ABSTRACT

CLADOPHORA PROMOTES *ESCHERICHIA COLI* GROWTH AND CONTAMINATION OF RECREATIONAL WATERS IN LAKE MICHIGAN

By Amy L. Vanden Heuvel

In recent years, thick mats of the green alga, *Cladophora*, have washed onto beaches along Lake Michigan. These mats become stranded on the shoreline, begin to rot, and are malodorous. The rotting mats are a nuisance to recreational beach users and shoreline property owners. *Cladophora* mats have the potential to harbor *Escherichia coli*, which is used as an indicator of recent fecal contamination to determine beach closures. *Cladophora* mats may allow bacteria to survive and replicate by providing nutrients, elevated temperatures, and protection from UV light. Bacteria-laden mats potentially increase bacterial concentrations in surrounding recreational waters. To test this hypothesis, stranded sampled stranded *Cladophora* mats were sampled for *E. coli* at two beaches along Lake Michigan in Door County, Wisconsin. Random and gradient water samples were collected to determine *E. coli* concentrations in water underlying the mat and in surrounding water. Algal samples were collected and washed to determine *E. coli* concentrations attached to *Cladophora*. Since *E. coli* in recreational water should correlate with increased concentrations of pathogenic bacteria (also found in fecal material), pathogen concentrations in and attached to *Cladophora* mats were examined. Standard microbial culture methods were used to identify and enumerate pathogens such as *Salmonella*, *Shigella*, and *Campylobacter*.

Escherichia coli concentrations in water underlying mats were significantly greater than surrounding water ($p < 0.001$). Below mat *E. coli* increased as the stranded mats persisted at the beach swash zone. Water adjacent to *Cladophora* mats had lower *E. coli* concentrations but surpassed EPA swimming criteria the majority of sampling days. A significant positive association was found between *E. coli* concentrations attached to *Cladophora* and in underlying water ($p < 0.001$). The attached *E. coli* likely acted as a reservoir for populating water underlying the mat. Removal of *Cladophora* mats from beach areas may improve aesthetic and microbial water quality at affected beaches. Fecal bacterial pathogens, however, could not be detected by microbiological culture methods either attached to mat biomass or in underlying water. These associations call into question the efficacy of using *E. coli* as a recreational water quality indicator of fecal contaminations.

CLADOPHORA PROMOTES *ESCHERICHIA COLI* GROWTH AND CONTAMINATION OF RECREATIONAL WATERS IN LAKE MICHIGAN

by

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A Thesis Submitted
In Partial Fulfillment of the Requirements
For the Degree of

Master of Science-Biology

Microbiology

at

The University of Wisconsin Oshkosh
Oshkosh WI 54901-8621

January 2010

COMMITTEE APPROVAL

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1/7/2010
Date Approved

FORMAT APPROVAL

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12/21/2009
Date Approved

ACKNOWLEDGMENTS

First of all, I would like to thank my advisor, Dr. Gregory Kleinheinz, for encouraging me to apply to graduate school and for allowing me to work in his lab. Thank you for all your support, guidance, teaching, encouragement, and humor for the past three years. I would not be where I am today if it were not for you. I would also like to thank my committee member, Dr. Colleen McDermott, for always being there for me and answering my many questions! All of your help and support is greatly appreciated. I would like to acknowledge my committee member, Dr. Robert Pillsbury, for his guidance, support, and statistical wisdom that played a large part in my thesis. Thank you to Dr. Todd Sandrin for all your help in getting me started with the lab portion of my project. I also would not have made it this far if it were not for Reyneé Kachur and Sonja Jeter. Thank you both for all of your support, encouragement, PowerPoint wizardry, grape tomatoes, and the many laughs during lunchtime in your office. Thank you to Kim Busse for friendship, support, and all your help with field sampling. A special thanks to Rhonda Daily for all of her help with field sampling and lab work. Thank you for the many laughs and for helping me keep my sanity! I could not have done this project without you. Last, but not least, I would like to thank my husband, Todd, for his support, encouragement, and most of all, patience throughout my graduate school experience. Thank you for pushing me along to see this project come to completion. I could not have done this without you.

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INTRODUCTION

The occurrence of the nuisance alga, *Cladophora*, in the form of mats along shorelines is an increasing problem for beach managers and private homeowners on the Great Lakes. This green alga (Chlorophyta: Cladophorales) is filamentous and grows best on firm substrates such as rocks, pilings, piers, and other hard surfaces in shallow waters (12) but can break free and form large floating mats that eventually accumulate on beaches (Figure 1). Wind and wave action, along with other unknown factors, cause these mats to wash up and become stranded on the shoreline. Once stranded, these mats can begin to decompose on the beach. The mats are unsightly and malodorous, often smelling like raw sewage.

Cladophora mat communities usually consist of a variety of organisms, including bacteria, diatoms, cyanobacteria, protozoa, rotifers, and other multicellular organisms (7, 19, 25). Other filamentous algae, such as *Spirogyra* sp., may also be comprise the ”*Cladopora* mats”. *Cladophora* may provide a variety of requirements for growth and survival of bacteria, such as nutrients and protection from predation, dessication, and UV light (13, 19). *Cladophora* may also provide a substrate for bacteria to attach to. In open waters, *E. coli* is in a battle for survival against sunlight, predation, and low nutrient availability (Figure 2) (31). *Cladophora* mats may also have positive effects on the survival of *E. coli* by providing a microenvironment characterized by minimal fluctuations in temperature and pH compared to ambient water (Figure 3) (31).

Recently there has been a resurgence in the growth of *Cladophora* along Lake Michigan shorelines to pre-1970 levels. High concentrations of phosphorus in agricultural runoff and urban sewage input were the main cause of algal blooms before the 1970s (21) and before the 1972 Amendments to the Clean Water Act (11). The resurgence of *Cladophora* growth has been since the 1990s (11). *Cladophora* growth appears to be stimulated by the addition of nutrients, such as phosphorus and nitrogen (25). This eutrophication of water is associated with increases in *Cladophora* mat production and accumulation (12, 25); however, conclusive evidence has not shown that the eutrophication of lakes is the only cause for stimulated growth of *Cladophora*. Water transparency also has been suggested as an important causative factor (2, 3). Since the invasion of zebra mussels and quagga mussels (*Dreissena* sp.), water clarity has greatly increased in the Great Lakes. Zebra mussels filter feed on microscopic algae and phytoplankton. By improving the water clarity, the mussels have allowed light to penetrate deeper into the lakes. *Cladophora* and other algae that grow attached to hard substrates are now thriving due to increased light conditions. Not only do zebra mussels increase water clarity, they also provide a hard substrate for *Cladophora* to attach and grow on (11). It has also been hypothesized that lower lake levels in recent years, as occurred in the 1960s, have contributed to the resurgence of *Cladophora* growth. Lower water levels could allow light to penetrate more easily into the nearshore zones where the alga grows.

As mats age and ultimately decay, communities of associated organisms change. High densities of *Escherichia coli* and enterococci have been recovered in *Cladophora* accumulations along Lake Michigan shorelines from Illinois to Indiana and into southern Wisconsin (9, 30). High densities of these bacteria have been attributed to *in situ* growth under favorable conditions (5). An association between the presence of *Cladophora* mats on a beach and bacterial contamination of water has been hypothesized (9, 30). This study shows a direct link between these two occurrences (29).

Escherichia coli is used as an indicator of recent fecal contamination of water. *E. coli* is a bacterium that inhabits the gastrointestinal tract of humans and other animals (22). Along with *E. coli*, there are pathogenic microbes that can be found in feces. High concentrations of *E. coli* in water should indicate a recent fecal contamination event that may contain pathogenic bacteria (e.g., *Salmonella*, *Shigella*, *Campylobacter*), viruses, (e.g., Norovirus), and/or protozoans (e.g., *Cryptosporidium*, *Giardia*) (5, 16, 18). Pathogenic microbes often are difficult to detect in the laboratory; therefore *E. coli* is used as an indicator organism because its detection is easier. There are many possible sources of fecal contamination at recreational beaches, such as boaters, bathers, agricultural runoff, domestic animals, waterfowl, and other wildlife. If people are swimming in and using recreational waters that are contaminated with feces, they are at risk for gastrointestinal illness (8). According to guidelines set by the U.S. Environmental Protection Agency (EPA), water samples at a recreational beach should not exceed a single sample limit of 235 *E. coli*/100 mL of water (27). The Wisconsin Department of Natural Resources (DNR) uses a two-tiered water quality criterion for recreational waters. Samples with *E. coli* concentrations between 235 and 999 Most Probable Number (MPN)/100 mL water result in issuance of a poor water quality advisory, and samples with *E. coli* concentrations greater than 1000 MPN/100 mL result in a beach closure (32).

If *E. coli* should survive for long periods of time or replicate associated with *Cladophora*, use of *E. coli* as an indicator organism would be challenged. As a fecal indicator, the presence of *E. coli* in recreational waters suggests that pathogenic microbes (i.e., *Campylobacter*, *Salmonella*, and *Shigella*) associated with fecal contamination also may be present. In a study by Ishii *et al*. (2006) *Campylobacter*, *Salmonella*, and *Shigella* were recovered from *Cladophora* mats in lower Lake Michigan (13). Additionally, the pathogenic bacterium, *Vibrio*, also has been found to associate with algal accumulations (14). It has been hypothesized that the pathogenic bacterium, *Clostridium botulinum*, may be able to grow in a decaying or dense *Cladophora* mat under anaerobic conditions (6). Since *E. coli* survives (and perhaps replicates) in *Cladophora* mats, the same may hold true for pathogenic bacteria; therefore, an increase in *Cladophora* in the Great Lakes may be a potential public health risk.

Conversely, should *E. coli* preferentially survive and reproduce for long periods of time in the mats, but the pathogens do not, then high concentrations of *E. coli* found at bathing beaches as a result of efflux of the bacterium from *Cladophora* mats would challenge the paradigm that *E. coli* is a suitable indicator of recent fecal contamination (i.e., does not replicate in the environment). Likewise, these elevated *E. coli* concentrations in beach water would not indicate a significant risk of gastrointestinal illness for recreational water users. In this case, beaches may be closed inappropriately and, thus, cause an unnecessary negative impact on tourism. In a laboratory microcosm study by Engelbert *et al*. (2008), *E. coli* was able to remain attached to *Cladophora* for 45 days; however, *Salmonella* and *Shigella* remained attached to *Cladophora* for 10 days and 2 days, respectively (10).

Door County, Wisconsin, a peninsula located on northen Lake Michigan, has been plagued by *Cladophora* accumulations for several years. In a preliminary study conducted by Engelbert *et al*. (2008), *E. coli* concentrations were shown to be elevated in *Cladophora* mats at beaches located in the geographical vicinity of the study area for this project (9). At those beaches there was no positive statistical correlation between the presence of stranded *Cladophora* and beach closures. In that study, stranded *Cladophora* mats and the beach water monitoring sites were often distant from one another. It was hypothesized that *E. coli* was unable to persist in open beach water after it was washed from *Cladophora* mats by wind and wave action and/or diffuse any distance to the beach monitoring site. For this project, two beaches (Whitefish Dunes State Park and Lakeside Park) were chosen as study sites because they historically have had *Cladophora* accumulations that have presented a problem for beach managers. In addition, *Cladophora* mats at these beaches typically float in close to the beach monitoring sites and strand for several days before disappearing. *E. coli* concentrations in water at these beaches have been relatively low compared to concentrations at beaches on southern Lake Michigan, but occasional beach closures have occurred due to elevated bacterial concentrations.

Figure 1. A stranded *Cladophora* mat at Whitefish Dunes State Park beach in Door County, WI in 2007.

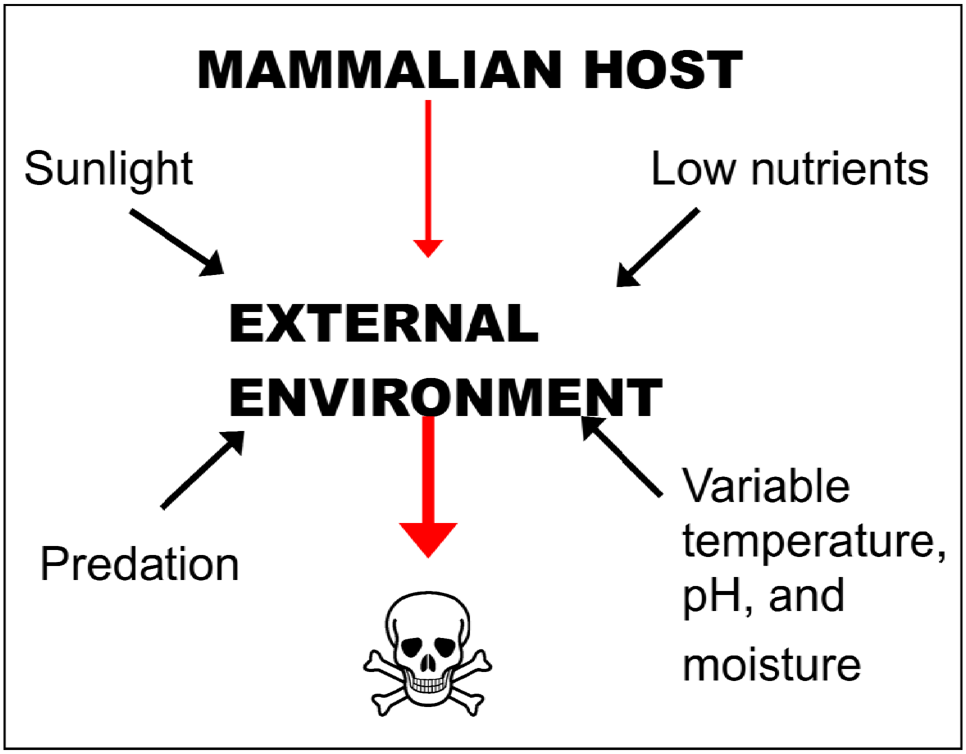

Figure 2. Once released from its mammalian host, *E. coli* is in a battle for survival in the external environment. (Adapted from Winfield and Groisman, 2003) (31).

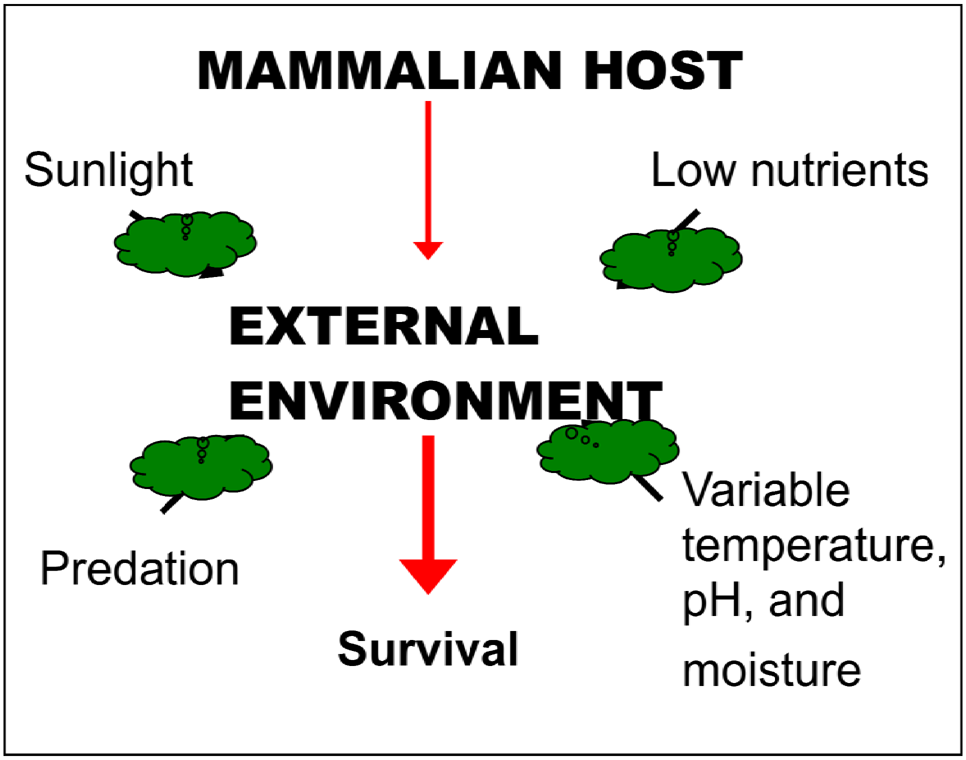

Figure 3. *Cladophora* may provide a variety of requirements for growth and survival of *E. coli* in the external environment. (Adapted from Winfield and Groisman, 2003) (31).

PROJECT OBJECTIVES

The overarching objective of this project was to determine if *Escherichia coli* concentrations associated with *Cladophora* mats influence *E. coli* concentrations in beach water adjacent to the mats. Specifically, the objectives of this project were:

1. Determine the spatial and temporal effects that *Cladophora* accumulations may have on the relative abundance of *E. coli* at bathing beaches.

2. Determine if the relative abundace of selected pathogens mimicked that of *E. coli* under the same conditions.

The underlying hypothesis for this project was that *Cladophora* mats may contribute to and allow for the persistence for *E. coli* in recreational beach water, but pathogens concentrations would not respond similarly.

MATERIALS AND METHODS

Field Site Selection

This project was conducted at two popular recreational beaches on Lake Michigan, located in Door County, WI, Whitefish Dunes State Park beach and Lakeside Park beach (Figure 4). These two locations both face east and are separated by a shoreline distance of 7.1 km. These beaches were chosen as study sites, because historically they have been subject to large accumulations of *Cladophora* mats during the recreational bathing season.

Cladophora Mat Selection and Sample Collection

The study sites were observed daily by student researchers for the ”arrival” of an algal mat. Within 24 hours of a mat becoming stranded on the beach, the mat was sampled for 3 consecutive days. Three consecutive sampling days are referred to as ”events”. Three separate sampling events occurred at both beaches during the summer months of 2007. At Whitefish Dunes State Park beach these three events occurred: July 16-18, July 31-August 2, and August 8-10, 2007. Three separate sampling events at Lakeside Park beach were July 16-18, August 1-3, and August 8-10, 2007. Samples collected included water underlying the *Cladophora* mat proper (within mats), outlying water on either side of the mat, and *Cladophora* biomass.

All sampling occurred before 10:00 a.m. in order to minimize insolation effects and disturbance by visitors. Upon arrival at the field site, the length of the mat proper was measured. Ten evenly spaced yellow flags were temporarily placed on the beach along the length of the mat to designate sampling locations. Ten evenly spaced red flags were temporarily placed on the beach to the right and left sides of the *Cladophora* mat to designate water sampling locations. The distance from the edge of the mat to the end of the red flags on either side varied from 3-10 m depending on the beach and its shoreline access.

Water samples were collected using 100-mL sterile bottles. Sampling within the mat was done by gently walking into the mat (approximately 3 m from shore); care was taken to prevent agitation of the mat. Bottles were capped with a 1 mm by 1 mm nylon screen to exclude *Cladophora* biomass. Samples were collected from water with a depth of approximately 45 cm and approximately 12 inches below the surface. *Cladophora* biomass was collected and placed into 7-oz sterile Whirl-pak bags (Nasco; Fort Atkinson, WI). All samples were analyzed within 2 to 6 hours of collection.

Random Sampling

Two different sampling protocols were employed to determine the impact that accumulated *Cladophora* mats have on *E. coli* concentrations in the surrounding beach water. Random and gradient sampling protocols were utilized. A random sampling strategy was used to determine the difference in *E. coli* concentrations in beach water collected within mats versus water collected on either side of the mats, specifically looking at mat water versus left water and mat water versus right water. This sampling strategy also was used to establish the effect of length of time mats are stranded on a beach on *E. coli* concentrations in beach water. Ten water samples were collected randomly from water on either side of the mat (1-10 m from the water-mat edge). A total of 30 random water samples were collected per beach per day of each sampling event (Figure 5B).

Gradient Sampling

The gradient sampling strategy was employed to determine if an *E. coli* concentration gradient existed in beach water within or on either side of the mat. A transect parallel to the shoreline, in water at a depth of 45 cm, was established across a mat and extending out to the right and left of the mat edge. Water and *Cladophora* biomass sampling took place at 10 evenly spaced locations across the transect (Figure 5A). Distance between the spaced samples within the mat depended on the length of the *Cladophora* mat (range = 1-18 m between samples). Distance between spaced samples on either side of the mat depended on shoreline access (range = 1.5-3 ft between samples). During the 2007 summer sampling season, one particularly large mat at Whitefish Dunes State Park beach (July 16-18) had more than 10 evenly spaced sample locations (17) designated. And for one mat at Lakeside Park beach (July 16-18) there were 12 designated sampling locations.

Sample Analysis of *Escherichia coli* Concentrations from Water

Escherichia coli concentrations from water were measured using defined substrate technology, Colilert® with Quantitray 2000 format (IDEXX Corp., Portland, ME) (1, 17). The Colilert defined substrate test is used to detect total coliforms and *E. coli* with the same test. The Colilert reagent contains 0-nitrophenyl bound to β-D-galactopyranoside (ONPG) as well as 4-methyl-umbelliferyl bound to β-D-glurcuronide (MUG). All coliform bacteria can metabolize ONPG by the enzyme β-galactosidase, thereby cleaving o-nitrophenyl to produce a yellow color. *E. coli* utilizes the enzyme β-glucuronidase to metabolize MUG which cleaves 4-methyl-umbelliferyl from β-D-glurcuronide producing fluorescence under UV light.

Incubation and *E. coli* enumeration were conducted following manufacturer's recommendations. Analyses were conducted at the University of Wisconsin-Oshkosh, Sturgeon Bay Laboratory. This facility, which is State-certified for analysis of fecal bacteria, is located within 15 min of the sampling locations. In order to determine the most probable number of *E. coli* per 100 mL of water without exceeding the test maximum, samples collected within the mat were processed using a 1:10 or 1:100 dilution based on the previous day's results. All results were reported as most probable number (MPN) of *E. coli* per 100 mL of water.

Laboratory Analysis of *Escherichia coli* Attached to *Cladophora*

For *Cladophora* samples, an initial bacterial elutriation step was necessary. One-gram subsamples of *Cladophora* biomass were placed in 15-mL centrifuge tubes, to which 9 mL of sterile phosphate-buffered water (PBW) diluent, pH 6.8, was added. The alga-PBW mixture was shaken vigorously for 2 min. The samples were then centrifuged for 10 min at 635 x *g* to allow large particles to settle to the bottom of the centrifuge tubes (30). Previous studies have shown that this elutriation method achieves an average rate of 55% bacterial recovery rate (30). The supernatant was decanted into another labeled sterile 15-mL centrifuge tube, and the *Cladophora* pellet was discarded. The centrifuge tube containing the supernatant was shaken vigorously for 20 sec to uniformly distribute the bacteria. The supernatant was further diluted (1/10, 1/30, 1/100, and 1/300) in PBW (7), and *E. coli* was enumerated utilizing traditional membrane filtration techniques (28). Filters were placed onto modified thermotolerant-*E. coli* agar medium (modified mTEC) (Becton Dickinson; Chicago, IL). Plates were first incubated at 35 ºC for 2 hours and then incubated at 44.5 ºC for 22 h (28). After incubation, the number of purple colonies on the membrane filters was recorded. Bacterial concentrations in algae were expressed as colony forming units per gram (dry weight) of algae (CFU/g). Approximately 1% of the presumptive colonies for *E. coli* were confirmed using the API 20E rapid identification system (bioMérieux; Marcy l'Etoile, France).

Laboratory Analysis for Presence of *Salmonella, Shigella,* and *Campylobacter* in Water

Water collected within the *Cladophora* mat was analyzed for the presence of the following pathogens: *Salmonella*, *Shigella*, and *Campylobacter*. These pathogenic bacteria were identifed and enumerated using standard microbiological methods.

Salmonella and *Shigella* were identified by using a modified membrane filtration technique (1, 33). Ten mL, 100 mL, and 1-L aliquots of water were filtered through sterile diatomaceous earth (DE). The DE plug was suspended in 50 mL Selenite broth

(Difco; Sparks, MD), and incubated at 42 ºC for 24 h. Ten µL of broth from each water aliqout were inoculated on xylose lysine desoxycholate medium (XLD) (Difco; Sparks, MD) and incubated at 35 ºC for 24 h. Red colonies with black centers (*Salmonella*) and red colonies without black centers (*Shigella*) were confirmed with API 20E (bioMérieux; Marcy l'Etoile, France) biochemical analysis. A most probable number (MPN) technique was used to estimate the number of organisms in the water samples (Table 1).

For *Campylobacter*, approximately 3 L water samples were filtered through 0.4-µm filters (Gelman Scientific; Ann Arbor, MI) and filters were placed on 7% Horse Blood Agar plates. Plates were incubated for up to 48 h in a microaerobic environment (Gas Pak without catalyst) (Oxoid; Hants, UK) at 42 ºC. *Campylobacter* colonies were identified by gram stain, motility, biochemistry, and agglutination with specific antibodies (Oxoid; Hants, UK) (1).

Laboratory Analysis of Presence of *Salmonella, Shigella,* and *Campylobacter* Attached to *Cladophora*

Four-inch diameter samples of *Cladophora* were manually collected from floating mats and placed into sterile Whirl-pak bags (Nasco; Fort Atkinson, WI). Five gram algal subsamples were placed into 50-mL centrifuge tubes to which sterile phosphate buffered water was added to equal 50 mL total volume. Samples were shaken vigorously for 2 min to remove bacteria from the algal mat and briefly centrifuged to remove debris (5). Supernatants were filtered onto membrane filters as described above for *Campylobacter*, *Salmonella*, and *Shigella* isolation. Identification of pathogens occurred as described for water samples. Counts are expressed as CFU/g dry weight.

Physical Parameters Measured Within and Outside *Cladophora* Mats

The following physical parameters were measured within and outside *Cladophora* mats: pH, conductivity, temperature (ºC), total dissolved solids (TDS), oxidation-reduction potential (ORP), and dissolved oxygen content (DO). These measurements were taken to see if environmental factors within the mat environment or in adjacent waters could be correlated with *E. coli* survival.

Statistical Analysis

Statistical analysis was performed using SYSTAT 11.0. *E. coli* concentrations were log transformed to conform to normal distribution. Analysis of Variance (ANOVA) was used to determine differences between samples, within and between transects. The null hypothesis was that there was no difference in the mean *E. coli* concentrations within and outside the *Cladophora* mats, and the alternate hypothesis was that there was a difference in these means. Alpha was set at 0.05 (34).

To determine if the concentration of *E. coli* was related to the location of the sample within the algae mat, a new independent variable, called ”position”, was created; whereby, the sampling position within the mat was assigned to three groups (1 = samples 8-10 [right of center], 2 = samples 4-7 [center of mat], and 3 = samples 1-3 [left of center]) (Figure 5A). To achieve equality with regard to gradient sample location and to offset unequal transect numbers, a standardized scale was created. A linear regression was then conducted using the new mat location scale as the independent variable and the log (CFU *E. coli*) and log (MPN *E. coli*) analyzed separately as the dependent variables. To determine if differences existed in the means of *E. coli* concentrations taken on different days of a *Cladophora* mat event, a two-way ANOVA was run using Beach (Whitefish Dunes or Lakeside Park) and Day (1^{st}, 2^{nd}, or 3^{rd}) of each sampling event (algal mat moving into shore) as independent factors, followed by a Tukey post-hoc test. The log (CFU *E. coli*) and log (MPN *E. coli*) were analyzed separately as dependent variables.

Laboratory Analysis for *Escherichia coli* Genetic Relatedness

Individual purple colonies (*E. coli*) were removed from modified mTEC plates using sterile inoculating loops. Care was taken to not touch any other colonies on the plate. No more than 6 isolates per *Cladophora* sample were collected. Each individual colony was streaked onto a Brain Heart Infusion agar slant (BHI) (Difo; Sparks, MD). The slants were incubated for 24 h at 35 ºC. Cultures were transferred from the slants into 9 mL of sterile BHI broth. The broth tubes were incubated for 24 h at 35 ºC. After incubation, 0.8 mL of the resulting culture was transferred to sterile 1.5-mL microcentrifuge tubes containing 0.3 mL sterile glycerol. Microcentrifuge tubes were vortexed and stored at -80 ºC. Also, from the BHI broth culture, 200 µL were transferred to a designated well in a sterile 96-well polystyrene microtiter plate (Nunc) (Nunc A/S; Roskilde, Denmark). Once a 6 X 8 block in the microtiter plate was filled, a 48-pin stamper (MULTI-BLOT™ REPLICATORS) (V & P Scientific; San Diego, CA), was placed in the wells of the microtiter plate and used to transfer 48 isolates onto a single petri plate containing Plate Count Agar (PCA) (Difco; Sparks, MD). The plate was marked to designate the location of the first isolate. The PCA plates were incubated for 24 h at 35 ºC. The 48-pin stamper was cleaned before transferring the second half of the 96 isolates in the microtiter plate. The pins were soaked in a 10% bleach solution for no longer than 30 min, rinsed twice with sterile water, and then disinfected with 95% ethanol. The PCA plates, along with a map of isolate location on the plates, were shipped on ice to the University of Minnesota to determine clonality or genetic diversity of *E. coli* within the *Cladophora* mats. The isolates were analyzed by a technique known as the horizontal, fluorophone-enhanced, repetitive extragenic palindromic-PCR (rep-PCR) DNA fingerprinting technique (HFERP) (15). This technique was performed with 1,492 isolates from the summer of 2007 sampling season.

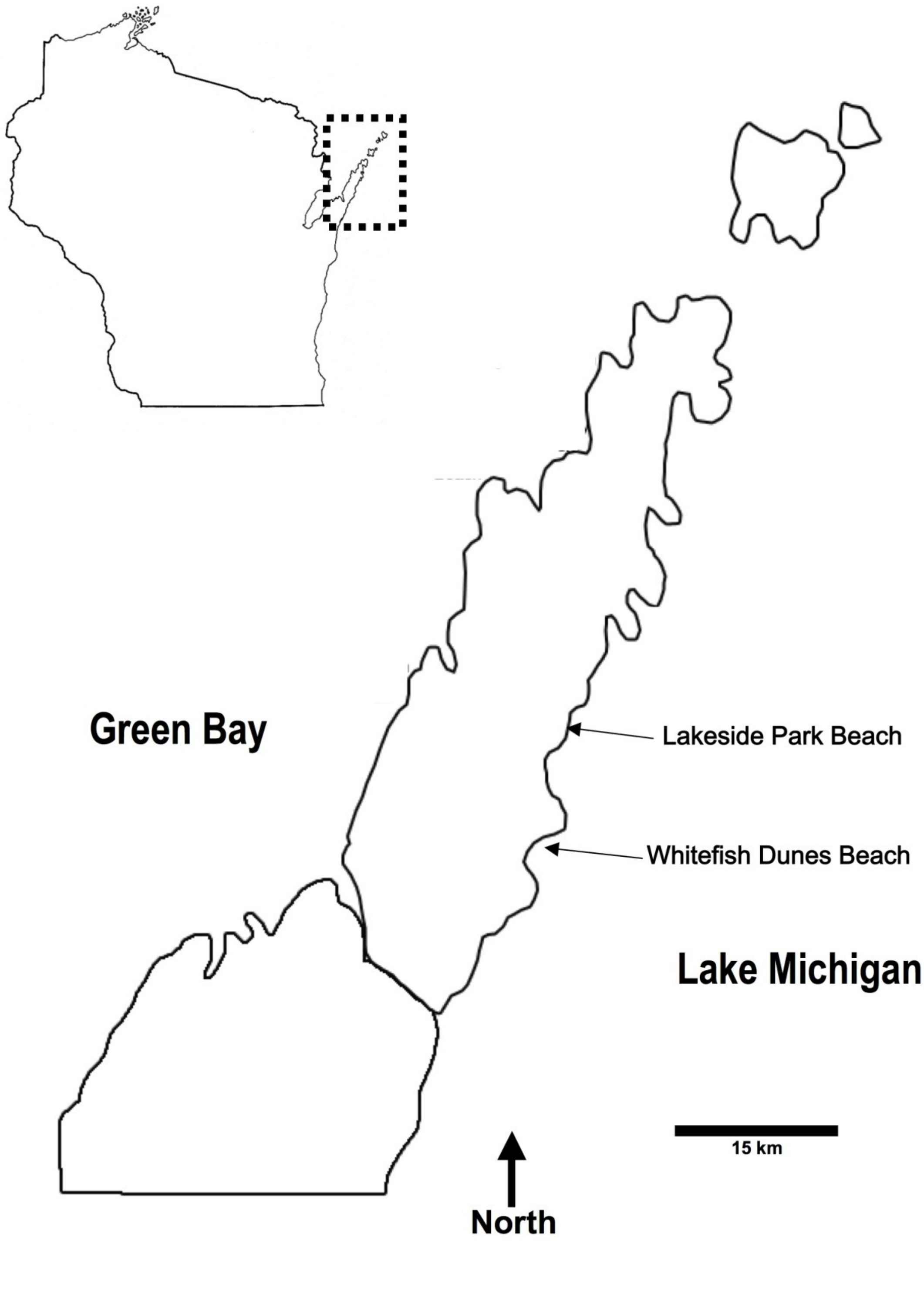

Figure 4. Map of Door County in relation to the State of Wisconsin and the location of the two study sites.

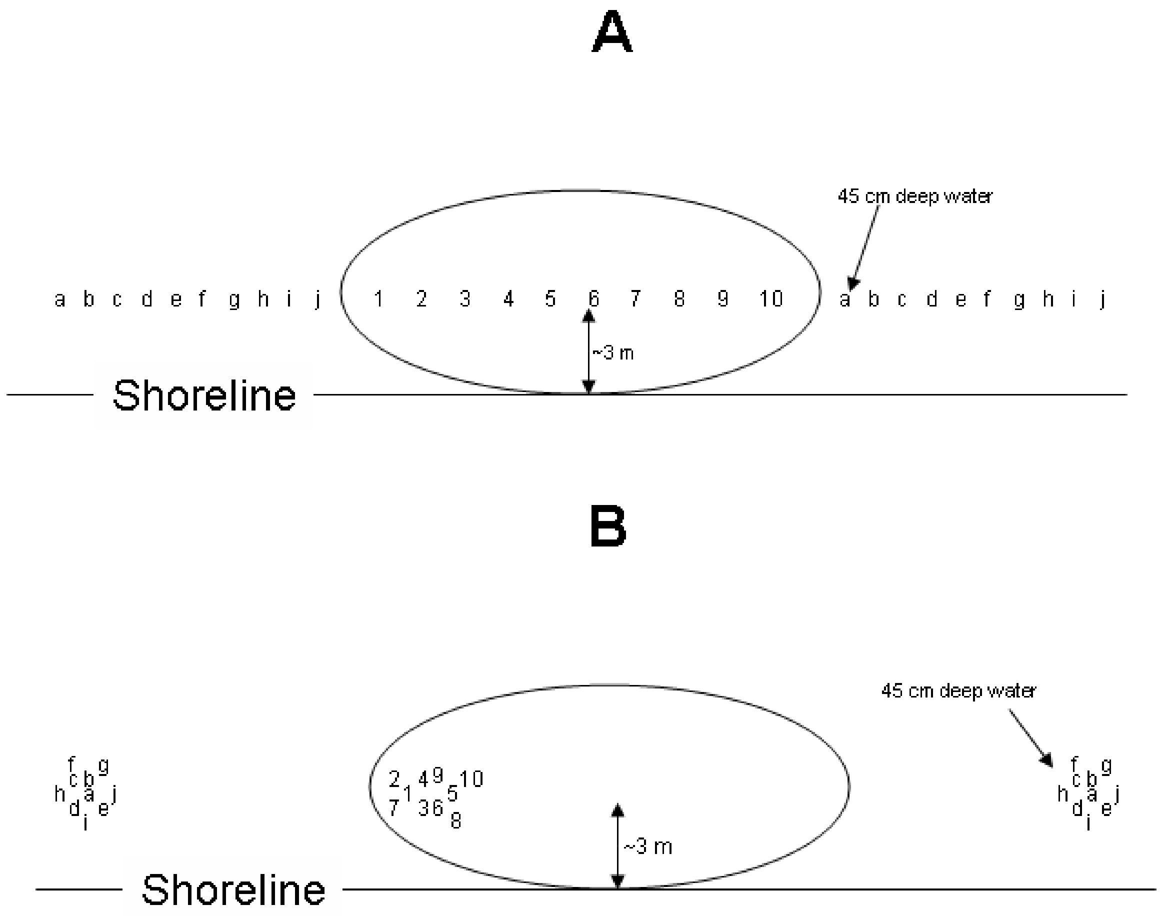

Figure 5. Sampling protocol for staked samples (A) and random water samples from within and outside the *Cladophora* mat (B). Oval = *Cladophora* mat, numbers and letters represent sample site locations (approximate).

Table 1. MPN technique used to estimate the number of *Salmonella* or *Shigella* in water samples.

+ Colonies from:	**MPN/100 ml water**
10 ml, 100ml, +1L	10
100ml, +1L	1.0
1L	0.1
All negative	<0.1

RESULTS

Escherichia coli in Water

Random Sampling

Escherichia coli concentrations from the random water sampling within the mat were significantly higher than concentrations of the bacterium found outside the mat ($p < 0.001$) (Figures 6-11). For Whitefish Dunes, mean *E. coli* concentrations (log MPN/100 mL) for water samples collected within the *Cladophora* mats for the three sampling events were 10 to 100 times greater than the mean *E. coli* concentrations detected in outlying water. The *E. coli* concentrations on either side of the mats also were significantly different from one another ($p < 0.001$), with concentrations from water samples to the left (north) of the mat generally greater than those to the right (Figures 6, 7, and 8). The prevailing longshore currents at this beach move north and may transport *E. coli* and other particulates from the mat into the surrounding water. The shoreline on the left (north) at this sampling location is rocky. *Cladophora* or other algae appear attached on the rocks. Any *E. coli* attached to *Cladophora* there could be released into the left water sampling area.

For Lakeside Park, mean *E. coli* concentrations (log MPN/100 mL) for water samples collected within the *Cladophora* mats for the three sampling events also were significantly higher than surrounding water ($p < 0.001$) (Figures 9, 10, and 11). *Escherichia coli* concentrations in randomly collected water samples to the left and right of the mats were not significantly different from one another ($p = 0.401$). There is little longshore current at this beach and *E.coli* may move by simple diffusion out of the mat proper.

These random samples not only establish that *E. coli* concentrations in water are greatest within *Cladophora* mats but also allow for day to day comparisons of *E. coli* concentrations. For both beaches (Figures 6, 8, 9, and 11) two of three sampling events (Events 1 and 3) showed an increase in *E. coli* concentrations in underlying water for consecutive days of the event (Day 1 < Day 2 < Day 3). Event 2 at both beaches (July 31-August 2, 2007 for Whitefish Dunes and August 1-3 for Lakeside Park) did not fit the same pattern as Events 1 and 3 (Figures 7 and 10). For Event 2, *E. coli* concentrations decreased over consecutive days of the event at Whitefish Dunes and showed no particular pattern at Lakeside Park.

At Whitefish Dunes State Park beach *E. coli* concentrations increased from 4.0 $\log_{10}$ on Day 1, to 4.3 $\log_{10}$ on Day 2, to 4.4 $\log_{10}$ on Day 3. A two-way ANOVA was significant for Beach ($p < 0.001$), and Day ($p = 0.001$), with no significant interaction between these two factors. A one-way ANOVA showed that *E. coli* concentrations in water over the three sampling days at Whitefish Dunes State Park beach were significantly different ($p = 0.036$), with Day 3 being significantly higher than Day 1. Similar results were seen for Lakeside Park with *E. coli* concentrations ranging from 3.6 $\log_{10}$ on Day 1 and Day 2 and increasing to 4.0 $\log_{10}$ on Day 3. Concentrations from Day 3 were significantly greater than both Day 1 and 2 ($p = 0.003$).

Gradient Sampling

Results of the random sampling established that *E. coli* concentrations in water were greatest within mats, and generally increased over time but could not help determine if a spatial gradient of *E. coli* concentrations existed within the mat and radiated outward. To answer this question water samples also were collected from evenly spaced positions within and outside of the *Cladophora* mat for each of the three (3 day) sampling events (Figure 5A).

For Whitefish Dunes, mean *E. coli* concentrations (log MPN/100 mL) from evenly spaced water samples collected within the *Cladophora* mats for the three sampling events were significantly greater than surrounding water ($p < 0.001$). The *E. coli* concentrations on either side of the mats also were significantly different from one another at $p < 0.001$, as was seen with the randomly collected water samples (Figures 12-14).

For Lakeside Park, mean *E. coli* concentrations (log MPN/100 mL) from evenly spaced water samples collected within the *Cladophora* mats for the three sampling events were significantly greater than in surrounding water ($p < 0.001$). In contrast to Whitefish Dunes, the *E. coli* concentrations in water collected to the left and right side of the mats at Lakeside Park were not significantly different from one another ($p = 0.151$) (Figures 15-17).

When *E. coli* concentrations in water from the edges of the *Cladophora* mat were compared with *E. coli* in water from the center of the mat, there was no consistent pattern in *E. coli* concentrations ($p = 0.447$, $r^2 = 0.003$). A significant ($p = 0.013$, $r^2 = 0.036$, $N =$

171) positive relation was found, however, using log (MPN *E. coli*) as the dependent variable and mat location. This suggests that there is more *E. coli* in water near the center of the *Cladophora* mat compared to the edges (Figures 12-17).

Escherichia coli Attached to *Cladophora* Mats

Stranded mats were comprised primarily of *Cladophora glomerata* at Whitefish Dunes State Park beach. *Spirogyra* sp. were a major component of the algal mats at Lakeside Park beach. *E. coli* concentrations exceeded 5.40 $\log_{10}$ *E. coli*/g dry weight of mat material during two sampling events (Events 2 and 3) at Whitefish Dunes State Park when *E. coli* was detached from mat biomass (Figure 18), and concentrations reached 5.42 $\log_{10}$ CFU/g dry weight during the first sampling event. This was the highest concentration of *E. coli* found in all samples. The second sampling event contained the highest average concentrations of *E. coli* with a mean of 5.03 $\log_{10}$ CFU/g dry weight. Lakeside Park demonstrated a similar relationship, with the second event having the highest mean concentration of *E. coli* at 4.04 $\log_{10}$ CFU/g dry weight (Figure 19). While the trends in *E. coli* concentrations were similar from event to event at both locations, the concentrations of *E. coli* were much lower in Lakeside Park mats. The highest concentration of *E. coli* was found to be 4.19 $\log_{10}$ CFU/g dry weight during the third event. At Lakeside Park only two mean *E. coli* concentrations from sampling event days averaged over 4.30 $\log_{10}$ CFU/g dry weight. In contrast, Whitefish Dunes State Park had 19 sampling event days where *E. coli* concentrations exceeded 4.70 $\log_{10}$ CFU/g dry weight.

Association between *Escherichia coli* in Water and *Cladophora*

For both beaches combined, there was a significantly positive association between the concentration of *E. coli* attached to *Cladophora* and *E. coli* concentrations from water samples collected within the mats ($p < 0.001$, $r^2 = 0.42$) (Figure 20). A similar pattern was revealed when the study beaches were analyzed separately. For Whitefish Dunes State Park beach there remained a significant relationship between the *E. coli* measurements (log (CFU) vs. log (MPN)) ($N = 76$, $r^2 = 0.365$, $p < 0.001$). Likewise, for Lakeside Park beach there was also a significant relationship between the *E. coli* measured attached to *Cladophora* and in free water underlying the mat ($N = 82$, $r^2 = 0.438$, $p < 0.001$).

Fecal Pathogens Associated with *Cladophora* Mats

Despite high *E. coli* concentrations in water and the algal mats at all sampling events, no *Salmonella*, *Shigella*, or *Campylobacter* were detected in these samples.

Physical Parameters Within and Outside of *Cladophora* Mats

The measurements of the physical parameters taken within the *Cladophora* mats were significantly different than the readings taken in water to the left and right of the mat for most parameters measured. At Whitefish Dunes State Park beach, the pH within mats was significantly lower compared to surrounding water ($p = 0.000$). Conductivity within mats was significantly greater compared to right and left water ($p = 0.025$) and ($p = 0.026$), respectively. Total dissolved solids within mats was significantly greater compared to right water (*p* = 0.014) and left water (*p* = 0.019). Oxidation-reduction potential was significantly lower within mats compared to right water (*p* = 0.000) and left water (*p* = 0.001). The dissolved oxygen content within mats was significantly lower compared to surrounding water (*p* = 0.000). The temperature within mats at Whitefish Dunes was significantly higher compared to right water (*p* = 0.001) and left water (*p* = 0.002). For Lakeside Park beach, pH within mats was significantly lower compared to surrounding water (*p* = 0.000). Conductitivity within mats was significantly greater within mats compared to right water (*p* = 0.003) and left water (*p* = 0.002). Total dissolved solids was significantly greater within mats compared to surrounding water (*p* = 0.000). Oxidation-reduction potential was significantly lower within mats compared to surrounding water (*p* = 0.000). Dissolved oxygen within mats was significantly lower compared to surrounding water (*p* = 0.000). Temperature within mats at Lakeside Park was significantly greater compared to right water (*p* = 0.014) and left water (*p* = 0.049). In general, the temperature (ºC), total dissolved solids (TDS), and conductivity (µS/cm) measurements were greater within the algal mats than for adjacent waters. The dissolved oxygen (DO), pH, and oxidation-reduction potential (ORP) measurements were generally lower within the *Cladophora* mats compared to right and left side waters (see Appendix A).

Genetic Relatedness of *Escherichia coli*

The HFERP data from the 1,492 *E. coli* isolates attached to *Cladophora* were analyzed using Multivariate Analysis of Variance (MANOVA). For Whitefish Dunes

State Park beach, *E. coli* populations change greatly between events, but *E. coli* genotypes are conserved (Figure B-1). For all three events at Whitefish Dunes large changes occured in the mat populations of *E. coli* over the three days, but some populations appear to be conserved from one day to the next (Figures B-2, B-3 & B-4). This suggests that *E. coli* can survive for at least 2 to 3 days within the algal mat. For Whitefish Dunes State Park beach there were 60 isolates that had 93.92% similarity found all three days of Event 1. All these isolates were not clones, but they are very similar (genetically related) (Figure B-5). These isolates are likely from a large point source. Similar results were found for *E. coli* populations at Lakeside Park beach (Figures B-6, B-7, B-8, and B-9). This suggests that *E. coli* populations within *Cladophora* mat environments must be very dynamic.

For both beaches combined, there were diverse populations of *E. coli*. There appears to be a lot of crossover in the population similarity between beaches, as well as uniqueness at each beach when looking at individual sampling dates (Figure B-10). Even though mats (events) at each beach are totally different and in separate locations, some of the population appears to be very similar (Figure B-11). This suggests that the input of *E. coli* within the mats at both beaches could be from the same source given the proximity of the beaches to one another.

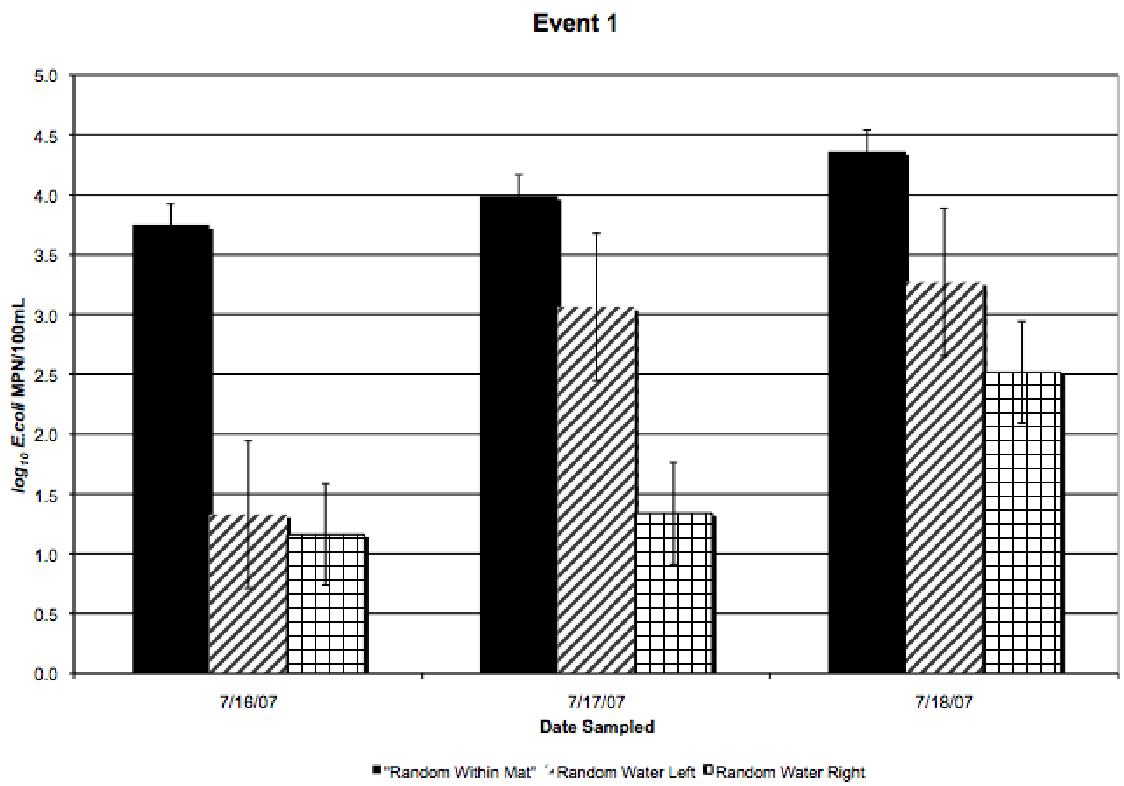

Figure 6. Log *E. coli* concentrations from ten randomly collected water samples from within and surrounding *Cladophora* mats during Event 1 at Whitefish Dunes State Park beach. Bars are means of ten random samples +/- standard error.

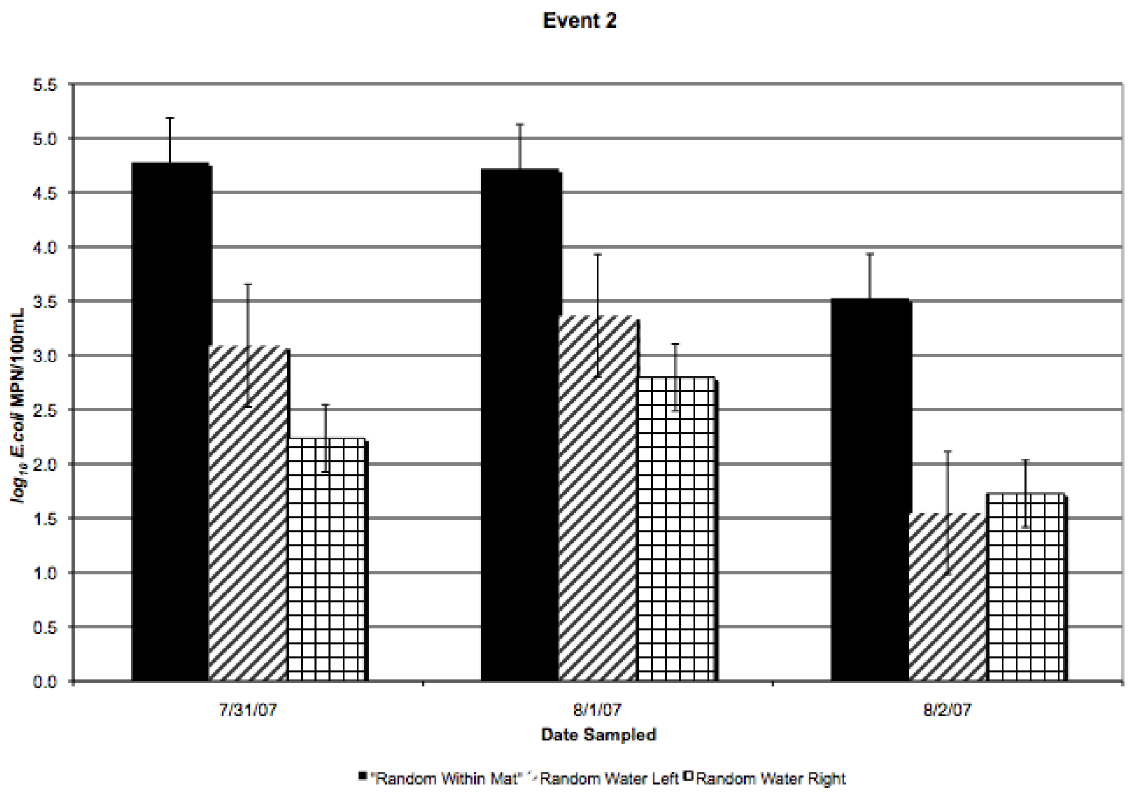

Figure 7. Log *E. coli* concentrations from ten randomly collected water samples within and surrounding *Cladophora* mats during Event 2 at Whitefish Dunes State Park beach. Bars are means of ten random samples +/- standard error.

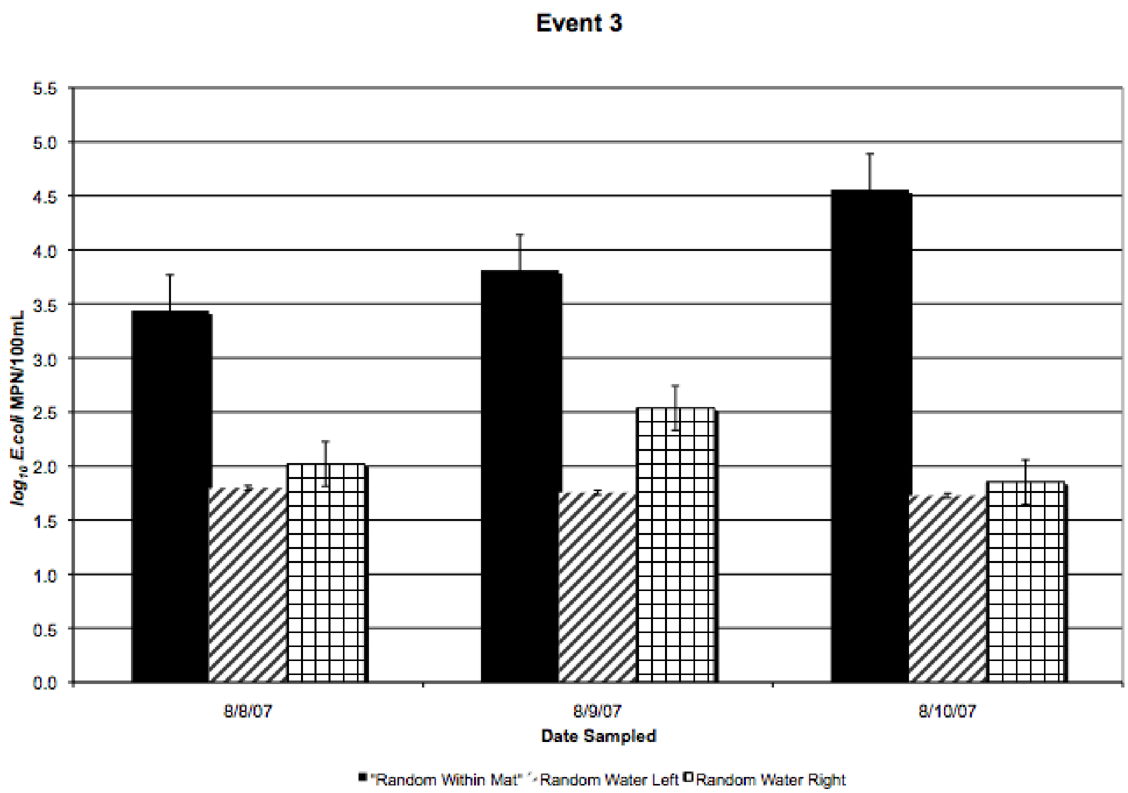

Figure 8. Log *E. coli* concentrations from ten randomly collected water samples from within and surrounding *Cladophora* mats during Event 3 at Whitefish Dunes State Park beach. Bars are means of ten random samples +/- standard error.

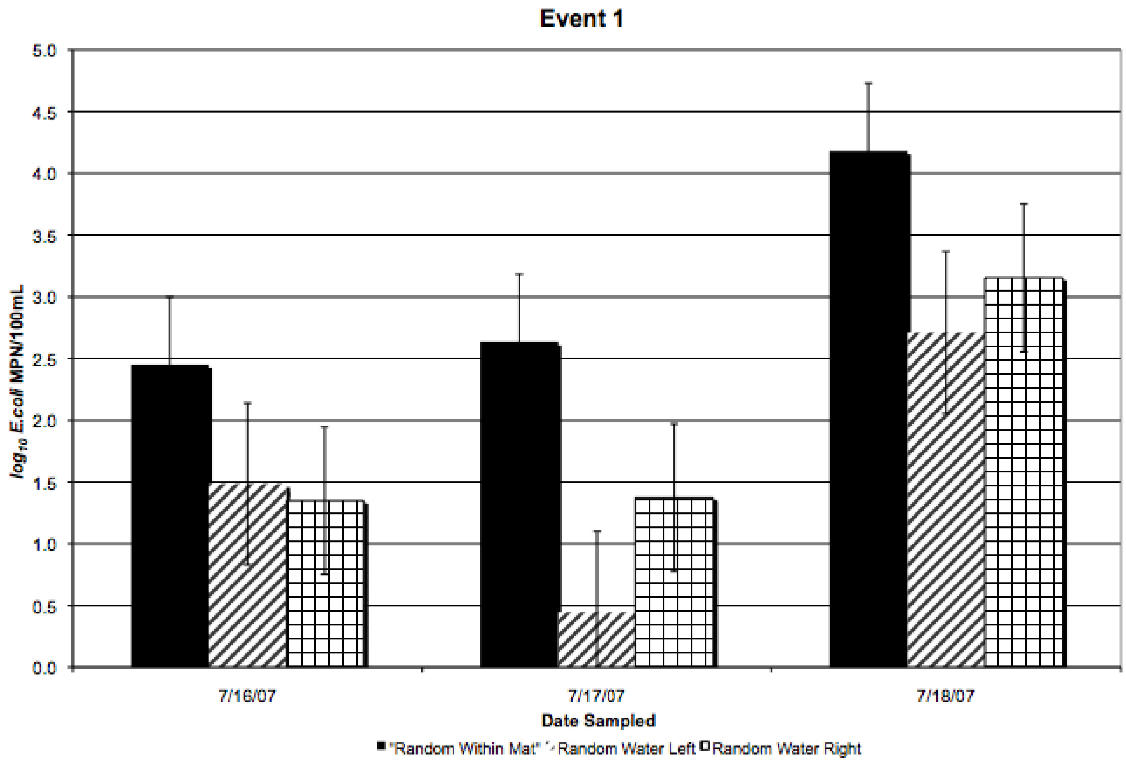

Figure 9. Log *E. coli* concentrations from ten randomly collected water samples from within and surrounding *Cladophora* mats during Event 1 at Lakeside Park beach. Bars are means of ten random samples +/- standard errror.

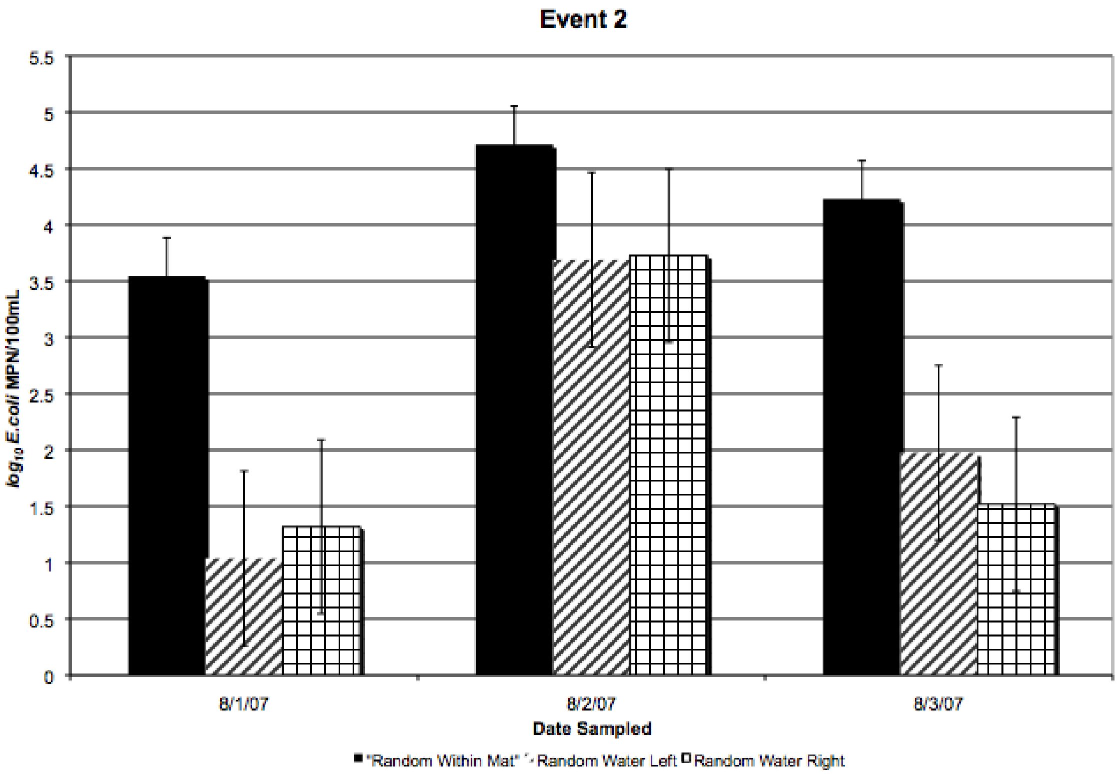

Figure 10. Log *E. coli* concentrations from ten randomly collected water samples from within and surrounding *Cladophora* mats during Event 2 at Lakeside Park beach. Bars are means of ten random samples +/- standard error.

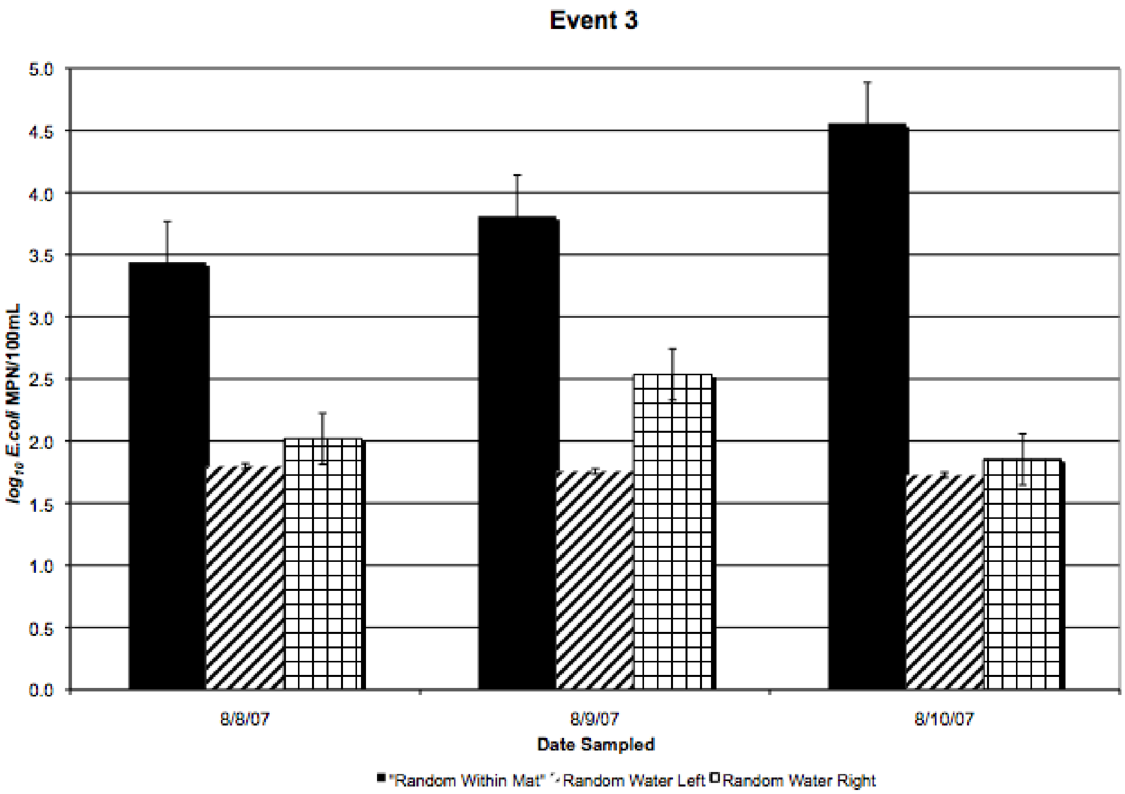

Figure 11. Log *E. coli* concentrations from ten randomly collected water samples from within and surrounding *Cladophora* mats during Event 3 at Lakeside Park beach. Bars are means of ten random samples +/- standard error.

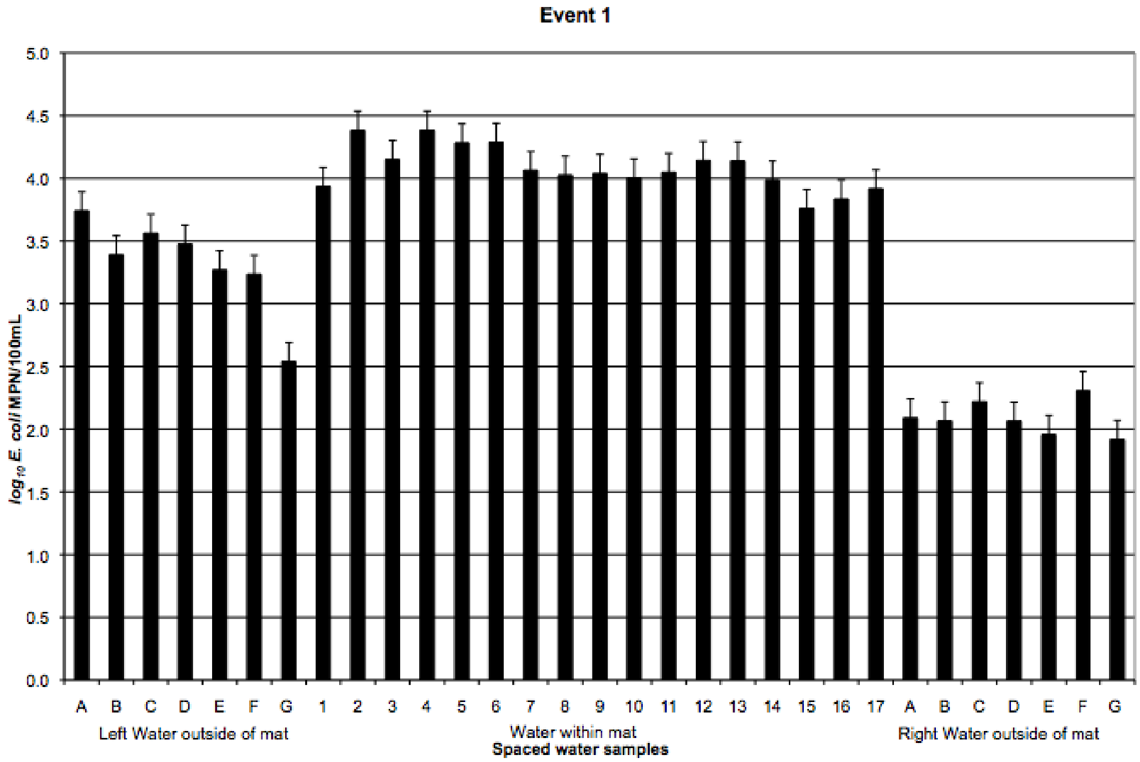

Figure 12. Log *E. coli* concentrations from 17 evenly spaced water samples from within the mat and ten evenly spaced water samples surrounding *Cladophora* mats during Event 1 at Whitefish Dunes State Park beach. Bars are means of three consecutive sampling days +/- standard error.

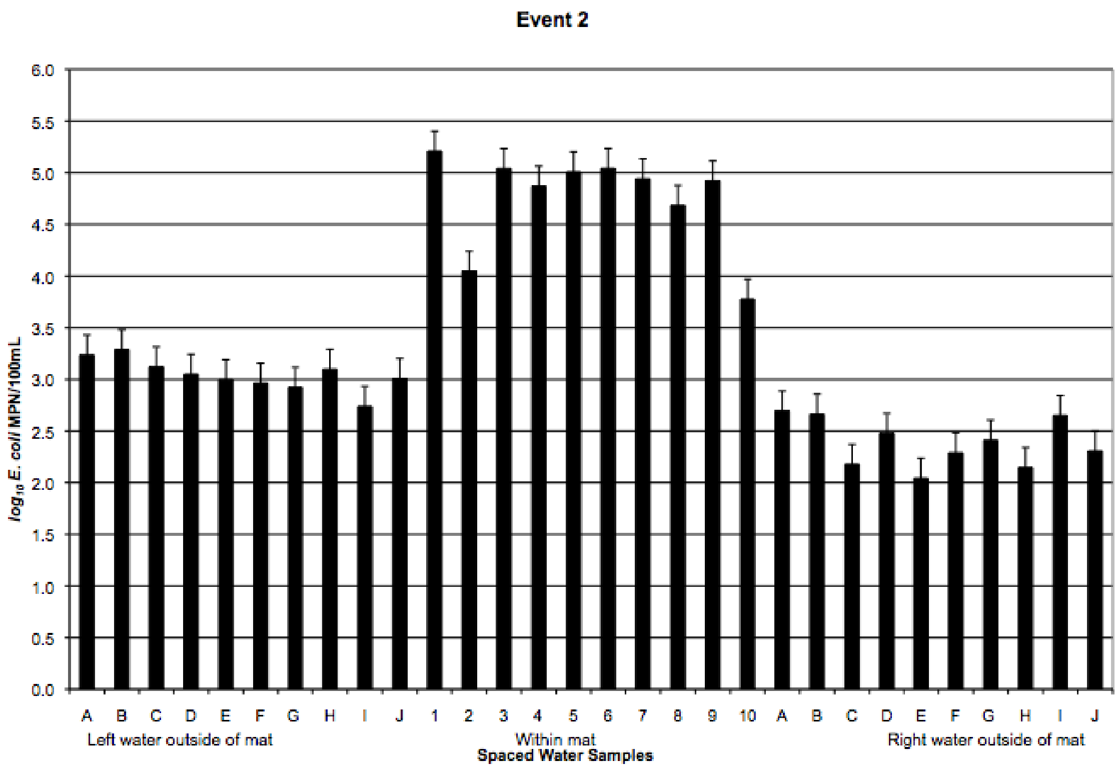

Figure 13. Log *E. coli* concentrations from ten evenly spaced water samples from within and surrounding *Cladophora* mats during Event 2 at Whitefish Dunes State Park beach. Bars are means of three consecutive sampling days +/- standard error.

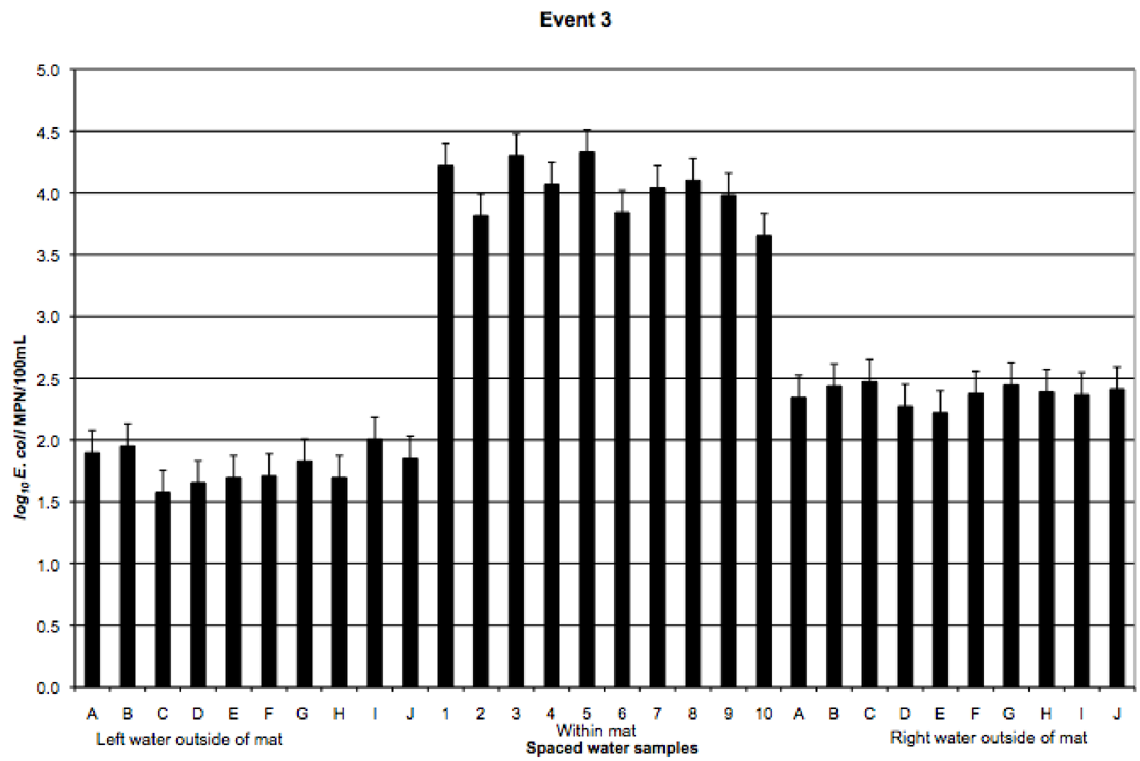

Figure 14. Log *E. coli* concentrations from ten evenly spaced water samples from within and surrounding *Cladophora* mats during Event 3 at Whitefish Dunes State Park beach. Bars are means of three consecutive sampling days +/- standard error.

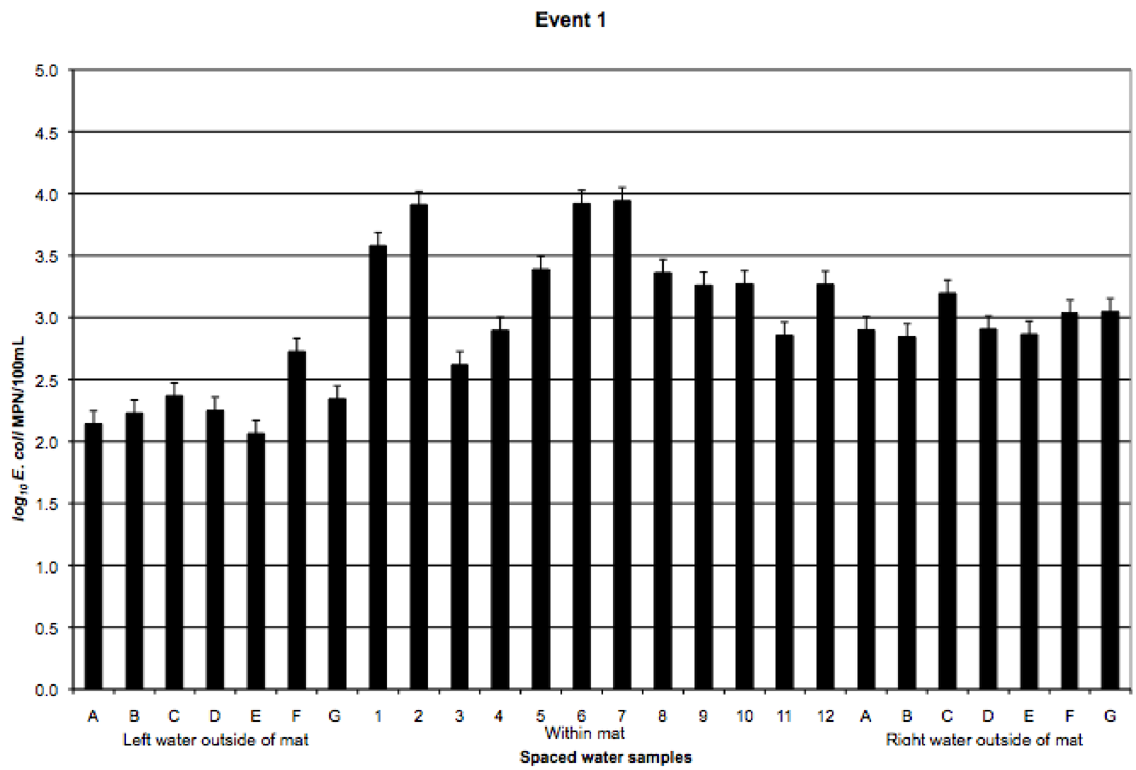

Figure 15. Log *E. coli* concentrations from 12 evenly spaced water samples from within the mat and ten evenly spaced water samples surrounding *Cladophora* mats during Event 1 at Lakeside Park beach. Bars are means of three consecutive sampling days +/- standard error.

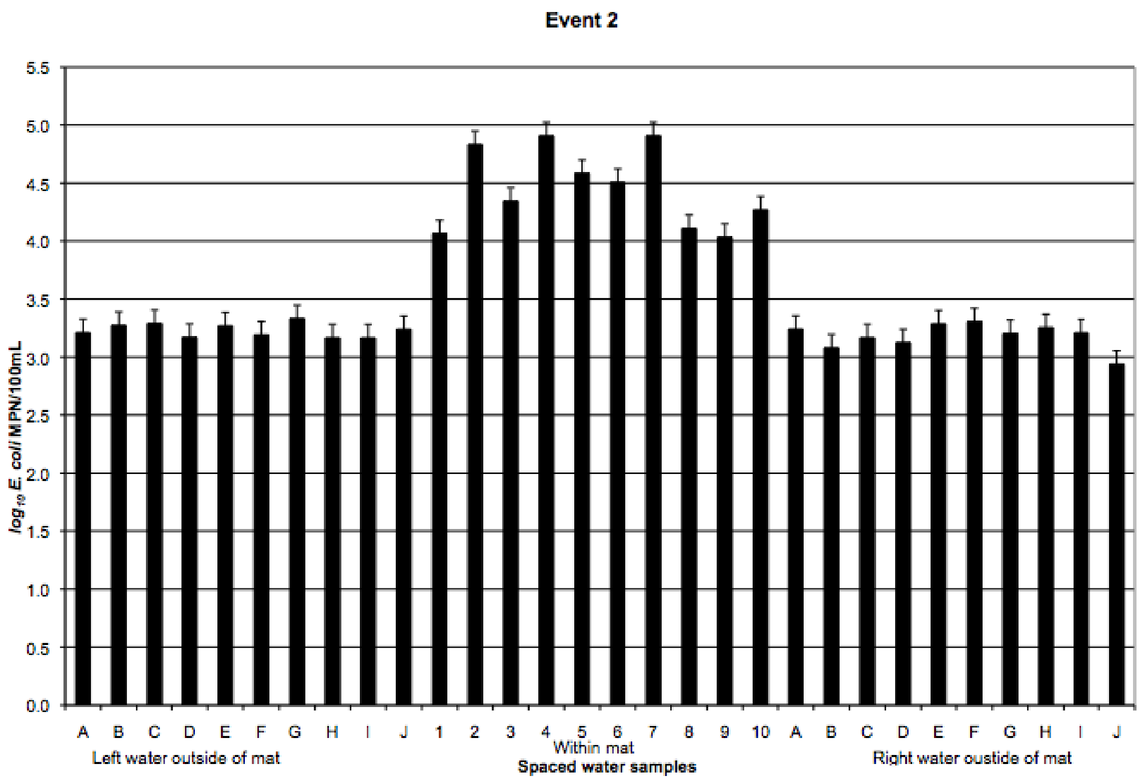

Figure 16. Log *E. coli* concentrations from ten evenly spaced water samples from within and surrounding *Cladophora* mats during Event 2 at Lakeside Park beach. Bars are means of three consecutive sampling days +/- standard error.

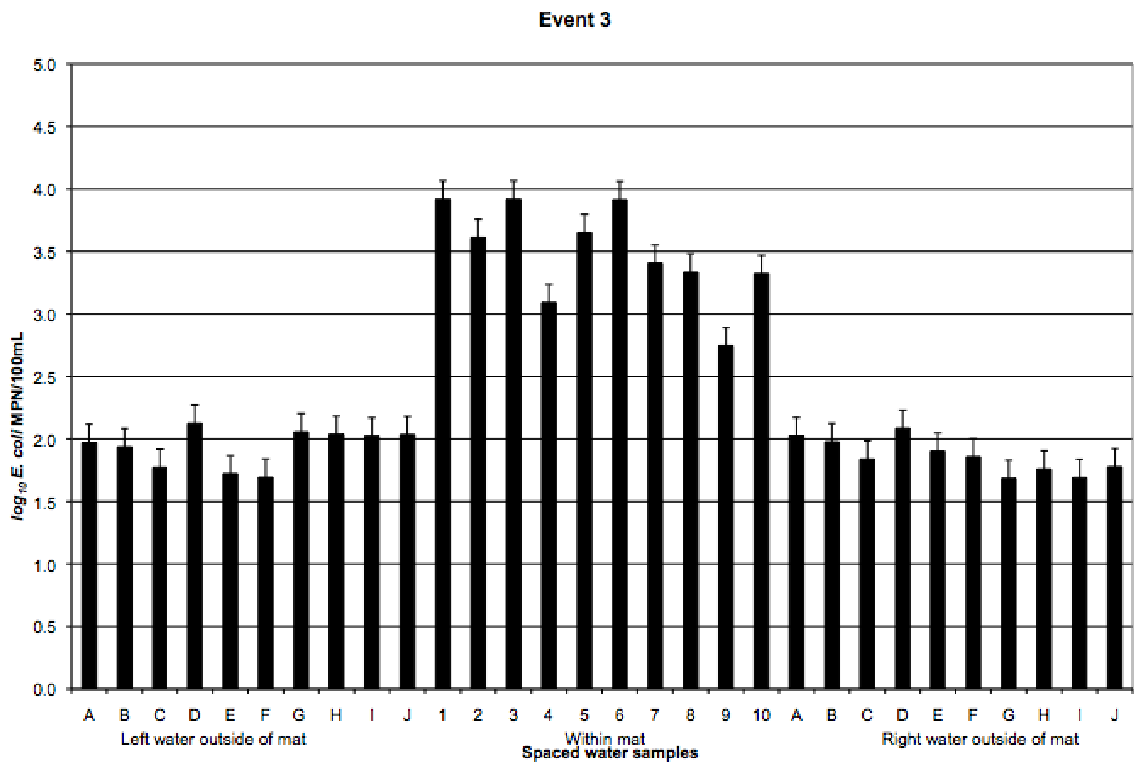

Figure 17. Log *E. coli* concentrations from ten evenly spaced water samples from within and surrounding *Cladophora* mats during Event 3 at Lakeside Park beach. Bars are means of three consecutive sampling days +/- standard error.

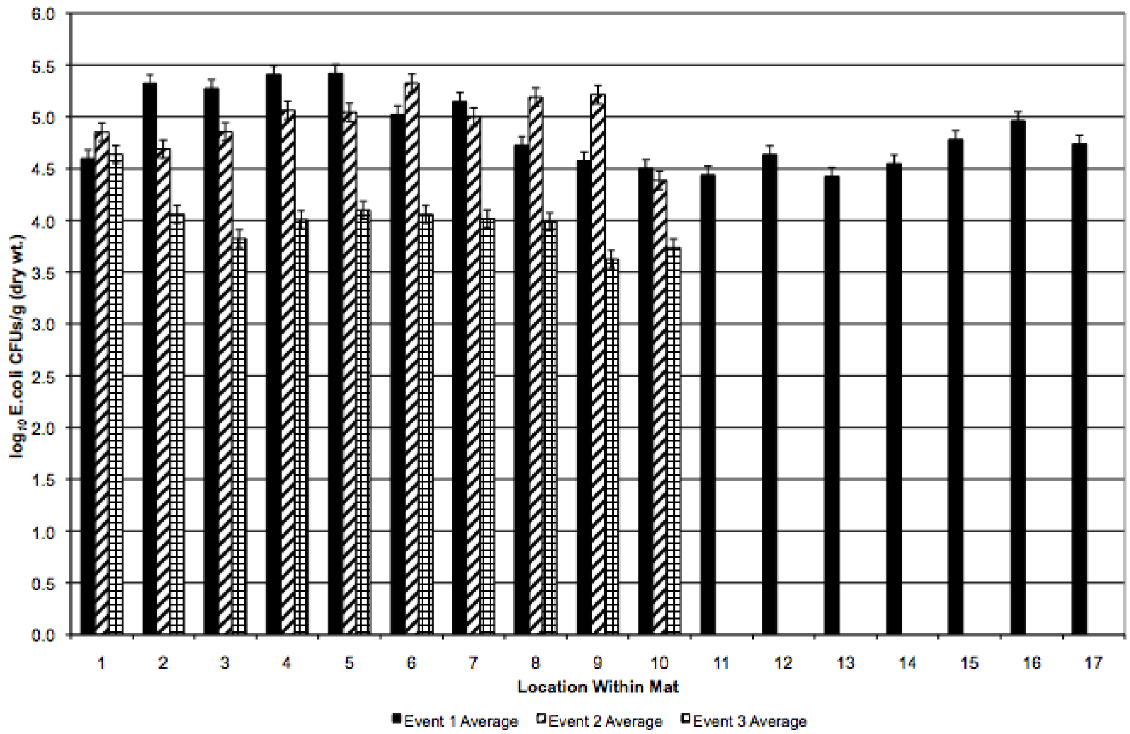

Figure 18. Log *E. coli* concentrations attached to the *Cladophora* mat at Whitefish Dunes State Park beach. Each bar represents the mean of three consecutive sampling days +/- standard error.

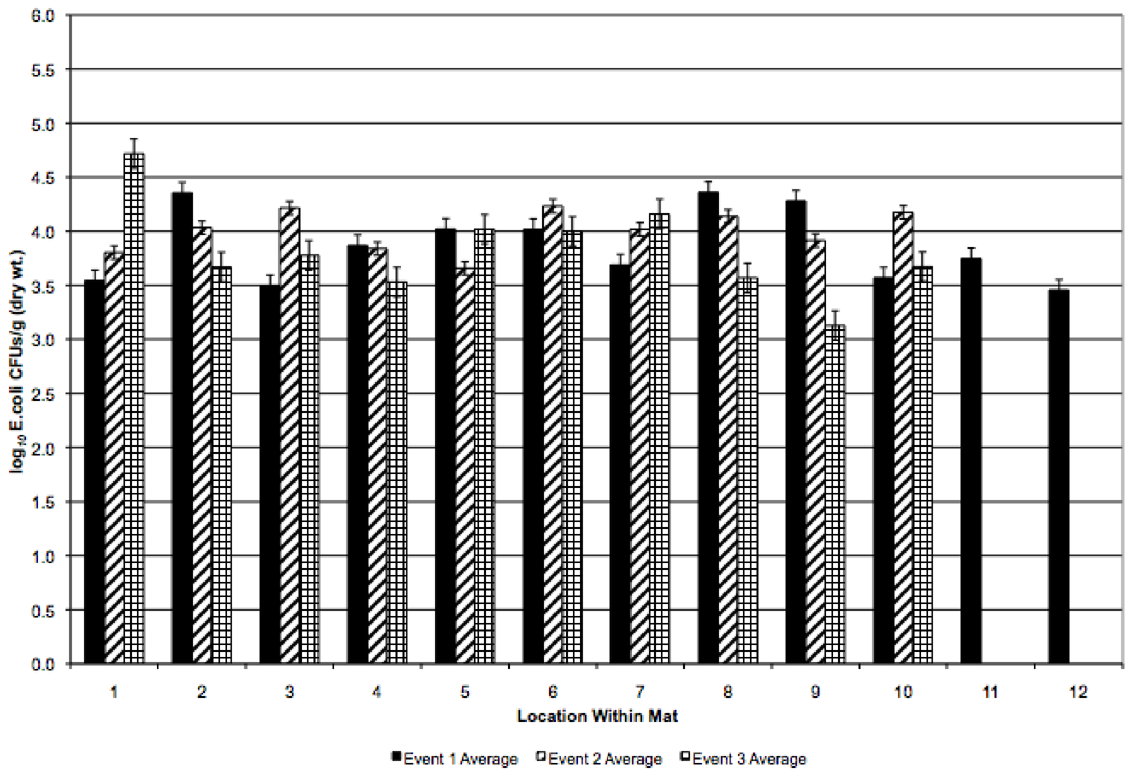

Figure 19. Log *E. coli* concentrations attached to the *Cladophora* mat at Lakeside Park beach. Each bar represents the mean of three consecutive sampling days +/- standard error.

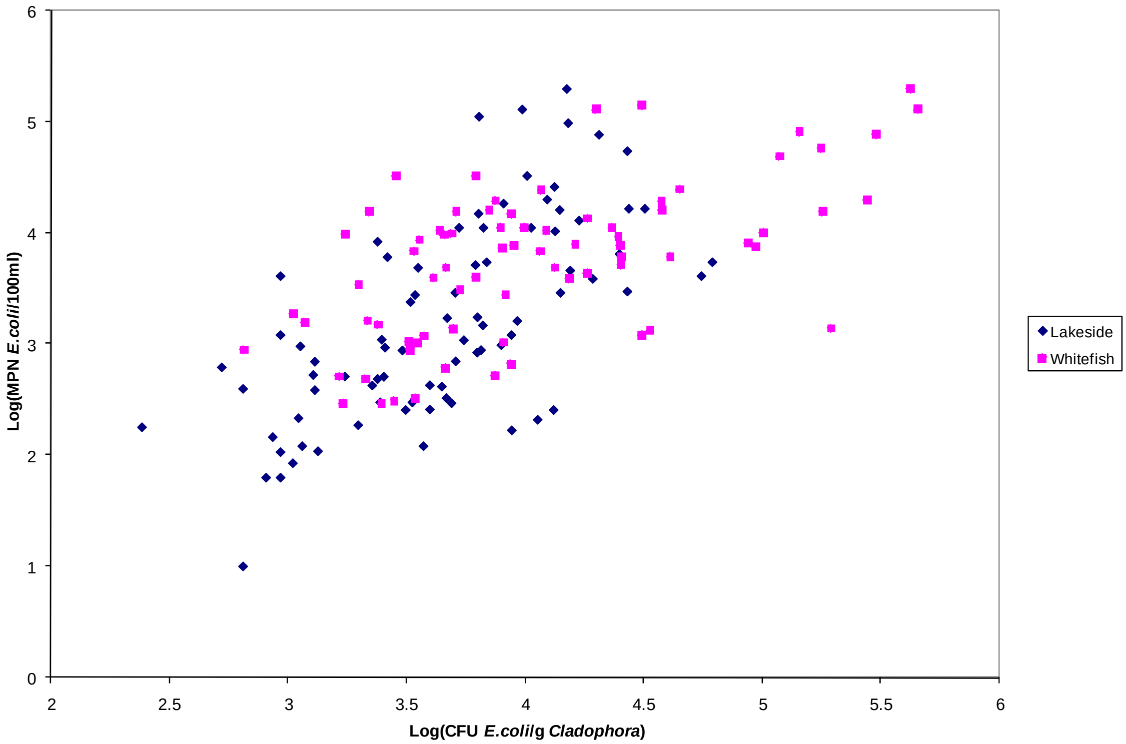

Figure 20. Relationship between *E. coli* concentrations found in water [log (MPN/100 mL)] and *E. coli* concentrations attached to *Cladophora* biomass [log (CFU/g dry wt)].

DISCUSSION

Escherichia coli concentrations in water underlying *Cladophora* mats were routinely higher than in water away from mats. The concentration of *E. coli* in outlying water to the left or right of the mat seemed to be dependent on the prevailing water current direction. Determination of current direction by beach managers (a simple process) could aid in predicting the *E. coli* concentration in adjacent beach water and thus aid in protecting public health.

Random sampling of water underlying and surrounding persistent *Cladophora* mats for consecutive days allowed identification of changes in *E. coli* concentrations with time. As the algae mat persisted on the beach, *E. coli* concentrations increased. This suggests that the algae mats provide a suitable habitat not only for the persistence, but also the growth of *E. coli*. This study supports the findings of others (5, 9, 30) that *Cladophora* mats can influence *E. coli* survival in the beach environment. The genetic analysis of the *E. coli* isolates recovered from algae also suggests that *E. coli* may be replicating within the *Cladophora* mats.

Generally, as *E. coli* concentrations within the *Cladophora* mats increased, *E. coli* in the surrounding water increased. While *E. coli* concentrations in the surrounding water were low relative to the concentrations within the mats, mean concentrations were greater than the allowable criteria for recreational freshwaters; 235 CFU/100 mL (27) for three of six (50%) random sampling events at Whitefish Dunes State Park (Figures 6, 7, and 8). Likewise, at Lakeside Park the mean *E. coli* concentrations outside the mats exceeded the allowable criteria for four of six (66.7%) random sampling events (Figures 9, 10, and 11). These findings suggest that the presence of stranded *Cladophora* mats in nearshore water or on beach sand may elevate *E. coli* concentrations in beach water used to determine beach openings and closures. Removal of these algal mats by beach managers may positively impact recreational water quality at beaches with *Cladophora* accumulations.

Collection of evenly spaced water samples underlying *Cladophora* mats allowed identification of patterns of *E. coli* distribution within a mat. In general, *E. coli* concentrations were greater in the center of the mat than at the periphery. The center of the mat is protected from wind and wave action, and *E. coli* from this area may not be moved to surrounding water as easily as *E. coli* located at the perimeter of the mat.

Escherichia coli attached to *Cladophora* biomass in mats was removed by vigorous washing and was enumerated and expressed as CFU *E. coli*/g dry weight *Cladophora* to determine if the algal biomass offered attachment points for the bacterium. At both beaches, extremely elevated concentrations of *E. coli* were removed from *Cladophora* biomass (4-5 $\log_{10}$/g dry wt) (Figures 18 and 19). Wind and wave action on the stranded *Cladophora* biomass should contribute to detachment of *E. coli* from the algae (as did mechanical shaking during the washing prodedure), seeding underlying water with the indicator organism and potentially allowing weather conditions to move *E. coli* into contact with bathers. The significant, positive correlation found between concentrations of *E. coli* attached to *Cladophora* and in free adjacent water (Figure 20) supports this. Since *E. coli* appears to persist in *Cladophora* mats for extended time periods and perhaps replicates, *Cladophora* mats on swimming beaches represent a challenge to maintaining beach water quality.

Surprisingly, common fecal pathogens (*Salmonella*, *Shigella*, and *Campylobacter*) were not detected in water underlying nor attached to *Cladophora* mats in this study. These bacterial pathogens have been detected in *Cladophora* mats, albeit in low densities, in other Lake Michigan beachsheds (4, 13). Two hypotheses may explain the lack of detection in 2007: First, the counts of these bacteria were too low to be enumerated by the culture methods used due to environmental conditions, such as dilution, temperature (23), solar radiation (20, 26), predation (23), and viable-but nonculturable (VBNC) state of the organisms (24). While *Cladophora* attached to its hard substrate in southern Lake Michigan was previously shown to contain these pathogens (13), both culturable and molecular methods have been unable to detect *Salmonella* from northern Lake Michigan, including Door County (4). The second hypothesis centers on the paucity of occurrence of these pathogens in northern Lake Michigan. The presence of these pathogens in southern Lake Michigan may be a factor of input from particular sources (wastewater) not commonly found in the northern areas of this lake.

If *E. coli*, the indicator of fecal contamination, is able to persist and perhaps replicate in *Cladophora* mats, while fecal pathogens are not present, the usefulness of *E. coli* as an indicator organism is called into question.

CONCLUSIONS

In summary, this study has measured some of the greatest concentrations of *E. coli* found in water underlying *Cladophora* mats (> 5.4 $\log_{10}$ MPN/100 mL), as well as attached to *Cladophora* biomass recovered from mats (> 5.4 $\log_{10}$ CFU/g dry wt). This study further demonstrated a direct relationship (at both beach sites) between attached *E. coli* concentrations within the mat and *E. coli* concentrations in water underlying the mat. Additionally, at both study sites the *E. coli* concentrations increased from Day 1 through Day 3 of the 3-day sampling events. This, coupled with the molecular analysis suggests that *E. coli* may be replicating within the *Cladophora* mats. The current lack of detection of fecal pathogens within *Cladophora* mats calls into question the ability of *E. coli* to serve as a good indicator of recent fecal contamination.

FUTURE WORK

The sampling protocols utilized on this project were repeated at two beaches during the summer seasons of 2008 and 2009. Whitefish Dunes State Park beach was sampled both years along with Newport State Park beach. Newport State Park beach was chosen as a new sampling location because *Cladophora* mats did not strand on the beach at Lakeside Park in subsequent years due to shoreline and/or lake level changes. Statistical analyses of the data from 2008 and 2009 needs to be performed. For the 2009 summer sampling season, molecular techniques will be used to analyzed water and *Cladophora* samples for pathogens (*Salmonella*).

Given the result from the first year of this study that *E. coli* appears to migrate from the edges of the mat to adjacent waters, it would be interesting to see the *E. coli* concentrations within the water after the mat has washed away. After a stranded *Cladophora* mat has washed off of the beach or been removed by beach managers, water samples could be collected from that location at various time points. This could provide information on how long *E. coli* may be present in waters where algal mats were previously stranded. This might help determine how long water may pose a threat to public health after a mat has washed away or been removed from the beach.

APPENDIX A

Physical Parameters Meaured Within *Cladophora* Mats and in Adjacent Waters

Table A-1. Measurements taken within the *Cladophora* mat and in adjacent waters at Whitefish Dunes State Park beach for Event 1.

Event 1		pH	Conductivity (μS/cm)	TDS (mg/L)	ORP (mv)	DO (ppm)	Temp. (^oC)
7/16/2007	**Left Water**	8.44	268	-	-	9.9	18.9
	Right Water	8.43	328	-	-	10.5	19
	Mat	7.89	550	-	58	2.1	24.6
7/17/2007	**Left Water**	6.74	263	1400.8	-	-	15
	Right Water	6.74	263	1400.8	-	-	15
	Mat	-	-1250	320	68	-	24.8
7/18/2007	**Left Water**	7.76	275	-	139	-	16.4
	Right Water	7.76	274	139	134	-	17.5
	Mat	6.68	-	-	108	-	20.9
	Mat vs. Right	($p = 0.000$)	($p = 0.025$)	($p = 0.014$)	($p = 0.000$)	($p = 0.000$)	($p = 0.001$)
	Mat vs. Left	($p = 0.000$)	($p = 0.026$)	($p = 0.019$)	($p = 0.001$)	($p = 0.000$)	($p = 0.002$)

N = 2-5 for Mat

Dash = No measurements recorded.

Table A-2. Measurements taken within the *Cladophora* mat and in adjacent waters at Whitefish Dunes State Park beach for Event 2.

Event 2		**pH**	**Conductivity (µS/cm)**	**TDS (mg/L)**	**ORP (mv)**	**DO (ppm)**	**Temp. (°C)**
7/31/2007	**Left Water**	7.35	343	166.4	79	8.26	21.6
	Right Water	8.17	283	141.8	98	8.56	21.8
	Mat	6.53 (0.34)	637.5 (286.7)	574.6 (305.9)	-286	2.1	29.8
8/1/2007	**Left Water**	7.57	322	163.5	37	7.83	22.5
	Right Water	7.81	279	138.9	163	8.19	21.8
	Mat	6.43 (0.26)	962.9 (593.4)	699.6 (412.9)	-83	1.61 (0.46)	27.15 (0.9)
8/2/2007	**Left Water**	7.3	279	136.3	110	8.63	20.3
	Right Water	7.7	278	138.9	110	8.07	20.1
	Mat	7.4 (0.05)	343 (97.9)	149.7 (10.7)	90 (10.5)	5.19 (1.98)	22
	Mat vs. Right	(p = 0.000)	(p = 0.025)	(p = 0.014)	(p = 0.000)	(p = 0.000)	(p = 0.001)
	Mat vs. Left	(p = 0.000)	(p = 0.026)	(p = 0.019)	(p = 0.001)	(p = 0.000)	(p = 0.002)

N = 2-5 for Mat

( ) = Standard deviation

Table A-3. Measurements taken within the *Cladophora* mat and in adjacent waters at Whitefish Dunes State Park beach for Event 3

Event 3		pH		Conductivity (µS/cm)		TDS (mg/L)		ORP (mv)		DO (ppm)		Temp. (ºC)
8/8/2007	**Left Water**	8.3		257		133.5		100		9.48		22.3
	Right Water	8.4		277		139.1		105		8.53		21.7
	Mat	7.95	(0.25)	301.4	(85.4)	172.1	(40.7)	97	(9.5)	8.15	(0.37)	23.2
8/9/2007	**Left Water**	8		227		138.9		128		8.24		21.1
	Right Water	8.15		298		150.6		67		8.01		21
	Mat	7.46	(0.64)	468.3	(73.7)	157.2	(134.7)	57	(64.8)	3.9	(0.91)	21.5
8/10/2007	**Left Water**	7.96		275		141.8		140		8.32		23
	Right Water	8.14		289		145.3		132		7.76		23.5
	Mat	6.95	(0.37)	501.7	(173.8)	258	(40.3)	-10		2.54	(2.81)	24.2
	Mat vs. Right	(p = 0.000)		(p = 0.025)		(p = 0.014)		(p = 0.000)		(p = 0.000)		(p = 0.001)
	Mat vs. Left	(p = 0.000)		(p = 0.026)		(p = 0.019)		(p = 0.001)		(p = 0.000)		(p = 0.002)

N = 2-5 for Mat

( ) = Standard deviation

Table A-4. Measurements taken within the *Cladophora* mat and in adjacent waters at Lakeside Park beach for Event 1.

Event 1		pH	Conductivity (µS/cm)	TDS (mg/L)	ORP (mv)	DO (ppm)	Temp. (ºC)
7/16/2007	**Left Water**	6.99	265	233	10.8	21.6	
	Right Water	6.99	265	233	10.8	21.6	
	Mat	5.93 (1.34)	643 (544.5)	-	-162	0.8 (1.13)	30.25 (0.9)
7/17/2007	**Left Water**	6.98	271	131.2	-	-	21
	Right Water	7.35	273	139	-	7.02	20.5
	Mat	-	520	251	-	6.41	31.2
7/18/2007	**Left Water**	-	-	-	-	-	-
	Right Water	-	-	-	-	-	-
	Mat	-	-	-	-	-	-
	Mat vs. Right	($p = 0.000$)	($p = 0.003$)	($p = 0.000$)	($p = 0.000$)	($p = 0.000$)	($p = 0.014$)
	Mat vs. Left	($p = 0.000$)	($p = 0.002$)	($p = 0.000$)	($p = 0.000$)	($p = 0.000$)	($p = 0.049$)

N = 2-5 for Mat

( ) = Standard deviation

Dash = No measurements recorded.

Table A-5. Measurements taken within the *Cladophora* mat and in adjacent waters at Lakeside Park beach for Event 2.

Event 2		pH		Conductivity (µS/cm)		TDS (mg/L)		ORP (mv)	DO (ppm)		Temp. (°C)
8/1/2007	**Left Water**	7.93		285		144.2		147	10.47		30
	Right Water	8.29		288		143.1		65	9.53		25
	Mat	6.69	(0.21)	766.7	(266.7)	389.3	(163.5)	-183	0.87	(0.24)	30.7
8/2/2007	**Left Water**	7.62		289		162.1		118	6.73		23
	Right Water	7.83		288		144.7		79	6.95		22.4
	Mat	7.31	(0.14)	436.3	(77.0)	221.7	(32.3)	-16	1.18	(0.15)	23
8/3/2007	**Left Water**	7.96		284		144.8		141	8.65		18.6
	Right Water	8.03		293		147		67	10.4		18.2
	Mat	7.16	(0.32)	294.8	(174.7)	222.4	(56.3)	-25	3.26	(1.19)	20.5
	Mat vs. Right	(p = 0.000)		(p = 0.003)		(p = 0.000)		(p = 0.000)	(p = 0.000)		(p = 0.014)
	Mat vs. Left	(p = 0.000)		(p = 0.002)		(p = 0.000)		(p = 0.000)	(p = 0.000)		(p = 0.049)

N = 2-5 for Mat

( ) = Standard deviation

Table A-6. Measurements taken within the *Cladophora* mat and in adjacent waters at Lakeside Park beach for Event 3.

Event 3		pH		Conductivity (µS/cm)		TDS (mg/L)		ORP (mv)		DO (ppm)		Temp. (ºC)
8/8/2007	**Left Water**	7.98		283		141.5		96		8.3		22.4
	Right Water	8.14		287		143.8		78		8.34		21.8
	Mat	7.36	(0.34)	372.4	(87.8)	234.4	(89.2)	58.8	(70.4)	4.35	(1.89)	22.4
8/9/2007	**Left Water**	8.29		281		142		132		9.13		23.4
	Right Water	8.48		285		140.7		120		8.74		23.3
	Mat	8.28	(0.04)	277.7	(2.08)	141.3	(1.48)	113.3	(2.5)	8.22	(0.46)	23.7
8/10/2007	**Left Water**	7.83		287		146.1		111		8.58		22.7
	Right Water	8.08		304		152.6		124		7.94		22.4
	Mat	7.44	(0.25)	332	(8.49)	170.7	(10.9)	123	(2.8)	4.58	(1.82)	22.9
	Mat vs. Right	(p = 0.000)		(p = 0.003)		(p = 0.000)		(p = 0.000)		(p = 0.000)		(p = 0.014)
	Mat vs. Left	(p = 0.000)		(p = 0.002)		(p = 0.000)		(p = 0.000)		(p = 0.000)		(p = 0.049)

N = 2-5 for Mat

( ) = Standard deviation

APPENDIX B

Genetic Relatedness of *Escherichia coli*

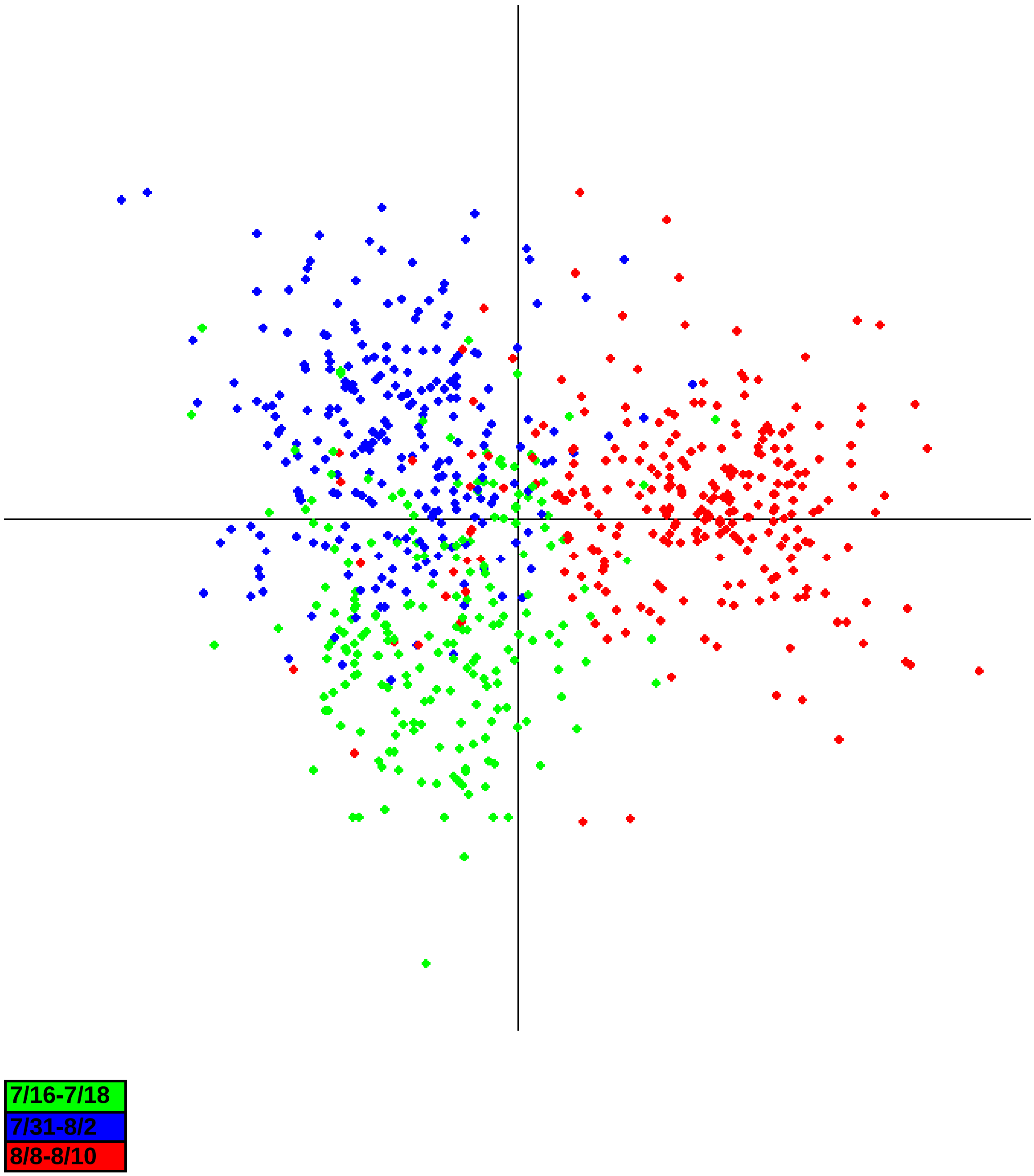

Figure B-1. MANOVA of *E. coli* isolates from *Cladophora* for each mat (event) at Whitefish Dunes State Park beach in 2007.

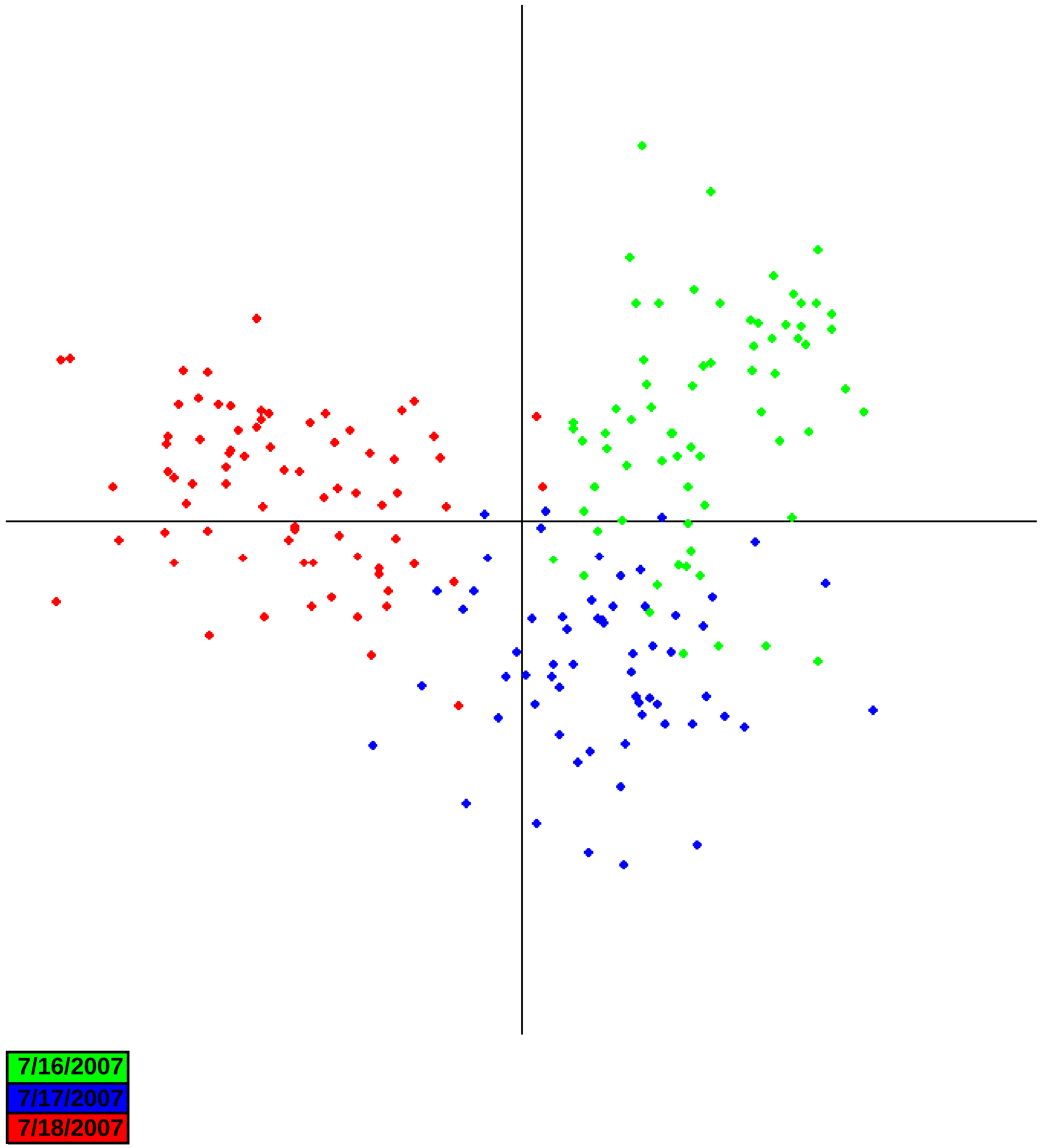

Figure B-2. MANOVA of *E. coli* isolates from *Cladophora* for Event 1 at Whitefish Duens State Park beach.

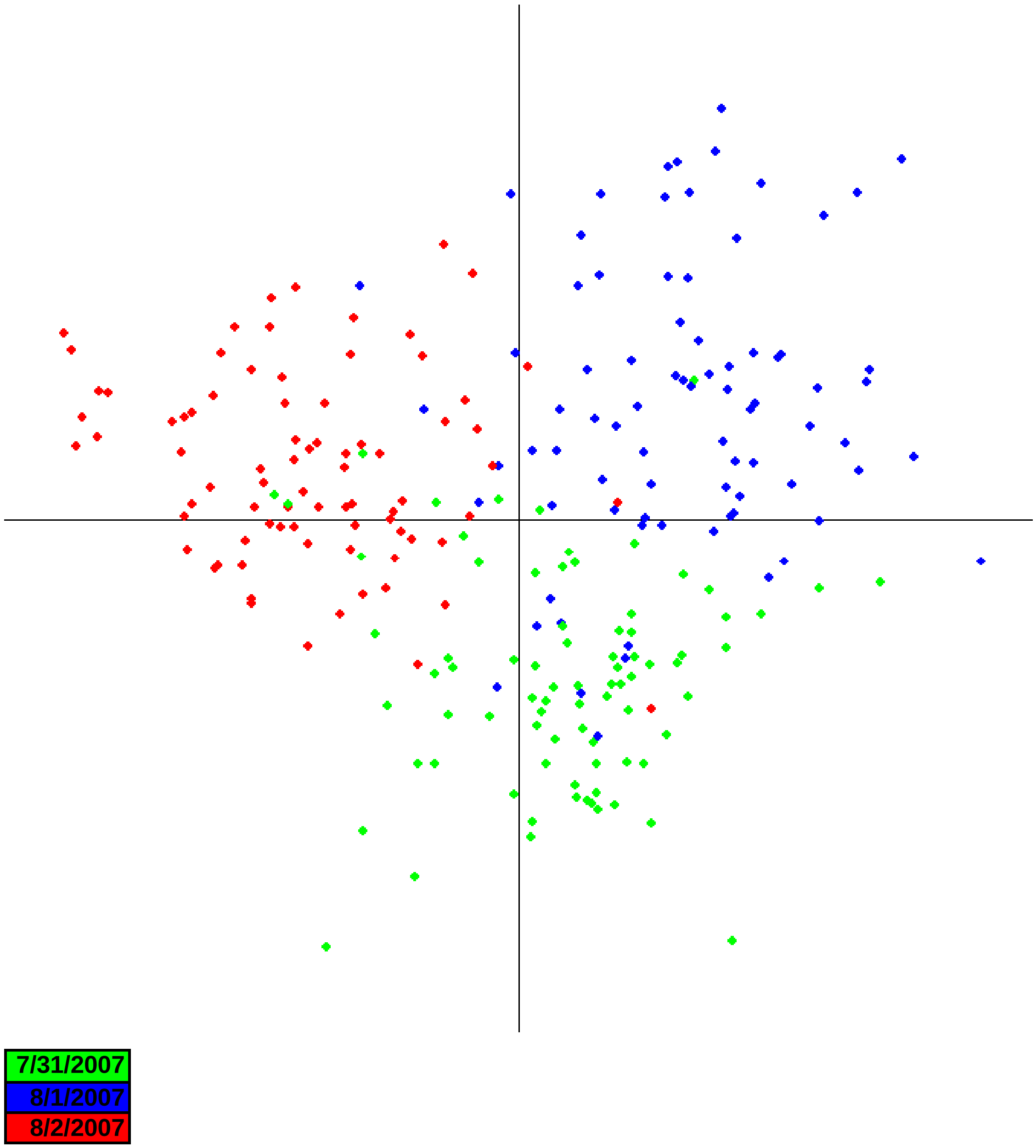

Figure B-3. MANOVA of *E. coli* isolates from *Cladophora* for Event 2 at Whitefish Dunes State Park beach.

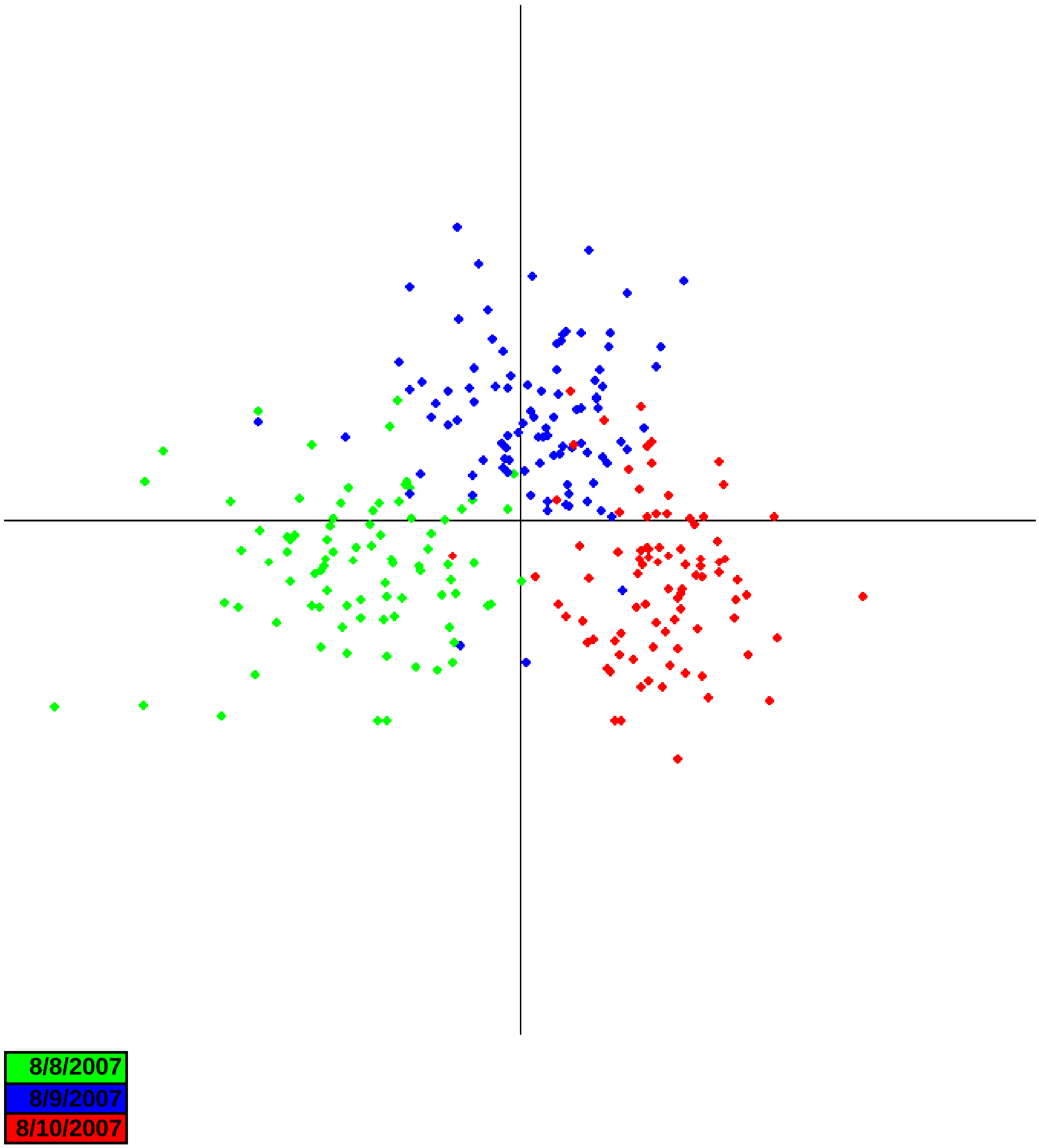

Figure B-4. MANOVA of *E. coli* isolates attached to *Cladophora* for Event 3 at Whitefish Dunes State Park beach.

{% Similarity}

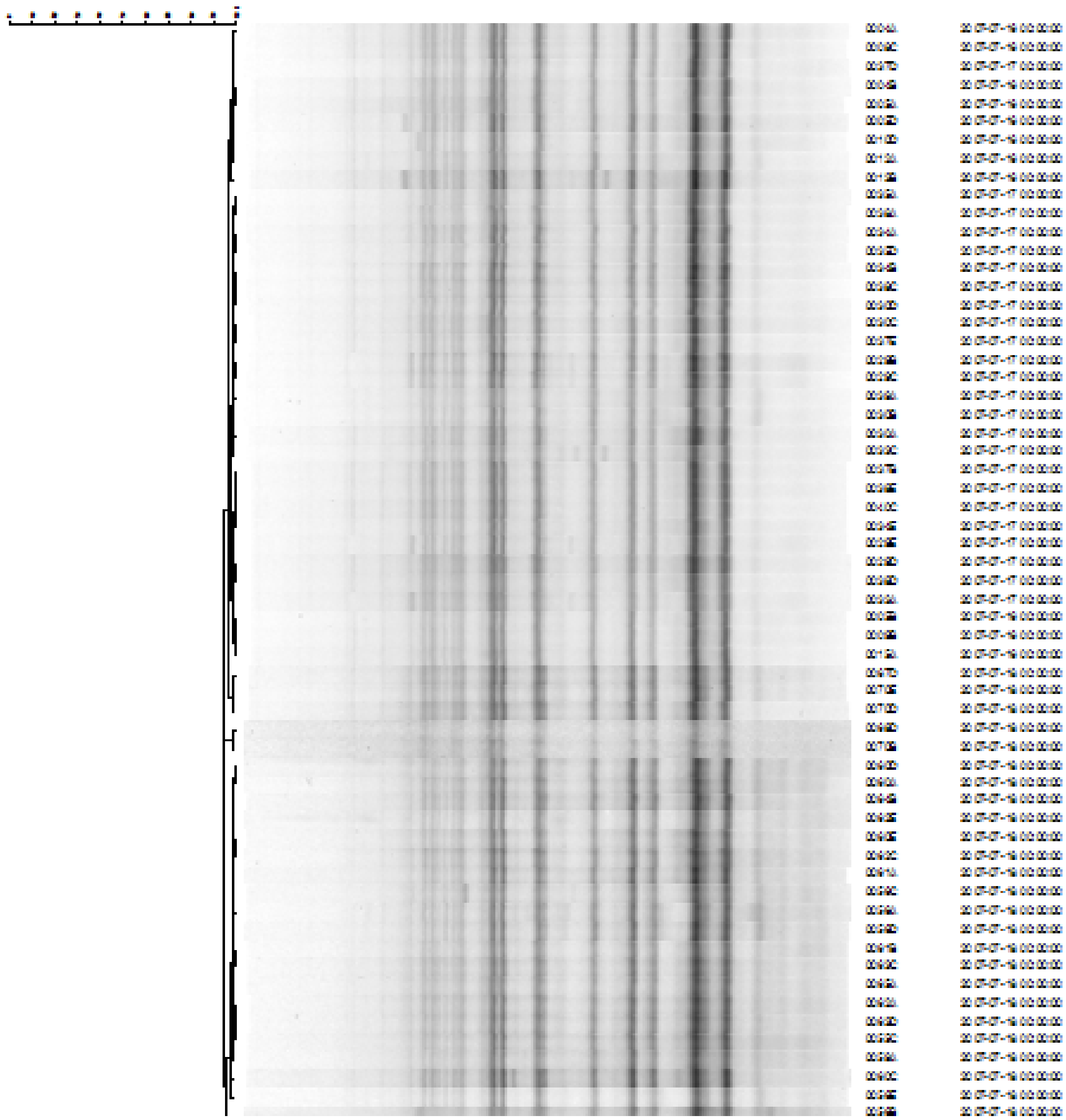

Figure B-5. DNA fingerprint patterns of *E. coli* isolates from all 3 days of Event 1 at Whitefish Dunes State Park beach generated by rep-PCR and HFERP.

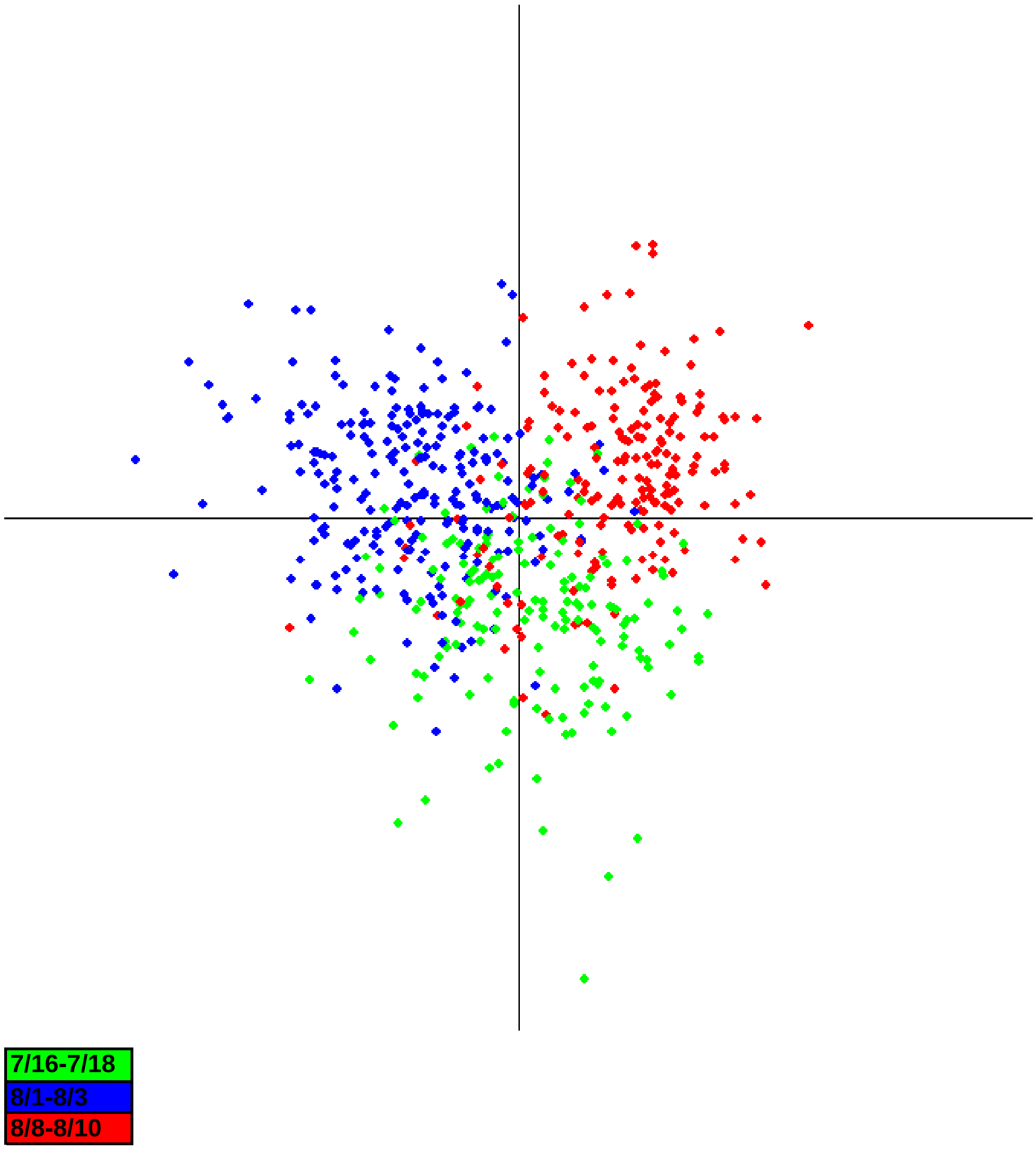

Figure B-6. MANOVA of *E. coli* isolates from *Cladophora* for each mat (event) at Lakeside Park beach in 2007.

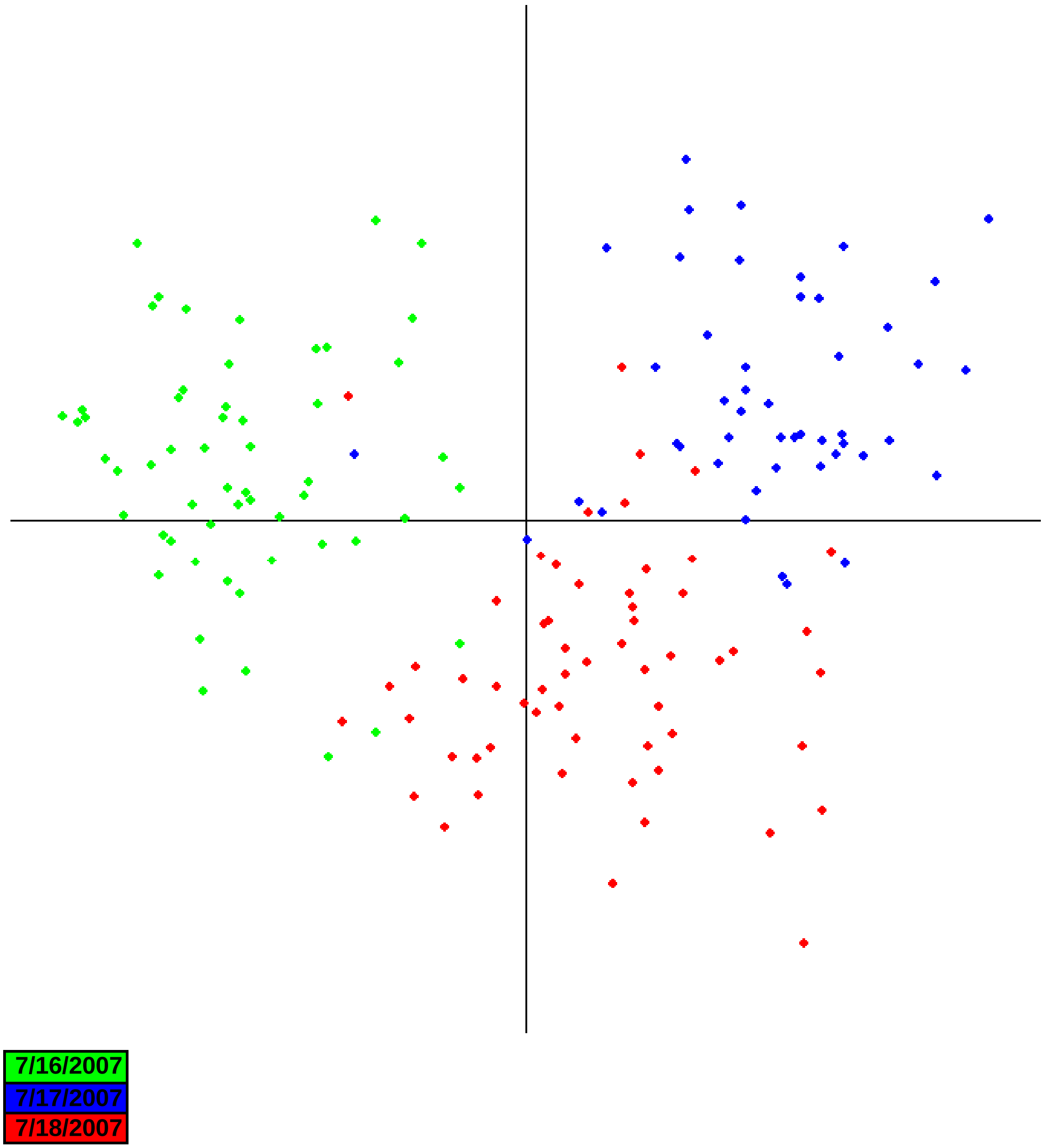

Figure B-7. MANOVA of *E. coli* isolates from *Cladophora* for Event 1 at Lakeside Park beach.

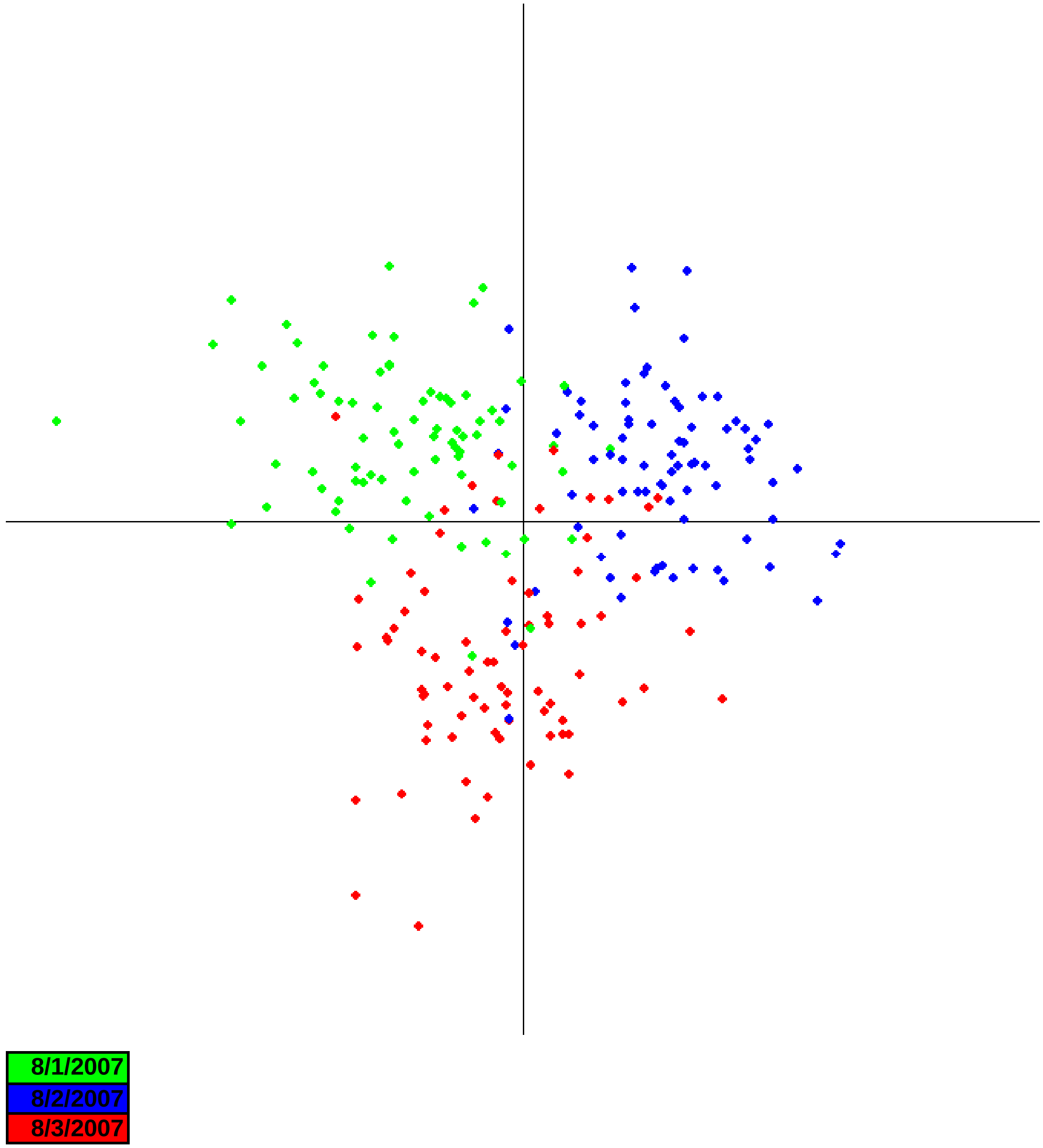

Figure B-8. MANOVA of *E. coli* isolates from *Cladophora* for Event 2 at Lakeside Park beach.

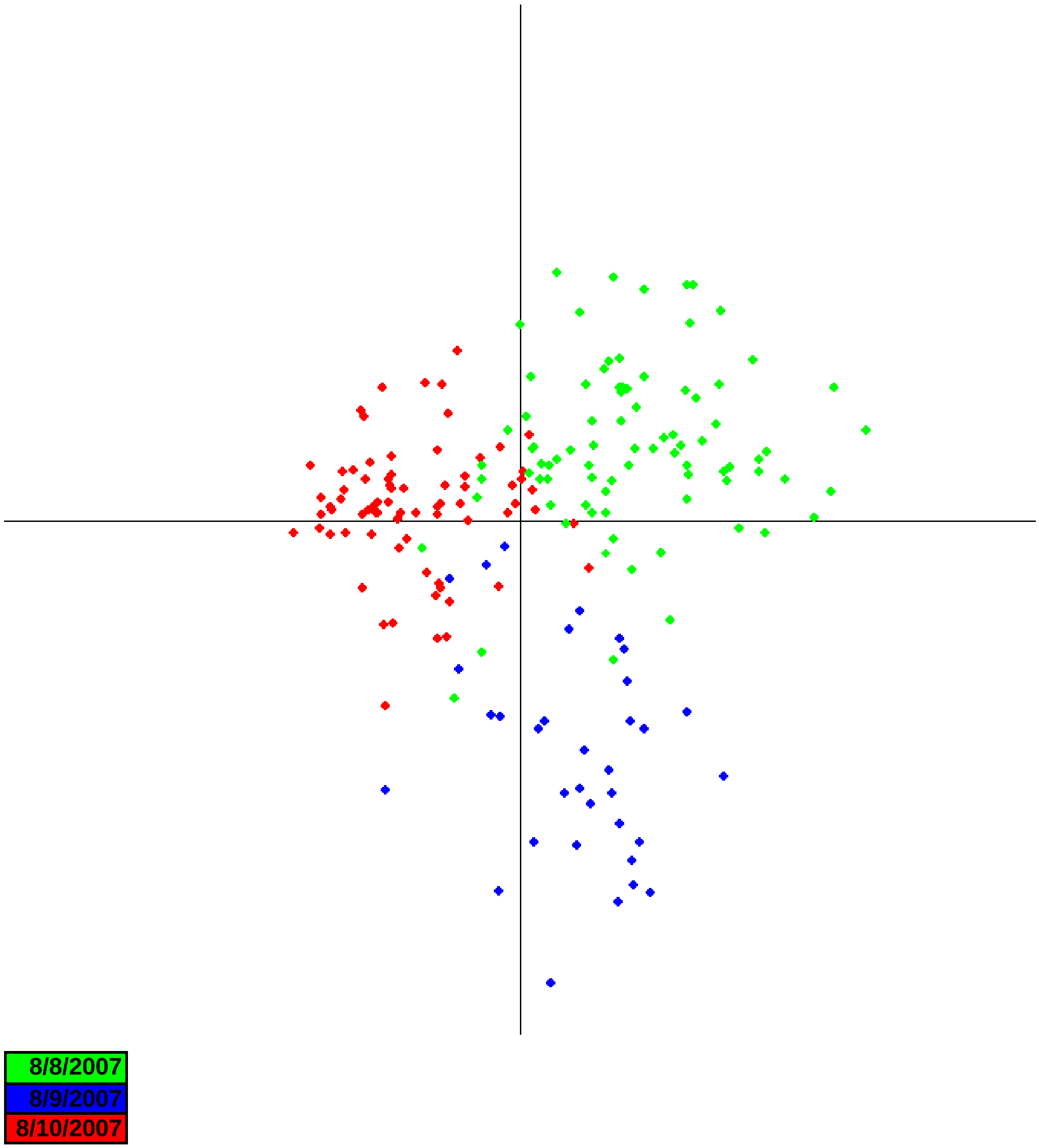

Figure B-9. MANOVA of *E. coli* isolates from *Cladophora* for Event 3 at Lakeside Park beach.

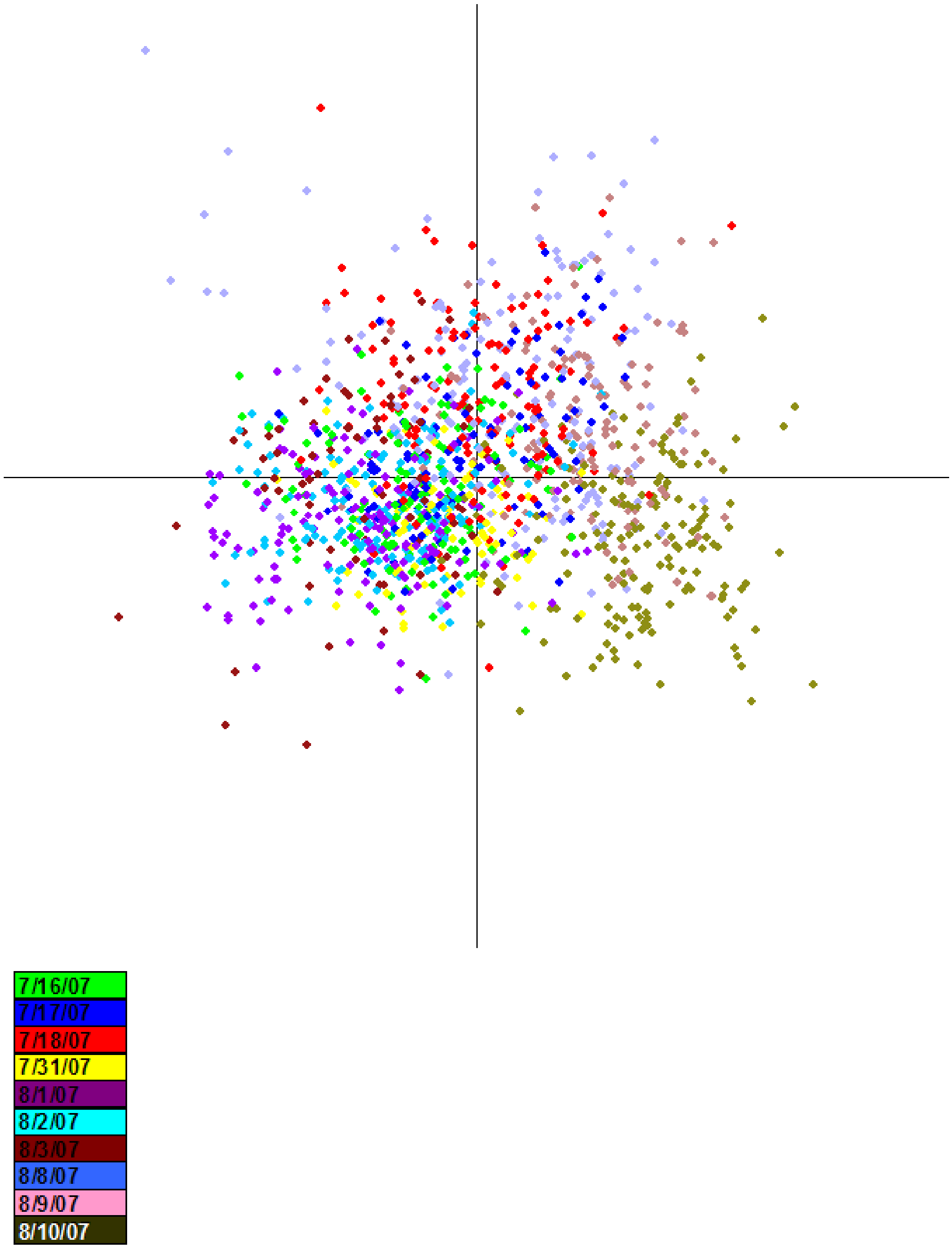

Figure B-10. MANOVA of *E. coli* isolates from *Cladophora* from individual sampling dates at Whitefish Dunes State Park and Lakeside Park beaches combined.

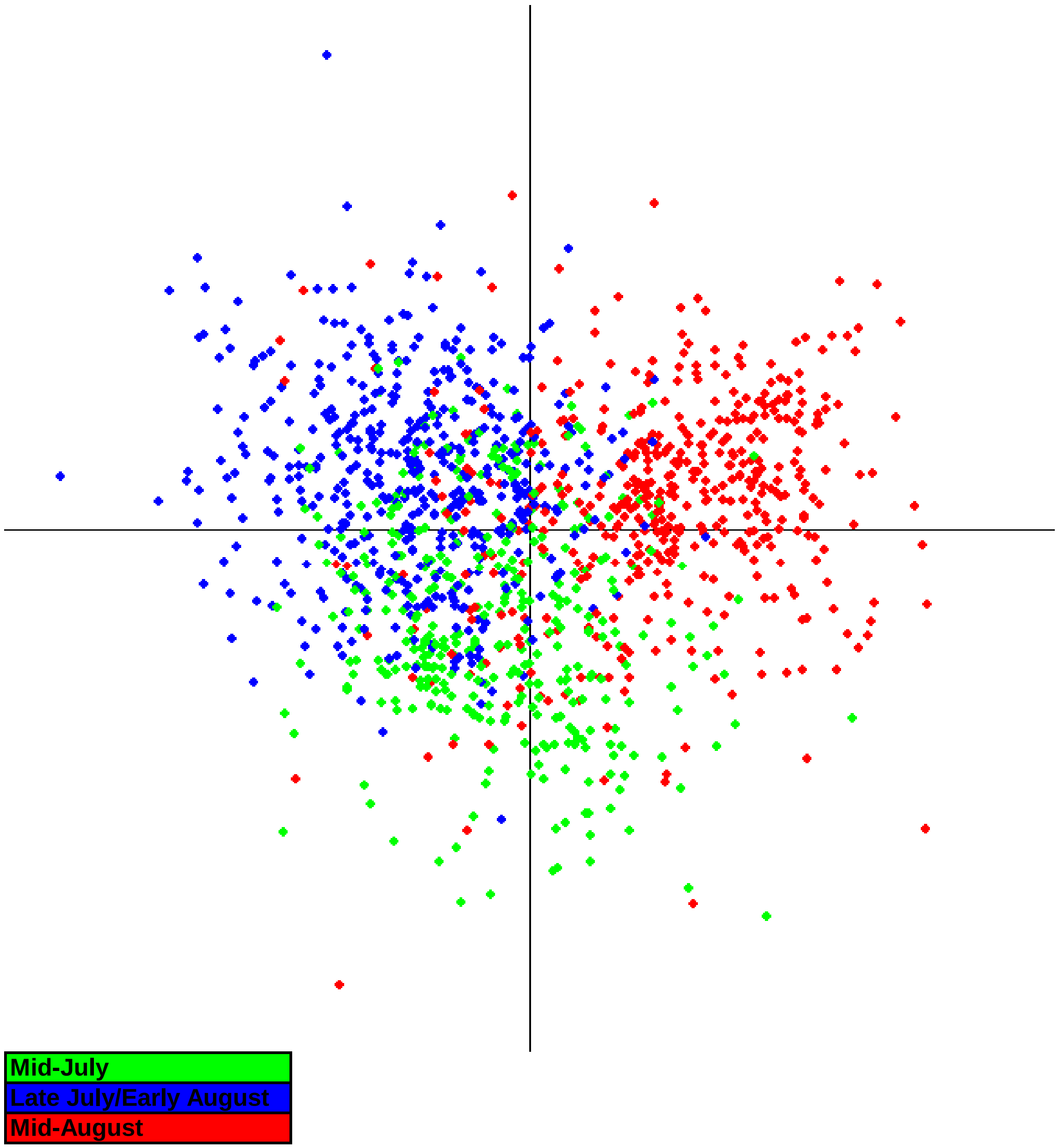

Figure B-11. MANOVA of *E. coli* isolates from *Cladophora* from all three events at Whitefish Dunes State Park and Lakeside Park beaches combined.

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