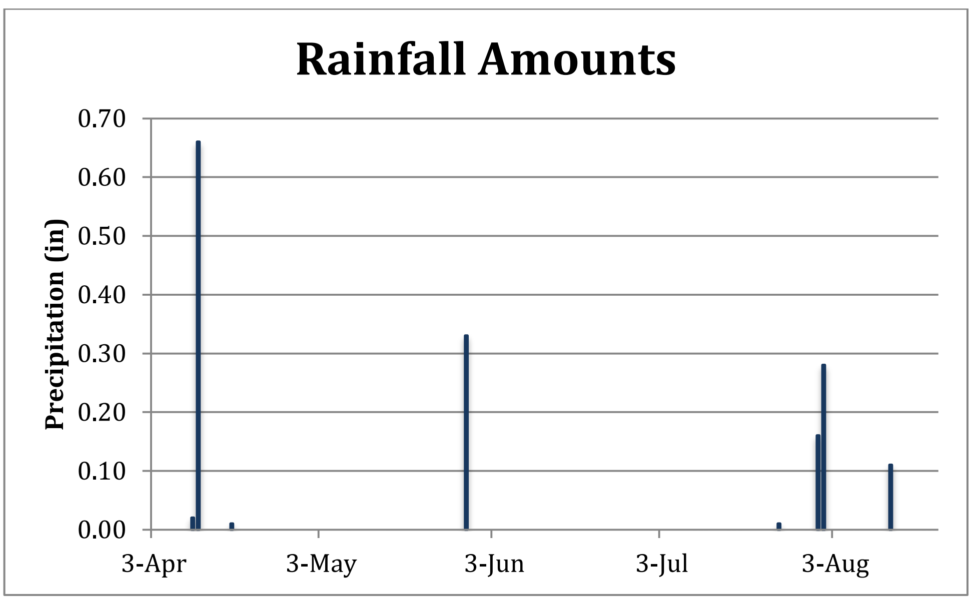
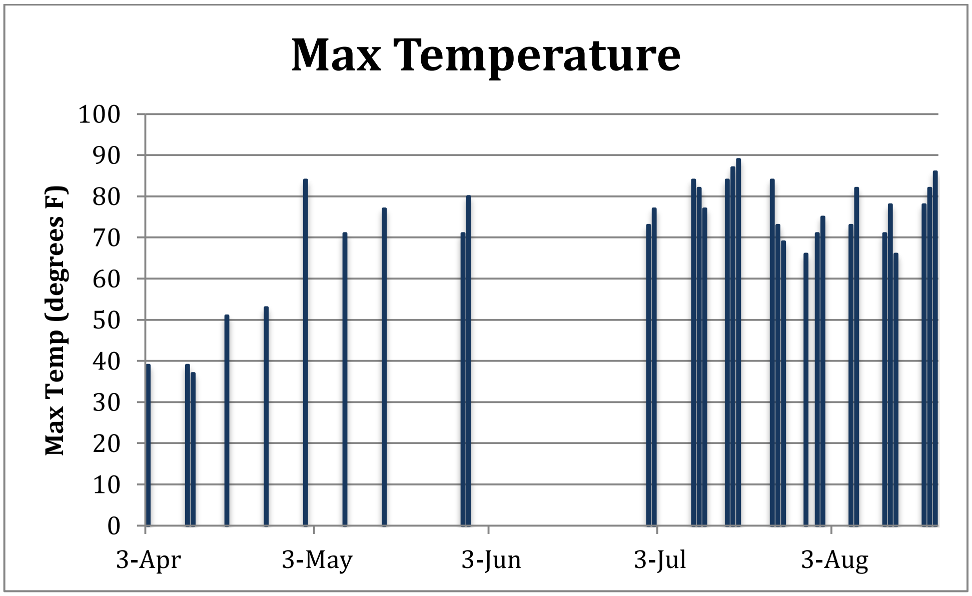


Figure 25: Maximum Daily Temperatures



Discussion

These results point to a positive correlation between temperature and bacteria levels in both recreational beach water and sand. At the beginning of the sampling period, in April, the *E. coli* and *Enterococci* counts were lower than the end of the period, in August. The bacteria counts are highest at the same time that average temperatures in Wisconsin are high. It is also important to note, however, that the variability of the samples taken in the summer was high. For future research projects, sampling should occur consistently throughout the year, either once per week or multiple times per week. The up shore and swash locations had very similar trend lines for both the *E. coli* and *Enterococci*. This is interesting because, due to the close proximity to the water, the swash zone would have more contact with the highly contaminated water. One reason that this could occur would be when there is a high tide or wave action on the beach, the up shore sand can also

come into contact with the near shore water and the bacteria could transfer to the sand. The *Enterococci* levels were consistently lower than the *E. coli* levels which supports the choice of the Wisconsin Health Department to test *E. coli* levels for beach closures and warnings. The water samples showed the largest increase in bacteria levels from the spring to summer months. Another study also showed year round presence of indicator bacteria (*Byappanahalli et al*, 2006). These results go against the use of *E. coli* and *Enterococci* as indicative of human source pollution. The city of Milwaukee had no wastewater overflows until April 11th, and yet their were measurable levels of bacteria already present in the beach sand. The bacteria appear to be able to survive the winter.

One of the biggest factors that can also contribute to the higher bacteria levels in the summer is the presence of *Cladophora*. For the water and swash zone samples, the data shows an increase in bacteria levels during higher degrees of algae contamination. Algal mats are full of nutrients that allow bacteria to grow and their presence near beaches has been shown to lead to higher bacteria counts in the near shore area (*Olapade et al*, 2006)(*Whitman et al*, 2003A). Beach cleaning crews were also observed over the summer to be raking the algal mats onto the swash zone beach to allow it to dry out in the sun. This practice could be leading to greatly increased levels of bacteria in the near shore sand due to migration of the bacteria from the algae to the sand. Children that are playing in the near shore area could be vulnerable to contamination from the sand itself. More research should be performed in order to determine if the practice of raking the algae onto the beach is a safe way to dispose of the algae. The statistical analysis for the relationship

between algae visual classification ratings and the average bacteria counts showed little statistical significance, however, this is most likely due to the small sample sizes. There were very few days in the summer that did not have any algae present so this sample set was much smaller than the days of high algae content. A larger sample volume could increase the statistical significance of this correlation. There were two relationships that were statistically significant. They were for the *E. coli* and *Enterococci* water samples and compared the low and high levels of algae. The high levels of algae in the water had statistically higher bacteria counts than the low levels of algae.

The rainfall amounts were taken from the day before the sampling day. The rainfall data did not seem to have an effect on our bacteria counts. The maximum daily temperatures were also taken from the day before the sampling day. This was done because the samples were taken early in the morning which means that the sampling day's temperature would have had little impact compared to the previous day. The maximum temperatures showed an increase when bacteria count increased. This corresponds with the other findings that as time progressed from spring to summer, the bacteria counts rose due to rising temperatures.

As we continue to learn more about recreational beach health, it is increasingly important to look at this issue holistically. There are many factors that contribute to bacterial contamination at a beach. It cannot be simply related to wastewater overflows. Bacteria appear to be able to thrive throughout the year in the dark, moist environment under the beach's surface. Nutrient addition from tidal wetting can lead to increased growth during the warmer weather months. The

presence of algae also appears to play a significant role in the levels of bacteria contamination. More research should be conducted to try and piece these various pieces of research into the larger picture of determining the overall health of recreational beaches.

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Appendix: Raw Data

Location	*200 mL PBS* **Sand Weight**	*Total Coliform* **MPN/100 mL**	**MPN/100 g**	*E. coli* **MPN/100 mL**	**MPN/100 g**	*Enterococci* **MPN/100 mL**	**MPN/100 g**
Beach	11.91	0	0.00	0	0.00	3	50.38
Beach	15.86	2	25.22	0	0.00	2	25.22
Beach	22.98	1	8.70	0	0.00	0	0.00
Water	-	49.6	-	2	-	2	-

Table 1-1: March 20, 2013 Sampling Data

Location	*250 mL PBS* **Sand Weight**	*Total Coliform* **MPN/100 mL**	**MPN/100 g**	*E. coli* **MPN/100 mL**	**MPN/100 g**	*Enterococci* **MPN/100 mL**	**MPN/100 g**
Swash	50.19	0	0.00	0	0.00	0	0.00
20'	50.21	0	0.00	0	0.00	0	0.00
40'	50.17	0	0.00	0	0.00	3	11.96
Water	-	6.3	-	0	-	1	-

Table 1-2: March 27, 2013 Sampling Data

200 mL DI water		*Total Coliform*		*E. coli*		*Enterococci*	
Location	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
Swash	81.55	35.9	88.04	23.1	56.65	9.8	24.03
20'	83.37	0	0.00	0	0.00	0	0.00
Water	-	7.2	-	7.2	-	6.3	-

Table 1-3: April 3, 2013 Sampling Data

200 mL DI water		*Total Coliform*		*E. coli*		*Enterococci*	
Location	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
Swash	77.85	33.6	86.32	2	5.14	10.8	27.75
20'	76.38	2	5.24	0	0.00	1	2.62
Water	-	579.4	-	24.1	-	63.1	-
*Note**	Samples were taken at 9:30 AM after prolonged rainfall. MMSD reported a CSO starting at 6:30 AM.						

Table 1-4: April 10, 2013 Sampling Data

200 mL DI water		*Total Coliform*		*E. coli*		*Enterococci*	
	Sand	**MPN/100**	**MPN/100**	**MPN/100**	**MPN/100**	**MPN/100**	**MPN/100**
Location	**Weight**	**mL**	**g**	**mL**	**g**	**mL**	**g**
Swash	71.99	6.3	17.50	0	0.00	2	5.56
20'	73.96	1	2.70	0	0.00	8.6	23.26
Water	-	195.6	-	7.5	-	52.8	-

*Note** Samples were taken a day after MMSD reported the start of a CSO. The CSO lasted from April 10 at 6:40 am to April 13 at 6:10 and dumped approx 594 million gallons.

Table 1-5: April 11, 2013 Sampling Data

200 mL DI water		*Total Coliform*		*E. coli*		*Enterococci*	
	Sand	**MPN/100**	**MPN/100**	**MPN/100**	**MPN/100**	**MPN/100**	**MPN/100**
Location	**Weight**	**mL**	**g**	**mL**	**g**	**mL**	**g**
Swash	75.37	57.6	152.85	14.6	38.74	1	2.65
20'	74.24	2	5.39	2	5.39	0	0.00
Water	-	201.4	-	68.9	-	2	-

Table 1-6: April 17, 2013 Sampling Data

200 mL DI water		Total Coliform		E. coli		Enterococci	
Location	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
Swash	63.63	13.5	42.43	1	3.14	1	3.14
20'	62.69	10.9	34.77	0	0.00	1	3.19
Water	-	93.3	-	4.1	-	1	-

Table 1-7: April 24, 2013 Sampling Data

200 mL DI water		Total Coliform		E. coli		Enterococci	
Location	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
Swash	76.32	9.8	25.68	5.2	13.63	0	0.00
20'	76.71	2	5.21	0	0.00	2	5.21
Water	-	52	-	3.1	-	0	-
*Note**	20' sample was taken at a depth of 12 inches.						

Table 1-8: May 1, 2013 Sampling Data

200 mL DI water		*Total Coliform*		*E. coli*		*Enterococci*	
Location	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
Swash	69.36	16	46.14	6.3	18.17	1	2.88
20' Bottom	70.56	46.4	131.52	25.6	72.56	5.2	14.74
20' Top	71.28	218.7	613.64	12.1	33.95	5.2	14.59
Water	-	4.1	-	1	-	1	-
*Note**	20' Bottom sample was taken at a depth of 9 inches.						

Table 1-9: May 8, 2013 Sampling Data

200 mL DI water		*Total Coliform*		*E. coli*		*Enterococci*	
Location	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
Swash	66.72	27.5	82.43	6.3	18.88	7.4	22.18
20' Bottom	64.97	139.6	429.74	79.4	244.42	13.5	41.56
20' Top	70.82	613.1	1731.43	435.2	1229.03	46.4	131.04
Water	-	142.1	-	23.1	-	2	-
*Note**	20' Bottom sample was taken at a depth of 6 inches.						

Table 1-10: May 15, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	47.07	67.7	287.66	2	8.50	1	4.25
1	*20'*	50	12.1	48.40	0	0.00	2	8.00
2	*Swash*	49.96	Max	Max	59.8	239.39	24.8	99.28
2	*20'*	49.52	3.1	12.52	0	0.00	0	0.00
3	*Swash*	48.56	77.1	317.55	4.1	16.89	0	0.00
3	*20'*	48.94	14.4	58.85	4.1	16.76	0	0.00
4	*Swash*	48.57	80.9	333.13	3.1	12.77	1	4.12
4	*20'*	48.14	39.5	164.10	11	45.70	1	4.15

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	49.33	52.9	214.47	5.2	21.08	0	0.00
1	*20'*	49.48	5.2	21.02	3.1	12.53	5.2	21.02
2	*Swash*	48.39	Max	Max	28.1	116.14	43.9	181.44
2	*20'*	49.91	4.1	16.43	1	4.01	0	0.00
3	*Swash*	48.99	41.7	170.24	6.3	25.72	4.1	16.74
3	*20'*	49.78	4.1	16.47	1	4.02	0	0.00
4	*Swash*	49.27	65	263.85	6.3	25.57	2	8.12
4	*20'*	48.1	14.6	60.71	13.4	55.72	2	8.32

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**
1	*Water*	Max	68.9	12
2	*Water*	Max	124.6	34.1
3	*Water*	2419.6	54.6	18.7
4	*Water*	Max	64.4	17.3

*Note** Max = MPN > 2419.6 and beyond measurable amount

Table 1-11: May 29, 2013 Sampling Data

200 mL DI water			Total Coliform		E. coli		Enterococci	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	49	178.9	730.20	6.2	25.31	0	0.00
1	*20'*	49.94	135.4	542.25	10.9	43.65	2	8.01
2	*Swash*	49.31	343.6	1393.63	7.5	30.42	4.1	16.63
2	*20'*	49.55	214.3	864.98	155.3	626.84	3.1	12.51
3	*Swash*	47.3	104.6	442.28	3.1	13.11	0	0.00
3	*20'*	47.5	26.2	110.32	16.1	67.79	2	8.42

200 mL PBS			Total Coliform		E. coli		Enterococci	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	49.55	133.3	538.04	7.5	30.27	4.1	16.55
1	*20'*	49.1	70.6	287.58	4.1	16.70	4.1	16.70
2	*Swash*	49.32	235.9	956.61	3.1	12.57	32.8	133.01
2	*20'*	49.39	172.3	697.71	98.7	399.68	2	8.10
3	*Swash*	48.15	63.8	265.01	3	12.46	1	4.15
3	*20'*	49.38	18.9	76.55	4.1	16.61	2	8.10

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**
1	*Water*	1203.3	41.4	12
2	*Water*	1299.7	37.9	17.1
3	*Water*	1986.3	47.1	8.4

Table 1-12: May 30, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.04	Max	Max	29.2	233.23	13.8	110.22
1	*20'*	26.04	980.4	7529.95	44.6	342.55	3.1	23.81
2	*Swash*	24.86	Max	Max	29.2	234.92	10.7	86.08
2	*20'*	25.69	124.6	970.03	35	272.48	3	23.36
3	*Swash*	25.62	285.1	2225.60	5.1	39.81	1	7.81
3	*20'*	26.35	5.2	39.47	2	15.18	0	0.00

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.19	691	5486.30	28.5	226.28	14.4	114.33
1	*20'*	25.25	435.2	3447.13	24.6	194.85	6.3	49.90
2	*Swash*	25.48	Max	Max	27.2	213.50	23.9	187.60
2	*20'*	26.06	74.9	574.83	32.7	250.96	12.2	93.63
3	*Swash*	26.41	218.7	1656.19	4.1	31.05	6.3	47.71
3	*20'*	25.5	5.2	40.78	3.1	24.31	1	7.84

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	727	Max	3
2	*Water*	Max	325.5	66.9	3
3	*Water*	Max	54.8	8.5	3
1 Diluted	*Water*	Max	602	74	-
2 Diluted	*Water*	Max	213	84	-
3 Diluted	*Water*	Max	63	31	-

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 10 in, 2B @ 14 in, 3B @ 18 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-14: July 1, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.12	1986.3	15814.49	30.9	246.02	4.1	32.64
1	*20'*	24.5	4.1	33.47	0	0.00	12.4	101.22
2	*Swash*	26.42	Max	Max	35.5	268.74	4.1	31.04
2	*20'*	25.8	14.6	113.18	0	0.00	2	15.50
3	*Swash*	25.32	275.5	2176.15	1	7.90	0	0.00
3	*20'*	25.38	2	15.76	0	0.00	0	0.00

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.01	1119.9	8955.62	31.3	250.30	9.2	73.57
1	*20'*	25.58	2	15.64	0	0.00	4	31.27
2	*Swash*	25.62	Max	Max	9.8	76.50	8.6	67.14
2	*20'*	24.72	25.9	209.55	0	0.00	1	8.09
3	*Swash*	25.14	178.5	1420.05	3.1	24.66	0	0.00
3	*20'*	25.95	1	7.71	0	0.00	2	15.41

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
2	*Water*	Max	143.9	18.5	3
3	*Water*	Max	16.1	3.1	3
2 Diluted	*Water*	24196	134	10	-
3 Diluted	*Water*	8164	20	30	-

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 11 in, 2B @ 12 in, 3B @ 17 in

Transect 1 had too much algae to take a water sample

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-15: July 2, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.95	290.9	2242.00	8.6	66.28	1	7.71
1	*20'*	25.39	Max	Max	21.6	170.15	13.4	105.55
2	*Swash*	25.42	547.5	4307.63	7.4	58.22	1	7.87
2	*20'*	25.72	13.4	104.20	4.1	31.88	0	0.00
3	*Swash*	24.97	1046.2	8379.66	19.7	157.79	6	48.06
3	*20'*	25.85	15.8	122.24	5.2	40.23	0	0.00

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.6	218.7	1708.59	5.1	39.84	1	7.81
1	*20'*	25.76	Max	Max	13.5	104.81	2	15.53
2	*Swash*	24.99	613.1	4906.76	10.9	87.23	3	24.01
2	*20'*	25.21	5.2	41.25	3	23.80	0	0.00
3	*Swash*	25.67	727	5664.20	11	85.70	9.3	72.46
3	*20'*	25.84	14.8	114.55	6.3	48.76	2	15.48

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	1046.2	21.3	1	1
2	*Water*	2419.6	98.8	3	1
3	*Water*	2419.6	22.8	1	3
1 Diluted	*Water*	1178	10	10	-
2 Diluted	*Water*	2098	63	0	-
3 Diluted	*Water*	1106	10	0	-

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 10.5 in, 2B @ 13 in, 3B @ 21 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-16: July 9, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.43	64.4	506.49	2	15.73	4	31.46
1	*20'*	25.94	365.4	2817.27	11	84.81	3	23.13
2	*Swash*	25.92	275.5	2125.77	5.2	40.12	4	30.86
2	*20'*	25.46	140.1	1100.55	2	15.71	0	0.00
3	*Swash*	25.05	Max	Max	39.9	318.56	Max	Max
3	*20'*	25.34	18.9	149.17	0	0.00	1	7.89

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.8	81.3	630.23	2	15.50	0	0.00
1	*20'*	24.98	325.5	2606.08	9.7	77.66	1	8.01
2	*Swash*	25.53	517.2	4051.70	2	15.67	4.1	32.12
2	*20'*	25.49	116.2	911.73	1	7.85	0	0.00
3	*Swash*	25.44	Max	Max	24.5	192.61	Max	Max
3	*20'*	25.96	24.3	187.21	1	7.70	2	15.41

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	547.5	6.3	5	1
2	*Water*	387.3	0	13	1
3	*Water*	261.3	2	5	2
1 Diluted	*Water*	857	0	10	-
2 Diluted	*Water*	457	0	50	-
3 Diluted	*Water*	479	10	20	-

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 13 in, 2B @ 12 in, 3B @ 21 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-17: July 10, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.39	80.9	637.26	1	7.88	2	15.75
1	*20'*	25.68	1299.7	10122.27	7.4	57.63	3	23.36
2	*Swash*	26.17	233.3	1782.96	4.1	31.33	1	7.64
2	*20'*	25.86	1986.3	15361.95	2	15.47	0	0.00
3	*Swash*	25.21	Max	Max	58.8	466.48	960.6	7620.79
3	*20'*	25.76	25.6	198.76	2	15.53	0	0.00

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.46	78	612.73	0	0.00	1	7.86
1	*20'*	25.56	648.8	5076.68	3.1	24.26	1	7.82
2	*Swash*	25.46	198.9	1562.45	4.1	32.21	2	15.71
2	*20'*	25.18	1203.3	9557.59	2	15.89	1	7.94
3	*Swash*	24.79	Max	Max	76.2	614.76	1011.2	8158.13
3	*20'*	25.58	30.5	238.47	2	15.64	0	0.00

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	980.4	8.6	3	1
2	*Water*	579.4	10.9	1	2
3	*Water*	866.4	3	5	3

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 14 in, 2B @ 13 in, 3B @ 21 in

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-18: July 11, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.5	178.5	1400.00	2	15.69	6	47.06
1	*20'*	25.96	1986.3	15302.77	20.1	154.85	2	15.41
2	*Swash*	25.39	2419.6	19059.47	17.1	134.70	5.2	40.96
2	*20'*	25.73	4.1	31.87	0	0.00	1	7.77
3	*Swash*	25.47	517.2	4061.25	18.5	145.27	5	39.26
3	*20'*	25.67	2	15.58	0	0.00	4	31.16

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.9	178.9	1381.47	1	7.72	4	30.89
1	*20'*	25.9	2419.6	18684.17	16.1	124.32	5.2	40.15
2	*Swash*	25.06	1732.9	13830.01	12.1	96.57	5.1	40.70
2	*20'*	25.41	6.3	49.59	0	0.00	0	0.00
3	*Swash*	25.01	224.7	1796.88	13.5	107.96	4.1	32.79
3	*20'*	25.93	1	7.71	0	0.00	4.1	31.62

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	49.5	6.2	1
2	*Water*	Max	38.4	4.1	2
3	*Water*	770.1	2	4	0

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 12 in, 2B @ 14 in, 3B @ 20 in

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-19: July 15, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.67	Max	Max	38.4	299.18	23.8	185.43
1	*20'*	25.51	167	1309.29	0	0.00	2	15.68
2	*Swash*	25.13	Max	Max	26.2	208.52	14.1	112.22
2	*20'*	25.61	1299.7	10149.94	0	0.00	0	0.00
3	*Swash*	25.96	816.4	6289.68	1	7.70	7.4	57.01
3	*20'*	25.6	3.1	24.22	0	0.00	0	0.00

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.26	Max	Max	27.5	217.74	29.8	235.95
1	*20'*	25.19	101.7	807.46	0	0.00	1	7.94
2	*Swash*	25.82	2419.6	18742.06	18.5	143.30	9.8	75.91
2	*20'*	25.18	290.9	2310.56	1	7.94	1	7.94
3	*Swash*	25.97	410.6	3162.11	2	15.40	8.4	64.69
3	*20'*	25.69	2	15.57	0	0.00	0	0.00

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	19.5	16.9	1
2	*Water*	Max	79.8	24.3	2
3	*Water*	2419.6	15.6	4.1	2

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 12 in, 2B @ 11.5 in, 3B @ 21 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-20: July 16, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.77	129.1	1001.94	1	7.76	2	15.52
1	*20'*	25.24	3.1	24.56	0	0.00	0	0.00
2	*Swash*	25.48	218.7	1716.64	2	15.70	1	7.85
2	*20'*	25.9	58.3	450.19	0	0.00	0	0.00
3	*Swash*	25.4	1553.1	12229.13	12.1	95.28	14.9	117.32
3	*20'*	25.74	0	0.00	0	0.00	0	0.00

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.97	83.6	643.82	3.1	23.87	0	0.00
1	*20'*	25.26	5.2	41.17	1	7.92	0	0.00
2	*Swash*	25.71	261.3	2032.67	0	0.00	2	15.56
2	*20'*	25.37	48	378.40	0	0.00	0	0.00
3	*Swash*	25.96	1119.9	8627.89	19.9	153.31	12.1	93.22
3	*20'*	25.33	0	0.00	0	0.00	4.1	32.37

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	579.4	2	3	0
2	*Water*	579.4	0	9.3	0
3	*Water*	1203.3	6.3	9.2	3

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 14 in, 2B @ 13 in, 3B @ 20.5 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-21: July 17, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.16	1732.9	13775.04	30.9	245.63	12.1	96.18
1	*20'*	25.79	49.6	384.65	7.4	57.39	6.3	48.86
2	*Swash*	25.58	1413.6	11052.38	30.9	241.59	8.4	65.68
2	*20'*	25.65	107.6	838.99	1	7.80	1	7.80
3	*Swash*	25.71	770.1	5990.67	11	85.57	3	23.34
3	*20'*	25.14	2	15.91	0	0.00	1	7.96

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.49	1203.3	9441.35	16.9	132.60	18.7	146.72
1	*20'*	25.8	143	1108.53	3	23.26	6.2	48.06
2	*Swash*	25.67	1299.7	10126.22	23.5	183.09	31.5	245.42
2	*20'*	25.87	50.4	389.64	1	7.73	2	15.46
3	*Swash*	25.78	770.1	5974.40	10.8	83.79	8.5	65.94
3	*20'*	25.55	1	7.83	0	0.00	1	7.83

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	82	14.5	2
2	*Water*	Max	135.4	25.6	3
3	*Water*	Max	80.1	9.7	1

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 12 in, 2B @ 15 in, 3B @ 18 in

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-22: July 23, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.86	829.7	6416.86	22.8	176.33	4	30.94
1	*20'*	25.58	1203.3	9408.13	137.6	1075.84	2	15.64
2	*Swash*	25.62	Max	Max	228.2	1781.42	7.5	58.55
2	*20'*	25.77	201.4	1563.06	5.2	40.36	1	7.76
3	*Swash*	25.75	1732.9	13459.42	30.5	236.89	8.5	66.02
3	*20'*	25.98	1413.6	10882.22	23.1	177.83	3.1	23.86

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.39	770.1	6066.17	22.6	178.02	18.7	147.30
1	*20'*	25.05	1986.3	15858.68	95.9	765.67	4.1	32.73
2	*Swash*	25.89	2419.6	18691.39	275.5	2128.23	13.2	101.97
2	*20'*	25.41	365.4	2876.03	1	7.87	3.1	24.40
3	*Swash*	25.35	1553.1	12253.25	21.6	170.41	42	331.36
3	*20'*	25.9	1046.2	8078.76	23.1	178.38	17.5	135.14

Water Samples		Total Coliform	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	517.2	214.2	2
2	*Water*	Max	261.3	68.3	1
3	*Water*	Max	1203.3	261.3	2

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 11 in, 2B @ 11 in, 3B @ 20 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-23: July 24, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.26	Max	Max	99	783.85	7.3	57.80
1	*20'*	25.72	2419.6	18814.93	12.2	94.87	3	23.33
2	*Swash*	25.38	2419.6	19066.98	53.8	423.96	3	23.64
2	*20'*	25.78	115.3	894.49	0	0.00	1	7.76
3	*Swash*	25.93	Max	Max	121.1	934.05	7.4	57.08
3	*20'*	25.72	Max	Max	3.1	24.11	7.3	56.77

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.28	Max	Max	57.1	451.74	19.5	154.27
1	*20'*	25.58	1986.3	15530.10	17.3	135.26	5.2	40.66
2	*Swash*	25.83	1413.6	10945.41	16.9	130.86	4.1	31.75
2	*20'*	25.75	93.3	724.66	0	0.00	2	15.53
3	*Swash*	25.74	Max	Max	122.3	950.27	45.5	353.54
3	*20'*	25.28	Max	Max	0	0.00	53.8	425.63

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	2419.6	2419.6	Max	3
2	*Water*	Max	172.6	1	2
3	*Water*	Max	488.4	43.7	3

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 14 in, 2B @ 11 in, 3B @ 20 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-24: July 25, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.31	1986.3	15695.77	23.3	184.12	4.1	32.40
1	*20'*	25.23	34.5	273.48	7.5	59.45	2	15.85
2	*Swash*	25.95	1553.1	11969.94	18.7	144.12	1	7.71
2	*20'*	25.34	30.9	243.88	1	7.89	0	0.00
3	*Swash*	25.75	727	5646.60	20.1	156.12	2	15.53

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.37	1553.1	12243.59	30.9	243.59	17.5	137.96
1	*20'*	25.97	56.5	435.12	4.1	31.57	4.1	31.57
2	*Swash*	25.55	1986.3	15548.34	16	125.24	6.3	49.32
2	*20'*	25.58	12	93.82	0	0.00	1	7.82
3	*Swash*	25.13	461.1	3669.72	4.1	32.63	8.6	68.44

Water Samples		Total Coliform	E. coli	Enterococci	Algae
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	648.8	179.3	3
2	*Water*	Max	218.7	122.3	3
3	*Water*	Max	79.8	16.9	2

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 16 in, 2B @ 14 in, 3B @ 20 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-25: July 29, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.63	461.1	3598.13	14.8	115.49	3	23.41
1	*20'*	25.56	8.5	66.51	0	0.00	0	0.00
2	*Swash*	25.34	1413.6	11157.06	28.5	224.94	5.2	41.04
2	*20'*	25.91	Max	Max	17.9	138.17	1	7.72
3	*Swash*	25.31	344.8	2724.61	45.5	359.54	6.3	49.78
3	*20'*	25.78	Max	Max	3	23.27	1	7.76

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	24.84	435.2	3504.03	13.1	105.48	4.1	33.01
1	*20'*	25.41	15.8	124.36	0	0.00	2	15.74
2	*Swash*	25.79	980.4	7602.95	17.1	132.61	9.7	75.22
2	*20'*	24.99	2419.6	19364.55	2	16.01	1	8.00
3	*Swash*	25.65	275.5	2148.15	17.1	133.33	9.6	74.85
3	*20'*	25.45	Max	Max	2	15.72	5.1	40.08

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	866.4	344.8	3
2	*Water*	Max	435.2	344.8	3
3	*Water*	2419.6	37.9	9.6	0

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 14 in, 2B @ 14 in, 3B @ 18 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-26: July 31, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.24	686.7	5441.36	12.2	96.67	4.1	32.49
1	*20'*	25	2419.6	19356.80	218.7	1749.60	1	8.00
2	*Swash*	25.17	686.7	5456.50	22.6	179.58	6.3	50.06
2	*20'*	25.36	7.4	58.36	0	0.00	0	0.00
3	*Swash*	25.03	135.4	1081.90	8.4	67.12	7.3	58.33
3	*20'*	25.3	14.6	115.42	1	7.91	0	0.00

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.08	648.8	5173.84	19.9	158.69	7.5	59.81
1	*20'*	25.04	1299.7	10380.99	209.8	1675.72	2	15.97
2	*Swash*	25.26	1203.3	9527.32	24.3	192.40	9.5	75.22
2	*20'*	25.03	21.3	170.20	0	0.00	3	23.97
3	*Swash*	25.37	74.9	590.46	13.4	105.64	7.4	58.34
3	*20'*	25.62	41.4	323.19	1	7.81	0	0.00

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	1413.6	2419.6	3
2	*Water*	Max	95.9	2419.6	3
3	*Water*	488.4	30.1	2	1

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 12 in, 2B @ 18 in, 3B @ 19 in

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-27: August 1, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.4	325.5	2562.99	24.9	196.06	7.3	57.48
1	*20'*	25.43	5.2	40.90	0	0.00	0	0.00
2	*Swash*	25.14	227.7	1811.46	9.6	76.37	1	7.96
2	*20'*	25.85	2	15.47	0	0.00	3	23.21
3	*Swash*	25.51	114.5	897.69	14.5	113.68	4.1	32.14
3	*20'*	25.17	19.9	158.12	0	0.00	1	7.95

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25	172	1376.00	14.8	118.40	3.1	24.80
1	*20'*	25.52	3.1	24.29	0	0.00	1	7.84
2	*Swash*	25.99	90.6	697.19	29.5	227.01	1	7.70
2	*20'*	25.55	4.1	32.09	0	0.00	1	7.83
3	*Swash*	25.06	129.1	1030.33	9.8	78.21	4.1	32.72
3	*20'*	25.67	21.1	164.39	0	0.00	0	0.00

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	70.3	218.7	1
2	*Water*	1046.2	101.7	8.4	1
3	*Water*	435.2	20.1	6.1	1

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 12 in, 2B @ 12 in, 3B @ 21 in

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-28: August 6, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.93	344.8	2659.47	23.3	179.71	2	15.43
1	*20'*	25.03	116.2	928.49	0	0.00	1	7.99
2	*Swash*	25.74	84.2	654.23	10.8	83.92	1	7.77
2	*20'*	25.17	95.9	762.02	1	7.95	1	7.95
3	*Swash*	25.45	88.4	694.70	11.9	93.52	0	0.00
3	*20'*	25.32	272.3	2150.87	1	7.90	1	7.90

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.27	206.4	1633.56	14.4	113.97	2	15.83
1	*20'*	25.73	41.4	321.80	0	0.00	0	0.00
2	*Swash*	25.32	81.6	644.55	6.3	49.76	5.1	40.28
2	*20'*	25.37	81.6	643.28	0	0.00	0	0.00
3	*Swash*	25.4	69.7	548.82	12.1	95.28	3	23.62
3	*20'*	25.29	87.6	692.76	0	0.00	2	15.82

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	298.7	35.9	5.1	0
2	*Water*	Max	435.2	78.9	1
3	*Water*	1986.3	107.6	16	0

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 13 in, 2B @ 11 in, 3B @ 19 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-29: August 7, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
			MPN/100		**MPN/100**	**MPN/100**	**MPN/100**	**MPN/100**
Transect	**Location**	**Sand Weight**	**mL**	**MPN/100 g**	**mL**	**g**	**mL**	**g**
1	*Swash*	25.06	260.3	2077.41	11.8	94.17	1	7.98
1	*0-2"*	25.22	3.1	24.58	0	0.00	0	0.00
1	*2-5"*	40.87	6.3	30.83	0	0.00	1	4.89
1	*5-8"*	40.51	3.1	15.30	0	0.00	1	4.94
1	*8-11"*	40.5	0	0.00	0	0.00	3	14.81
1	*11-14"*	40.12	960.6	4788.63	77.1	384.35	1299.7	6479.06

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
			MPN/100		**MPN/100**	**MPN/100**	**MPN/100**	**MPN/100**
Transect	**Location**	**Sand Weight**	**mL**	**MPN/100 g**	**mL**	**g**	**mL**	**g**
1	*Swash*	25.83	108.1	837.01	3.1	24.00	2	15.49
1	*0-2"*	25.46	6.2	48.70	0	0.00	1	7.86
1	*2-5"*	40.65	6.3	31.00	0	0.00	0	0.00
1	*5-8"*	40.27	0	0.00	0	0.00	0	0.00
1	*8-11"*	40.53	0	0.00	0	0.00	1	4.93
1	*11-14"*	40.02	501.2	2504.75	3	14.99	1046.2	5228.39

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
			MPN/100	**MPN/100**	
Transect	**Location**	**MPN/100 mL**	**mL**	**mL**	**(0-3 scale)**
1	*Water*	2419.6	32.7	5.1	1

*Note** *1B @ 14 in*

Table 1-30: August 8, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.99	920.8	7085.80	27.9	214.70	16.7	128.51
1	*0-3"*	40.2	18.7	93.03	5.2	25.87	2	9.95
1	*3-6"*	40.07	2	9.98	1	4.99	1	4.99
1	*6-9"*	40.72	2	9.82	0	0.00	1	4.91
1	*9-12"*	40.4	2	9.90	0	0.00	1	4.95

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	816.4	36.8	3	2

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 17 in

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-31: August 12, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.1	980.4	7811.95	17.3	137.85	9.5	75.70
1	*20'*	25.54	829.7	6497.26	3.1	24.28	25.9	202.82
2	*Swash*	25.71	222.4	1730.07	8.5	66.12	3	23.34
2	*20'*	25.5	1046.2	8205.49	579.4	4544.31	10.9	85.49
3	*Swash*	25.85	574.8	4447.20	26.2	202.71	4.1	31.72
3	*20'*	24.97	88.6	709.65	74.3	595.11	3	24.03

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.11	549.3	4375.15	15.8	125.85	7.4	58.94
1	*20'*	25.69	686.7	5346.05	2	15.57	24.3	189.18
2	*Swash*	25.57	222.4	1739.54	8.5	66.48	5.2	40.67
2	*20'*	25.42	1203.3	9467.35	727	5719.91	78.9	620.77
3	*Swash*	25.82	524.7	4064.29	25.9	200.62	13.5	104.57
3	*20'*	25.43	71.2	559.97	62.4	490.76	5.1	40.11

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	209.8	193.5	2
2	*Water*	Max	275.5	166.4	1
3	*Water*	Max	248.1	172.2	1

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 7 in, 2B @ 9 in, 3B @ 19 in

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-32: August 13, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.71	1119.9	8711.79	40.4	314.27	8.4	65.34
1	*20'*	25.25	1986.3	15733.07	142.1	1125.54	11	87.13
2	*Swash*	25.05	83	662.67	1	7.98	6.2	49.50
2	*20'*	25.67	128.4	1000.39	0	0.00	14.7	114.53
3	*Swash*	25.42	829.7	6527.93	22.8	179.39	0	0.00
3	*20'*	25.41	121.1	953.17	0	0.00	1	7.87

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.27	1046.2	8280.17	43.7	345.86	35.9	284.13
1	*20'*	25.21	920.8	7305.04	123.6	980.56	17.1	135.66
2	*Swash*	25.48	96	753.53	0	0.00	0	0.00
2	*20'*	25.36	133.3	1051.26	0	0.00	4	31.55
3	*Swash*	25.48	387.7	3043.17	12	94.19	5.1	40.03
3	*20'*	25.68	83.6	651.09	0	0.00	1	7.79

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	613.1	228.2	2
2	*Water*	Max	151.5	25.6	1
3	*Water*	Max	387.3	74.9	2

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 13 in, 2B @ 14 in, 3B @ 20 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-33: August 14, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.75	1553.1	12062.91	7.4	57.48	1986.3	15427.57
1	*20'*	25.82	156.5	1212.24	11	85.21	0	0.00
2	*Swash*	25.32	980.4	7744.08	5.2	41.07	1	7.90
2	*20'*	25.57	0	0.00	0	0.00	2	15.64
3	*Swash*	25.59	2419.6	18910.51	53.8	420.48	8.6	67.21
3	*20'*	25.76	32.7	253.88	4.1	31.83	0	0.00

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.26	357.8	2832.94	5.2	41.17	14.5	114.81
1	*20'*	25.57	123.6	966.76	4.1	32.07	5.1	39.89
2	*Swash*	25.25	1203.3	9531.09	0	0.00	1	7.92
2	*20'*	25.41	1	7.87	0	0.00	0	0.00
3	*Swash*	25.26	2419.6	19157.56	25.6	202.69	10.7	84.72
3	*20'*	25.63	16	124.85	2	15.61	1	7.80

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	461.1	1986.3	3
2	*Water*	Max	1046.2	2419.6	3
3	*Water*	2419.6	166.4	10.8	1

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 16 in, 2B @ 17 in, 3B @ 19 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-34: August 19, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.14	261.3	2078.76	6.3	50.12	3	23.87
1	*20'*	25.52	40.4	316.61	4.1	32.13	4.1	32.13
2	*Swash*	25.54	Max	Max	1203.3	9422.87	15.8	123.73
2	*20'*	25.14	228.2	1815.43	1	7.96	0	0.00
3	*Swash*	25.71	Max	Max	55.6	432.52	76.5	595.10
3	*20'*	25.5	36.4	285.49	4.1	32.16	2	15.69

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.15	272.3	2165.41	4.1	32.60	6.2	49.30
1	*20'*	25.16	45.5	361.69	5.2	41.34	3	23.85
2	*Swash*	25.22	2419.6	19187.95	1413.6	11210.15	37.9	300.56
2	*20'*	25.69	20.3	158.04	0	0.00	0	0.00
3	*Swash*	25.85	Max	Max	18.1	140.04	62.9	486.65
3	*20'*	25.17	20.1	159.71	1	7.95	2	15.89

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	86	0	3
2	*Water*	Max	547.5	67	3
3	*Water*	Max	129.6	9.7	2

*Note** Max = MPN > 2419.6 and beyond measurable amount
1B @ 16 in, 2B @ 20 in, 3B @ 19 in
Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-35: August 20, 2013 Sampling Data

200 mL DI water			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.56	866.4	6779.34	5.2	40.69	4.1	32.08
1	*20'*	25.14	101.7	809.07	2	15.91	1	7.96
2	*Swash*	25.87	770.1	5953.61	25.9	200.23	3	23.19
2	*20'*	25.52	0	0.00	0	0.00	0	0.00
3	*Swash*	25.81	Max	Max	82.3	637.74	7.3	56.57
3	*20'*	25.41	325.5	2561.98	4.1	32.27	2	15.74

200 mL PBS			*Total Coliform*		*E. coli*		*Enterococci*	
Transect	**Location**	**Sand Weight**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**	**MPN/100 mL**	**MPN/100 g**
1	*Swash*	25.04	980.4	7830.67	8.5	67.89	6.3	50.32
1	*20'*	25.07	125.9	1004.39	3.1	24.73	1	7.98
2	*Swash*	25.7	648.8	5049.03	6.2	48.25	4.1	31.91
2	*20'*	25.34	0	0.00	0	0.00	1	7.89
3	*Swash*	25.43	Max	Max	22.1	173.81	7.2	56.63
3	*20'*	25.08	517.2	4124.40	3	23.92	4.1	32.70

Water Samples		*Total Coliform*	*E. coli*	*Enterococci*	*Algae*
Transect	**Location**	**MPN/100 mL**	**MPN/100 mL**	**MPN/100 mL**	**(0-3 scale)**
1	*Water*	Max	1986.3	325.5	3
2	*Water*	Max	1553.1	238.2	3
3	*Water*	Max	61.3	12	2

*Note** Max = MPN > 2419.6 and beyond measurable amount

1B @ 17 in, 2B @ 19 in, 3B @ 20 in

Algae scale: 0=none, 1=mild, 2=mediocre, 3=high

Table 1-36: August 21, 2013 Sampling Data