Investigation of Water Quality and Source Tracking at
Port Crescent State Park day use Beach
Huron 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 bacteria 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 Huron County 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 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.

One beach chosen for further investigation was Port Crescent State Park Day Use beach. This beach is located outside of Port Austin, Michigan and has a reach length greater then 2,600 meters (Figures 1 and 2). The Port Crescent State Park Day Use beach is located on the northeast shore of outer Saginaw Bay. The Saginaw Bay Watershed has mixed land use of 56% agriculture, 25% forested, 9% rangeland, 6% urban, and 4% water/wetlands. The beach at Port Crescent State Park 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 geographic, weather conditions, and hydrologic data specific to this site. The Huron County Health Department has reported data on the Michigan Department of

Environmental Quality BeachGuard website since 2001 for this location. One water quality exceedance has been reported (8/13/2007) resulting in a beach closure of two days.

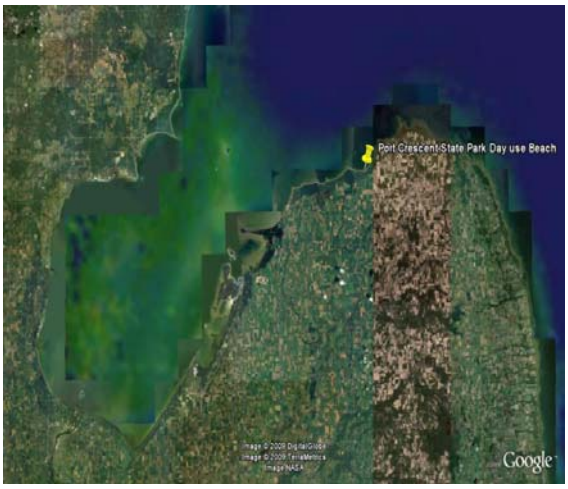

Figure 1: Port Crescent State Park day beach, Port Austin, Michigan

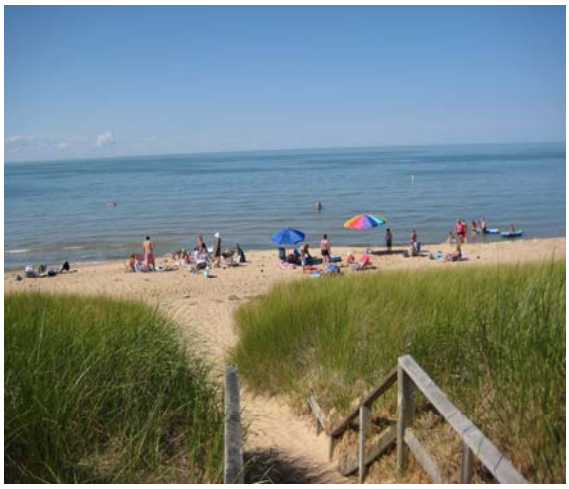
Figure 2: Port Crescent State Park day use use beach 8/12/2008

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 muck and sediments as well as in shallow waters where children play;
3. Identify potential sources of fecal pollution at Port Crescent State Park beach area.

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

2. MATERIALS AND METHODS

2.1 *Sample location, type, and strategy*

Tests performed by the Water Quality and Environmental Microbiology Laboratory on samples from Port Crescent State Park 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) as indicated in Table 1. Deep water samples were initially collected twice (7-10-2008 and 7-15-2008). The Huron County Health Department

performed routine beach monitoring for *E. coli* concentrations at the same time as the source tracking samples were collected (n=5)

Table 1: Port Crescent State Park day use beach monitoring dates and sample types, Saginaw Bay, Michigan

Water Sample ID	Location Description	Dates Collected	Types of Samples Collected
Port Crescent State Park day use beach	Park entrance near Loosemore Rd and Port Austin Rd. 44.00175, -83.07320	7-10-2008	Deep and shallow
		7-15-2008	Deep, shallow, and sediment
		7-22-2008	Shallow and sediment
		7-29-2008	Shallow and sediment
		8-5-2008	Shallow, and sediment
		8-12-2008	Shallow and sediment
		9-6-2008	Shallow and sediment
		9-30-2008	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 ***Muck sampling***

Muck samples were collected from large masses in the nearshore water area. A sterile Whirl-Pak® was inverted and plunged into the muck. Three grab samples were taken during each event and composited as one sample for analysis in the laboratory. Samples were stored and transported as previously described.

2.6 ***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.

One hundred grams of wet weight of muck was placed into a sterile blender, pulsed until homogenized, and diluted to a 1% w/v with sterile Phosphate Buffer Water (PBW). From the mixture, 100 ml was assayed for *E. coli* and Enterococci using Colilert and Enterolert, respectively. Bacterial levels were reported as colony forming units/100 grams wet weight of material.

Similarly 100 grams of wet weight of sediment were diluted to a 10% w/v with sterile PBW and 100 ml of the mixture 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.

Sediment samples were diluted to a different w/v percentage than the muck samples to obtain countable results. The muck required a greater dilution than the sediment to obtain countable results and to assure membrane filters were not too thick to mask colonies.

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°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 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

2.7 ***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.

2.8 ***Sample analysis using Molecular methods***

2.8a *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 4000xg. 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.

2.8b *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*.

2.9 ***Data analysis***

The geometric mean for each microorganism was calculated for each sample type (muck, 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 and muck 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 Port Crescent State Park day use beach in the swimmable waters (n=3; n=5 for *E.coli* only sampled by the 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). Swimmable water samples were initially collected on July 10th and July 15th but bacteria levels were low enough to discontinue source tracking efforts. The Huron County Health Department collected routine beach monitoring samples at the same time source tracking samples were collected. Throughout the summer, *E. coli* concentrations in the swimmable waters had a geometric mean average of 12 CFU/100 ml and ranged from 1 to 112 CFU/100 ml. The other fecal indicators ranged from <0.33 to 12 CFU/100 ml for enterococci, <0.22 to 2.0 CFU/100 ml for *C. perfringens*, and <10 PFU/100 ml to 60 and 90 PFU/100 ml for

Coliphage F+amp and CN-13, respectively. The geometric means and ranges per sample location and assay are given in Table 3. The raw data as collected in the swimmable waters are given in Appendix 2.

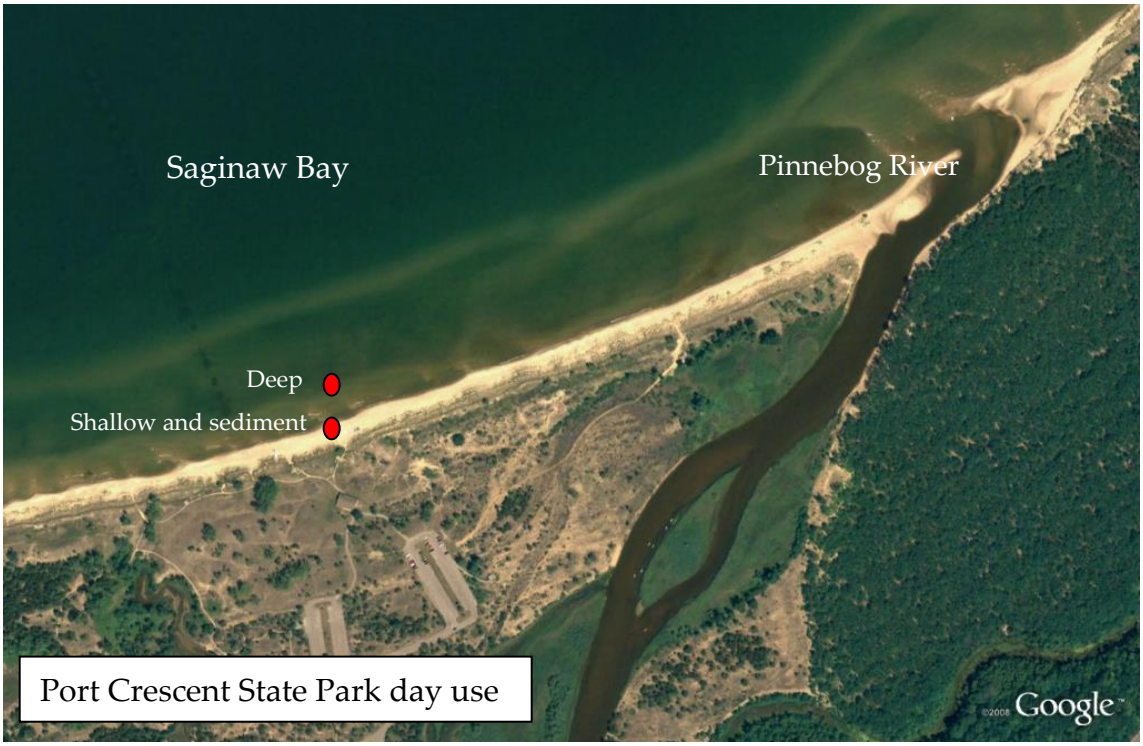

Figure 3: Sampling parameter locations at Port Crescent State Park day use beach

Table 3: Spatial sample analysis from Port Crescent State Park day use beach, No muck was found

Site		*E. coli*	Enterococci	*C. perfringens*	Coliphage (F^+amp)	Coliphage (CN-13)
Shallow (a) n=8	Geometric mean	13.2	2.8	1.2	11.6	27.1
	Range	4.1-225	<0.22-133	<0.25-5.1	<10-20	<10-970
	Percent +	100	14	86	12	37.5
Swimmable (a,c) n=3;n=5	Geometric mean	12.0	1.6	0.76	18.2	20.8
	Range	1-112	<0.33-12	<0.22-2.0	<10-60	<10-90
	Percent +	100	33	66	33	33
Sediment (b) n=7	Geometric mean	31.97	21.39	2.869	109.9	181.2
	Range	<9.0-7834	<9-1928	<2.0-6.0	<90-150	<90-720
	Percent +	86	43	60	67	33

a: CFU/100 ml or PFU/100 ml

b: CFU/100 g wet weight or PFU/100 g wet weight

<: Below method detection limits

c: Deep water includes n=7; 3 samples collected in this study and 5 *E. coli* samples collected by health department

The geometric means of *E. coli*, enterococci, *C. perfringens*, Coliphage F+amp, and Coliphage CN-13 in the shallow water were 13.2, 2.8, 1.2, 11.6, and 27.1 organisms/100 ml, respectively. Shallow water bacteria concentrations (CFU/100 ml) ranged from 4.1 to 225 for *E. coli*, <0.22 to 133 for enterococci, and <0.25 to 5.1 for *C. perfringens*. Coliphage concentrations in the shallow water ranged from below detection limits (10 PFU/100 ml) to 20 and 970 PFU/100 ml for F+amp and CN-13, respectively. The raw data collected from the shallow water at Port Crescent State Park day use beach are given in Appendix 3.

The geometric mean, as detected in the sediment, of *E. coli*, enterococci, *C. perfringens*, Coliphage F+amp, and Coliphage CN-13 were 31.97, 21.39, 2.869, 109.9, and 181.2 organisms/100 g wet weight, respectively. Bacteria concentrations (CFU/100 g wet weight) in the sediment ranged from <9.0 to 7834 for *E. coli*, <9.0 to 1928 for enterococci, and <2.0 to 6.0 for *C. perfringens*. Coliphage concentrations in the sediment ranged from below detection limits (90 PFU/100 g wet weight) to 150 and 720 PFU/100 g wet weight for F+amp and CN-13, respectively. The raw data collected from the sediment at Port Crescent State Park day use beach are given in Appendix 4.

3.2 Temporal sampling analysis

Samples were collected at Port Crescent State Park day use beach from Mid-July through the end of September (n=8). A total of eighty-three fecal indicator assays were processed. Water samples collected on July 22nd, 2008 had a higher *E. coli* arithmetic average (168.9 CFU/100 ml) than any other sampling date. *E. coli* concentrations in the sediment were also highest on July 22nd with an average of 7.8×10^3 CFU/100 g wet weight.

On September 6th, 2008 a sample was collected from Port Crescent State Park day use beach in the shallow water. This sample was tested for the presence of *Cryptosporidium* and *Giardia*. At the lower detection limit (.05 (oo)cysts/l), neither organism was detected.

Due to limited data no analyses have been undertaken.

3.3 Physical data analysis

Physical data collected at Port Crescent State Park day use beach at time of sampling are giving in Appendix 5. Air temperature at time of sampling ranged from 15.4°C on September 30th to 24.9°C on August 5th. Water temperatures also were lowest on September 30th (16.9°C) and highest on August 5th (24.2°C). Wind speed ranged from 1

to 5 mph with variable directions. Wave height ranged from .21′ to 1.5′ during sampling.

On two sampling dates (July 10th, 2008 and August 12th, 2008) 6 and 12 bathers were present at the time samples were collected. An average of seven birds were counted during five sampling events. Algae was present in 20% of the nearshore water during four sampling events and was present on less than 20% of the shoreline area during six sampling events, but no muck was detected or sampled.

Precipitation data collected August 12th had the largest 72 hour total precipitation volume recorded (1.89″), September 6th had the largest 24 hour and 48 hour precipitation volume recorded (.17″ and .88″, respectively). Samples collected on August 5th had 0.0″ of precipitation recorded in the previous 72 hours.

3.4 Molecular analysis

The enterococci surface protein (esp) gene was used as one source tracking marker at Port Crescent State Park day use beach. Analysis for esp was performed on seven samples (one swimmable, one sediment, and five shallow water samples). No samples tested positive for the esp gene. Results from the esp testing at Port Crescent State Park day use beach are given as present/absence in Appendix 7.

Samples were also assayed for the human and bovine Bacteroides marker through the use of PCR and qPCR. Six samples (one swimmable and five shallow) collected at Port Crescent State Park day use 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 Appendices 8 and 9, respectively.

The sample collected on July 29th, 2008 was 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 Port Crescent State Park day use beach.

4. CONCLUSIONS

Port Crescent State Park day use beach was very clean and there was no muck detected or sewage or bovine markers. However, the shallow waters still had evidence of 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). We have suggested that the coliphage represent more recent pollution or environments that allow for greater survival. In deeper waters, the *E. coli* was found at low levels 100% of the time, the enterococci and *Clostridium* were not found as frequently but interestingly the coliphage were found on occasion in the swimmable waters. It must be kept in mind that only 3 samples were processed. In the shallow waters, the *E. coli* was found 100% of the time but the other bacteria and phage were found only 14 to 27% of the time. The coliphage (CN-13Host) was the most stable of the two phages. The bacteria including the *Clostridium* were also found between 43 and 87% of the time in the sediment, and the coliphage was found 67% and 33% of the time in the sediments.

The sediments appear to be the major source of bacterial inputs at this beach.

The molecular source tracking results did not detect the presence of the enterococci surface protein gene in any of the Port Crescent State Park day use beach samples tested. The esp gene is an emerging tool for source tracking in surface waters but its reliability has been contested. Byappanahalli et al. (2008) suggest the esp gene is not a dependable indicator of human fecal inputs. Their research indicates that the esp gene is not unique to human feces but can also be detected in dog feces. In contrast, Ahmed (2008) reported that the esp gene was detected in 67% of human septic samples and in 100% of sewage samples but never detected in non-human source samples (i.e. cattle, avian, swine, etc.). The esp gene was not detected at Port Crescent State Park Day use beach in 2008 muck and shallow water samples. Further, development and research is required to determine the specificity and sensitivity of the esp gene to human fecal contamination and to develop a standard method.

Future research should focus on the hydrodynamics of the Pinnebog River and Lake Huron and the way these waterbodies may influence the water quality at Port Crescent State Park day use beach.

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

INDICATOR	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 concentration in the deep/ swimmable water samples collect at Port Crescent State Park day use beach (microorganism/100 ml)

DATE	*E. coli*	Enterococci	*C. perfringens*	Coliphage F+amp	Coliphage CN-13
7/10/2008	NT	12.5	2.00	<10.0	<10.0
7/15/2008	1.000*	<0.330	1.000	<10.0	<10.0
7/22/2008	112.400*	NT	NT	NT	NT
7/29/2008	17.930*	NT	NT	NT	NT
8/5/2008	10.090*	NT	NT	NT	NT
8/12/2008	5.560*	NT	NT	NT	NT
9/6/2008	NT	NT	NT	NT	NT
9/30/2008	25.700	<1.0	<0.22	60.0	90.0
Geometric	11.95	1.60	0.76	18.17	20.80

NT: Not tested (**E. coli* data collected by local health department)
<: Below method detection limits

Appendix 3: Fecal indicator concentration in the shallow water samples collect at Port Crescent State Park day use beach (microorganism/100 ml)

DATE	*E. coli*	Enterococci	*C. perfringens*	Coliphage F+amp	Coliphage CN-13
7/10/2008	NT	2.0	5.14	<10.0	<10.0
7/15/2008	4.38	<0.22	<0.25	20.0	<10.0
7/22/2008	225.30	NT	NT	<10.0	970.0
7/29/2008	NT	133.30	1.00	<10.0	<10.0
8/5/2008	26.80	8.40	1.70	<10.0	60.0
8/12/2008	4.10	1.50	1.70	<10.0	<10.0
9/6/2008	4.10	2.00	1.00	<10.0	<16.67
9/30/2008	12.00	1.00	0.87	<10.0	30.0
Geometric	13.22	2.84	1.18	11.62	27.10

NT: Not tested
<: Below method detection limits

Appendix 4: Fecal indicator concentration in the sediment water samples collect at Port Crescent State Park day use beach (microorganism/100 g wet weight)

DATE	*E. coli*	Enterococci	*C. perfringens*	Coliphage F+amp	Coliphage CN-13
7/15/2008	9.0	<9.0	NT	NT	NT
7/22/2008	7834	1928	NT	90	720
7/29/2008	<9.0	<9.0	3.0	<90	<90
8/5/2008	36.9	<9.0	2.7	180	<90
8/12/2008	18	18	<2.0	<90	<90
9/6/2008	9.0	9.0	6.0	150	<150
9/30/2008	9.0	<9.0	<2.0	90	450
Geometric	31.97	21.39	2.869	109.9	181.2

NT: Not tested
<: Below method detection limits

Appendix 5: Physical data collected from Port Crescent State Park day use beach on sampling dates.

DATE	Air temp. (°C)	Water temp. (°C)	Wind speed (MPH)	Wave height (feet)	24 hour precipitation (inches)	48 hour precipitation (Inches)	72 hour precipitation (inches)	Bather load	Algae on nearshore	Algae on beach	Bird count	Wind direction
7/10/2008	22.2	22.8	1.6	0.21	0	0	0.33	6	1	1	0	NNW
7/15/2008	23.3	20.9	5	1	0	0	0.02	0	0	0	0	SW
7/22/2008	21.2	21.1	1	1.5	0.02	0.58	0.76	0	1	1	4	NW
7/29/2008	22.5	22.5	3.1		0	0.28	0.47	0	0	1	16	SE
8/5/2008	24.9	24.2	4	0.5	0	0	0	0	0	0	5	NNW
8/12/2008	19.9	24.1	1.6	0.5	0	0.48	1.89	12	1	1	0	NNW
9/6/2008	16.5	21.4	1	0.5	0.17	0.88	0.88	0	1	1	5	variable
9/30/2008	15.4	16.9	1	1.5	0.07	0.34	0.34	0	0	1	5	WNW

Appendix 7: Analysis of esp testing from Port Crescent State Park day use beach

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

NT: Not tested for esp

Appendix 8: Human and bovine Bacteroides results as detected in the deep water at Port Crescent State Park day use beach.

DATE	Volume Assayed	Final Concentration Volume	Human Bacteroides	Bovine Bacteroides
7/10/2008	3500 ml	2 ml	-	-

Appendix 9: Human and bovine Bacteroides results as detected in the shallow water at Port Crescent State Park day use beach.

DATE	Volume Assayed	Final Concentration Volume	Human Bacteroides	Bovine Bacteroides
7/10/2008	2200 ml	2 ml	-	-
7/15/2008	3000 ml	2 ml	-	-
7/22/2008	1700 ml	2 ml	-	-
7/29/2008	2150 ml	2 ml	-	-
8/5/2008	2000 ml	2 ml	-	-