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The Movement Of Escherichia Coli And Enterococci Among Beach Sand, Lyngbya Wollei, And The Water Column: Implications For Human Health

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THE MOVEMENT OF *ESCHERICHIA COLI* AND ENTEROCOCCI AMONG BEACH SAND, *LYNGBYA WOLLEI*, AND THE WATER COLUMN: IMPLICATIONS FOR HUMAN HEALTH

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DEDICATION

I dedicate this research to my parents, Clyde and Sharyl Bakkila, who supported my exploration of the natural world around me and all of the organisms within it. Their endless encouragement has helped me along the path of this research.

I also dedicate this research to my sister, Melissa Bakkila, who has experienced much of the life experiences with me that drove my interest in the field of ecology.

Lastly, I would like to dedicate this research to my grandparents, Eric and Signe Bakkila who live in the upper peninsula of Michigan, and the late Richard Bunton who lived in northern Michigan. My grandparents have always lived in places of pure wonder, surrounded by life and nature, and my experiences with them as a child have instilled me with the curiosity that put me on this path from a young age.

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INTRODUCTION

Blooms of nuisance benthic cyanobacterial species have increased in occurrence over the last decade in the Laurentian Great Lakes region, causing concerns over the potentially negative impacts to ecosystems and human health (Vis et al. 2008, Bridgeman and Penamon 2010, Vijayavel et al. 2013). Agriculture, industry, and urban development around the Great Lakes basin have led to anthropogenic loading of nutrients to the connected freshwater systems. Eutrophication associated with nutrient loading can lead to algal blooms characterized by a few or a single species that dominate an aquatic ecosystem. In addition to changes in the watershed which have affected the nutrient and phytoplankton dynamics in the lakes, the invasion of the dreissenid mussels which arrived and established in the region in the 1980s (Hebert et al. 1989), further changed these relationships. For example, the prolific filtering of dreissenids has resulted in a concentration of nutrients in the littoral zones of lakes, which has contributed to the maintenance of eutrophic conditions near shore long after allogenic inputs have been managed (Hecky et al. 2004). Additionally the filtering capacity of dense colonies of dreissenids in near shore habitats has resulted in a decrease in light attenuation by as much as 88% (Fahnenstiel et al. 1995). Sunlight penetrating to greater depths has allowed filamentous benthic algae, such as *Cladophora*, to proliferate and cover a much larger area than before (Hecky et al. 2004). Furthermore, cyanobacteria, such as *Lyngbya wollei*, which require less substrate for attachment than *Cladophora* and less light, may give it a competitive advantage in some areas (Bridgeman and Penamon 2010). These changes in the Great lakes system in

part, have likely contributed to an increased frequency of benthic algal and cyanobacterial blooms in the Great Lakes region.

Historically the most commonly reported benthic bloom forming algae in the Great Lakes region has been *Cladophora* (Herbst 1969, Auer et al. 2010), but recently documented blooms of the benthic cyanobacteria *L. wollei* have increased in occurrence, first in the St. Lawrence River, then in Lake Erie (Vis et al. 2008, Stewart 2008, Bridgeman and Penamon 2010), and recently in Lake St. Clair (Vijayavel et al. 2013). Historically *L. wollei* has been documented in the Great Lakes region since the early 1900's (Miller 1915), under various names including *Plectonema wollei*, *Lyngbya aestuarii*, and *Lyngbya majuscula* (Speziale and Dyck 1992). Detailed descriptions of these historical documentations have led scientists to agree that all occurrences were in fact the same species now known as *L. wollei* (Speziale and Dyck 1992, Hudon et al. 2014).

Lyngbya wollei is a non-heterocystous, filamentous, benthic cyanobacterium that is commonly found in the southeastern United States (Philips et al. 1992). *Lyngbya wollei* is capable of nitrogen fixation, a process that converts biologically unavailable atmospheric nitrogen gas to a biologically available form, ammonia, using the enzyme nitrogenase (Philips et al. 1992). Nitrogen fixation can provide a competitive advantage for this cyanobacteria species when nitrogen is limiting for other native algae species under conditions of increased phosphorus and micro nutrients such as calcium, potassium, and iron (Cowell and Botts 1994, Ahern et al. 2007, Ahern et al. 2008). *Lyngbya wollei* is also known to produce hepatotoxins, neurotoxins, and cause epidermal problems, although toxin strength and production varies among regions and

different strains of the cyanobacteria (Foss et al. 2012, Carmichael et al. 1997, Osborne et al. 2001). *Lyngbya wollei* in the St. Lawrence River is recorded to produce an analog of saxitotoxin, commonly known as paralytic shellfish toxin (Gélinas et al. 2012, Lajeunesse et al. 2012), while the *L. wollei* found in Lake Erie lack other hepatotoxin or neurotoxin compounds (Bridgeman and Penamon 2010).

Cyanobacteria blooms can have cascading impacts on ecosystems. When *L. wollei* dominates a water body it can cause a decrease in the typical diversity of the submerged aquatic vegetative community which can impact the entire food web, including decreasing species diversity and abundance of the benthic invertebrates, and thus impacting fish assemblages (Hudon et al. 2012). In addition to altering biotic diversity, cyanobacterial blooms can impact water quality by increasing turbidity and decreasing dissolved oxygen (Robertson and Saad 2011).

Lyngbya wollei is becoming an increasingly common nuisance species of cyanobacteria, even in areas where it has been a natural component of the aquatic vegetation community for decades. For example, off the coast in Florida, *Lyngbya* has been observed as a non-dominant population of the benthic algal community since the early 1990's, however over the 2002-2003 winter the population persisted and has become increasingly abundant in following years (Paul et al. 2005). In temperate climates nuisance blooms of *Lyngbya* occur in summer months under conditions of high light and warm water temperatures and begin with the development of a benthic mat that can span an area as great as a square kilometer (Albert et al. 2005), however in more moderate or tropical climates, blooms can occur year round (Speziale et al. 1991). Although the optimal biomass growth for *L. wollei* has been found to occur at 26°C,

biomass growth begins to decrease at 10°C (Yin et al. 1997). In the St. Lawrence River *L. wollei* mats can persist beneath ice in the winter season and would maximally increase in biomass as water temperatures rise into the summer (Hudon et al. 2012). Under summer conditions photosynthesis occurs at a high rate and oxygen released from this process forms bubbles that become trapped within the benthic mat resulting in *Lyngbya* becoming buoyant and detaching from the benthos (Albert et al. 2005). The *Lyngbya* then becomes a floating mat at the water surface and can be carried to and deposited on the shoreline by wind or wave action. These deposited shoreline mats of *Lyngbya* decay and become aesthetically displeasing, malodorous, and can impair local waterways for navigational and recreational use (Albert et al. 2005, Speziale and Dyck 1992, Ahern et al. 2007).

As the detached and fragmented *Lyngbya* accumulates and decay along the shoreline they can harbor potentially harmful bacteria, such as *E. coli* and enterococci (Vijayavel et al. 2013). In recent years the Great Lakes region has experienced an increase in the frequency and extent of benthic filamentous green algae blooms of *Cladohpora*, which is structurally similar to *Lyngbya* and also detaches from the benthos to wash ashore and form thick detrital mats along the shoreline (Byappanahalli et al. 2003, Byappanahalli et al. 2007, Whitman et al. 2003). Submerged *Cladophora* have been shown to harbor fecal indicators and potentially harmful bacteria such as *E. coli*, *Campylobacter*, *Shigella*, and *Salmonella* (Ishii et al. 2006). Byappanahalli et al. (2003) demonstrated that sun-dried *Cladophora* mats could harbor and propagate the growth of *E. coli* to a level of 8 log *E.coli* colony forming units (CFU)/g even after eight months of storage at 4°C. Furthermore, *Cladophora* leachate has been shown to contribute to

E. coli growth, indicating that *Cladophora* can promote bacteria growth (Byappanahalli et al. 2003). Thickness of detrital mats can impact *E. coli* persistence as thicker mats prevent desiccation and provide protection from UV sunlight sterilization (Whitman et al. 2003). Even though these mats are frequently removed from public beaches and recreation areas through regular beach grooming activities, it is likely that contact with detrital mats can transfer potentially harmful bacteria to underlying sand left behind. Microcosm studies have shown that *E. coli* inoculated on sterilized sand can increase in growth from 1 CFU/g to 4 x 10^5 CFU/g in 24 hours, and can persist at these or higher levels above this for up to 36 days (Alm et al. 2006). This indicates that natural beach sand can contain essential nutrients and compounds needed to support bacterial growth. However, a further understanding of the ability of *L. wollei* to transfer *E. coli* and enterococci to both underlying beach sand and the near shore water column could provide more insight on how *L. wollei* can impact human health. The objectives of this research is to determine if *E. coli* and enterococci associated with detrital mats of *L. wollei* can be transferred from the mats into the water column and the underlying sand.

METHODS

Two laboratory microcosm experiments were coupled with a field experiment to evaluate the movement of *E. coli* and enterococci from *L. wollei* between sand and water. The first laboratory microcosm experiment quantified the transfer of *E. coli* and enterococci from *L. wollei* to the water column, while the second laboratory experiment evaluated the water column transfer of these fecal indicators from underlying sand that was previously exposed to *L. wollei*. The field experiment assessed the transfer and persistence of *E. coli* and enterococci to underlying beach sand from a mat of *L. wollei* in natural environmental conditions over seven days.

Study Site – Lake St. Clair Metropark Beach

Lake St. Clair Metropark Beach is located on Lake St. Clair, which is a part of the waterway between Lake Huron and Lake Erie within the Great Lakes Basin. The public access beach is located on a peninsula that extends into Lake St. Clair and has approximately 300 meters of sandy shoreline and is near a spill way of the Clinton River which is the main drainage for the St. Clair watershed that empties into Lake St. Clair (Figure 1). The Clinton River is listed as an Area of Concern (AOC) with eight Beneficial Use Impairments (BUIs), one of which is related to beach closures. Lake St. Clair Metropark Beach experiences relatively frequent beach closures, as a result of elevated *E. coli* levels (Michigan Department of Environmental Quality 2010).

Laboratory Microcosm Experiments

Collection of Lyngbya and sand

Lyngbya wollei was collected from floating shoreline mats in approximately 0.5m of water at the western edge of the public beach (Figure 1). Sand was collected from beneath what appeared to be fresh detrital deposits of *L. wollei* approximately one meter inland from the shoreline. The underlying sand was collected by scraping away the overlying *L. wollei* and collecting sand immediately beneath to a depth of approximately 15 centimeters. The *L. wollei* and sand were transported back to the laboratory within four hours of collection in Ziploc bags in a cooler that was cleaned with 90% ethanol and stored in the dark at 4°C.

For the laboratory experiments water was collected from a site on the Detroit River (southern side of Belle Isle in Detroit, Michigan) with low *E. coli* and enterococci levels (both <1 MPN/100ml) and close proximity to Lake St. Clair. The water was collected in clean 5L carboys and stored in the dark at 4°C. Dissolved Oxygen (YSI: ProODO optical oxygen), temperature, pH (Thermo Scientific: Orion 4 Star), and conductivity (Mettler Toledo: FG3/EL3) were measured at both Lake Saint Clair and the Detroit River at the time of sampling.

Upon arrival at the laboratory half of the sand was sterilized by autoclaving. The autoclaved sand was then thoroughly mixed and measured into 200g wet weight and placed in clean 250ml beakers. The sand collected from under the *L. wollei* mats was prepared by transferring all Ziploc bags into a small, sterilized, cooler and stirred continuously for five minutes. The underlying sand was re-stirred between removals of

each 200g sub-sample. All equipment was sterilized with 90% ethanol before and after contact with *Lyngbya*.

The *L. wollei* was prepared by transferring all Ziploc bags to a sterilized cooler and then continuously mixed using a sterilized metal spatula for five minutes in the cooler to achieve a homogenous mixture. The *L. wollei* was then pressed by gloved hands in a sterilized metal strainer to remove excess water. The pressed *Lyngbya* was weighed out to a wet weight of 200g and placed in individual beakers. Once in the beaker the pressed *Lyngbya* was re-stirred to maintain consistency.

Microcosm experiment design

Two microcosm experiments were conducted to quantify the transfer of *E. coli* and enterococci from detrital *L. wollei* deposits, to the underlying sand and the water column. The first experiment evaluated the transfer of *E. coli* and enterococci to the water column from *L. wollei*. The experiment consisted of four treatments, 1) *L. wollei*, 2) *L. wollei* plus sterilized sand, 3) sterilized sand, and 4) a control with water (N=5).

The second experiment was designed to quantify the transfer of *E. coli* and enterococci from sand collected from beneath *Lyngbya* mats to the water column. The four treatments included 1) *L. wollei* plus underlying sand, 2) *L. wollei* plus sterilized sand, 3) underlying sand, and 4) sterilized sand as a control (N=5).

For both experiments, all treatments were placed in artificial lake microcosms (76 x 46 x 14 cm) with 12L of water from the Detroit River. Water in the microcosms were lightly circulated with a MN404 120V~60Hz 5W Z.P. 106gph Hmax 29in Mini-Jet water pump that was set at maximum speed and angled against the microcosm wall at approximately 45° angle to promote water flow. This simulated the gentle flow of water

over detrital *Lyngbya* along the beach shoreline. The 20 artificial lakes were randomly assigned a treatment.

Measurement of E. coli and enterococci

On day zero of the experiment, *L. wollei* and sand samples were measured for *E. coli* and enterococci using an altered elutriation method described by Whitman et al. (2003), while water samples were processed daily using traditional IDEXX QuantiTray-2000 methods (IDEXX, Westbrook, Maine). After homogenization of *L. wollei* as previously described, 1g portions of *L. wollei* were removed from the microcosm and placed in sterilized Nalgene bottles with 100ml of phosphate buffered water (pH 6.8). The bottles were mechanically shaken for five minutes and allowed to settle for one minute. The supernatant was used for *E. coli* and enterococci enumeration using the IDEXX Colilert and Enterolert in QuantiTray-2000 methods (IDEXX, Westbrook, Maine). This same process was repeated for sand, using 1g of sand added to 100ml of phosphate buffered water and measuring *E. coli* and enterococci from the supernatant using IDEXX methods. To quantify the daily bacteria levels suspended in the water column, 100ml samples of water were obtained from each microcosm for *E. coli* and enterococci using a plastic 60ml syringe cleaned with 90% ethanol. These samples were processed using the standard IDEXX Colilert and Enterolert QuantiTray-2000 methods (IDEXX, Westbrook, Maine). For both experiments, *E. coli* and enterococci were measured every 24 hours for 3 days. Additionally measurements of dissolved oxygen, temperature, pH, and conductivity were also taken at the time of water sample collection from each microcosm.

Data Analysis

The data, including log-transformed data, lacked normality and heterogeneity of variance among treatments; Therefore, non-parametric Friedman test was performed on log transformed data to test for the main effect of time across all treatments for all days. When time had a significant main effect, between-day comparisons were made using the Wilcoxon-Signed Rank Test with a Bonferroni correction. A Kruskal-Wallis H Test with a post-hoc Mann-Whitney U Test including a Bonferroni correction was used to determine the differences between treatments of each day with respect to the dependent variable, *E. coli* or enterococci levels. All data was analyzed using SPSS.

Field Experiment

A seven day field mesocosm experiment was conducted from August 7th through August 13th 2013 on Lake St. Clair Metropark Beach to quantify the transfer of *E. coli* and enterococci from mats of *L. wollei* to the underlying sand. This experiment was set up by inserting square mesocosms constructed of Plexiglas (30 x 30 x 30cm) with a 200micron mesh bottom and open top into the beach sand, approximately six meters from the shoreline, at an approximate depth of 15cm and spaced approximately 30cm apart from each other and testing various combinations of treatments of sand and *L. wollei* (Figure 2). Each mesocosm contained approximately 3,500g of autoclave sterilized sand, which had a depth of approximately 2cm inside the mesocosm (Figure 2). The sterilized sand was initially obtained from Lake Saint Clair Metropark beach, brought back to the lab, autoclaved, then measured out into the 3,500g increments and stored in Ziploc bags for no more than 1 week prior to the experiment. Treatments were randomly assigned between 4 treatments; 1) *L. wollei* and deionized (DI) water, 2) *L.*

wollei and site water, 3) site water, and 4) a control of DI water, resulting in a 2x2 factorial design. Fresh *L. wollei* was obtained from the wash zone on the day the experiment was set up, day zero, and placed in a large autoclave sterilized container from which subsamples were taken and excess water was removed by pressing, with clean gloved hands, the *Lyngbya* into a sterilized metal strainer. A volume of 1,500 ml (avg wet weight 901.42g) of *L. wollei* was placed into assigned treatment mesocosms, this was enough to completely cover the sand in a thin (approximately 2cm) layer. All mesocosms were watered daily after sampling, depending on the assigned treatment, with 500ml of either DI water or site water from Lake St. Clair obtained that day from knee depth water. This amount of water was sufficient to moisten the overlying *Lyngbya* and simulate a daily light rain event.

Each mesocosm was subdivided into 8 equal grid sections (Figure 2) and each day a small (approximately 5ml volume) sample of sand was taken at random from a grid cell within each mesocosm, care was taken not to disturb adjacent grid cells. In the mesocosms that contained *L. wollei*, the *Lyngbya* was carefully scraped aside with forceps so as not to disturb the underlying sand and the sand was collected using sterile disposable wooden tongue depressors and stored in sterile 15ml falcon tubes or Ziploc bags. The *Lyngbya* was then carefully placed back over the remaining sand in that transect. Sand samples were taken back to the laboratory in a cooler on ice and processed within 2 hours. Enumeration of *E. coli* and enterococci followed the same methods as previously stated for the laboratory microcosm experiment. On day zero samples of *L. wollei*, autoclaved sand, site water, and DI water were tested for *E. coli* and enterococci. Each day samples of site water were collected at knee depth and

assessed for *E. coli* and enterococci and measurements of dissolved oxygen, temperature, pH, and conductivity were taken using a multi-parameter meter, which included an optical oxygen probe (YSI: 556 MPS).

Data Analysis

A Two-Way Repeated Measures ANOVA was performed to test the main effect of time across all treatments. Data was log transformed to meet the assumptions of normality and heterogeneity of variances among treatments. Mauchly's Test of Sphericity was used to confirm that data met the assumptions of ANOVA. Differences among treatments within each day were determined using a 2x2 Factorial ANOVA with a Bonferroni adjustment. Data between treatments for day five were analyzed using non-parametric tests, Kurskal-Wallis and Mann-Whitney U tests with a Bonferroni adjustment, as data did not meet assumptions of normality and heterogeneity of variances among treatments. Data analysis was performed using SPSS.

RESULTS

Laboratory Microcosm Experiments

During the course of the experiment there was little variability in the water quality parameters (Table 1). For example water temperature ranged between 23.08°C and 24.74°C. The greatest variation among the parameters was in dissolved oxygen (DO). In the treatments with *L. wollei* the average DO level was 5.7 ± 0.48 sd mg/L compared to readings around 8.3 ± 0.08 sd mg/L in treatments without *L. wollei* present (Table 1). *Escherichia coli* levels differed significantly across the three day experiment ($\chi^2(2, N = 5) = 13.739$, $p = 0.001$). During all time points monitiored, sterilized sand did not transfer *E. coli* or enterococci to the overlying water ($U > 7.500$, $p > .050$; Figure 3).

After 24 hours, treatments that contained *L. wollei* had significantly higher levels of *E. coli* compared to the control ($U = 0.000$, $p = 0.005$ for both *Lyngbya* only and sterilized sand plus *Lyngbya* treatments; Figure 3). Only the *L. wollei* plus sterilized sand treatment exceeded the Michigan water quality standard (WQS) of 300 *E. coli*/100ml after 24 hours (Figure 3). By 48 hours the levels of *E. coli* present in the water column in the treatments with *L. wollei* dropped to levels similar to the control and autoclaved sand ($U = 2.500$, $p > 0.008$ for both *Lyngbya* only and sterilized sand plus *Lyngbya* treatments; Figure 3).

The trend for enterococci was similar to that of *E. coli* for all treatments throughout the experiment. The sterilized sand was comparable in enterococci levels compared to the control throughout the experiment ($U = 9.500$, $p > 0.050$), and after the first 24 hours, enterococci levels were significantly higher in both treatments containing *Lyngbya* compared to the control ($U = 0.000$, $p = 0.007$ for both *Lyngbya* only and

sterilized sand plus *Lyngbya* treatments; Figure 3). After 48 hours the average levels of enterococci dropped to barely detectible numbers for both treatments containing *L. wollei*, and they were no longer significantly different from the control ($\chi^2(3, N = 5) = 0.979$, $p = 0.806$; Figure 3). Overall enterococci were present at much lower numbers than *E. coli* for all treatments.

The second laboratory microcosm experiment, which was designed to quantify the transfer of *E. coli* and enterococci from sand collected from beneath *Lyngbya* mats to the water column, had the highest *E. coli* levels in the first 24 hours in the water column for all treatments compared to the control ($U = 0.000$, $p = 0.008$ for underlying sand only, sterilized sand plus *Lyngbya*, and underlying sand plus *Lyngbya*; Figure 4). After 24 hours the *E. coli* levels dropped substantially, most notably in the two treatments with the underlying sand, but still were significantly higher than the control ($U = 0.000$, $p = 0.007$ for underlying sand only and underlying sand plus *Lyngbya*). The *L. wollei* plus underlying sand treatment dropped 153 MPN/100ml. *Escherichia coli* levels collected from the water column from the underlying sand treatment were significantly higher than the control ($U = 0.000$, $p = 0.007$) after 48 hours and was over five times lower and not significantly different from the control after 72 hours ($\chi^2(3, N = 5) = 5.219$, $p = 0.156$; Figure 4). *E. coli* levels were also significantly higher in both *L. wollei* treatments for the first 24 hours ($U = 0.000$, $p = 0.008$ for both sterilized sand plus *Lyngbya* and underlying sand plus *Lyngbya*; Figure 4). After 72 hours the levels of *E. coli* in both *L. wollei* treatments dropped such that they were no longer significantly different from the control ($\chi^2(3, N = 5) = 5.219$, $p = 0.156$), although they were detectible

(Figure 4). For the first 48 hours, *E. coli* levels were highest in treatments with underlying sand present (Figure 4).

Similar to *E. coli* trends, all treatments differed from the control with respect to enterococci levels for the first 24 hours ($U = 0.000$, $p = 0.005$ for underlying sand, sterilized sand plus *Lyngbya*, and underlying sand plus *Lyngbya* treatments; Figure 4). Unlike *E. coli* trends, the underlying sand did not have a significant impact on the transfer of enterococci to the water column after 48 hours ($U = 3.500$, $p > 0.008$; Figure 4). Also, unlike *E. coli* trends, the enterococci levels were highest in both treatments containing *Lyngbya*, which were significantly higher than the control after 48 hours ($U = 0.000$, $p = 0.008$ for both sterilized sand plus *Lyngbya* and underlying sand plus *Lyngbya*; Figure 4). Although by 72 hours the levels of enterococci in both treatments containing *L. wollei* had dropped more than 70 fold and were so low that only the treatment of sterilized sand plus *Lyngbya* was significantly different from the control ($U = 0.000$, $p = 0.005$; Figure 4).

Field Experiment

Some of the basic water quality parameters differed at the site over the seven day experiment (Table 2). The water temperature varied by a maximum of 5.32°C over the course of the experiment, while pH ranged from 8.3 to 9.3, and conductivity had a maximum difference of 58µS/cm between the maximum and minimum reading. The greatest variation among the parameters was in dissolved oxygen, which ranged from 8.65mg/L to 15.25mg/L (Table 2). Fecal indicator bacteria in Lake St. Clair site water varied greatly throughout the experiment (Table 2). *Escherichia coli* levels at their lowest was 28.5MPN/100ml and at their highest were 913.9MPN/100ml, while

enterococci experienced a difference of >2000MPN/100ml over the course of the experiment (Table 2). Initial levels of both *E. coli* and enterococci present in *L. wollei*, taken from day zero measurements for both experiments, were > 2419 MPN per gram in 100ml of phosphate buffered water.

There was no significant change of *E. coli* levels throughout the experiment within similar treatments ($F(1.832, 9.161) = 0.785$, $p = 0.474$; Figure 5). *Escherichia coli* levels were consistently significantly higher in the sands from all treatments containing *L. wollei* compared to the control ($p < 0.002$) and the sand that was watered with water from Lake St. Clair ($p < 0.001$; Figure 5). Neither the water from Lake St. Clair nor the DI water had a significant impact on *E. coli* levels in the sand ($p > 0.050$; Figure 5). *Escherichia coli* levels showed a slight decline for six days, but showed a slight increase immediately after rain events (Figure 5). Rainfall occurred on day six of the experiment, recorded as 2.11cm from the Mount Clemens Selfridge Field station (National Oceanic and Atmospheric Administration 2013), approximately 11 kilometers from the site.

Throughout the duration of the experiment enterococci levels in the sand remained higher when *L. wollei* was present compared to the control ($p < 0.030$; except the *Lyngbya* plus site water treatment on day seven, $p = 0.087$; Figure 5). As with *E. coli,* the treatment of site water collected from Lake St. Clair did not affect enterococci levels in the sand when compared to the control during this seven day experiment ($p > 0.050$; Figure 5). Unlike *E. coli*, enterococci levels were not significantly different in treatments with *Lyngbya* present when compared to the treatment of site water for days three and four ($p > 0.050$; Figure 5).

DISCUSSION

In 2010, Vijayavel et al. (2013) observed the first documented case of *L. wollei* in Lake St. Clair, although there were previous anecdotal sightings of possible *Lyngbya* mats in years prior by Lake St. Clair Metropark rangers (Vijayavel et al. 2013). Since then, seasonal mats of *Lyngbya* have been occurring along the public access beach throughout the swimming season. Vijayavel et al. (2013) also identified fecal indicator bacteria, including *E. coli* and enterococci associated with *Lyngbya* mats. Although there are daily efforts to remove freshly deposited *Lyngbya* mats during the beach use season (Muelle 2011), thus removing a significant portion of *E. coli* and enterococci from the beach, there still remains the possibility that the persistent deposition of *Lyngbya* along the shoreline can contaminate near shore waters.

Our results indicate that *L. wollei* can transfer *E. coli* and enterococci to the water column. The 200g wet weight of *L. wollei* added to 12L of Detroit River water for the microcosm experiments was about the size of a fist, and even this small of an amount transferred significantly measurable levels of *E. coli* and enterococci to the water column for both experiments after 24 hours (Figures 4 and 5). In some cases the levels of *E. coli* were even above the Michigan water quality standard of 300 *E. coli* per 100ml of water (Figures 4 and 5). The scale of the deposits of *L. wollei* along the shoreline is substantially larger, such that a bulldozer is used to remove deposits daily. It is possible that the reoccurring deposition of *L. wollei* along the shoreline can contribute enough fecal indicator bacteria to impact water quality and beach closures.

Cladophora mats have been shown to provide a favorable environment of ideal temperatures, nutrients, and protection from UV-B radiation to harbor potentially harmful pathogens (Byappanahalli et al. 2003, Whitman et al. 2003), and it is possible that mats of *Lyngbya* can act similarly. There have been previous studies that have shown the filamentous green alga *Cladophora* can both harbor fecal indicator bacteria (Whitman et al. 2003, Byappanahalli et al. 2003), and propagate the growth of these bacteria when dried algae is rehydrated (Whitman et al. 2003). Although *L. wollei* is a cyanobacterium, it has characteristics similar to *Cladophora* in that is also filamentous and benthic and can harbor *E. coli* and enterococci (Vijayavel et al. 2013). A laboratory microcosm study performed by Englebert et al. (2008) revealed that *E. coli* was able to survive up to 45 days in the water column in the presence of *Cladophora* mats. The rapid decline in *E. coli* and enterococci levels in our laboratory experiment is likely the result of the source water that we used, as it was likely limited in nutrients and unable to sustain such high levels of bacterial growth, which suggests it reached the carrying capacity provided by the limited nutrient sources and small amount of *Lyngbya* in the treatment.

A study performed on marine beaches in which wrack (decaying lacustrine and marine plants) containing fecal indicators, including *E. coli* and enterococci, was groomed from the beach over a season demonstrated that the physical act of grooming can even result in an increase of these microbes to the water column (Russell et al. 2014). Our results also demonstrate that the sand underlying *Lyngbya* mats can transfer *E. coli* and enterococci to the water column, which suggests that even daily beach grooming to remove *Lyngbya* may not be enough to prevent water contamination.

Our field experiment demonstrated that *Lyngbya* can transfer *E. coli* to the underlying sand over the seven day duration of the experiment; however, it is unclear how long this transfer can persist. Previous studies have shown that *E. coli* growth in sand is correlated to organic content contained within sand, and that continuous rewetting of dry sand can result in an increase in fecal indicator bacteria (Desmarais et al. 2002). Our study supports this work in that there was a significant increase in *E. coli* levels following a natural rain event. Furthermore, the act of beach grooming can physically introduce organic matter from *Lyngbya* mats into the sand, potentially increasing the organic content within beach sand and rewetting of this sand can increase fecal indicator growth.

Results from this study indicate that *E. coli* and enterococci can move among the various substrates from the *L. wollei* to the sand and water column. Although it was not the intent of the study to investigate the impacts or rain on the transfer of pathogens, *E. coli* and enterococci were detected in higher levels in the sand in treatments following a natural rain event. The mechanism is not clear as to the direction of movement as water could move both laterally or vertically through the sand, as the bottom of the mesocosm was not sealed to allow drainage and the natural movement of water. Although we applied small amounts of water to our treatments daily, it did not simulate storm events where a beach would likely experience lateral water flow beneath/through the sand and temporary changes in the water table during storm events. Although, the likely pathway of movement was through the rain rinsing the indicator bacteria into the sand, other pathways of movement cannot be ruled out. Regardless, the implications are the same, when a beach has deposits of *Lyngbya* on it the sand is likely

contaminated with fecal indicator bacteria, and likely other pathogens, above levels which are typically deemed safe and are higher than allowed in water samples by State Agencies. Likewise, following a rain event bacterial levels are likely to increase, as often observed in beach monitoring programs of water quality.

Beach closures are based on fecal indicator levels present in the water column and do not take into account the levels of these microbes in the sand. A study by Alm et al. (2003) revealed that *E. coli* levels ranged from 3-38 times higher in the top 20cm layer of beach sand compared to the water column at six different beaches. This study also showed a correlation between *E. coli* and enterococci levels in sand and adjacent water (Alm et al. 2003). People that utilize public beaches are typically exposed to both the water and sand, therefore, bacteria and pathogen concentrations in both mediums could have direct impacts on human health. To our knowledge our research is the first to evaluate the impact that *Lyngbya* has on transferring *E. coli* to the water column, and beach sand. Our research indicates that not only can *L. wollei* harbor and transfer *E. coli* and enterococci to the water column, but can transfer these fecal indicators and other potentially harmful pathogens to the sand, resulting in a risk for recreational beach users.

Conclusion

Although *L. wollei* has been reported as becoming increasingly common, and is considered a nuisance species that restricts recreation use of public beaches due to its prolific nature, ability to clog water intakes, toxin production, unaesthetic appearance, and offensive odor associated with the decaying shoreline mats (Speziale et al., 1988; Speziale and Dyck 1992), little research has been done on its capacity to harbor

potentially harmful bacteria (Vijayavel et al. 2013). This is the first study to document the movement of *E. coli* and enterococci from shore deposits of *L. wollei* mats to beach sand and the adjacent water column. Results from this study demonstrated that *L. wollei* and the underlying beach sands contain high levels of *E. coli* and enterococci, that can be indirectly transferred to the near shore water and beach sand even after removal of the *Lyngbya* mats. Recreational beach users may be exposed to *L. wollei* directly and the potential health risks associated from direct exposure to the cyanobacteria and potentially harmful pathogens that it harbors. Climate change is likely to result in increased water temperature of lakes and when combined with increased nutrient loading, blooms of cyanobacteria, such as *L. wollei*, are likely to increase in frequency (Carey et al. 2012). Further research in preventing the occurrence of *L. wollei* blooms and in implementing alternative beach management practices to ensure less exposure and transfer of microbes associated with *L. wollei* mats is needed to provide better protection for the public in areas where *L. wollei* remains a problem.

APPENDIX A

Tables

Table 1. Water quality parameters and fecal indicator density of laboratory microcosm experiments 1 and 2, and Detroit River water. Values are the means and standard deviations obtained from daily samples of microcosm treatments from 5 samples (n=5).

Laboratory Experiment 1		Characteristics				Fecal Indicators	
Day	Treatment	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Conductivity (µS/cm)	*E. coli* (MPN/100ml)	Enterococci (MPN/100ml)
1	Control	24.74 ± 0.4	8.37 ± 0.1	7.98 ± 0.1	239.4 ± 3.5	0	0.2 ± 0.4
	Sterilized sand	23.92 ± 0.2	8.21 ± 0.2	8.11 ± 0.1	244.8 ± 2.6	0.4 ± 0.5	0.6 ± 0.9
	Lyngbya	23.74 ± 0.3	5.09 ± 1.1	7.72 ± 0.1	289.4 ± 6.5	224.36 ± 81.9	46.3 ± 9.0
	Sterilized sand + *Lyngbya*	23.94 ± 0.1	5.73 ± 0.6	7.75 ± 0.1	301.6 ± 8.2	324.96 ± 79.2	39.72 ± 23.1
2	Control	24.06 ± 0.3	8.43 ± 0.1	8.14 ± 0.2	241.8 ± 4.7	0	0.8 ± 1.1
	Sterilized sand	23.52 ± 0.04	8.33 ± 0.1	8.21 ± 0.03	251 ± 2.9	0.4 ± 0.5	0.8 ± 1.8
	Lyngbya	23.72 ± 0.1	5.82 ± 0.4	7.92 ± 0.1	311.8 ± 6.6	7.32 ± 6.3	0.6 ± 0.9
	Sterilized sand + *Lyngbya*	23.44 ± 0.1	6.54 ± 0.8	7.93 ± 0.03	328.4 ± 6.9	2.6 ± 1.8	0.5 ± 0.6
3	Control	23.44 ± 0.7	8.34 ± 0.2	7.0 ± 0.3	253.8 ± 5.9	0	0.6 ± 0.5
	Sterilized sand	23.34 ± 0.3	8.28 ± 0.1	7.93 ± 0.3	261.8 ± 3.0	0	1 ± 1.7
	Lyngbya	23.6 ± 0.3	5.62 ± 0.9	8.03 ± 0.1	341.2 ± 8.3	9.6 ± 9.8	1.8 ± 1.6
	Sterilized sand + *Lyngbya*	23.62 ± 0.1	5.45 ± 0.9	8.08 ± 0.02	353.8 ± 5.4	3.1 ± 1.7	1.8 ± 2.2
Laboratory Experiment 2		**Characteristics**				**Fecal Indicators**	
Day	Treatment	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Conductivity (µS/cm)	*E. coli* (MPN/100ml)	Enterococci (MPN/100ml)
1	Control (Sterilized sand)	23.08 ± 0.4	8.52 ± 0.1	7.14 ± 0.2	235.2 ± 3.4	0.6 ± 0.5	0
	Underlying sand	22.32 ± 0.1	8.52 ± 0.1	7.85 ± 0.1	238.4 ± 3.2	127.16 ± 48.9	6.65 ± 3.4
	Sterilized sand + *Lyngbya*	23.44 ± 0.8	6.06 ± 0.5	8.01 ± 0.02	282.4 ± 3.9	64.34 ± 25.2	172.16 ± 179.3
	Underlying sand + *Lyngbya*	24.54 ± 0.2	5.86 ± 0.3	8.05 ± 0.01	285.2 ± 13.4	186.18 ± 96.7	140.88 ± 107.3
2	Control (Sterilized sand)	23.54 ± 0.6	8.25 ± 0.2	7.25 ± 0.2	239.6 ± 4.4	0.2 ± 0.4	0.4 ± 0.5
	Underlying sand	23.12 ± 0.1	8.31 ± 0.1	7.86 ± 0.1	237.8 ± 6.2	40.6 ± 17.3	7.9 ± 12.1
	Sterilized sand + *Lyngbya*	23.44 ± 0.1	6.57 ± 0.5	8.03 ± 0.03	290.6 ± 7.8	26.34 ± 31.4	40.6 ± 40.7
	Underlying sand + *Lyngbya*	23.66 ± 0.1	6.17 ± 0.4	8.07 ± 0.03	297.2 ± 12.4	32.64 ± 28.1	33.06 ± 31.5
3	Control (Sterilized sand)	24.58 ± 0.7	8.11 ± 0.2	7.18 ± 0.3	250.6 ± 9.1	0.2 ± 0.4	0
	Underlying sand	23.8 ± 0.1	8.04 ± 0.2	7.88 ± 0.2	248.0 ± 6.1	1.86 ± 2.6	0
	Sterilized sand + *Lyngbya*	24.08 ± 0.2	6.27 ± 0.3	8.12 ± 0.1	314.0 ± 6.0	0	4.18 ± 2.8
	Underlying sand + *Lyngbya*	24.52 ± 0.04	6.13 ± 0.4	8.22 ± 0.03	325.8 ± 10.4	0.4 ± 0.5	1.4 ± 1.1
Detroit River water		25.5	8.25	7.26	217	2.03 ± 1.8	1.7 ± 2.1

Table 2. Water quality parameters and fecal indicator density of Lake St. Clair site water used on each corresponding day to water treatments designated “site water”.

	Characteristics				Fecal Indicators	
Day	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Conductivity (µS/cm)	*E. coli* (MPN/100ml)	Enterococci (MPN/100ml)
0	22.39	8.65	8.3	427	274.2	>2419.6
1	25	9.97	8.85	482	40.2	343
2	25.48	15.25	9.33	457	231	461.1
3	27.59	13.15	9.21	485	61.3	65.3
4	25.77	13.46	9.29	453	28.5	25.5
5	23.07	8.78	8.78	457	33.6	33.3
6	22.27	10.66	9.01	435	913.9	>2419.6
7	23.21	12.27	9.24	429	866.4	>2419.6

Figures

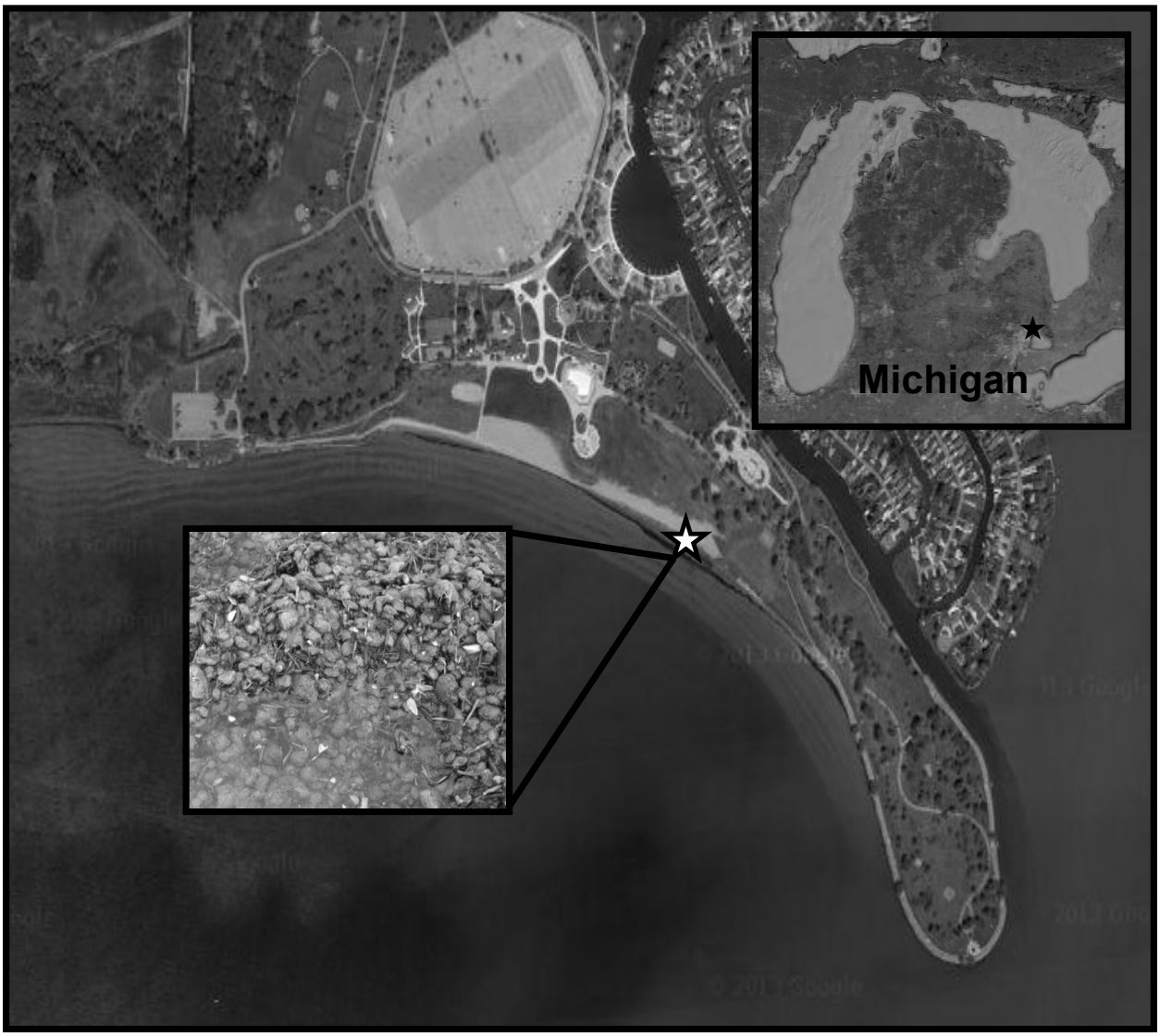

Figure 1. Location of sampling sites at Lake St. Clair Metropark Beach.

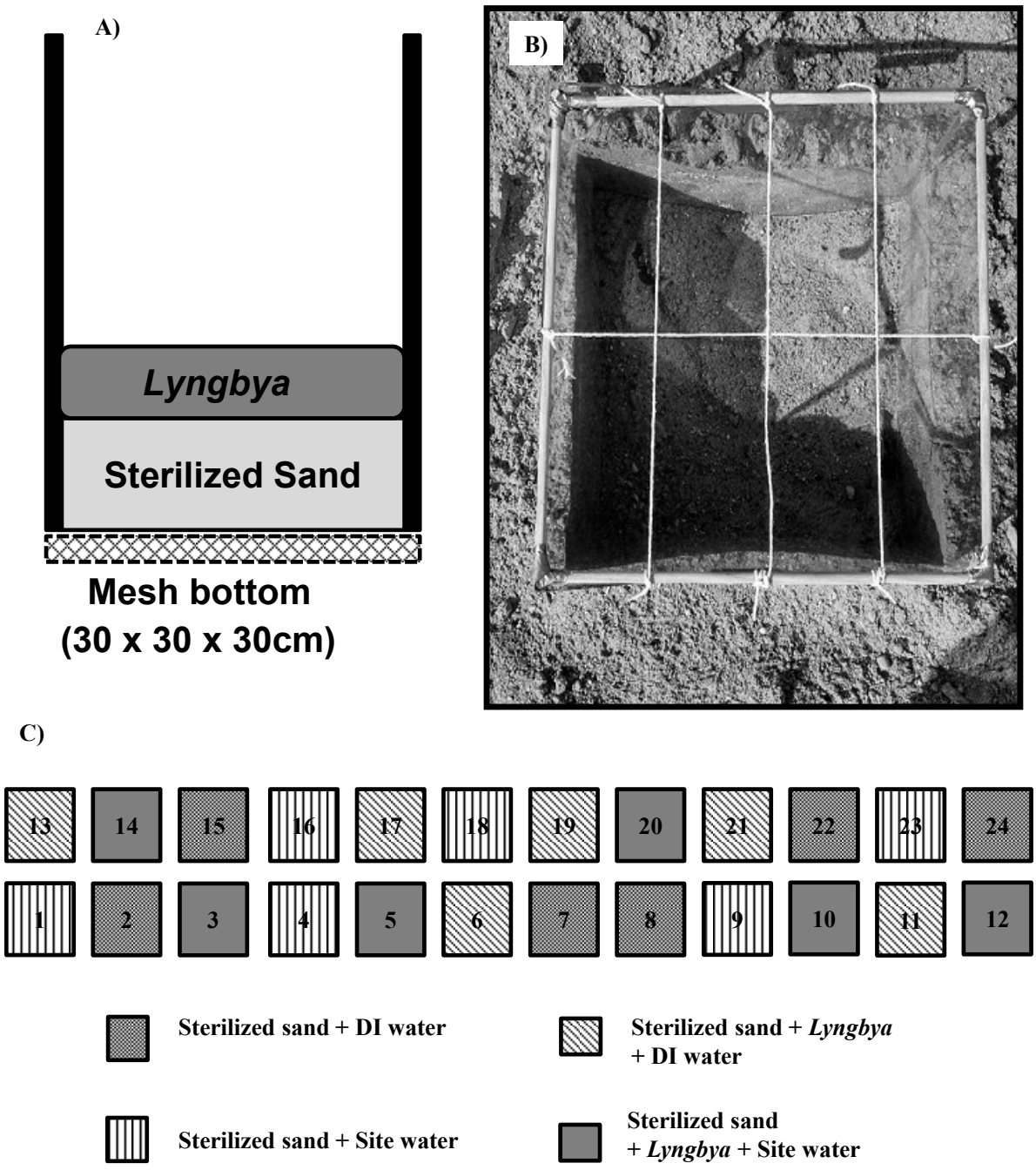

Figure 2. Depiction of a field mesocosm designed to quantify the transfer of *E. coli* and enterococci from mats of *Lyngbya wollei* to the underlying sand. A) Cartoon of a cross-section of the mesocosm. B) Picture of the mesocosm including the sampling grid. C) Mesocosm treatment set-up 1) Sterilized sand + DI water, 2) Sterilized sand + Site water from Lake St. Clair, 3) Sterilized sand + *Lyngbya* + DI water, 4) Sterilized sand + *Lyngbya* + Site water from Lake St. Clair.

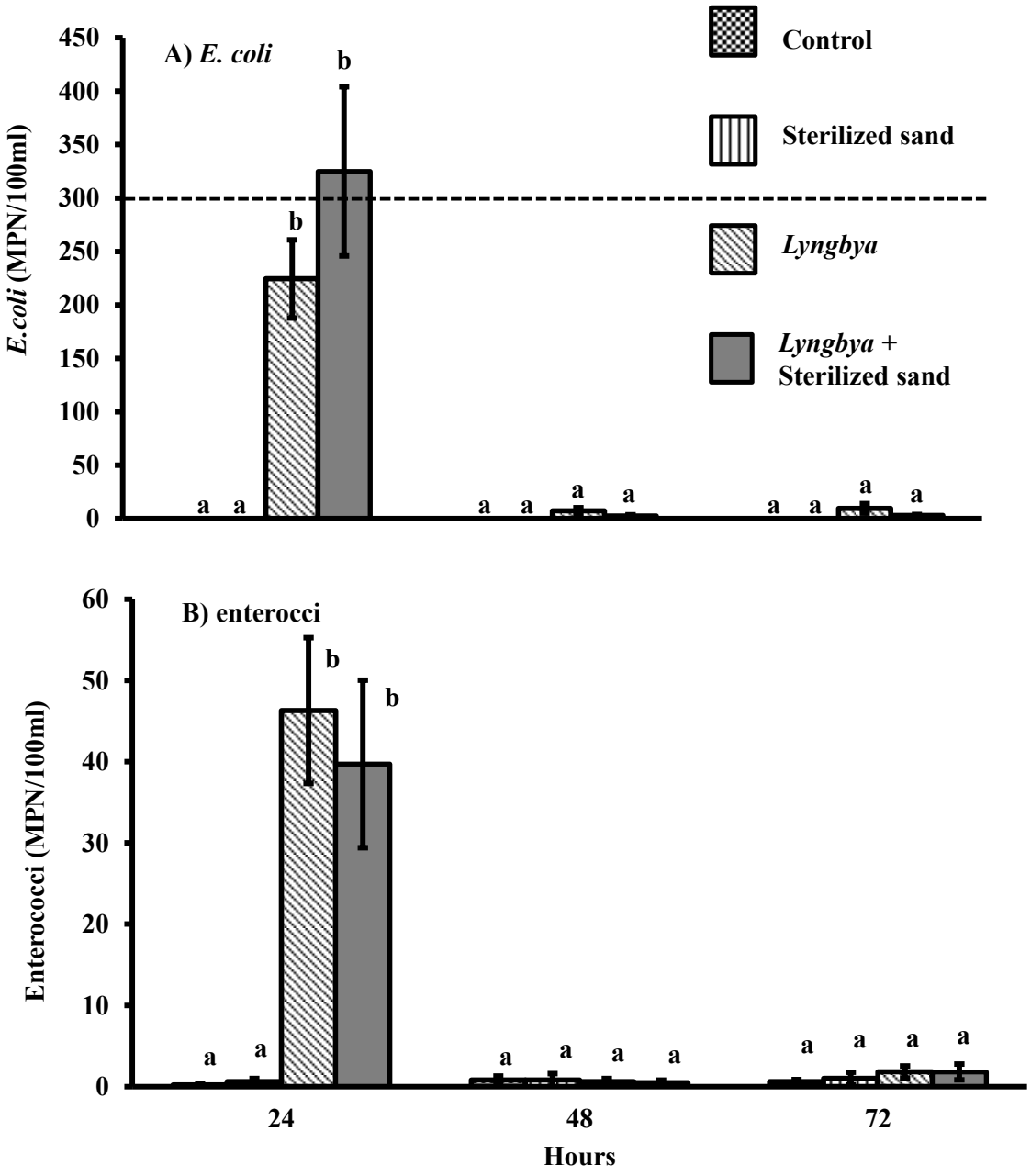

Figure 3. Mean most probable number (MPN) per 100ml (± 1 Standard Error) of water per day of A) *Escherichia coli* and B) Enterococci in the water column from microcosm treatments. The dashed line in panel (A) represents the Michigan water quality standard of 300 *E. coli* per 100ml water for beach closures.

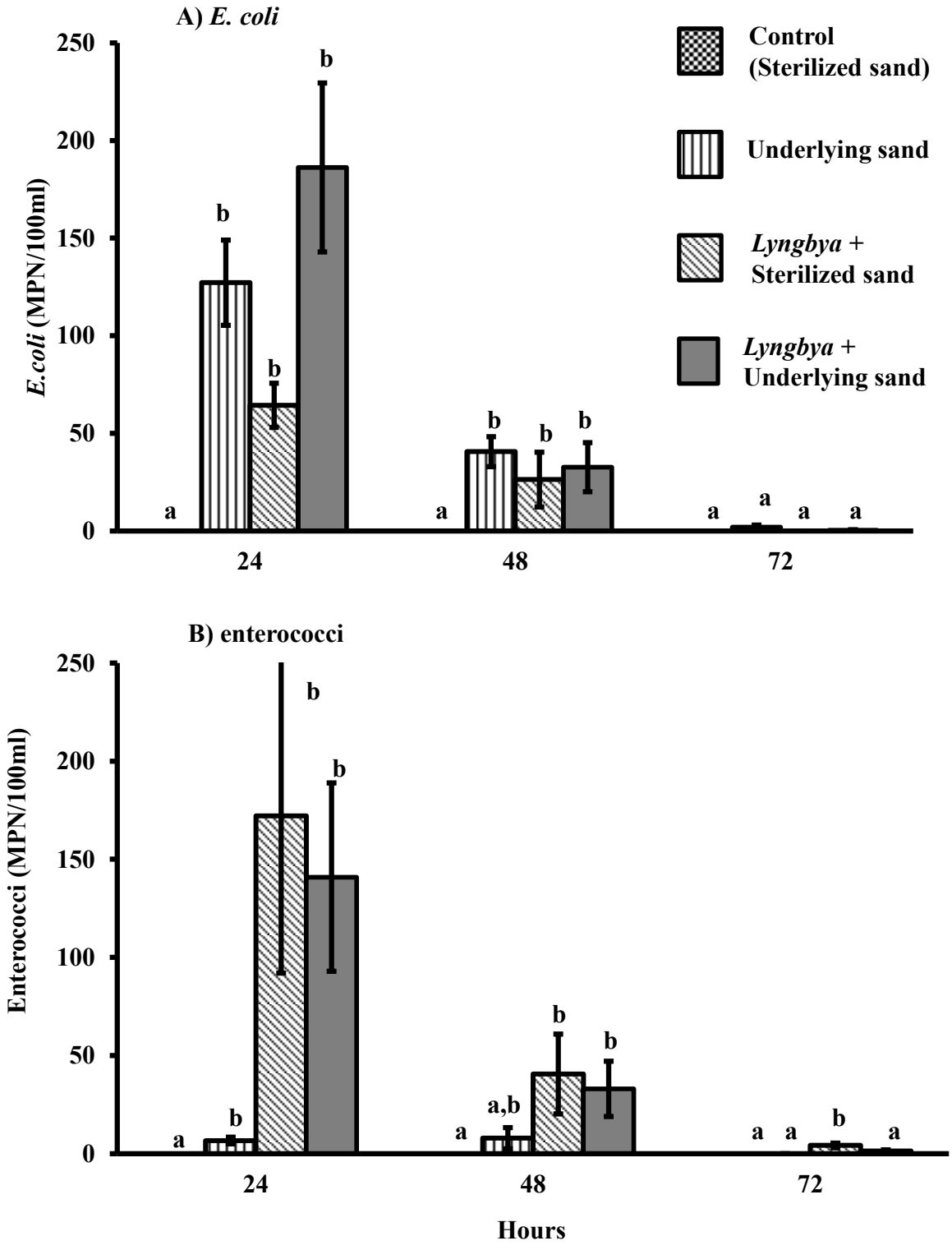

Figure 4. Mean most probable number (MPN) per 100ml (± 1 Standard Error) of water per day of A) *Escherichia coli* and B) Enterococci in the water column from microcosm treatments.

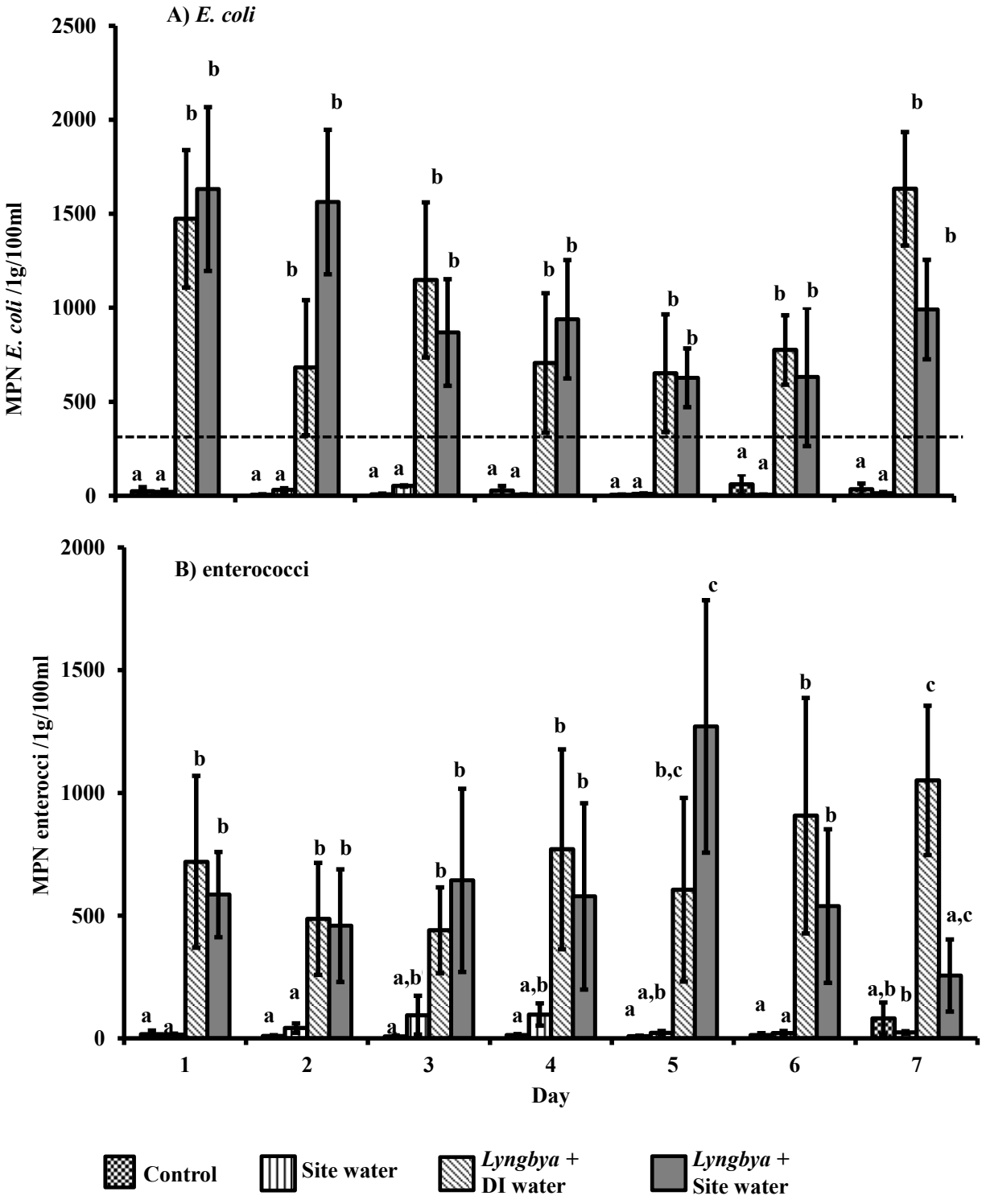

Figure 5. Mean most probable number (MPN) of 1g of sand per day in 100ml (± 1 Standard Error) of phosphate buffered water among various treatments A) *Escherichia coli* and B) Enterococci in the water column from field microcosm treatments.

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ABSTRACT

THE MOVEMENT OF *ESCHERICHIA COLI* AND ENTEROCOCCI AMONG BEACH SAND, *LYNGBYA WOLLEI*, AND THE WATER COLUMN: IMPLICATIONS FOR HUMAN HEALTH

by

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May 2014

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Major: Biological Sciences

Degree: Master of Science

Occurrence of the filamentous cyanobacteria *Lyngbya wollei* has become an increasing concern in the Great Lakes region. Prior to the early 1900's, *L. wollei* had been occasionally documented in the Great Lakes region, but in the last decade it has been observed with increasing frequency. In the Great Lakes *L. wollei* has been proliferating, fragmenting, and accumulating ashore, where it decays forming thick detrital mats harboring potentially harmful bacteria such as *Escherichia coli* and enterococci. While the filamentous green algae species *Cladophora glomerata* has been well studied in this region, very little research has been done on the cyanobacteria *L. wollei* and the potential for it to harbor and transfer fecal microbes to the water column and underlying sand on beaches. Complementary laboratory and field experiments were performed to quantify the transfer of *E. coli* and enterococci to the water column from *L. wollei* mats and to the beach sand underlying the mats. Laboratory microcosm experiments demonstrated significant concentrations of *E. coli* (p

= 0.005) and enterococci (p = 0.007) were transferred to the water column from *L. wollei* mats, and from the sand collected immediately beneath the mats (p= 0.008 for *E. coli* and p = 0.005 for enterococci) after a 24 hour exposure. Field mesocosm experiments conducted for one week on a publicly accessible beach showed that *L. wollei* mats can persistently transfer *E. coli* ($p < 0.005$) and enterococci ($p < 0.008$ for the first five days) to underlying beach sand. Our results indicate that surface waters as well as the sand on public beaches can be negatively impacted by harmful bacteria where decaying mats of *L. wollei* persist along the shoreline, resulting in potential human health risks.

AUTOBIOGRAPHICAL STATEMENT

As a child I spent my summer vacations at my grandparents' farm in the upper peninsula of Michigan, which has instilled me with an insatiable curiosity for the natural world. I have always been interested in the biological sciences, and in 2010 I earned my Bachelor's degree in this field from Wayne State University. While finishing up my undergraduate course work, I enrolled in an ecology course and wanting to learn more about research in this field I followed up as a directed study to work in Dr. Donna Kashian's laboratory. Ecology research has given me to opportunity to learn even more about the natural world around me and has given me the opportunity to ask my own questions and discover the answers to those questions about the environment around me. Having the opportunity to explore various topics within this broad field of science has led me to an unexpected passion for aquatic macroinvertebrates and their role in aquatic ecosystems. In the future I hope to pursue a PhD in which I can further indulge myself in the curiosities of various freshwater ecosystems and the significant impacts that aquatic macroinvertebrates have on the world around us.