Investigation of Water Quality and Source Tracking at
Whites Beach
Arenac County, Michigan

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1. INTRODUCTION

1.1 *Background*

Throughout the United States, recreational waters are expected to meet swimmable standards as defined under the Clean Water Act. However, each year, thousands of beaches are closed due to elevated bacteria levels (NRDC 2008). It is critical that the sources of contamination are identified in order to develop solutions to water quality problems that reduce the number of beach closures and protect human health.

In 1986, the United States Environmental Protection Agency (EPA) developed water quality criteria for recreational waters based on indicator bacteria. These criteria were based on public health studies conducted in the 1950s-1980s. EPA recommended the use of two indicators: enterococci for marine waters and *Escherichia coli* (*E. coli)* or enterococci for freshwaters. *E. coli* and Enterococci are used as water quality indicators because they have been linked to human gastroenteritis through the use of epidemiological studies (EPA 1986, Wade et al. 2008). Each state is responsible for developing and adopting standards for "swimmable" waters under the Clean Water Act that are as protective, based on the risk of illness, as the EPA criteria. Michigan's *E. coli* total body contact standard has been set at 300 CFU/100 ml, which was approved as equally protective of human health as the EPA level of 235 CFU/100 ml.

In Michigan, beach managers conduct routine beach monitoring of *E. coli* concentrations to assess water quality conditions at recreational beaches. Samples are collected in waist-deep water at least once per week during the swimming season. More recently, managers have added more extensive data collection, through the use of beach sanitary surveys, to routine beach monitoring in order to improve the understanding of the source and transport associated with the *E. coli*. Beach managers are beginning to undertake sanitary surveys which will collect physical data, such as water temperature, air temperature and wave height, for every water sample collected, potentially enabling development of a predictive model in the future to address conditions when *E. coli* concentrations are high.

Scientists have also conducted research on recreational waters in order to better understand risks to human health from fecal contamination. Elevated bacteria levels have been detected at some beaches during high energy periods such as increased wind and wave action. This has led researchers to investigate microbial contamination, survival, and transport in the nearshore zones (Garrido-Perez et al. 2008, Whitman 2003). Researchers have also found high levels of indicator bacteria in the sediment and sand of nearshore beaches which can be suspended into the water column during high energy inputs. Algal mass accumulation on recreational beaches prompted researchers to explore pathogens and indicators in algae (Englebert et al. 2008a, Englebert et al. 2008b, Ishii et al. 2006, Olapade et al. 2006).

There are multiple indicators for fecal contamination in water. In addition to *E. coli* and enterococci, *Clostridium perfringens* (*C. perfringens*) and Coliphage have emerged as useful alternative fecal indicators. Appendix 1 describes the indicators and advantages/disadvantages to using each. When used together, the fecal indicators can provide a better understanding of water quality than *E. coli* alone. Fecal indicators are based on the premise that each indicator will be found consistently in fecal waste and have similar survival and transport properties as pathogens known to cause disease (Colford 2007). However, survival rates and regrowth potential of some bacteria varies depending on water temperature, sunlight, nutrient status, and turbidity (McLellan 2007) and bacterial indicators have been poor predictors of the presence of viruses and parasites. In addition, the indicators are not able to identify the source of the contamination. Thus, fecal indicators have multiple limitations.

The inability of indicators to identify pollution sources has come to the forefront of water quality in recent years and has lead to the development of microbial source tracking. Microbial source tracking is a field of study that seeks to identify the origin of fecal waste. There are two types of source tracking methods: library-dependent and library-independent. Library-dependent methods involve creating a large dataset of particular target indicator bacteria (predominantly *E. coli* and enterococci) present in one area (i.e. watershed, county, etc.) from a variety of sources (human, bovine, avian, etc.). Water samples are then assayed and the results are compared to the identified organisms in the library to determine the origin of the fecal pollution. The disadvantage of library-dependent methods is that it requires a great deal of time and resources to collect, identify, catalog, and store all of the organisms that may be present in a given area. This method also returns a large number of false positives and false negatives (Santo Domingo et al. 2007).

Library-independent methods involve the detection of a marker specific to one species to identify the source of microbial contamination. Library-independent methods include the use of chemicals, sterols, viruses, bacterial genes, and toxins. In the case of bacterial genes, the DNA sequences first are identified and then undergo validation testing to evaluate the specificity of the sequence as unique to a particular species. Water samples are then assayed for the source markers, generally through a non-culture based method (Santo Domingo et al. 2007) and DNA amplification through polymerase chain reactions (PCR). The library-independent method can use conventional or real-time PCR (which is a quantitative approach) to detect DNA sequences. One set of specific source markers found in the anaerobic bacteria *Bacteroides* have the potential to identify fecal inputs from human, bovine, birds, pigs, elk, and dogs and correlates with other fecal indicator bacteria (Field and Samadpour 2007). Another useful tool for source tracking is the enterococci surface protein gene (esp, Scott et al. 2005). This human-specific marker has been shown to be present in sewage and septage and absent in all tested animals (Ahmed et al. 2008). Library-

independent methods return less false positive and false negative results than the library dependent method. However, like all methods for source tracking, library-independent methods do have disadvantages. There are few specific markers available (Field and Samadpour 2007) and these markers may not be present in large quantities in the environment (Scott et al. 2005). Source tracking has been gaining use in the Great Lakes region as identifying the source of contamination becomes more critical for maintaining or improving water quality.

1.2 *The Problem*

In recent years, the Saginaw Bay and its beaches have experienced elevated bacteria levels, algal masses awash on the beach (muck), and fish kills in surrounding rivers. Multiple groups, from local health departments to universities, have studied Saginaw Bay to provide a more comprehensive understanding of the causes of these water quality problems. Routine beach monitoring has identified several beaches which exhibit chronically high bacteria levels. A sanitary survey project identified rain, wind, combined sewer overflow systems, and septic systems as potential factors impacting Saginaw Bay beaches. The Water Quality and Environmental Microbiology Laboratory of Michigan State University (East Lansing, Michigan) performed preliminary source tracking on water and muck samples from Huron County on August 13th, 2007. The results indicated high levels of bacteria and the presence of human *Bacteroides* and esp markers as well as bovine *Bacteroides* in the muck and water samples. The results raised further concern on the role of muck in causing human illness and the sources of impact to area beaches.

Muck was present on Saginaw Bay beaches in 2007 which prompted the Department of Environmental Quality to characterize the muck. Results indicate that it consisted of dead and decaying green algae, blue-green algae, *Cladophora*, and macroinvertebrate (B. Walker, personal communication, January 16, 2009). On August 13, 2007 the Water Quality and Environmental Microbiology Laboratory of Michigan State University sampled muck from Saginaw Bay and found *E. coli*, Enterococci, and *Clostridium perfringens*. The esp gene was detected in about half of the muck samples while the human and bovine *Bacteroides* were each found once in the muck. Further investigation into the movement and origin of the muck is being planned.

One beach that was chosen for further investigation was Whites Beach. Whites Beach was selected because of a lack of understanding about potential pollution contributions to the beach and because it was part of a beach sanitary survey project that provided geography, weather conditions, and hydrology data specific to this site. This beach is located in Whites Beach, Michigan. The beach has a reach length of approximately 18 meters (Figures 1 and 2). The park has a large open grass area between Whites Beach Road end and the waters edge. Whites Beach is located on the western shore of inner Saginaw Bay. The Saginaw Bay

Watershed has mixed land use of 56% agriculture, 25% forested, 9% rangeland, 6% urban, and 4% water/wetlands. The Central Michigan District Health Department has reported data on the Michigan Department of Environmental Quality BeachGuard website since 2001 for this location. Sixteen water quality exceedances have been reported since 2001 resulting in twelve beach closures or advisories totaling 110 action days.

1.3 *Study Objectives*

The goals of this project were to:

1. Explore the environmental factors and their relationship (i.e. wind, rain, temperature) with elevated *E. coli* concentrations
2. Address the level of fecal contamination in sediments and shallow waters where children play
3. Identify potential sources of fecal pollution at Whites Beach.

To achieve our objectives we used alternative and conventional fecal indicators, source tracking markers and beach monitoring, of the shallow waters, deep/swimmable waters, and sediments.

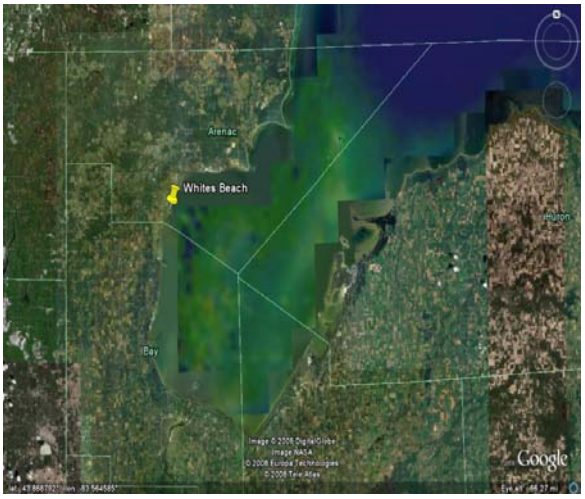

Figure 1: Whites Beach
Whites Beach, Michigan


Figure 2: Whites Beach on 8/12/2008

2. MATERIALS AND METHODS

2.1 *Sample location, type, and strategy*

Tests performed by the Water Quality and Environmental Microbiology Laboratory on samples from Whites Beach included fecal indicators (*E. coli*, enterococci, *C. perfringens*, and Coliphage) and microbial source tracking markers (Human and Bovine *Bacteroides* markers and Enterococcus Surface Protein (*esp*)). Environmental samples were collected eight times at one location and included sediment samples (n=7), muck samples (n=0), shallow water samples (n=8), and waist deep water samples (n=3; deep

water also includes 2 *E. coli* samples collected in this study and 4 *E. coli* samples collected by health department [*E. coli* n=6]) as indicated in Table 1. Deep water samples were initially collected twice (7-10-2008 and 7-15-2008). The Central Michigan District Health Department performed routine beach monitoring for *E. coli* concentrations at the same time as the source tracking samples were collected.

Table 1: Whites Beach monitoring dates and sample types, Saginaw Bay, Michigan
No muck was present during sampling dates.

Water Sample ID	Location Description	Dates Collected	Types of Samples Collected
Whites Beach	Beach at end of Whites Beach Road in Whites Beach, Michigan 43.92861, -83.89051	7-10-2008 7-15-2008 7-22-2008 7-29-2008 8-5-2008 8-12-2008 9-6-2008 9-30-2008	Deep and shallow Deep, shallow, and sediment Shallow and sediment Shallow and sediment Shallow, and sediment Shallow and sediment Shallow and sediment Deep, shallow, and sediment

2.2 *Physical data*

Physical parameters were collected at the same time water samples were collected and included bather load, animals present on the beach, debris in the water and on the beach, wave height, and water and air temperature. Other data (precipitation, wind speed/direction, etc.) were collected from local weather stations on-line (weatherunderground.com). Once in the laboratory pH and turbidity were also measured.

Wave height data were collected using a yard stick and measured from the trough to the crest of the wave. Debris and algae amounts in the water and on the beach were estimated and later quantified using the following approach: 0% present was given a 1, 1-20% material per area present was given a 2, 21-50% material per area present was given a 3, and 51-100% material per area present was given a 4. Birds in the water or at the swash zone were counted by sight and if species could be identified the count was noted. Bather load was determined by counting the number of individuals in and out of the water and noting their activity.

2.3 *Water sampling*

Grab samples were collected at the beach in ankle deep water (approximately 15-20 cm) using sterile sample bottles for shallow water sampling. Care was given to not

disturb the surrounding sediment during collection. Grab samples were obtained at the beach in waist deep water (3′) using sterile sample bottles. Inverted sample bottles were plunged to a depth of 6″-12″ below the surface, turned up, and capped underwater to avoid surface water from being collected. All Samples, regardless of type, were placed on ice (4° C) and brought to the MSU Water Quality and Environmental Microbiology Laboratory for analysis. The samples were kept at 4° C and processed within 24 hours of collection.

2.4 ***Sediment/sand sampling***

Sediment samples were collected in the swash zone via sterile Whirl-Pak ®. Sediment samples were collected by inverting the Whirl-Pak®, grabbing a handful of sediment from three points on the beach, and then compositing all subsamples into one bag. Samples were placed on ice at 4° C and brought to the MSU Water Quality and Environmental Microbiology Laboratory for analysis. The samples were kept at 4° C until processed the same day as collection.

2.5 ***Sample analysis for culture based methods***

2.6a *Bacterial analysis*

Water samples were analyzed for *E. coli* and Enterococci via membrane filtration and the mTEC agar method (US EPA 2005) and mEI agar method (US EPA 2002), r*esp*ectively. Water, muck, and sediment samples were analyzed for *Clostridium perfringens* by using membrane filtration and mCP agar method (US EPA 1995, Bisson 1979). Sample volume ranged from 1 ml to 100 ml of undiluted sample. Negative controls were run using sterile PBW and plating on each agar. Positive controls were also set up and assayed by membrane filtration using dilutions of stock cultures in PBW and plating on the r*esp*ective selective agar.

Seventy-five grams of wet weight of sediment were diluted to a 10% w/v with sterile Phosphate Buffer Water (PBW). From the mixture, 100 ml was used to assay for *E. coli* and Enterococci using Colilert and Enterolert, r*esp*ectively. Bacterial levels were reported as colony forming units/100 grams wet weight of sediment.

2.6b *Coliphage analysis*

Agar overlays were utilized to detect coliphage following EPA methods 1601 and 1602 (EPA 2001a and EPA 2001b). Non-filtered water samples were used to enumerate coliphage. Two bacterial hosts were used in the overlays including *E. coli* F^+amp and *E. coli* CN-13. The F^+amp is known as a host that supports growth of the male specific coliphage as the phage infects the host at the F-pili. The (CN-13) host bacteria supports somatic coliphage where these phage attach at the outer cell wall.

In order to achieve a log phase of host bacteria, 1 ml of stock culture *E. coli* CN-13 and F+amp stocks were added to 9 ml of sterile TSB and 1% total volume of appropriate antibiotic, either Naladixic acid for CN-13 or Streptomycin Ampicillin for F+amp. Hosts were then placed in a 36 ^{o} C shaking incubator at 100 rpm for approximately four hours. One-half ml of log phase host *E. coli* CN-13 or F+amp and 2 ml of water sample were added to melted top agar (at 1.5% agar, maintained in a liquid state at 48^{0} C) the samples were then immediately mixed and poured onto a tryptic soy agar plate (TSA), these were allowed to solidify, inverted and incubated for 24 hours in a 37^{0} C incubator. Coliphage samples were analyzed using five replicate plates per host. Thus, 20 ml of sample per site were assayed for coliphage during each sampling event. Two negative control plates were made, one with each host, by adding 1.5 ml host to the top agar, mixing and pouring onto a TSA plate. A positive control was run for each host type by adding 1.5 ml host to the top agar, mixing and pouring onto a TSA plate. Stock phage was spotted onto the hardening agar layer. Overlays were incubated at 37°C for 24 hours, and then assessed for plaque formation.

Incubation times, temperatures, and EPA standards are for the fecal indicator culture based methods discussed above are summarized in Table 2.

Table 2: Media and methods used for microbial indicator testing

Test	**Media**	**Incubation**	**Reference**	**EPA Recreational Standards**
E. coli	mTEC	24-28 hours at 37°C	US EPA Method 1603 (US EPA. 2005)	235 *E. coli*/ 100 ml
Enterococci	mEI agar	24 hours at 41°C	US EPA Method 1600 (US EPA. 2002)	61 Enterococci/100 ml
Clostridium perfringens	mCP	24 hours at 45°C	EPA 1995, Bisson 1979	Not established
Coliphage	Tryptic Soy Agar	16 – 24 hours at 37°C	US EPA Method 1601/1602 (US EPA 2001)	Not established

2.6 *Sample analysis for Cryptosporidium/Giardia*

Analysis for *Cryptosporidium* and *Giardia* were done following the EPA approved method 1623. Water samples were filtered and the (oo)cysts (*Giardia* cysts and *Cryptosporidium* oocysts) and extraneous materials were retained on a HV Gelman filter. The material on the filter was eluted and then centrifuged to pellet the (oo)cysts. The supernatant fluid was aspirated. The (oo)cysts were further concentrated via attachment of magnetic beads conjugated to anti-*Cryptosporidium* and anti-Giardia

antibodies. The (oo)cysts were separated from the extraneous materials using a magnet. The extraneous material was then discarded. The magnetic bead complex was then detached from the (oo)cysts. The (oo)cysts were stained on well slides with fluorescently labeled monoclonal antibodies and 4',6-diamidino-2-phenylindole (DAPI). The stained sample was examined using fluorescence and differential interference contrast (DIC) microscopy. Quantitative analysis was performed by scanning each slide and counting all (oo)cysts that met the size, shapes, and fluorescence criteria of *Cryptosporidium* and *Giardia* (oo)cysts. A percentage of the (oo)cysts were assessed through DAPI staining characteristics and DIC microscopy to record any internal features observable.

2.7 *Sample analysis using Molecular methods*

2.7a *Bacteroides analysis*

One liter of water was filtered through a membrane filter, placed into a 50 ml centrifuge tube, and vortexed for five minutes. The tube was then centrifuged for 30 minutes at 4000 xg. Mobio Mega soil DNA kit was used to extract the DNA from the pellet. PCR amplification was performed on the extracted DNA. Primers for both human and cow *Bacteroides* sequences were used as previously described (Bernhard 2000). Gel electrophoresis was performed on the PCR product, run on a 1.2% w/v agarose gel at 95 V for approximately one hour.

2.7b *Enterococci esp analysis*

The enterococci bacteria which grew up on the membrane filter on MEI as described in the culture based methods were washed off the membrane, centrifuged for 15 minutes and DNA was extracted from the pellet (Kumar, L. 2007, Scott et al. 2005) using Qiagen QIAmp DNA mini kit. The primers specific for the *esp* gene in *E. faecium* previously developed and examined for specificity to human fecal pollution were used in a polymerase chain reaction [PCR] (Scott et al. 2005). The forward primer: (5′-TAT GAA AGC AAC AGC ACA AGT-3′) and the conserved reverse primer (5′ –ACG TCG AAA GTT CGA TTT CC-3′) were used for all reactions. Gel electrophoresis was performed on the PCR product and run on a 1.2% w/v agarose gel at 95 V for approximately one hour. Samples with bands at 680 bp were recorded as positives for *esp*.

2.8 *Data analysis*

The geometric mean for each microorganism was calculated for each sample type (shallow, deep, and sediment) at each site. When organisms were not detected, the lower detection limit value was used in calculations. Detection limits were calculated by dividing 1 by the total sample volume processed to return a detection limit (converted to < per 100 ml). In sediment samples, the lower detection limit was

determined by dividing 1 by the total volume/g processed, including the dilution. Results that exceeded the upper detection limit were used at the upper number.

Mean air temperatures were calculated as averages of hourly observations as recorded at local weather stations over a 24 hour period on the sampling date.

3. RESULTS

3.1 Spatial sampling analysis

Samples were collected throughout the summer at Whites Beach in the deep/ swimmable waters (n=3; [*E. coli* n=6: 2 samples by MSU and 4 samples by health department]), shallow waters (n=8), and sediment in the swash zone (n=7) as depicted in Figure 3. Samples were processed the same day for *E. coli*, enterococci, *C. perfringens*, and coliphage (CN-13 and F+amp). The geometric means and ranges per sample location and assay are given in Table 3. Deep water samples were initially collected on July 10th and July 15th but bacteria levels were low enough to discontinue source tracking efforts. The Central Michigan District Health Department collected routine beach monitoring samples at the time source tracking samples were collected. The Central Michigan District Health Department collected ten samples throughout the 2008 summer with an arithmetic average *E. coli* concentration of 29.02 CFU/100 ml and ranged from below detection limits (0.5 CFU/100 ml) to 58.0 CFU/100 ml. The other fecal indicator concentrations in the swimmable waters ranged from <0.6 to 15.2 CFU/100 ml for enterococci, and <0.6 to 1.44 CFU/100 ml for *C. perfringens*. The Coliphage virus was never above the lower limits of the method detection (10 PFU/100 ml) in the swimmable waters. Raw data collected from Whites Beach in the swimmable waters are detailed in Appendix 2.

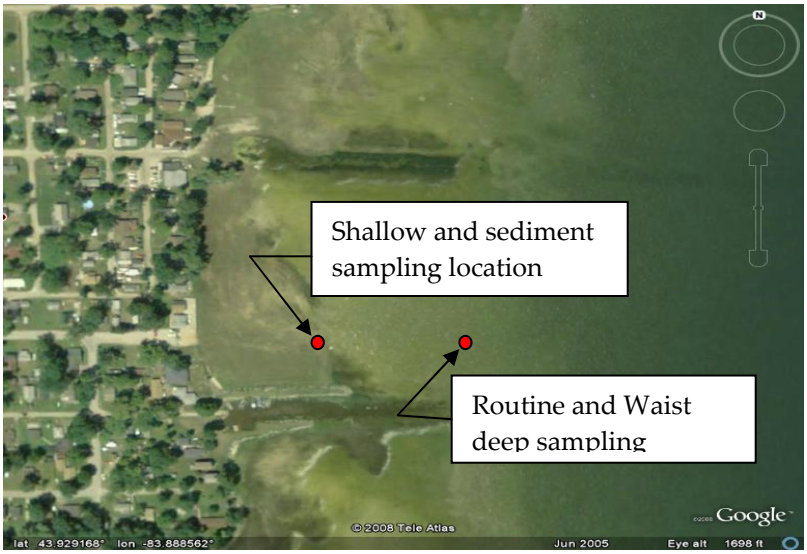

Figure 3: Sampling parameter locations at Whites Beach

Table 3: Microbial Quality of Shallow, Deep/Swimmable and Sediments at Whites Beach

Site		*E. coli*	Enterococci	*C. perfringens*	Coliphage (F^+amp)	Coliphage (CN-13)
Shallow (a) n=8	Geometric mean	62.1	53.3	2.4	10.0	47.5
	Range	5.2 - 3067	6.2 – 2233	<0.5- 6.13	<10.0 - 10.0	10.0-660
	Percent +	100	100	87	13	100
Deep (a; c) n=3	Geometric mean	11.3	0.8	0.4	<10.0	<11.9
	Range	1.76- 58.0	<.006 - 15.2	<0.7 - 1.44	<10.0	<10.0-<16
	Percent +	100	66	33	0	0
Sediment (b) n=7	Geometric mean	736.0	750.3	232.9	90.0	166.4
	Range	333.9-9101	27-3917	102-657	<90-90	<90-450
	Percent +	100	100	100	17	67

a: CFU or PFU/100 ml

b: CFU or PFU/100 g wet weight

<: Below method detection limits

c: Deep water E. coli data includes n=6; 2 samples collected in this study and 4 by health department

The geometric means of *E. coli*, enterococci, *C. perfringens*, Coliphage F+amp, and Coliphage CN-13 in the shallow water were 62.1, 53.3, 2.4, 10, and 47.5 organisms/100 ml, respectively. Shallow water bacteria concentrations (CFU/100 ml) ranged from 5.2 to $3.1x10^3$ for *E. coli*, 6.2 to $2.2x10^3$ for enterococci, and <.5 to 6.13 for *C. perfringens*. Coliphage concentrations in the shallow water ranged from below detection limits (10 PFU/100 ml) to 10 and 660 PFU/100 ml for F+amp and CN-13, respectively. Raw data collected from the shallow water at Whites Beach are detailed in Appendix 3.

Seven samples were collected from the sediment in the swash zone. The geometric mean concentration for *E. coli*, enterococci, *C. perfringens*, Coliphage F+amp, and Coliphage CN-13 as detected in the sediment was 736.0, 750.3, 232.9, 90.0, and 166.4 organisms/100 g wet weight, respectively. Bacteria concentrations (CFU/100 g wet weight) in the sediment ranged from 333.9 to 9,101 for *E. coli*, 27 to 3,917 for enterococci, and 102 to 657 for *C. perfringens*. Coliphage concentrations in the sediment ranged from below detection limits (<90 PFU/100 g wet weight) to 90 and 450 PFU/100 g wet weight for F+amp and CN-13, respectively. Raw data collected from the sediments at Whites Beach are detailed in Appendix 4.

3.2 Temporal sampling analysis

Samples were collected at Whites Beach from mid-July through the end of September (n=8). A total of eighty-five indicator assays were processed as part of this project. Not

one specific sampling event appeared to have an overwhelming level on all indicators. On July 10th, tests in the shallow water found that *E. coli* levels were greater than $3.0x10^3$ CFU/100 ml, enterococci levels greater than $2.2x10^3$ CFU/100 ml, and coliphage CN-13 was present at a concentration of 100 PFU/100 ml. The physical data on wave height were not collected but will be examined later using data from NOAA's stations. There was some rain (0.33") that preceded these findings 72 hours earlier.

There were no beach closures or advisories in response to *E. coli* concentrations at Whites Beach during the sampling period.

Clostridium perfringens assays were performed on seventeen samples. The highest levels of *C. perfringens* were detected in the sediment on July 22nd (657 CFU/100 g wet weight) which was preceded by .53" of precipitation in the previous 72 hours. The highest concentrations of the somatic coliphage detected in the sediment during this project and the only date the F+amp coliphage was detected occurred on July 29th.

On September 6th, 2008 a sample was collected from Whites Beach in the shallow water and tested for the presence of *Cryptosporidium* and *Giardia*. At the lower detection limit (.5 (oo)cysts/l), neither organism was detected.

3.3 Physical data analysis

Physical data (temperatures, wind speed, wave height, precipitation, etc) collected at Whites Beach are detailed in Appendix 5. The lowest average daily temperature was recorded on September 30th (15.0°C) and the highest on August 5th (24.4°C). The lowest water temperature recorded at time of sampling was on July 29th (18.1°C) and the highest on July 22nd (25.3°C). Wave height was measured at 0.5 feet during three sampling events and calm (< 0.5') during all other events. Wind speed and direction were light and variable with an average of 1.2 MPH for the project.

Precipitation data collected at Whites Beach on September 6th had the largest 48 hour and 72 hour rainfall of this project (1.09" and 1.10", respectively). On July 22nd, 0.34" of rainfall was recorded in the 24 hours prior to sampling.

No algae/muck were present in the nearshore or on the beach during any sampling event of this project.

3.4 Molecular analysis

The enterococci surface protein (esp) gene was used as a source tracking marker at Whites Beach. Analysis for esp was performed on eleven samples (two swimmable, four sediment, and five shallow water samples). This source tracking marker was not

detected in any of the samples collected and processed from Whites Beach during this project. Results from the esp testing at Whites Beach are given as present/absence in Appendix 6.

Samples were also assayed for the human and bovine *Bacteroides* marker through the use of PCR and qPCR. Seven samples (two swimmable and five shallow) collected at Whites Beach were assayed for the presence of *Bacteroides* (human and bovine) using conventional PCR methods. No samples tested posted for either of the *Bacteroides* indicators. *Bacteroides* results from the swimmable and shallow waters are given as presence/absence in appendix 7 and 8, respectively.

The samples collected on July 10th and July 22nd were further assayed using a large sample volume (500 µl) extraction method and a qPCR marker for human *Bacteroides*. This method did not detect the presence of human *Bacteroides* at Whites Beach.

4. CONCLUSIONS

Whites Beach water quality in the shallow and deeper waters met *E. coli* and enterococci standards and criteria. There were no muck, sewage, or bovine markers detected during the summer 2008 sampling . However, the shallow waters still exhibited some evidence of water quality degradation, perhaps due to accumulating pollution which was likely a result of the sediment and shallow water interactions.

Four indicators were used, two that could possibly regrow (*E. coli* and enterococci) and one that does not but can accumulate and survive (C*lostridium*) and the coliphage which can not regrow and do die-off, (with the DNA phage which would show up on the CN-13 host surviving a bit better in surface water than the other f-specific phage). We have suggested that the coliphage represent more recent pollution or environments that allow for greater survival. In deeper waters while the *E. coli* was found at low levels 100% of the time, the enterococci and *Clostridium* were not found as frequently and the coliphage were not found at all. In the shallow waters the bacteria were found 100% of the time as was the coliphage (CN-13 host). The bacteria, including the *Clostridium*, were also found 100% of the time in the sediment, and the somatic coliphage was found 67% of the time in the sediments.

Based on the results from the multiple indicator results taken from across the beach transect at Whites Beach there may be some consistent shoreline source of pollution influencing the shallow waters and the sediments at Whites Beach. We recommend further investigation be taken to identify the transport mechanisms in which bacteria are entering this beach.

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Appendix 1: Indicators and their applications to assessing recreational water quality

INDICTOR	DEFINITION	RECREATIONAL USE STANDARD OF MICHIGAN	ADVANTAGE OF USE AS AN INDICATOR	DISADVANTAGE OF USE AS AN INDICATOR
E. coli	A type of coliform bacteria that naturally occurs in the human intestinal tract Many strains exist but only a few are pathogenic	A geometric mean of at least 5 samples may not exceed 130 CFU/100 ml A single sample may not exceed 300 CFU/100 ml	Used as an indicator of bacteriological quality in both drinking and recreational waters Found to have a high correlation with gastroenteritis associated with bathing in freshwater Source tracking methods have bee developed	May grow in the soil of tropical locations Found to be poorly correlation to gastroenteritis in marine waters *E. coli* presence does not always correlate with the presence of enteric viruses and parasites
Enterococci	A gram positive, non-spore forming member of the Streptococci bacteria Commonly found in the feces of warm blooded animals Multiple strains, many of which are not harmful	A geometric mean of at least 5 samples may not exceed 33 CFU/100 ml A single sample may not exceed 61 CFU/ 100 ml	Enterococci may die at a slower rate than fecal coliforms in water and sediments, providing more reliable indications of recent pollution Multi-site epidemiological studies have shown that enterococci have a higher correlation with gastroenteric disease related to swimming in fresh and marine waters than fecal coliforms	Can regrow in the environment Not as well researched as *E. coli*
Clostridium perfringens	Obligate anaerobic gram-positive bacteria that forms endospores and does not carry out dissimilatory sulfate reduction Found in sewage and highly impacted waters An opportunistic pathogen that produces enterotoxin	FRESHWATER STANDARDS USED IN HAWAII: A single sample may not exceed 50 CFU/100 ml	*C. perfringens* spores could be an index parameter for the occurrence of persistent intestinal pathogens like viruses and oocysts of protozoa Useful in such specific situations as the examination of chlorinated waters or industrial waters that may contains compounds lethal to non-spore forming bacterial indicators, samples that cannot be processed within 12 hours and the detection of recent or long term inputs of fecal pollution.	May be too conservative an indicator which may not adequately protect human health Often found in low concentrations
Coliphage	Viruses whose hosts are strains of the bacteria *E. coli* Found wherever fecal contamination occurs	A single sample may not exceed 100 pfu/100 ml	A good indicator of enteroviruses due to similar seasonal variation, propensity for removal and resistance to environmental stress	Coliphage is not specific to human sewage

Appendix 2: Fecal indicator concentrations in the deep/swimmable water samples collected at Whites Beach (microorganisms/100 ml)

DATE	*E. coli*	Enterococci	*C. perfringens*	Coliphage CN-13	Coliphage F+amp
7/10/2008	5.00	<0.556	<0.6667	<10.0	<10.0
7/15/2008	1.76*	5.23	<0.6606	<16.667	<10.0
7/22/2008	16.86*	NT	NT	NT	NT
7/29/2008	6.03*	NT	NT	NT	NT
8/5/2008	57.95*	NT	NT	NT	NT
9/30/2008	39.3	15.2	1.44	<10.0	<10.0
Geometric	11.26	0.762	0.387	<11.856	<10.0

NT: Not tested

<: Below method detection limits

*: Samples collected by Central Michigan District Health Department and reported as the geometric mean of triplicate samples per event

Appendix 3: Fecal indicator concentrations in the shallow water samples collected at Whites Beach (microorganisms/100 ml)

DATE	*E. coli*	Enterococci	*C. perfringens*	Coliphage CN-13	Coliphage F+amp
7/10/2008	3067	2233	<0.5	100	<10.0
7/15/2008	73.3	102	4	30	<10.0
7/22/2008	45.7	116	2.8	130	<10.0
7/29/2008	NT	56.3	2.4	660	10.0
8/5/2008	NT	54	1.7	40	<10.0
8/12/2008	5.2	18.3	1.6	12.5	<10.0
9/6/2008	17.7	17.7	4.29	20	<10.0
9/30/2008	60.5	6.2	6.13	10	<10.0
geometric	62.1	53.3	2.4	47.5	10.0

NT: Not tested

<: Below method detection limits

Appendix 4: Fecal indicator concentrations in the sediment samples collected at Whites Beach (microorganisms/ g wet weight)

DATE	*E. coli*	Enterococci	*C. perfringens*	Coliphage CN-13	Coliphage F+amp
7/15/2008	9101	3248	NT	NT	NT
7/22/2008	333.9	2688	657.0	360	<90
7/29/2008	757.8	27.00	171.4	450	90
8/5/2008	645.3	720.9	128.6	90	<90
8/12/2008	498.6	3103	210.0	<90	<90
9/6/2008	198.9	3917	102.0	180	<90
9/30/2008	793.8	64.80	514.3	<90	<90
Geometric	736.0	750.3	232.9	166.4	90.0

NT: Not tested

<: Below method detection limits

Appendix 5: Physical data collected at time of sampling at Whites Beach

DATE	Daily mean temp. (°C)	Air temperature (°C)	Water Temperature (°C)	Wind speed (MPH)	Wave height (feet)	24 hour precipitation (inches)	48 hour precipitation (inches)	72 hour precipitation (inches)	Bather load (# in water)	Algae in nearshore (% area)	Algae on beach (% area)	Birds on beach	Wind Direction
7/10/2008	20.4					0	0	0.33					variable
7/15/2008	19.56	13.5	19.1	0	0.5	0	0	0.05	0	0	0	1	variable
7/22/2008	20	25.6	25.3	3	0	0.34	0.34	0.53	2	0	0	0	S
7/29/2008	22.19	22.9	18.1	1	0.5	0	0	0	0	0	0	0	NE
8/5/2008	24.4	23.3	9	0	0.5	0	0	0	0	0	0	0	variable
8/12/2008	18.36	18.9	25	2.5	0	0	0	0.87	0	0	0	0	NNW
9/6/2008	15.28	20.5	19.6	2	0	0	1.09	1.1	0	0	0	30	NE
9/30/2008	15	21.4	18.6	0	0	0.21	0.21	0.21	0	0	0	0	variable

Appendix 6: Analysis of esp testing from Whites Beach

DATE	DEEP	SHALLOW	SEDIMENT
7/10/2008	-	-	NT
7/15/2008	-	-	NT
7/22/2008	NT	-	-
7/29/2008	NT	-	-
8/5/2008	NT	-	-
8/12/2008	NT	NT	-

NT: Not tested for esp

Appendix 7: Human and bovine *Bacteroides* results as detected in the swimmable water at Whites Beach.

DATE	Volume Assayed	Final Concentration Volume	Human *Bacteroides*	Bovine *Bacteroides*
7/10/2008	4000 ml	2 ml	-	-
7/15/2008	2000	2m	-	-

Appendix 8: Human and bovine *Bacteroides* results as detected in the shallow water at Whites Beach.

DATE	Volume Assayed	Final Concentration Volume	Human *Bacteroides*	Bovine *Bacteroides*
7/10/2008	2000 ml	2 ml	-	-
7/15/2008	3000 ml	2 ml	-	-
7/22/2008	2200 ml	2 ml	-	-
7/29/2008	2200 ml	2 ml	-	-
8/5/2008	2000 ml	2 ml	-	-