TERRELL'S ISLAND: A CASE STUDY IN BIOLOGICAL AND CHEMICAL EFFECTS OF WATERFOWL ON FRESHWATER ENCLOSURES

by

Asbah Z. Hadi

Although American White Pelicans (*Pelecanus erythrorhynchos*) (AWP) typically reside in the western plains of North America, within the last 30 years they have migrated to unexpected locations, perhaps due to climate change. One area of Wisconsin that has encountered unexpectedly high numbers of AWP (and other waterfowl) is Terrell's Island on Lake Butte des Morts, estimated to have over 1,500 nesting AWP. The presence of pelicans and other waterfowl is likely to have a significant, negative impact on recreational water quality in this area.

Water samples were collected twice weekly from two sites around Terrell's Island on Lake Butte des Morts from inside the breakwall, directly adjacent to nesting islands, and from a "control" area in Shubert Marsh, away from pelican populations. A total of 24 samples were collected throughout the summer of 2012. Fecal indicator bacteria (FIB) were measured from both locations using the Colilert® and Enterolert® assays (for *E. coli* and enterococci, respectively). Each sample type was also filtered through nitrocellulose and polycarbonate filters for further microbial testing. Nitrocellulose filters were plated in triplicate onto various selective media to qualitatively identify potential pathogens (*Salmonella, Campylobacter*). Each set of polycarbonate filters was frozen at -80°C to enumerate *C. jejuni* using quantitative PCR (qPCR). Equal numbers of samples were collected for both culture-based enumeration as well as molecular-based enumeration.

Average culturable FIB concentrations were notably greater in waters adjacent to pelican nesting areas (*E. coli*: 480 vs. 76 MPN/100 ml, enterococci: 637 vs. 290 MPN/100 ml). Fecal pathogens (*Salmonella, Campylobacter*) were undetectable in water samples by traditional culture methods and by quantitative PCR (qPCR).

On average, chlorophyll *a* concentrations were also considerably higher in the breakwall area compared to chlorophyll *a* concentrations in Shubert Marsh (119.83 vs. 92.74 μg/L). In summary, AWP have a negative impact on water quality in Lake Butte des Morts and may impair recreational water quality at other locations with high waterfowl densities.

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10/23/13
Date Approved

DEDICATION

To my husband, mother, father and brothers who have supported me throughout this journey.

BIOGRAPHY

Asbah Z. Hadi was born on May 18, 1987 in Hyderabad, India and grew up in the United States. She joined the undergraduate program in Microbiology at the University of Minnesota-Twin Cities in September 2005 and received the Bachelors of Science in Microbiology in August 2009. She worked for two years as a microbiologist in industry as well as academia before joining the Master's program at the University of Wisconsin-Oshkosh in September 2011.

Hadi's research interests include environmental microbiology (i.e. within water quality) as well as microbial disease causing bacteria.

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CHAPTER 1. INTRODUCTION

1.1 Terrell's Island Wetland Restoration Area

Recreational water activity is a favorite pastime for many, including residents and tourists of Wisconsin. Water recreation is a major source of income for Wisconsin, making up a significant portion of the tax base, as well as the $12.3 billion tourism industry (Asplund *et al.* 2012). Good water quality adds to the enjoyment of these activities and recreational water users often see water as the most important feature of their chosen setting (Zube *et al*. 1975). The amount of land/water edge and surface water present in a setting are positively correlated to an increase in the scenic value of an area (Zube *et al.* 1975). Not only does clean water add to the aesthetic enjoyment, but it also prevents illness in recreational water users.

Terrell's Island Wetland Restoration Area (TIWRA) is a 1,200 acre property, located on Lake Butte des Morts, near the Fox River (Figure 1). It is one of Wisconsin's many nature preserve destinations, acquired by the Lake Butte des Morts Conservation Club (LBDMCC). Originally, the property was an island and it wasn't until the 1960s that it was finally connected to the surrounding land via a road.

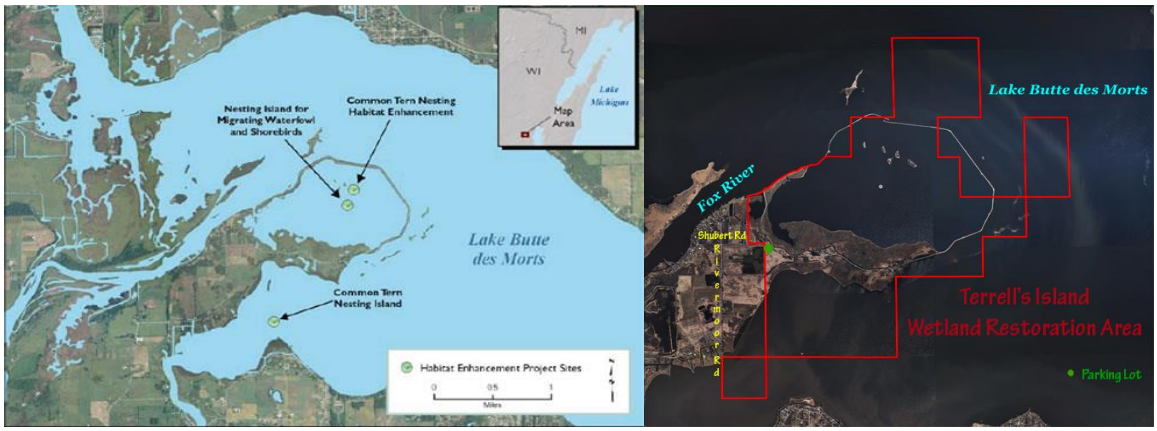


Figure 1 Terrell's Island Wetland Restoration Area in comparison to the state of Wisconsin (BDMCC 2013, FRNRTC 2013)

Beginning in the late 1950's and into the 1960s, the first breakwall was built at TIWRA where the Fox River enters Lake Butte des Morts. This was meant to improve the land and prevent soil erosion. The project was completed with the help of the Wisconsin Department of Natural Resources (WI DNR), along with Winnebago County and the Terrell's Island Hunting Club and the project cost was an estimated $10,000 (LBDMCC website 2013).

When LBDMCC bought the TIWRA property in 1998, it was at the same time the WI DNR began construction of the present breakwall at TIWRA. This breakwall was completed in 1999. No birds were observed to have nested at TIWRA prior to the construction of the breakwall (Personal Communication with A. Sabai 2013). However, in the early 2000s, high numbers of waterfowl, especially the American White Pelican (*Pelecanus erythrorhynchos*) (AWP) have been observed at TIWRA (LBDMCC website 2013).

1.2 Global Climate Change and its Impact on Waterfowl Migration

Global climate change has impacted weather patterns in many areas, altering precipitation, raising sea levels, changing season timing and lengths, and changing frequency and intensity of weather patterns (IPCC 2012). In part, due to these changes, North American wetland and waterfowl have greatly been impacted (Cox 2010). Low water levels in North American lakes have affected migration patterns of waterfowl, such as the AWP, which typically reside in the western plains of North America (Gibbons and Withers 2006). As a result, these birds are now nesting in unexpected areas, such as Wisconsin and other parts of the upper Midwest (Figure 2). One particular area of Wisconsin that has had a surprisingly high number of waterfowl is at the TIWRA.

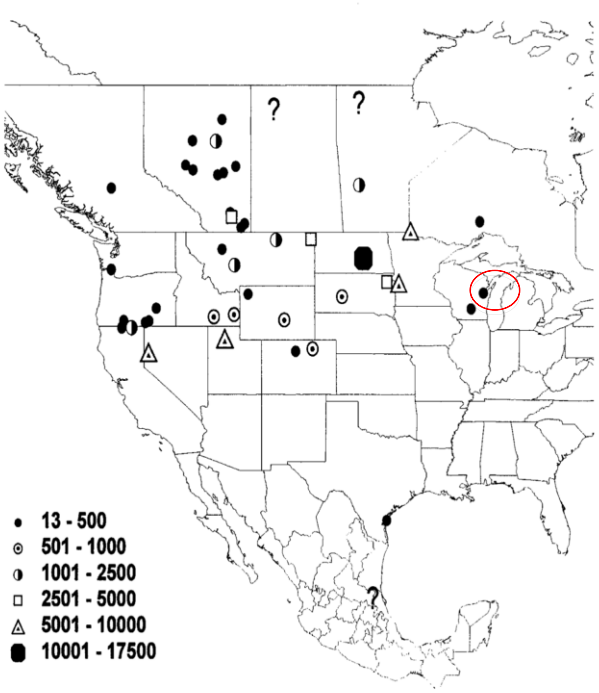


Figure 2 American White Pelican breeding colonies and their relative sizes from the years 1998-2001 (Anderson and King 2005)

1.3 Fecal Indicator and Pathogenic Bacteria in Waterfowl Feces

Contact with small amounts avian fecal matter has minimal impact on human health. High numbers of waterfowl, when concentrated in a specific area, however, create a potential public health hazard (Girdwood *et al*. 1985). Waterfowl feces can contain pathogens such as *Campylobacter spp.* and *Salmonella spp*., causing Campylobacteriosis and Salmonellosis, respectively (Bolton *et al*. 1999, Wilson *et al*. 2008). People can become infected from contaminated recreational water. Hence, it is important to study the impact waterfowl have on recreational water quality (Pond *et al*. 2005).

One of the two pathogenic bacteria that this study focused on was *Campylobacter jejuni*. The genus *Campylobacter* includes Gram-negative bacteria that are spiral, with a corkscrew appearance, which are microaerophilic. These organisms are motile, with either unipolar or bipolar flagella (Ryan and Ray 2004). *Campylobacter jejuni* is one of the common causes of bacterial foodborne illness (Campylobacteriosis) in developed countries and a number of species of *Campylobacter* have been associated with disease in humans. Campylobacteriosis causes symptoms of gastrointestinal inflammation, including bloody diarrhea or dysentery syndrome (CDC 2013). People become infected via consuming contaminated food or water, eating raw meat, or via the fecal-oral route (USEPA 2000).

The other pathogen that this project studied was *Salmonella enterica* due to its role in causing gastroenteritis. The genus *Salmonella* is rod shaped, Gram-negative, non-spore forming, primarily motile bacteria of the family *Enterobacteriaceae* (Ryan and Ray 2004). Salmonellosis, the disease caused by *Salmonella* species, can result from

consuming contaminated food or water or transferred between humans and other animals. Healthy adults are mildly affected while infants and young children are more susceptible to even a small dose of the bacteria (via contaminated food or water) (CDC 2012).

Wisconsin has 15,000 lakes, approximately 88,000 stream miles, 1,000 miles of Great Lakes shoreline, 5 million acres of wetland and 1.2 quadrillion gallons of groundwater (Asplund *et al*. 2012). With such extraordinary amounts of water resources, it is important to sufficiently care for them and this is done by regularly assessing water quality in order to maintain or treat the present water. Maintaining water quality becomes especially important due to runoff from various sources (human, wildlife, etc.).

There are numerous ways water quality can be evaluated. Testing methods include secchi disk measurement for water clarity, testing chlorophyll *a* concentration in water for estimation of algal growth, phosphorus concentrations in water and sediment, and measuring concentrations of fecal indicator bacteria (FIB).

Since fecal pollution is one of the most common source of surface water pollution, it was imperative to this study to measure FIB concentrations. Hence, FIB testing was one of the ways water quality was measured in this study to test for the presence of feces (likely avian) in water. Enumerating FIB assesses microbial contamination of water in an area or selected sample. FIB indicate fecal contamination and potential presence of waterborne pathogens, but do not necessarily equate to disease (Myers *et al*. 2007). Avian feces can contain FIB, some of which are pathogens (Abulreesh *et al*. 2006), which can be potentially harmful for recreational water users. FIB concentration limits are present for a person to safely engage in full-body contact

recreational activities and consumption (Myers *et al*. 2007). Approximately 235 *E. coli* per 100 ml of water and 35 enterococci per 100 ml are known to cause swimming-associated gastrointestinal illnesses per 1000 full body contact swimmers (Dufour 1984).

Warm-blooded animals, including waterfowl, carry a variety of intestinal microbes (viruses, bacteria, and/or protozoa) that are pathogenic to humans (Myers *et al*. 2007). When fecal matter is passed through their system into their surroundings, it is then that humans can come in contact with these pathogens. Examples of bacterial pathogens that cause disease include those of the genus *Bacteroides*, *Salmonella*, *Shigella*, and *Vibrio*. With significant exposure, each can cause gastroenteritis, dysentery, typhoid fever or cholera in humans (Myers *et al*. 2007).

Common FIB in warm-blooded animals include *E. coli*, enterocci, *Bacteroides spp.*, and streptococci. In order to test for these FIB, bacteriological tests are employed to measure the sanitary quality of water and sediments. This allows for rapid identification of indicator organisms and public health risks, such as gastrointestinal pathogens. FIB used for these purposes are ranked for suitability, according to the following list of criteria (Myers *et al*. 2007):

Preferred FIB:

- Is easily tested for
- Is of human or animal origin
- Survives equally as long, or perhaps longer than pathogens
- Present at densities equivalent with fecal contamination
- Can be used as a proxy for a variety of different pathogens
- Is equally viable in both freshwater and marine environments

When officially testing for fecal contamination, the types of FIB used depend on regulations that apply to the type of water being tested (Table 1).

Table 1 Fecal indicator bacteria used to assess fecal contamination (Myers *et al*. 2007).

Type of water	Description of water type and its use	Federally required indicator bacteria
Ambient water	Any water body encountered in the environment, regardless of use designation.	(Depends on use.)
Recreational water	Water bodies where people engage in, or are likely to engage in, activities that could lead to ingestion of the water or immersion in the water. Recreational water is designated as such in State and Tribal water-quality standards.	Enterococci and *E. coli* — required for ocean and Great Lakes beaches (coastal waters). Requirements for inland beaches are subject to State regulations.
Shellfish-growing water	Any site that supports or could support the propagation and harvesting of shellstock (molluscan shellfish, such as oysters, clams, mussels, and scallops) in the natural environment or at fish farms.	Total coliform and fecal coliform.
Potable (drinking) water	A water supply that meets the requirements of the Safe Drinking Water Act, as administered by the U.S. Environmental Protection Agency and any applicable State or local jurisdictions.	Total coliform. Detection requires follow-up testing for fecal coliform and *E. coli*.
Treated drinking water	Potable water from a public water supply that has been treated by physical or chemical means to improve water quality.	The U.S. Environmental Protection Agency Ground Water Rule for public supply systems includes testing for total coliform, *E. coli*, enterococci, and coliphage viruses.
Public water system	A water system that serves 25 or more people or that has 15 or more service connections and operates at least 60 days per year.	

E. coli, enterococci, *Campylobacter spp.*, and *Salmonella spp.* were selected to use in this study and were detected using the culture based methods Colilert® and Enterolert®, as well as selective microbiological media. *E. coli* and enterococci are the most common FIB used to measure sanitary quality of recreational waters because both can be used to predict swimming-associated gastroenteritis and are stable in freshwater and marine environments (Myers *et al*. 2007).

Numerous studies have shown that FIB are able to persist in the environment, even without a host (Byappanahalli and Fujioka 1998, Byappanahalli *et al*. 2006, Davies *et al*. 1995). On average, enterococci have a longer half life (in hours) than coliform bacteria (e.g. *E. coli*) and *Salmonella spp*. Enterococci have, on average, a half life of 22 h while coliform bacteria, on average, have a half life of 17 hours, which is similar to *Salmonella spp*. average of 17.06 hours (McFeters *et al*. 1974).

In addition to culture-based methods, quantitative PCR (qPCR) was used, but specifically to quantify the potential fecal pathogen, *Campylobacter jejuni*. This was used as a more sensitive method to tabulate the present pathogenic bacteria. Quantitative PCR is a more sensitive method than culture-based methods due to its ability to target specific genes or DNA sequences. Hence, qPCR can detect subtle differences in various bacteria, providing accurate results.

1.4 Analyzing Phosphorus Content in Water and Sediment at TIWRA

Phosphorus is one of Wisconsin's most common pollutants. Even small increases in phosphorus to Wisconsin surface waters can substantially increase aquatic plant growth, toxic algae booms, as well as algae (*Cladophora*). This plant growth results in decreased levels of oxygen, which is needed for healthy aquatic ecosystems (Asplund *et al*. 2012).

Nutrients, including carbon, nitrates and phosphates, are necessary nutrients for plants and healthy surface waters (USEPA 2000). They are also the molecules that can lead to eutrophication of an aquatic system, especially since they are abundant in human derived products (fertilizers, detergents, cleaners, soaps, shampoos) as well as fecal sources. Avian fecal matter contains nutrients, of which, carbon, nitrogen and phosphorus are of the most concern (Manny *et al*. 1994).

Although both nitrate and phosphate contribute to eutrophication, phosphorus is the main concern. Estimating phosphorus-loading rates is a good indicator of lake productivity (Portnoy *et al*. 1989), which is limited by phosphorus (Brierly *et al*. 1975). Nutrient enrichment encourages aquatic plant growth, reduces water quality and contributes to increased sedimentation in lakes and reservoirs (NAS 1969).

A study by Brierly *et al.* (1975) on waterfowl refuge effects on bacterial water quality in the Bosque Refuge, in southern New Mexico, suggests that an increased number of waterfowl also has an impact on average concentration of suspended bacterial populations. The study concludes that bacterial populations were larger in times of ample nutrient availability and higher temperature. The lowest bacterial populations were in the winter months of December-January and the highest in were in the mid-summer months

of July-August. Hence, temperature and nutrient availability (i.e. phosphorus) can be monitored in order to determine overall water quality of a system.

The purpose of this study was to investigate the impact that waterfowl have on the breakwall enclosed water reservoir at TIWRA (the 'breakwall'). The WI DNR intended this area to be a restoration site that would improve water quality overall at Lake Butte des Morts (Addis *et al*. 1993). When this study began in 2012, the water was visibly turbid with high numbers of waterfowl loafing, feeding and nesting in the breakwall and its adjacent islands. Based on these observations, it has been hypothesized that waterfowl may have a negative impact on the water quality at TIWRA, increasing fecal indicator and fecal pathogenic bacterial concentrations, making it unsafe for recreation.

1.5 Objectives for this Study

The primary objectives for this study include the following:

1. To study high populations of birds at TIWRA, in particular AWP, in order to determine if they have an impact on the fecal indicator bacteria *E. coli* and enterococci, in the water inside the breakwall.
2. To determine if AWP are releasing *significant* amounts of pathogenic bacteria, (*Campylobacter spp.* and *Salmonella spp.*) via their feces, making recreational water hazardous.
3. To apply a qPCR protocol for determining *Campylobacter jejuni* in water samples.
4. To determine if a large AWP population is correlated with elevated phosphorous levels, resulting in high algal productivity.
5. To determine if chlorophyll *a* levels were elevated, especially with elevated levels of phosphorus.

CHAPTER 2. MATERIALS AND METHODS

2.1 Study Location Selection

Samples were collected from two locations within the Terrell's Island Wetland Restoration Area (TIWRA) located in Winnebago County on Lake Butte des Morts, WI (Figure 1). The test site was located within the breakwall (Figure 3). The square dots in Figure 3 refer to the two sampling sites in this study. The other locations, shown with arrows, in Figure 3 are small islands where various birds nest.

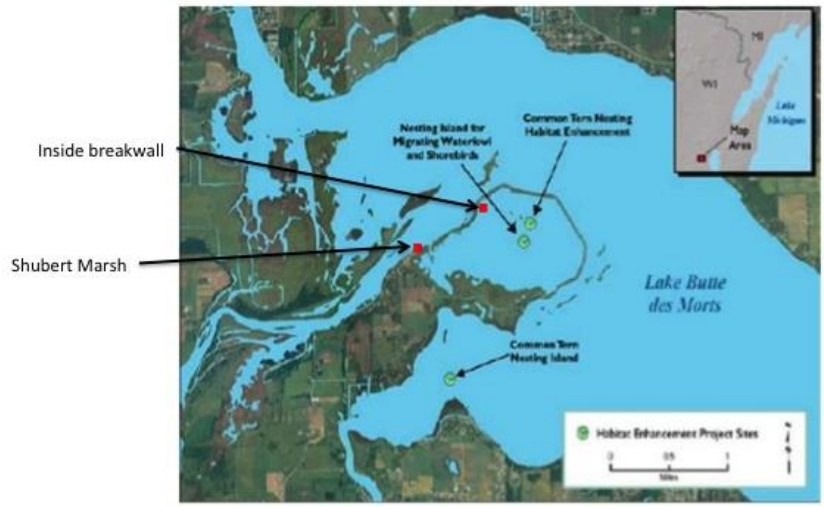


Figure 3 Collection sites at TIWRA during the summer of 2012 (Original map: FRNRTC 2013).

In order to have a valid comparison, a non-impacted water (control) site was identified. Ideally, a control location would be in proximity to the breakwall (to compare similar site conditions), but its water would have little to no intermingling with breakwall water. With these considerations in mind, Shubert Marsh was chosen as the control location. It is situated about two-thirds of a mile away from the breakwall, near the mouth of the Fox River, but still within Lake Butte des Mort. Few birds were seen in the Marsh, especially those species that were observed in the breakwall (e.g. pelicans, gulls, cormorants). Those that were observed in the marsh predominantly were ducks (i.e. mallards).

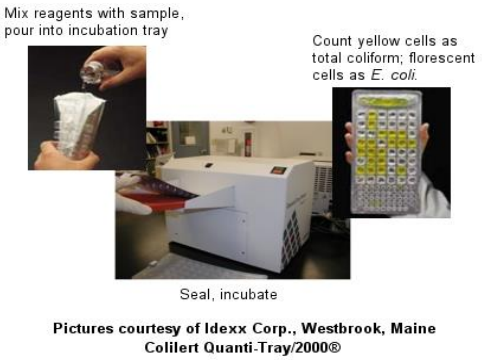
Preliminary studies using culture based methods for enumerating *E. coli* and enterococci (Colilert® and Enterolert® respectively, from IDEXX), as well as visual observations, confirmed that water quality was significantly more impaired in the breakwall area than in Shubert Marsh, confirming the appropriateness of the chosen control location. The breakwall was selected as an appropriate test site as a result of the conclusions drawn from the preliminary data collected from the visual and culture based methods mentioned above. Additionally, its location in relation to nesting water birds such as the AWP, cormorants, geese and gulls, further contribute to the possibility of the breakwall containing contaminated water.

2.2 Sample Collection

Water samples were collected twice weekly (except for two weeks during the total sampling period), starting May 30 through August 31, 2012, from Shubert Marsh and the breakwall at TIWRA. One additional sample was collected during September as a comparison sample to the summer samples for a total of 24 samples for each site. Approximately 3.5 to 7.5 liters of water were collected from each site, from water with a depth of approximately 30 to 60 cm, into sterile containers. The time that samples were collected varied, but generally during the hours of 9 am to 1 pm. Once collected, samples were held on ice and transported back to the lab for immediate processing and analysis.

2.3 Sample Processing and Analysis

Samples were analyzed for both fecal indicator bacteria (FIB) (*E. coli* and enterococci) and fecal pathogens (*Campylobacter spp.* and *Salmonella spp.*). Defined substrate analysis using the Colilert® and Enterolert® (IDEXX) assays was used to quantify the number of FIB, *E. coli* and enterococci, respectively, present per 100 mL of water sample (Figure 4).



Colilert and Enterolert assays

Membrane Filtration

Figure 4 Methods of culture based quantification

2.4 Identifying Fecal Pathogens

To identify fecal pathogens, 100 mL of each water sample type were filtered in triplicate through sterile 0.45 μm nitrocellulose filters (Millipore in Billerica, MA). These were then plated in triplicate on the selective media, XLD and Campy Blood CVA Agar (BD Diagnostic Systems, Franklin Lakes, NJ), and incubated at 37°C and 42°C and 10% CO_2, respectively. If the water sample was turbid (i.e. with vegetation or particulate matter), it was pre-filtered using a sterile coffee filter (Melita brand #4) before being filtered through the nitrocellulose filter. Organisms were identified after 24 hours and additional biochemical tests (oxidase, catalase, antibody kit for *Campylobacter jejuni*) were conducted to identify any potential *Campylobacter spp.* or *Salmonella spp.* isolates, as needed.

Before samples for this study were collected, filter capacity was roughly determined with various filtering volumes. Even after filtering excess debris and vegetation, any volume greater than 100 mL significantly clogged the filter. Hence, 100 mL was determined as a preset filtering volume for filters used in identifying FIB as well as for those used for qPCR.

2.5 qPCR Detection of Fecal Pathogens

It is difficult to quantify pathogens (found in low concentrations) via culture-based methods, especially from water. Hence, a more sensitive molecular technique, qPCR, was used to detect potential pathogen DNA. For qPCR identification, water samples from both the Shubert Marsh and breakwall were each additionally filtered in triplicate onto sterile 0.4 μm polycarbonate filters (Millipore in Billerica, MA) and frozen at -80°C until assayed.

In order to be used for qPCR, DNA needed to be extracted from the polycarbonate filters. This was done using a soil DNA extraction kit (MoBio, Carlsbad, CA). The extracted DNA was frozen at -20°C until used. Extracted DNA samples were used within three days of extraction for best results.

Campylobacter jejuni DNA was used as a standard (calibrator) in this study. The *C. jejuni* strain used was *Campylobacter jejuni*, sub species jejuni, #29428 from the American Type Culture Company (Manassas, VA). Manufacturer's instructions were followed to bring up the *C. jejuni* cells, which were grown on Campy CVA medium (Remel, Lenexa, KS) at 42°C and 10% CO_2 for 24-48 hours. Calibrators were made by

aliquoting a pure culture of *C. jejuni* into 10 µl aliquots and storing at -20°C. The original concentration of the culture was $3.8x10^7$ CFU/mL. To determine the CFU/mL, the original culture was serially diluted and plated on a series of Campy CVA media plates. When qPCR was to be run, an aliquot of *C. jejuni* was thawed and DNA was extracted using the same soil DNA extraction kit used for the polycarbonate filters.

The qPCR method used to identify *Campylobacter jejuni* in the samples was developed using the method published in Nogva *et al.* 2000, as adapted by B. Meka (Personal Communication with B. Meka 2013). This method used primers (Table 2) that targeted the *Campylobacter jejuni* oxidoreductase gene, which is highly specific to the organism (Nogva *et al.* 2000).

Various adaptations were made to the original protocol, including substitution of SYBR Green I chemistry (Applied Biosystems, Grand Island, NY), for TaqMan probe chemistry (Applied Biosystems, Grand Island, NY). Also, thermal cycling settings were changed: the denaturation step was changed to 95°C for 10 minutes; the annealing step was changed to 95°C for 20 seconds, followed by 60°C for 60 seconds; finally, the extension step was eliminated since it was not helpful in amplification. Other adaptations included changing the total reaction volume from 50 µl to 25 µl, doubling the amount of template DNA added from 0.5 µl per 25 µl reaction to 1 µl per 25 µl reaction, adding $MgCl_2$ to the qPCR master mix (in addition to what was already present) and lastly, conducting a melt curve analysis for each experiment.

The adaptations were made because the original TaqMan probe chemistry was not providing interpretable results on the qPCR thermocycler used in this study (Applied

Biosystems, Grand Island, NY). Substituting SYBR Green I chemistry allowed for potentially interpretable results.

Table 2 *Campylobacter jejuni* oxidoreductase primers (Nogva *et al.* 2000).

Probe or primer	**Sequence (5' to 3')**	**Denaturation temp (°C)**
Primers		
Forward	CTG AAT TTG ATA CCT TAA GTG CAG C	60.4
Reverse	AGG CAC GCC TAA ACC TAT AGC T	60.3

2.6 Chlorophyll *a* Concentration Measurement in Water

Chlorophyll *a* was measured to test for algal presence, an indirect measure of nutrient loads (feces) in water. Water samples were collected on various dates, but due to processing error, only two dates could be analyzed, which were those of September 25, 2012 and October 23, 2012. When samples were collected, they were placed immediately on ice and transported to the lab for further processing. The United States Environmental Protection Agency (USEPA) ESS Method 150.1 (online method LMMB 086) was modified for this study as per availability of equipment and upon consultation with Dr. R. Pillsbury at the University of Wisconsin-Oshkosh.

For the chlorophyll *a* study both Shubert Marsh and breakwall water samples were filtered in 100 mL aliquots onto 0.45 μm nitrocellulose filters (Millipore) in triplicate and stored in aluminum foil in the dark at -20° C until further processed. When filters were ready to process, the filters were thawed and dissolved in 8 mL of 90% acetone and centrifuged. Freezing, thawing and dissolving the filters in acetone helped the cells lyse and release the chlorophyll *a*. The absorbance of the resulting supernatant was measured at various wavelengths, as specified by the EPA protocol. The absorbance readings were used to calculate concentration of chlorophyll, as per the equation provided in the EPA protocol (ESS Method 150.1 1991). Only uncorrected values were used to calculate chlorophyll *a* concentration. See Appendix for additional details on this chlorophyll *a* study.

2.7 Phosphorus Concentration Measurement in Water

Phosphorus concentrations were measured in sediment, as well as from water samples collected from Shubert Marsh and inside the breakwall. Water samples tested for phosphorus were taken on the following dates: August 9, 10, 14, 15, 21, and 22 as well as October 23, 2012. Water samples that were collected for FIB and fecal pathogen analysis were aliquoted for additional testing for phosphorus concentration at the Environmental Research and Innovation Center (ERIC, UW-Oshkosh). With the help of the groundskeeper at TIWRA, Pete Gukenberg, sediment was collected approximately one meter from the paved road from water with an approximate depth of 65 cm, which was then transported on ice back to the ERIC for phosphorus detection.

For both water and sediment samples, ortho phosphorous was measured using the United States Environmental Protection Agency (USEPA) method EPA-119-B Rev-0, operated on a SEAL Analytical brand instrument (SEAL Analytical, Inc. 2011). The phosphorus samples were processed at the ERIC, UW-Oshkosh by R. Bartell.

This instrument uses absorbance readings to compare standardized phosphorous samples to test samples (SEAL Analytical, Inc. 2011). The sample is first digested using 0.4 grams of ammonium peroxydisulfate, 50 mL of the test sample (1 g in 50 mL Millipore water for sediment samples) and 1 mL of 11N sulfuric acid. This digestion takes place even before the sample is placed in the instrument and serves to convert all of the ‘bound’ phosphorus (in organic molecules such as DNA and proteins) into ‘free’ phosphate (ortho-phosphate).

Two reagents are used in the AQ1 Total Phosphorus method: one, a working color reagent and two, an ascorbic acid solution. Both of these reagents are stored in a separate reagent wedge on the instrument. The instrument then uses an autosampler to aspirate the appropriate amounts of each reagent and sample, which are mixed in a reaction segment wedge (which are disposable and located on the machine). Next, the instrument pauses the appropriate amount of time to allow for blue color development (approximately eight minutes). The amount of phosphorus present is directly correlated to the absorbance at 880 nm. Hence, a more intense blue color means more phosphorus is present. The instrument measures the absorbance at 880 nm by aspirating the developed sample/reagent mixture into a cuvette located inside the instrument.

The instrument has a lamp to provide a beam of light, which passes through a filter, filtering out all wavelengths of light except for 880 nm. At this point the absorbance is detected after the light passes through the sample, and a result appears on the screen. It compares the absorbance of the sample to the standard curve to provide a phosphorus concentration. A new standard curve is run twice (with six points on the curve) a year or when a reagent with a different lot number is used in the assay.

2.8 Statistics

Water samples were collected from two locations, Shubert Marsh (low waterfowl concentration) and from inside the breakwall (high waterfowl concentration). Samples were tested for bacterial concentrations (*E.* coli and enterococci) and phosphorus concentrations (water only). The means from each set for each type of parameter

(bacterial, phosphorus, etc.) with N=3 or greater, were compared using the student's t-test (Zar 1999). Further, in order to be able to use the student's t-test, data must be normally distributed. The *E. coli* and enterococci bacterial concentration data were not normally distributed, hence, they were log transformed in order to use the student's t-test (Figure 5, Figure 6).

CHAPTER 3. RESULTS

3.1 Fecal Indicator Bacterial Analysis

Collected water samples were analyzed for *E. coli* and enterococci using the Colilert® and Enterolert® assay systems, respectively, measured in units of most probable number (MPN) per 100 mL of water. Both assays have a maximum value output of 2419.6 MPN/100 mL of water and any value higher than this concentration is written as >2419.6 MPN/100 mL. Hence, this study was constrained to the limits of these assays for any concentrations above this stated value.

Overall, *E. coli* concentrations (Figure 5, Table 3) and enterococci concentrations (Figure 6, Table 4) from the breakwall water were, on average, greater than for water for Shubert Marsh (Figure 7): *E. coli*: 480 ± 584 vs. 76 ± 145 MPN/100 ml ($p<0.01$) and enterococci: 637 ± 734 vs. 290 ± 596 MPN/100 ml ($p<0.01$). These samples showed a peak in FIB concentrations between mid-July and early August. The figures that depict the FIB data of *E. coli* and enterococci data included some values that exceeded the maximum value of the assays used. In this case, the value was simply written as the maximum concentration measurable by the assays, >2419.6 MPN/100 mL.

FIB concentrations for safe water recreation limits *E. coli* to 235 MPN/100 mL and enterococci to 35 MPN/100 mL (Dufour 1984). Out of 24 sample days, two days in Shubert Marsh exceeded *E. coli* water quality levels while the breakwall had 10 days that

exceeded *E. coli* water quality levels. For enterococci however, both Shubert Marsh and the breakwall had more sampling days that exceeded water quality levels, 11 days and 20 days, respectively. Intra-correlation values were calculated for *E. coli* and enterococci concentrations within Shubert Marsh and the breakwall and no strong correlations could be determined (r^2=0.04 and r^2=0.28, respectively). This helped determine that *E. coli* and enterococci did not affect each other's concentrations within the specified locations.

Important to note was that the waterfowl had all migrated or left the TIWRA by the September 25, 2012 sample collection date. Interestingly, breakwall *E.* coli and enterococci were also lower than for Shubert Marsh for this sample date.

The student's t-test was performed on log-transformed *E. coli* and enterococci concentrations to determine if there was a statistical difference between the means of each set of values. Both *E. coli* and enterococci concentrations were significantly greater in water from within the breakwall than from water from Shubert Marsh ($p<0.01$ for both *E. coli* and enterococci) (Figure 7).

Precipitation events have the potential to affect FIB concentrations in water, especially those of *E. coli* (Kleinheinz *et al*. 2010). This is due to the runoff material from land flowing into nearby water bodies, including fecal matter from waterfowl and other wildlife. Hence, to investigate a potential correlation between precipitation events and FIB concentrations, precipitation data was compiled for days previous to, of and following sample collection dates (Table 5). Analyzing precipitation data and FIB concentrations for both *E. coli* and enterococci concentrations for Shubert Marsh and

breakwall (Table 3 and Table 4), no strong correlations were found (Shubert Marsh r^2=0.03 and r^2=0.61, and for Breakwall, r^2=0.20 and r^2=0.01, respectively).

3.2 qPCR Detection of Pathogenic Bacterial DNA

No *Campylobacter spp.* or *Salmonella spp.* were recovered using XLD and Campy CVA agar media from any of the water samples collected from Shubert Marsh or from within the breakwall. Since culture based techniques may not be sensitive enough to detect potential *Campylobacter spp.* in samples, qPCR was used as an alternative detection method. *Salmonella spp.* may be identified by qPCR in future studies, but was not evaluated in this research. At this time, data from enumeration of *Campylobacter* by qPCR cannot be provided. The data obtained was erratic. The control results were not consistent and often the test samples of the same type would give varied C_T values. Even after numerous rounds of qPCR, set up meticulously, the data collected was inconclusive. Additional work is needed to further improve the method for consistent use in laboratory experiments.

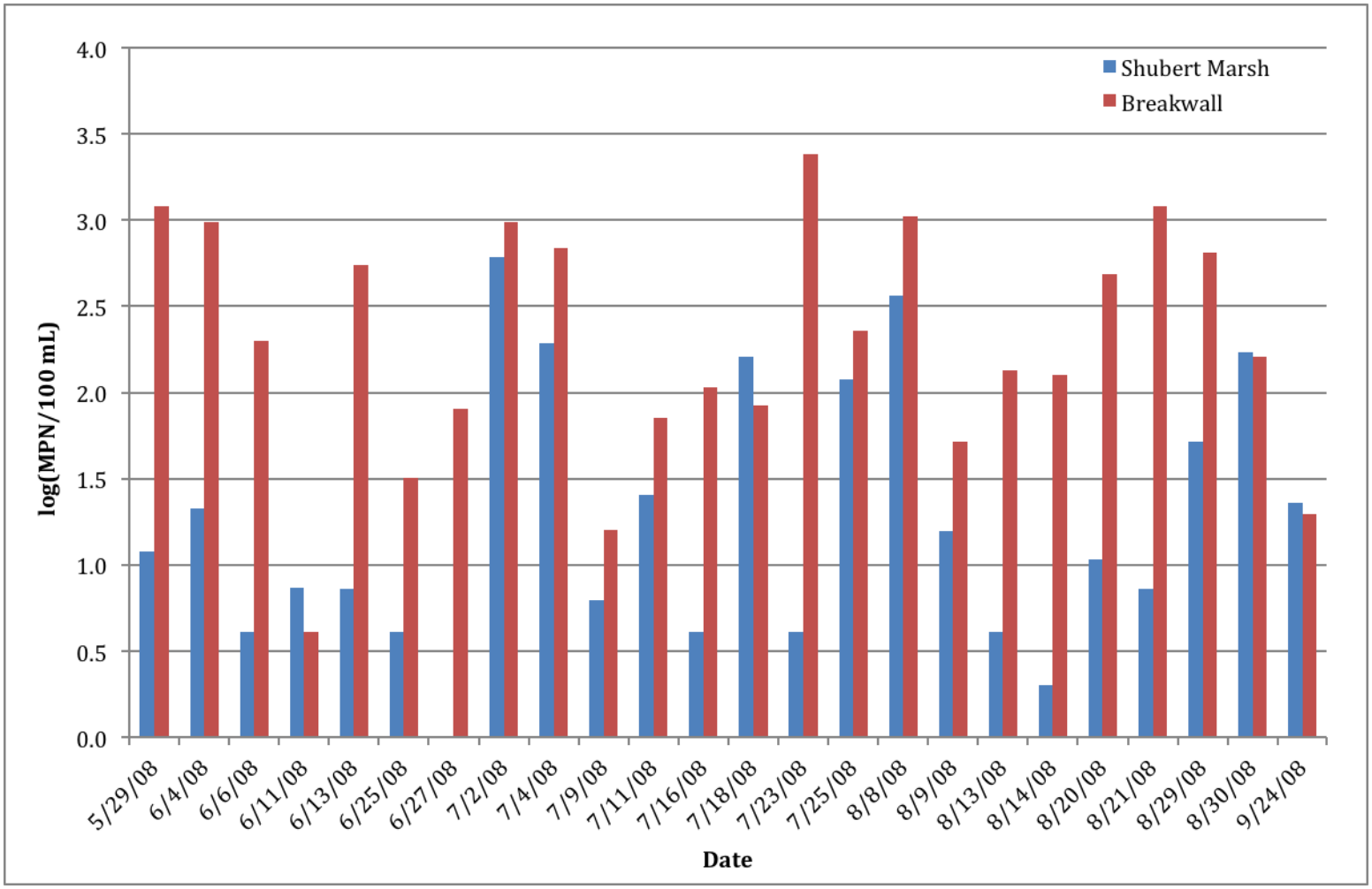


Figure 5 *E. coli* concentrations from water samples (N=24) collected from Shubert Marsh and from within the breakwall in summer 2012.

Table 3 *E. coli* concentrations in water samples determined by the Colilert® assay.

	MPN/100 mL	
Date	**Shubert Marsh**	**Breakwall**
5/30/12	11.9	1203.3
6/5/12	21.1	980.4
6/7/12	4.1	198.9
6/12/12	7.4	4.1
6/14/12	7.3	547.5
6/26/12	4.1	31.8
6/28/12	1.0	79.8
7/3/12	613.1	980.4
7/5/12	191.8	686.7
7/10/12	6.2	16.0
7/12/12	25.6	71.2
7/17/12	4.1	106.7
7/19/12	161.6	83.6
7/24/12	4.1	>2419.6
7/26/12	119.8	228.2
8/9/12	365.4	1046.2
8/10/12	15.6	52.1
8/14/12	4.1	135.4
8/15/12	2.0	127.4
8/21/12	10.8	488.4
8/22/12	7.3	1203.3
8/30/12	52.1	648.8
8/31/12	172.3	160.7
9/25/12	22.8	19.7

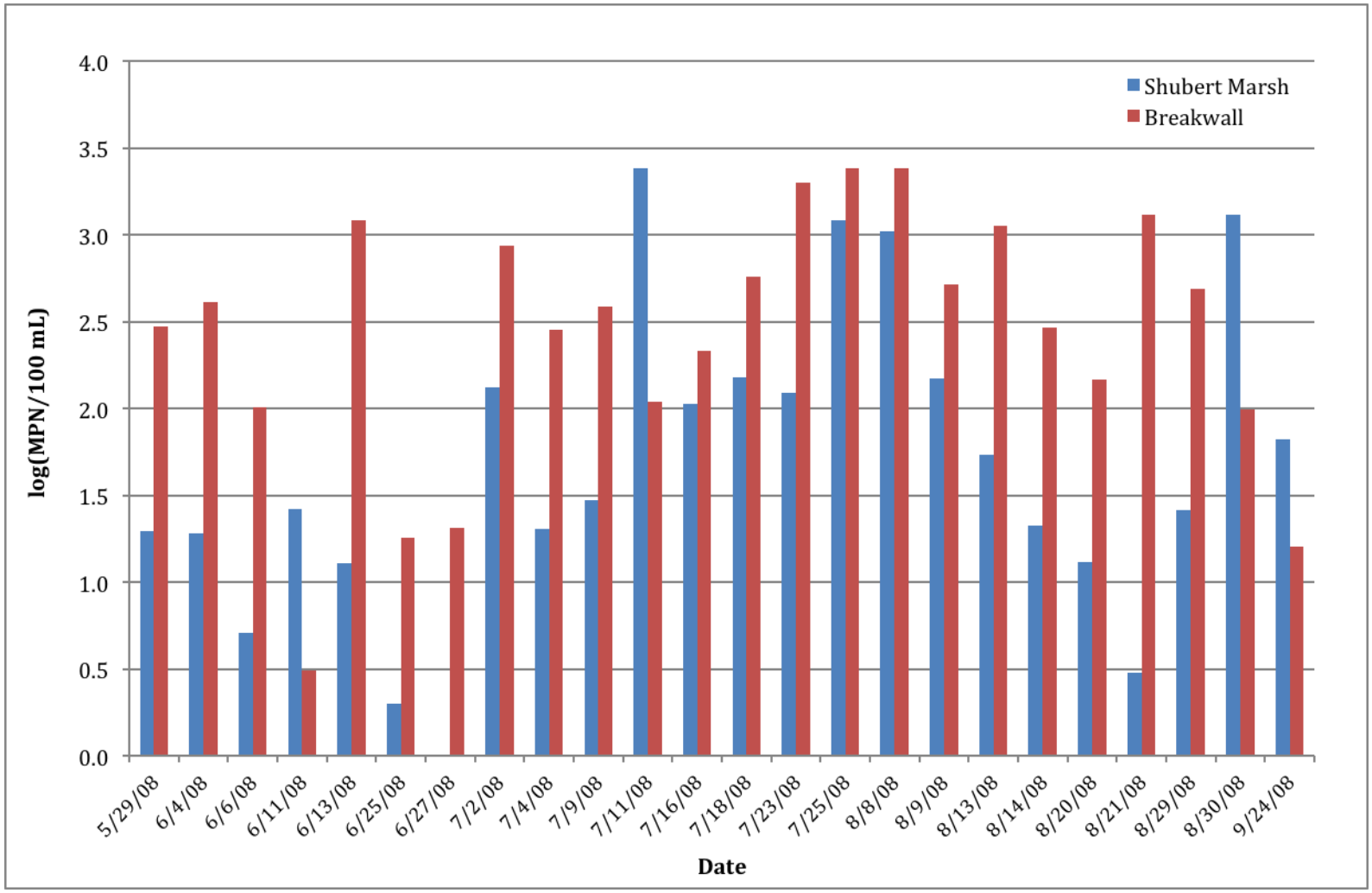


Figure 6 Enterococci concentrations from water samples (N=24) collected from Shubert Marsh and from within the breakwall in summer 2012.

Table 4 Enterococci concentrations from water collected from Shubert Marsh and from within the breakwall.

	MPN/100 mL	
Date	**Shubert Marsh**	**Breakwall**
5/30/12	19.6	298.7
6/5/12	19.1	410.6
6/7/12	5.1	101.4
6/12/12	26.3	3.1
6/14/12	12.8	1203.3
6/26/12	2.0	18.1
6/28/12	0.0	20.6
7/3/12	131.7	866.4
7/5/12	20.2	285.1
7/10/12	29.8	387.3
7/12/12	>2419.6	109.5
7/17/12	106.0	216.4
7/19/12	150.3	574.8
7/24/12	124.0	1986.3
7/26/12	1203.3	>2419.6
8/9/12	1046.2	>2419.6
8/11/12	149.7	517.2
8/14/12	54.5	1119.9
8/15/12	21.1	290.9
8/21/12	13.1	145.9
8/22/12	3.0	1299.7
8/30/12	26.2	488.4
8/31/12	1299.7	98.5
9/25/12	66.3	16.0

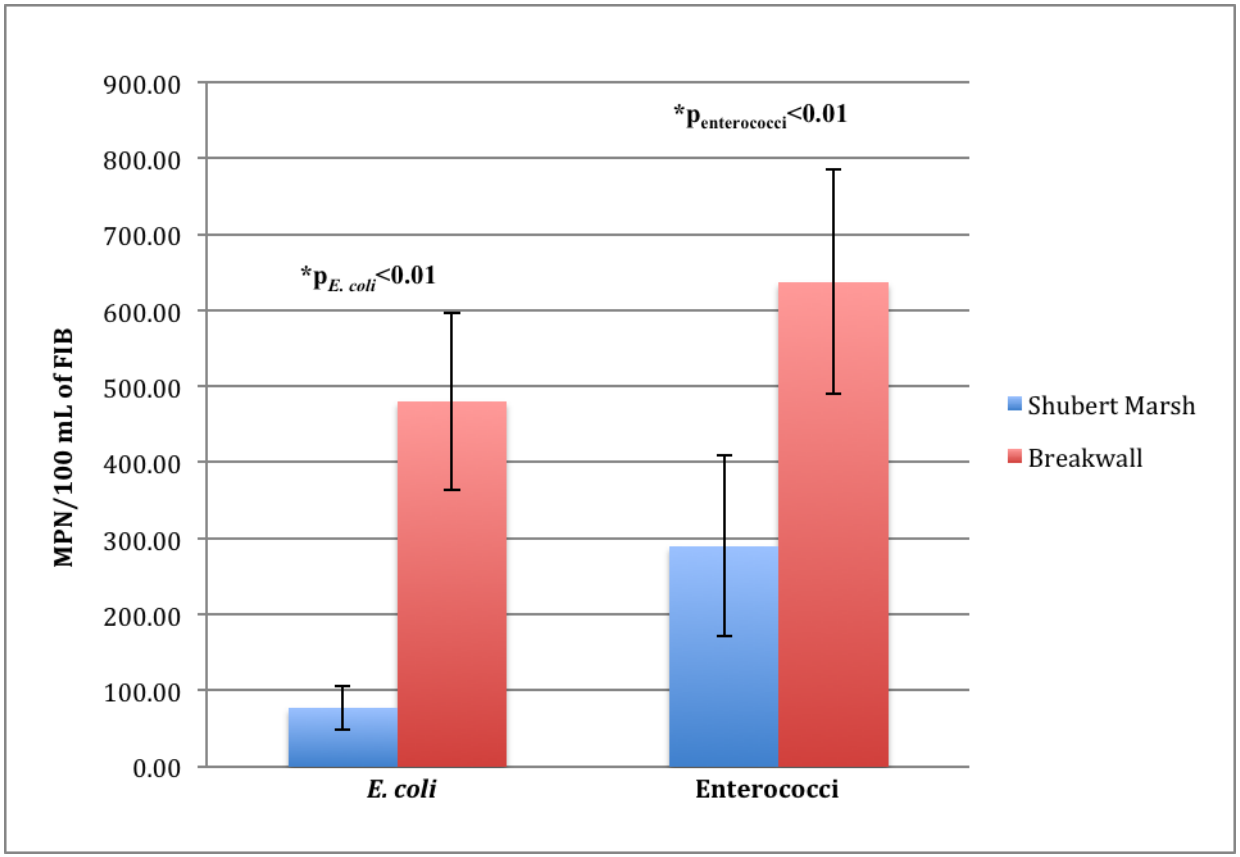


Figure 7 Seasonal means of concentrations of *E. coli* and enterococci from water samples (N=24) collected from Shubert Marsh and from within the breakwall in summer 2012

Table 5 Precipitation data from up to 72 hours prior to sampling.

Date	Precipitation (cm)	Mean temperature (°C)
5/27/12	1.27	21.7
5/28/12	0.13	23.9
5/29/12	0.00	17.8
5/30/12	**0.08**	**11.1**
6/2/12	0.00	15.6
6/3/12	0.00	19.4
6/4/12	0.00	18.9
6/5/12	**0.00**	**17.8**
6/6/12	0.00	16.7
6/7/12	**0.00**	**19.4**
6/9/12	0.00	26.1
6/10/12	0.00	24.4
6/11/12	0.00	24.4
6/12/12	**0.00**	**16.7**
6/13/12	0.00	15.6
6/14/12	**0.00**	**17.2**
6/23/12	0.00	19.4
6/24/12	0.08	22.2
6/25/12	0.00	17.2
6/26/12	**0.00**	**18.3**
6/27/12	0.00	23.9
6/28/12	**0.00**	**27.8**
6/29/12	0.00	25.0
6/30/12	0.00	25.6
7/1/12	0.00	24.4
7/2/12	4.57	26.7
7/3/12	**0.05**	**27.2**
7/4/12	0.05	27.2
7/5/12	**0.00**	**28.9**
7/7/12	0.00	23.9
7/8/12	0.00	23.9
7/9/12	0.00	23.3
7/10/12	**0.00**	**21.1**
7/11/12	0.00	21.1
7/12/12	**0.00**	**22.2**
7/14/12	0.00	25.6
7/15/12	0.00	26.7
7/16/12	0.00	30.0
7/17/12	**0.53**	**29.4**

7/18/12	0.00	23.9
7/19/12	**0.43**	**21.1**
7/21/12	0.00	24.4
7/22/12	0.00	26.7
7/23/12	0.00	30.0
7/24/12	**0.13**	**25.6**
7/25/12	1.12	27.2
7/26/12	**0.97**	**25.6**
8/6/12	0.00	21.1
8/7/12	0.00	23.3
8/8/12	0.05	22.2
8/9/12	**3.00**	**17.8**
8/10/12	**0.00**	**18.3**
8/11/12	0.00	18.3
8/12/12	0.00	17.8
8/13/12	0.00	20.0
8/14/12	0.00	20.0
8/15/12	0.00	21.1
8/18/12	0.00	16.7
8/19/12	0.10	18.3
8/20/12	0.03	15.6
8/21/12	**0.00**	**19.4**
8/22/12	**0.00**	**21.1**
8/27/12	0.03	23.3
8/28/12	0.00	21.1
8/29/12	0.00	22.2
8/30/12	**0.00**	**26.7**
8/31/12	**0.00**	**22.8**
9/22/12	0.00	8.9
9/23/12	0.00	7.8
9/24/12	0.00	12.8
9/25/12	**0.00**	**12.2**

***Sampling dates are bolded.**

3.3 Chlorophyll *a*

Evidence suggests that the large amount of birds at TIWRA has negatively affected water quality. Fecal matter, especially that of birds, encourages algal activity and the production of phytoplankton biomass (Lillie and Mason 1983). Chlorophyll *a*, present as a pigment in algae, can be quantified and used as an indicator of algal activity in lakes, rivers and oceans. Hence, additional parameters, such as chlorophyll *a* concentrations, were used in order to measure water quality.

This study found that on average, chlorophyll *a* concentrations were considerably higher in the breakwall area compared to chlorophyll *a* concentrations in Shubert Marsh (119.83 vs. 92.74 μg/L). This supports the hypothesis in that a buildup of avian feces in the water may be a cause of increased chlorophyll *a* concentrations. See Appendix for complete analysis of chlorophyll *a* concentrations in Shubert Marsh and the breakwall.

3.4 Phosphorous Concentrations in Water and Sediment

Phosphorous concentrations were measured from water and sediment sampled from Shubert Marsh and the breakwall in the late summer and fall of 2012. Similar to FIB results, phosphorous concentrations also were greater in the breakwall than in Shubert Marsh in water ($p<.001$) (Figure 8, Table 6). Mean phosphorus concentration values were compiled for Shubert Marsh and breakwall water samples (Figure 9). When sediment phosphorus concentrations were measured, the same trend was not observed. For one sampling date, the sediment phosphorus concentration in Shubert Marsh was greater than that in the breakwall (Figure 10, Table 7). As mentioned earlier, the

waterfowl present during the summer months were not visible after late September. Hence, it is important to note that additional fecal inputs were greatly reduced in September.

The student's t-test was performed only on the water samples tested for phosphorus concentrations. Phosphorus concentrations from water collected within the breakwall were significantly greater than those from water from Shubert Marsh ($p<0.01$) (Figure 8, Table 6).

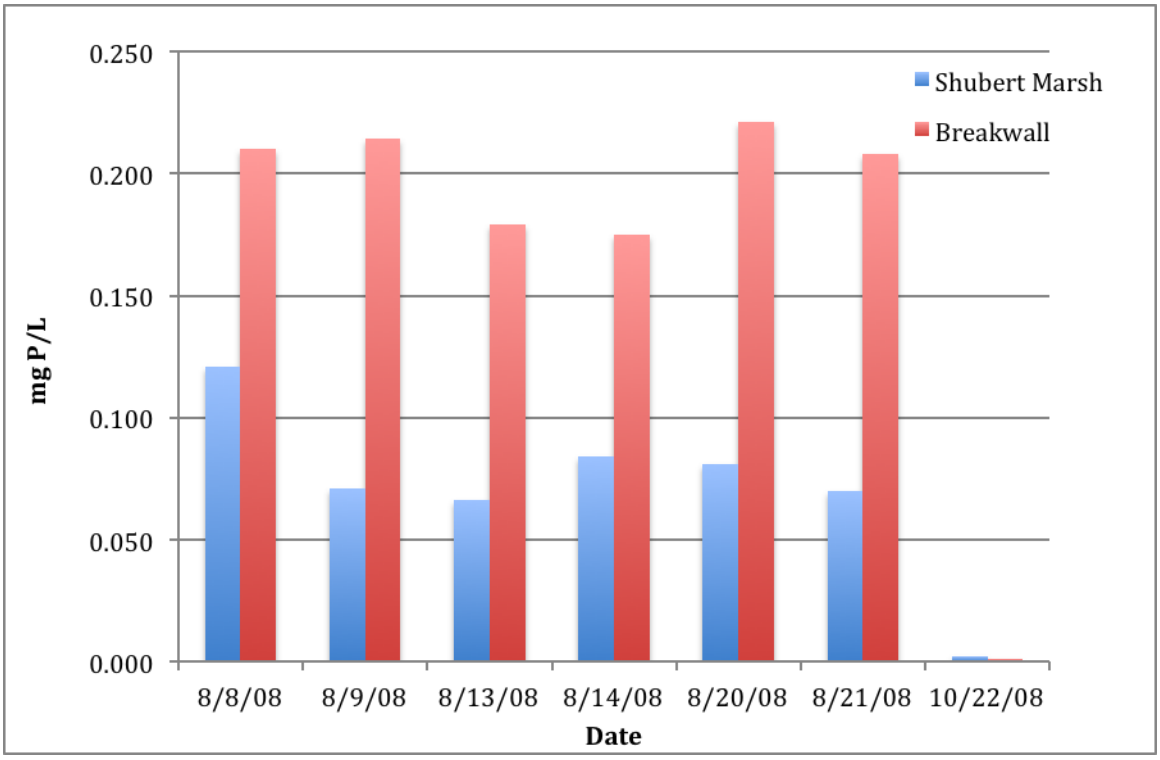


Figure 8 Phosphorous concentrations, measured in milligrams of phosphorous per liter water, from samples (N=7) collected from Shubert Marsh and breakwall from summer 2012.

Table 6 Phosphorous concentrations measured in milligrams of phosphorous per liter water from Shubert Marsh and the breakwall

	mg P/L	
Date	**Shubert Marsh**	**Breakwall**
8/9/12	0.121	0.210
8/10/12	0.071	0.214
8/14/12	0.066	0.179
8/15/12	0.084	0.175
8/21/12	0.081	0.221
8/22/12	0.070	0.208
10/23/12	0.002	0.001

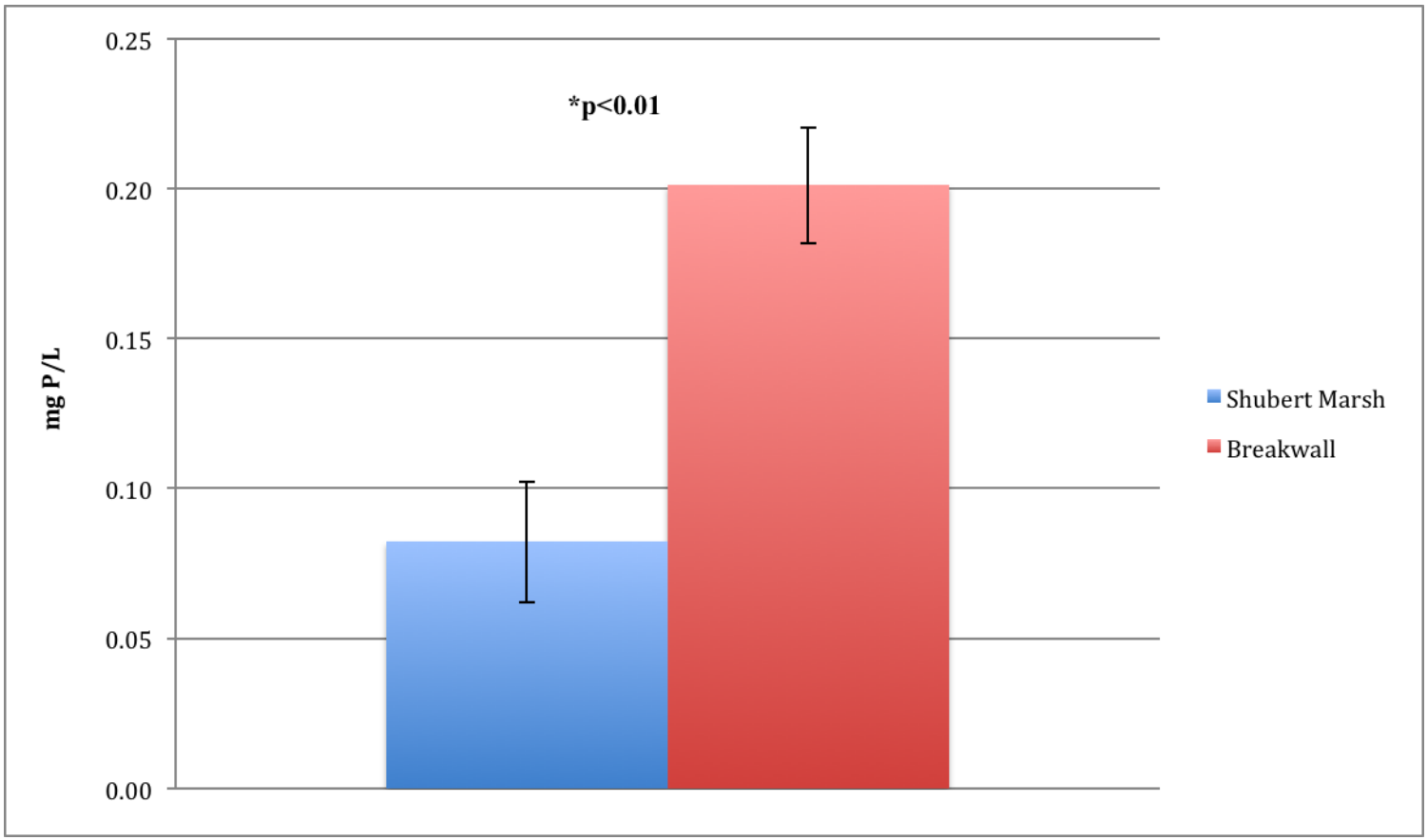


Figure 9 Means of phosphorus concentrations from water samples (N=7) collected from Shubert Marsh and from within the breakwall in summer 2012.

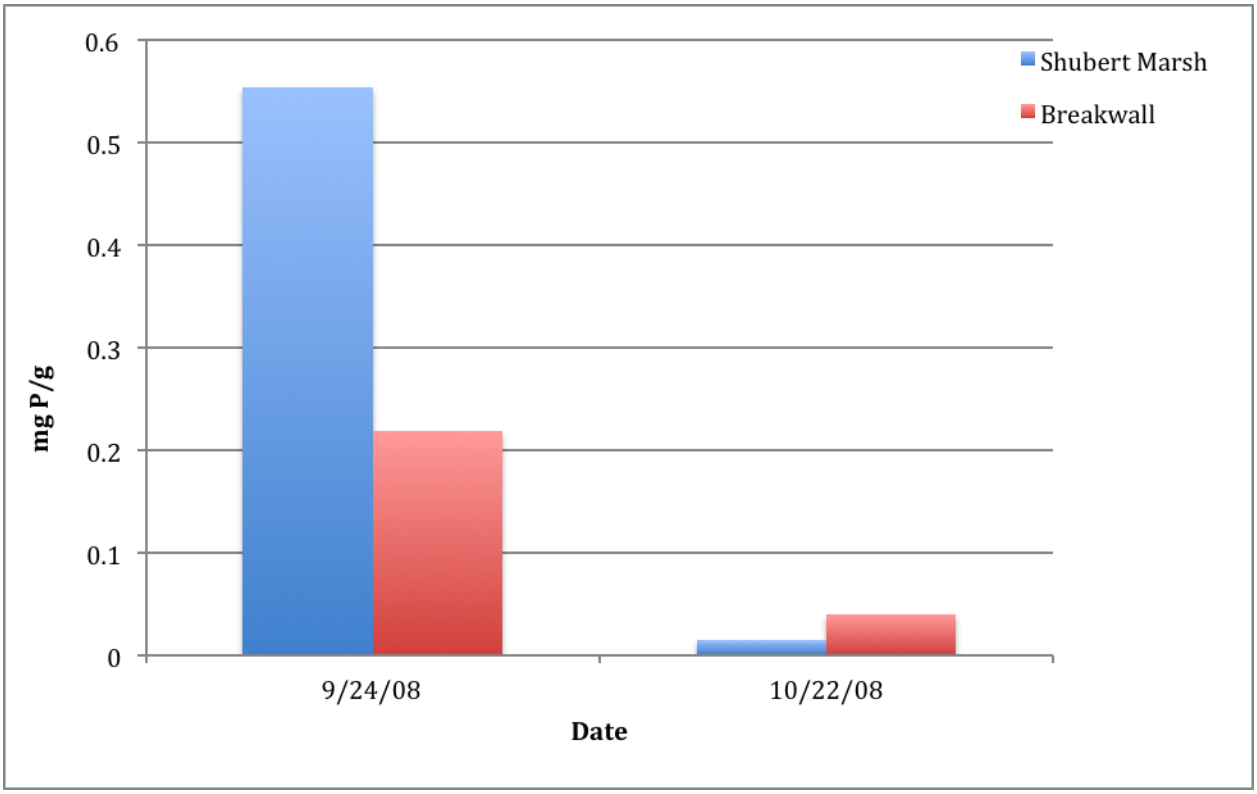


Figure 10 Phosphorous concentrations in sediment samples (N=2) from Shubert Marsh and breakwall from fall 2012.

Table 7 Phosphorous concentrations measured in milligrams of phosphorous per gram of sediment from Shubert Marsh and the breakwall.

	mg P/g	
Date	**Shubert Marsh**	**Breakwall**
9/25/12	0.554	0.219
10/23/12	0.015	0.039

CHAPTER 4. DISCUSSION

According to a WI DNR bird count from 2012, approximately 1,500 bird nests were spotted in and around TIWRA (Personal Communication with A. Sabai 2012). With such high numbers, the waterfowl it is likely that these birds have a negative impact on the recreational water quality in and around the breakwall area by increasing fecal indicator bacterial and fecal pathogenic bacterial concentrations and making water unsafe for recreational water users.

4.1 Impact of Avian Fecal Matter on Bacterial Levels in Water at Terrell's Island

With the high numbers of waterfowl present at TIWRA (especially AWP, cormorants and gulls), it was necessary to study the impact these birds might have on water quality. As was hypothesized, these data do support the idea that waterfowl presence has a negative impact on water quality in this area. On average, FIB levels in water were significantly elevated ($p<0.01$) within the breakwall when compared to Shubert Marsh for summer of 2012. Both *E. coli* (Table 3) and enterococci (Table 4) concentrations were greater inside the breakwall than in Shubert Marsh.

The elevated FIB concentrations from water within the breakwall could have been due to many reasons. Avian fecal matter contains bacteria, which can easily get into water. The birds will defecate in the water while loafing and feeding or flying over the

water. Some FIB can persist up to three to four days in water, so, using culture based methods to detect these organisms was reasonable (McFeters *et al*. 1974).

There were certain dates in 2012 (June 12, August 31, September 25 for enterococci and June 12, July 19, August 31, September 25 for *E. coli*) in which the measured FIB concentrations were greater in Shubert Marsh than within the breakwall. One explanation for this could be that the drought of 2012 lowered water levels. Some days, the water levels were lower in Shubert Marsh than in the breakwall, concentrating the bacteria present. On this note, it is also possible that FIB concentrations from both Shubert Marsh and the breakwall area were higher than in non-drought years.

To our knowledge, no study is currently available to compare FIB concentrations at TIWRA from previous years. However, other studies provide conclusions similar to those presented in this study. In an earlier study, Hill and Grimes (1984) examined water in Lake Onalaska near La Crosse, WI during the months of July and October of 1981. Water samples were analyzed for FIB as well as transmission of *C. jejuni* via avian fecal matter. The authors concluded that waterfowl feces does elevate FIB, however, *C. jejuni* is not necessarily spread in enclosed water bodies.

Certain types of weather (such as rain) may have affected bacterial concentrations around TIWRA. Rain and winds may cause a number of elements to mix (water, sediment, runoff), resulting in elevated bacterial concentrations (Ackerman and Weisberg 2003). But, when comparing precipitation amounts to bacterial concentrations for both *E. coli* and enterococci for each of the sampling dates, no strong correlations could be determined (Table 3, Table 4, Table 5).

As mentioned earlier, it is interesting that pelicans and most other waterfowl had left TIWRA by the September 25, 2012 sampling date (due to migration or otherwise). The data reflect a subsequent drop in FIB concentrations, further supporting the hypothesis that waterfowl are indeed affecting water quality at TIWRA.

Overall, the data support the idea that high numbers of waterfowl are negatively affecting water quality at TIWRA, especially inside the breakwall. The information from this study can be used by the WI DNR to manage waterfowl populations, as necessary.

4.2 Impact of Avian Fecal Matter on Phosphorus Levels at Terrell's Island

Every day phosphorus was tested from water samples collected from Shubert Marsh and the breakwall (except for one date), phosphorus concentrations were higher in the breakwall water than in Shubert Marsh water. The phosphorus concentrations overall were significantly lower in the September 25, 2012 sampling date (after the waterfowl were observed to be gone) ($p<0.01$), although the Shubert Marsh water phosphorus concentration was slightly higher than that of the breakwall water (average of 0.0822 mg P/L vs. 0.201 mg P/L, respectively). This again supports the hypothesis that waterfowl are having a negative impact on water quality on the breakwall water at TIWRA.

The primary reason that overall phosphorus was higher in the breakwall may have been due to the large amounts of fecal matter in the water. In freshwater as well as marine aquatic systems, phosphorus is in a particulate or dissolved state. It occurs in organic and inorganic forms, both of which can be dissolved in water. It settles out into the sediment within a matter of days (River Watch Network 1992, *Methods for chemical*

analysis of water and wastes 1983). Despite this, a high level of phosphorus was measured from the water inside the breakwall. This again supports the hypothesis that the waterfowl are responsible for repeated inputs of fecal material and thus phosphorus into water, negatively impacting the water quality inside the breakwall at TIWRA.

When phosphorus was measured in sediment, the opposite was observed. The phosphorus level in Shubert Marsh was higher than in the breakwall water (Figure 10, Table 7). This seems the opposite of what was predicted, but there is a possible explanation for this phenomenon. Shubert Marsh contains much more vegetation than does the area inside the breakwall. There may have been a settling of decaying plant material, hence elevating phosphorus levels in the sediment, especially since the sediment was sampled in late September.

Samples were taken near the shore with a depth of approximately 30 to 60 cm. To get a full scope of the water quality around the breakwall, it may be useful to collect water in the center of the breakwall area. Also, pseudo-replication was used in this study during filtration and for the Colilert® and Enterolert® assays. Collecting separate samples from the water would have given genuine sample replication for accuracy and statistical purposes.

There are future studies that may be done to gain further insight into the affect of waterfowl at TIWRA. Since pelicans were the overwhelming majority of the waterfowl population present at TIWRA, it would be relevant to analyze pelican feces for its bacterial content, as well as its phosphorous content. This was attempted in this study, but difficulty in correctly identifying pelican feces led us to abandon this aspect of the

project. Currently, there is no study that fully explores the implications of pelican fecal matter on water quality.

The data from this study suggest that the increased number of waterfowl species have had a negative impact on the water inside the breakwall at TIWRA. TIWRA is a closed system, hence, a build up of nutrients (i.e. from waterfowl) is detrimental to natural vegetation and wildlife in the area. Controlling bird populations via habitat destruction, oiling eggs and culling become necessary to minimize the impact these birds have at TIWRA. If a bird is an endangered species and protected by law, permission by the federal government is necessary in order to cull the population. AWP as well as the Double Crested Cormorants (DCC) (*Phalacrocorax auritus*) would be the main targets for population reduction at TIWRA, since their populations have been the highest at TIWRA (Personal Communication with A. Sabai 2012). The federal government did cull the DCC population at TIWRA and 1,500 DCC were shot and eggs oiled in summer 2013 (Personal Communication with A. Sabai 2013).

Water is crucial not only for consumption, but also aesthetic and recreational enjoyment. Since water recreation can occur in a variety of lakes, rivers, and coastal waters, it is important to maintain excellent water quality in these areas. Measuring water quality can involve a number of methods, including those that are quantitative or qualitative in nature. This study included testing water quality via quantitative and qualitative methods for a better understanding of the present water quality at TIWRA. This study was able to conclude that the water quality within the breakwall at TIWRA is indeed poor. Since TIWRA is located on Lake Butte des Morts where recreation is

common, it is important to improve the water quality at TIWRA to keep recreationalists safe and healthy.

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APPENDIX

Chlorophyll *a* in the Waters Surrounding Terrell's Island

Introduction

In photosynthetic organisms, one or more organic pigments are present which are capable of absorbing visible radiation (i.e. from the sun), initiating the photochemical reactions of photosynthesis. Found inside chloroplasts, the photosynthetic center in plants and algae, chlorophylls are one of the three major classes of photosynthetic pigments. One of the five types of chlorophylls, chlorophyll *a*, is what gives plants and algae their characteristic color (bluish-green) (Hall and Rao 1999).

Chlorophyll *a* concentration in water can be quantified and used as a measure of phytoplankton (algal) biomass (Lillie and Mason 1983). Phytoplankton biomass is an indicator of algal activity in all types of water bodies including lakes, rivers and oceans. An overabundance of algal numbers is detrimental for water body health because it can lead to eutrophication. Eutrophication is the ecosystem's response to excess, artificial or natural, nutrients such as nitrates and phosphates in an aquatic system (Schindler and Vallentyne 2004).

Nitrates and phosphates are necessary nutrients for plants to survive. These two nutrients are also the main concerns in eutrophic systems, especially since they are abundant in human derived (detergents, cleaners, soaps, shampoos) as well as wildlife sources (fecal matter). Although both these nutrients contribute to eutrophication, phosphorous is generally regarded as the main source. In freshwater ecosystems, phosphorous is the primary limiting growth factor (among micronutrients) for photosynthetic organisms (Schindler 1977).

There are multiple nutrient sources that may contribute to eutrophication, known as point and non-point sources. Point sources are pollution sources from an identified source while non-point sources can be multiple or unknown sources of pollution. Some sources are human (agricultural runoff and sewage) while others are from wildlife (fecal matter from animals). Combined, these nutrient sources can lead to excessive plant growth, mostly that of algae and plankton, commonly known as harmful algal bloom (HABs).

There are two types of HABs, both of which can be harmful due to different reasons. One type of HAB is toxic and produces toxins and harmful metabolites while the other type of HAB is non-toxic but has a high production of biomass leading to foam/scum, hypoxia as blooms decay, or fish/shellfish habitat destruction by shading of submerged vegetation (Anderson *et al*. 2002). Negative effects of these algal blooms are many but one of the most severe is hypoxia, where oxygen is depleted in the water, leading to the death of fish and other aquatic organisms. Hypoxia occurs through a series of events. As algae die, they sink to the bottom of the water body where they are decomposed by bacteria, a process which also converts organic matter into inorganic matter. Oxygen is required by this decomposition process, which deprives the deeper waters of oxygen, resulting in the death of fish and other aquatic life (Bartram *et al*. 1999). Additionally, the water body becomes cloudy, colored green, yellow, brown or red, as a result of eutrophication.

Since some HABs can produce toxins, they can lead to mass mortalities of wild and farmed fish as well as shellfish; human illness and death due to consumption of toxic

seafood; exposure to toxins via inhalation or water contact; sickness and death of marine mammals, seabirds and other animals; and a change in marine habitats and trophic structures (Anderson *et al*. 2002).

Overall, eutrophication negatively affects recreational water activities including boating, fishing, hunting and aesthetic enjoyment. Additionally, it can affect human health via contamination of freshwater bodies, making it hazardous to consume water from such sources (Bartram *et al*. 1999).

As mentioned earlier, eutrophication can occur through human or natural (wildlife) sources. It can especially plague lakes and rivers that are home to increasing numbers of waterfowl. Avian feces, rich in nutrients such as phosphorous and nitrogen, can either enter freshwater bodies via defecation in the water or run off from surrounding bird habitats (USEPA 1997). Hence, it is important to monitor water quality when many waterfowl are present.

Terrell's Island, located on Lake Butte des Morts in Winnebago County, WI is one such location where large numbers of birds, including waterfowl, nest. At Terrell's Island, a large breakwall exists, composted of rock and dirt, enclosing a small body of water. It has a carp gate, which is the only entrance and allows one boat to pass at a time. There are bars on the portion of the gate that sits beneath the water to keep carp out. Construction was completed in 1998 to restore wildlife habitat as well as water quality within a significant part of Lake Butte des Morts, in which it is situated (Asplund and Lenz 2000). Population of numerous avian species, in particular a type of waterfowl, the American White Pelican (*Pelecanus erythrorhynchos*) (AWP), has increased in the past

15 years. The AWP predominatly reside in wetland regions with high amounts of precipitation (Keith 2005). Large nesting populations means birds will fish as well as bathe and defecate in the water, affecting water quality. Evidence suggests that the large amount of birds here has negatively affected water quality, prompting the DNR and conservation groups to assess the situation (unpublished data).

The purpose of this study was to investigate the water quality of the breakwall at Terrell's Island, using chlorophyll *a* as a measure of algal activity. Due to the high numbers of waterfowl present, nitrogen and phosphorous levels should be elevated when compared to other areas in Lake Butte des Morts where the waterfowl do not reside.

Materials and Methods

Study Location Selection

Chlorophyll *a* concentrations were measured using water samples from two spots along Terrell's Island in Winnebago County, WI during the early fall months of 2012. The two locations chosen were Shubert Marsh and the breakwall, as a "clean" water (control) and test spot, respectively. Careful consideration was given when selecting a control location and was selected based upon its distance from the breakwall. An ideal control spot would be far from the breakwall with potential for little to no intermingling with breakwall water. Preliminary studies using culture based methods for indicating *E. coli* and enterococci numbers (Colilert® and Enterolert® (IDEXX)) as well as visual observations confirmed that water quality was significantly lower in the breakwall than in Shubert Marsh, hence this spot was chosen. The breakwall was selected as the test site due to the

visual and culture based methods mentioned above as well as its location in which water birds such as the AWP, cormorants, geese, gulls, and etc. nest.

Water Sample Collection and Processing

Water samples were collected, stored immediately on ice and transported back to the lab for further processing. Upon arrival, Shubert Marsh and breakwall samples were processed for various assays, including chlorophyll *a* testing. The United States Environmental Protection Agency (USEPA) ESS Method 150.1 (online method LMMB 086) was modified for this study as per availability of equipment and upon consultation with Dr. R. Pillsbury at the University of Wisconsin-Oshkosh (USEPA 1997).

Both Shubert Marsh and breakwall water samples were filtered in 100 mL aliquots onto 0.45 µ nitrocellulose filters in triplicate and stored in aluminum foil in the dark at -20° C until further processed. Freezing the filters replaced the sonification step in order to break open the cells to release the chlorophyll *a*. When filters were ready to process, they were thawed and dissolved in 8 mL of 90% acetone and centrifuged as listed in the protocol. The absorbance of the resulting supernatant was measured in a 1 cm cuvette at various wavelengths, as specified by the protocol. The absorbance readings were used to calculate concentration of chlorophyll, as per the equation provided in the ESS 150.1 protocol. Only uncorrected values were used to calculate chlorophyll *a* concentration.

Results

Due to processing error, only samples from two dates were properly analyzed. Filters were processed and absorbance readings were measured at various wavelengths for Shubert Marsh and breakwall samples for the dates of September 25, 2012 and October 23, 2012. The absorbance readings were used to calculate uncorrected chlorophyll *a* concentrations as per the USEPA ESS 150.1 protocol (Table 1 A and B) (USEPA 1997).

For both sampling dates, on average, Shubert Marsh chlorophyll *a* concentrations were lower than in the breakwall (Figure 1). It is important to note that waterfowl were present on September 25 but none present on October 23 (perhaps due to migration).

Overall, Shubert Marsh and breakwall sample concentrations were higher in October than in September.

Chlorophyll *a* concentrations were higher in 2012 than in 1999, when the Wisconsin Department of Natural Resources (WI DNR) conducted a water quality study, shortly after the breakwall was completed (Table 2). The Shubert Marsh sampling site was not used in the WI DNR study (Asplund 2000), nevertheless, it is located near the Fox River mouth, thus being a comparable site in chlorophyll *a* comparison.

Table 8 Chlorophyll *a* (Chl *a*) concentrations as measured on (A) 25-Sep-12 and (B) 23-Oct-12, respectively, from Shubert Marsh and Breakwall.

(A)

25-Sep-12

Sample	Absorbances measured at different wavelengths (nm)					Chl *a* concentration (µg/L)
	750	665	663	645	630	
Marsh 1	0.1422	0.1078	0.1075	0.1207	0.1192	80.20
Marsh 2	0.1556	0.1216	0.1213	0.1363	0.1353	90.48
Marsh 3	0.1661	0.1329	0.1326	0.1469	0.1456	99.26
Breakwall 1	0.1786	0.1473	0.1433	0.1593	0.1587	107.2
Breakwall 2	0.1943	0.1588	0.1584	0.1739	0.1732	118.8
Breakwall 3	0.1771	0.1447	0.1442	0.1558	0.1547	108.6

(B)

23-Oct-12

Sample	Absorbances measured at different wavelengths (nm)					Chl *a* concentration (µg/L)
	750	665	663	645	630	
Marsh 1	0.1855	0.1370	0.1366	0.1641	0.1637	100.2
Marsh 2	0.1807	0.1250	0.1245	0.1567	0.1565	90.11
Marsh 3	0.1728	0.1306	0.1303	0.1527	0.1525	96.17
Breakwall 1	0.1905	0.1657	0.1658	0.1755	0.1748	125.5
Breakwall 2	0.1907	0.1664	0.1664	0.1758	0.1750	126.0
Breakwall 3	0.1984	0.1752	0.1752	0.1837	0.1830	132.9

Table 2 Date wise comparison of average chlorophyll *a* concentration (µg/L) (Asplund and Lenz 2000).

Sample	Average chlorophyll *a* concentration (µg/L)		
	Sep-12	Oct-12	Jul-99
Shubert Marsh	89.98	95.48	NA
Fox River mouth	NA	NA	40.5
Breakwall	111.5	128.1	34.05

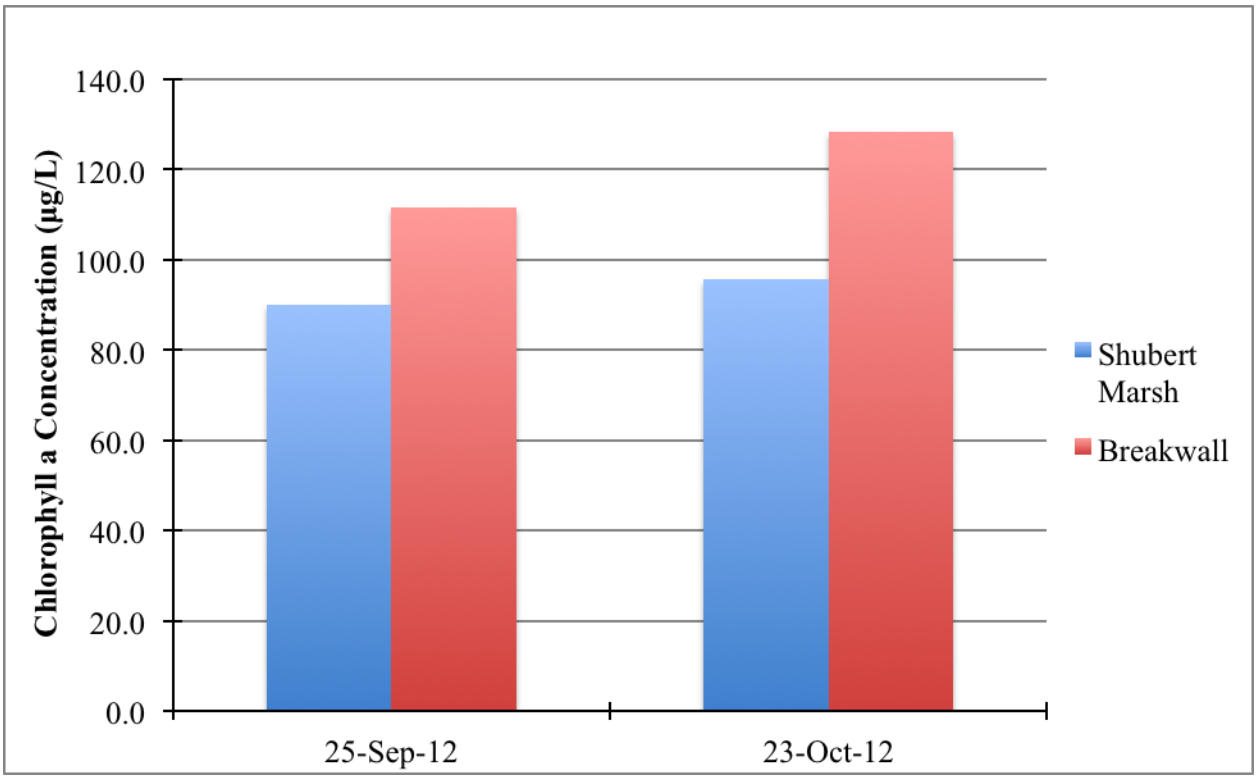

Figure 11 Average chlorophyll *a* concentrations from multiple dates.

Discussion

Chlorophylls are one of the three types of major photosynthetic pigments capable of initiating photosynthesis. Among the five types of chlorophylls, chlorophyll *a* in particular is what gives plants and algae their characteristic blue-green color (Hall and Rao 1999). Since chlorophyll *a* concentration can be measured and used as an indicator of algal activity, it is an important indicator of water body health (Lillie and Mason 1983). Excessive algae are damaging to water bodies such as lakes, rivers and oceans because of their potential to cause eutrophication. Essentially, a higher chlorophyll *a* concentration means a higher amount of algae present in the water.

Algae thrive on micronutrients such as nitrogen and phosphorus, which are abundant in avian fecal matter (Bazely and Jefferies 1985). Hence, it is important to monitor water bodies that host large numbers of waterfowl, which can indirectly contribute to eutrophication by allowing algal blooms through excessive fecal loads.

As predicted, the chlorophyll *a* concentration in Shubert Marsh (for both sampling dates) was lower than the water inside the breakwall, indicating the large quantity of waterfowl that nest there do indeed have an impact on its water quality.

Chlorophyll *a* concentration inside the breakwall at Terrell's Island had not been studied since its completion in 1998. The recently measured chlorophyll *a* concentration in the breakwall was nearly three times higher than values measured in 1999. Large populations of waterfowl, especially the AWP migrated to the region in the years following and have been nesting on the breakwall or islands surrounding it. Considering this population increase, there seems a correlation between the number of birds and water

quality inside the breakwall (Anderson and King 2005). The large amount of birds bathe, feed and defecate in the water inside the breakwall due to its location away from predators and humans. The nutrients from the fecal matter, with their soluble nitrogen and phosphorus content, contribute to algal growth. And with substantial bird numbers, the fecal load will be high, making HABs inevitable.

Overall, the chlorophyll *a* values from September were lower than in October. It is important to note that the majority of waterfowl that were present during the September date were no longer present during this second sampling date. Speaking with the groundskeeper at Terrell's Island, the birds had left only a week or so before the October samples were collected. It is possible that the algae were using the nutrients from the fecal matter present in the water inside the breakwall, hence, resulting in a higher chlorophyll *a* concentration from the collected sample.

The 2012 Shubert Marsh and breakwall samples together were overall higher than the Fox River mouth and breakwall samples from 1999 (Table 2). This is an interesting observation and a number of factors may have contributed to this phenomena. Waterfowl populations were higher in 2012 than in 1999. The waterfowl may have nested in various habitats surrounding Lake Butte des Morts and the Fox River, contributing to higher fecal loads throughout the area (albeit higher in some areas than others). Also, 2012 was considered to be a drought year, thus chlorophyll concentrations may have appeared higher than in a non-drought year (WI Dept. of Agriculture 2012).

Using data from this study will help conservation managers as well as the WI DNR to properly manage water quality at Terrell's Island. This is important especially

due to the high recreational activity present in Lake Butte des Morts and the Fox River in general as well as conserving the aesthetic quality of the region.

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