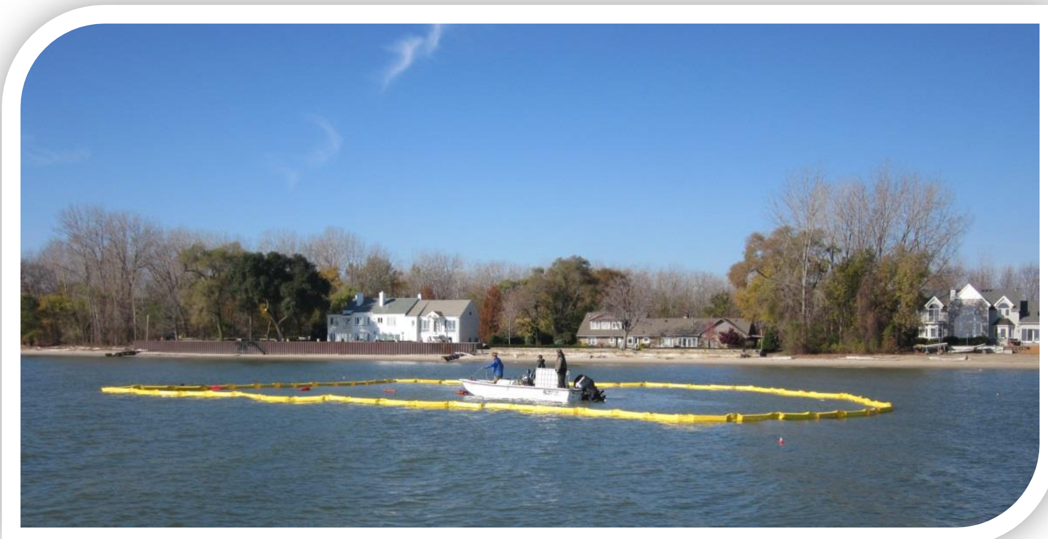
Zequanox Application Technique Pilot Study on Lake Erie



MBI Project Number 401_0053

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Prepared for:

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Report Date:

1/30/2015

Table of Contents

1.0 Acknowledgements

Marrone Bio Innovations, Inc. (MBI) would like to thank Michigan Department of Environmental Quality (DEQ) for initiating and funding this project. Sarah LeSage and Jennifer Johnson at DEQ, in particular, devoted significant time to support site selection, project logistics, pilot study execution, and permitting assistance. James Luoma and Todd Severson of the United States Geological Survey's Upper Midwest Environmental Science Center generously provided their time and assistance, including the use of a barge, for barrier installation and treatment execution. We thank PLM Lake and Land Management Corp. for their work to implement an application system for this project. Finally, a very special thanks to Tom Trout and Trout's Yacht Basin for providing a staging and mixing area for the project, use of their boat launch and slips during the project, and devoting time (and a forklift) to moving and loading equipment during the execution of the project.

2.0 Executive Summary

This pilot study was part of an effort by Michigan Department of Environmental Quality (MDEQ), working with Marrone Bio Innovations (MBI), to test an application strategy in high energy (high wave and wind activity) waters for Zequanox®. Zequanox is a biopesticide developed and registered by MBI for control of invasive zebra and quagga mussels (no other species) while having no lasting impact to water quality. This project is part of MDEQ's response to reduce the impacts and occurrences of harmful algal blooms in the Great Lakes.

The pilot study occurred within La Plaisance Bay along the western shore of Lake Erie in early November 2014. The study team injected a Zequanox solution into the bottom layer of water within a treatment site enclosed by PVC barriers. Zequanox concentrations were monitored at the bottom, middle, and surface of the enclosed waters to test the application technique's ability to create a stable treatment layer at the bottom without treating the full water column. The purpose of this pilot study was to help guide future development of a Great Lakes zebra mussel control strategy.

Project Objectives:

- Conduct a Zequanox application to evaluate methods for a large-scale invasive mussel treatment program within the Great Lakes.
- Determine the ability of a 15% - 20% (mass/volume) stock solution of Zequanox to maintain a benthic (bottom) treatment layer in the water column for 6 hours in conditions common to Lake Erie, within a barrier.
- Monitor turbidity, conductivity, dissolved oxygen, and pH during the treatment to identify any impacts to water quality.

Results Summary:

- The tested technique of benthically applying a 15% stock solution of Zequanox was successful in setting up a stratified treatment in the bottom layer of the water column.
- Mixing of the water column occurred by 3 hours 20 minutes after application.
- The treatment had no lasting impacts to water quality parameters, including turbidity, conductivity, dissolved oxygen, and pH.
- Additional evaluation of application techniques, including testing in summer months, evaluating additional methods, and development of improved formulations would benefit Zequanox treatment programs for high energy and deep water environments.

3.0 Introduction

Zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*), bivalves native to Eastern Europe, are invasive species that were first discovered in North America in the late 1980s in the Great Lakes. Since then, these destructive invaders have spread throughout North America and have caused significant ecological and economic damage to areas they have infested. Because of their ability to reproduce quickly, and in large numbers, these filter feeders can rapidly take over water systems they invade, outcompeting native species for food and space.

An issue that has become of increasing concern in the Great Lakes, especially Lake Erie, is the mussels' contribution to harmful algal blooms (HABs). HABs are capable of producing toxins that can impact water quality and create taste and odor issues and potentially unsafe conditions for drinking water. This impact was brought into the spotlight in August of 2014 when a *Microcystis* (blue-green algae) bloom near Toledo caused a "Do Not Drink" order to be placed on the drinking water of half a million residents (Frankel 2014, August 11). Although multiple factors contribute to HABs, including phosphorus and other nutrient input from runoff into lakes, these mussels' ability to selectively feed can create conditions that support HABs. The mussels are able to selectively reject food items while filter feeding, including toxin producing *Microcystis*, which creates conditions that support the growth and proliferation of blooms of those species (Vanderploeg et al. 2001).

Until recently, there were no commercially viable treatments for invasive zebra and quagga mussels in open water systems like lakes and streams. In June of 2014, the United States Environmental Protection Agency (EPA) approved the registration of Zequanox® for control of zebra and quagga mussels for recreational and environmental restoration in lakes, reservoirs, and other open waters. This has provided an environmentally compatible option for control efforts without impacts to non-target species, or lasting impacts to water quality.

Early testing in open waters with Zequanox occurred in inland lakes. In these calm waters, evaluation of target treatments to the bottom layer of water (the area inhabited by most adult mussels) using 5% Zequanox stock solutions, have been successful in inducing mussel mortality levels as high as 99% (with no lasting water quality impacts). This reduces the overall treatment within a lake from one in which the whole water column is treated, while still reaching the same mussel control objective.

In contrast to the early inland lake testing, the complex high wind and wave environment found in the Great Lakes presents different challenges for this application technique, with waves and currents that can induce mixing into the upper layers of water. In 2014, on Lake Minnetonka in Minnesota, United States Geological Survey scientists experienced these challenges on days with strong wind fetch while conducting Zequanox application trials. Although these challenges can be overcome by treating the entire water column, in lakes with deep water this is not as efficient of an approach as treating only the bottom layer where the mussels are located.

This report presents the design, results, and conclusions of a pilot study testing an application strategy for Zequanox to target the bottom layer of water in a section of Lake Erie. During this study, scientists used a thick stock solution (15%) to determine if the treatment could maintain concentrations in a benthic layer in the high energy waters of Lake Erie. The objective was to maintain 75% of the target concentration (the target was 100 milligrams active ingredients per liter [mg a.i./L] for at least six hours after application. This treatment duration was identified as providing the highest efficacy in the shortest treatment time when treating mussels in flow-through systems. Turbidity was used as an indicator for Zequanox concentration and was monitored along with other water quality parameters before, during, and the day following the application to assess the success of this technique.

4.0 Methods

This pilot study took place along the western shore of Lake Erie in Michigan waters during early November 2014. Section 4.1 describes the study site and its selection in more detail. The study team used a PVC barrier system (Section 4.2) to contain the treatment site and tested a Zequanox application technique targeting the bottom layer of water with a thick stock solution (Section 4.3). Zequanox concentrations were estimated during treatment with a turbidity correlation curve (Section 4.4) and were monitored to evaluate the success of the application technique, along with other water quality parameters including dissolved oxygen, pH, conductivity, and temperature to identify any impacts of the treatment (Section 4.5). Water temperature and ice formation at the time of this trial were not conducive to safe monitoring of mussel mortality which occurs over a period of 30+ days after Zequanox treatments.

4.1 Study Site

La Plaisance Bay is located along the western basin of Lake Erie near the town of Monroe, Michigan. A treatment site was selected within the bay approximately 80 meters (m) off-shore midway between the outflows for Otter Creek and Woodchuck Creek (Appendix A). Coordinates for the approximate center-point of the treatment area are 41°51'4.49"N, 83°23'47.62"W. Depths within the treatment area were mostly uniform, approximately 1.4 m. This site area was selected because of its proximity to potential staging locations and the long, shallow sloping bottom with sandy to small cobble substrate which was ideal for the team to deploy the turbidity curtains without the need for SCUBA equipment. A control location was designated with a buoy approximately 35 m southwest (upwind) of the treatment area. Zebra mussels were observed at the treatment site, indicating the selected location was also representative of an area that could be targeted for invasive mussel control treatments.

4.2 Barrier System

A 31.5 m by 25.5 m area was enclosed by custom-built Type II turbidity curtains (barriers) manufactured by Elastec/American Marine. The day prior to treatment, the team installed the barriers around the treatment area, adjusting the shape and depth of the treatment area from original plans (27.5 m x 27.5 m square in approximately 2 m depths) due to the high wind speeds and resulting rough wave conditions (Figure 1).

4.3 Zequanox Mixing and Application

Trout's Yacht Basin in Monroe, MI served as the mixing and staging location for this project. MBI staff pumped raw water drawn from the harbor into a 1000 liter (L) tote. Zequanox was mixed into the raw water using a ZC1 model powder disperser (Quadro Engineering) and transferred to a second tote. The mixed stock solution was then transferred to a tote onboard the application boat using a ¾ hp flexible impeller pump (Appendix B). Initially, 120 kilograms of Zequanox (Lot # 401P140904MC) was mixed into 600 L of water (final volume) to reach a 20% weight per volume (w/v) stock solution. The resulting stock solution was difficult for the pumps to transfer, so an additional 150 L of water was mixed with the stock solution to form a final stock solution of 15% w/v, which was easily transferred with the pumps available.

The application boat then traveled to and entered the treatment site and anchored in the center of the enclosure. PLM Lake and Land Management (PLM) staff deployed a drop-hose system with four injection points from the boat to the edge of the barriers. The drop-hoses were adjusted to deliver product to the bottom 0.75 m of the water column. PLM staff continuously pumped the stock solution through the drop hose system while circulating the drop-hose line counter-clockwise around the treatment area (Figure 1). Application of the product was complete within 20 minutes, which was equal to three passes of the hose system over the area.

Figure 1 - Zequanox application within the treatment site. Drop hoses are located at each of the orange floats within the barrier. Photo credit: James Luoma, United States Geological Survey

4.4 Turbidity Correlation Standards

Zequanox concentration has a linear relationship with turbidity, and so turbidity measurements are used to determine the concentration of Zequanox in treated waters. A location specific correlation between turbidity and Zequanox concentration was created by filling three beakers with 100 milliliters (mL) of water from the study area and dispensing a small measured aliquot of stock solution to each beaker to reach the target concentration in the beaker water. The resulting average turbidity recorded from the three beakers was the turbidity standard for that concentration. Turbidity standards were created for the following concentrations: 0 milligrams of active ingredient per liter of water (mg a.i./L), 25 mg a.i./L, 75 mg a.i./L, 100 mg a.i./L, and 200 mg a.i./L. MBI staff completed a linear regression of the readings to generate an equation for calculating the concentration of Zequanox in treated waters at any sampled turbidity, which was used to monitor the concentration during the treatment and evaluate the application technique.

4.5 Water Quality Monitoring

A water quality monitoring station was established at each of four mid-points along the edges of the treatment area. One additional station was established at an untreated control site (Figure 2). An In-Situ Troll 9500 Profiler was used to collect temperature, pH, turbidity, conductivity, and dissolved oxygen data from the bottom, middle, and surface at each station. Readings were taken prior to treatment, immediately after application, every 30 minutes for the first two hours after application, then approximately every hour thereafter until dark, and then once on the day following the treatment.

Figure 2 - Water quality monitoring stations at the study site. The yellow lines represent the barrier and perimeter of the treatment area. Orange diamonds represent monitoring stations within the treatment site and the green diamond represents the untreated control site monitoring location.

5.0 Results

5.1 Zequanox Concentration Standards

Turbidity standards indicated that background (0 mg a.i./L) turbidity of the study area was 15.1 nephelometric turbidity units (NTU) ± 2.5 NTU. Turbidity of the standard for the target treatment concentration (100 mg a.i./L) was 128.0 NTU ± 2.6 NTU. These two points, along with the results for the 25 mg a.i./L, 75 mg a.i./L, and 200 mg a.i./L standards were plotted on a graph. A linear regression of these data points with an R^2 value of 0.9307 (indicating high correlation) provided an equation that was then used to determine Zequanox concentration from turbidity readings during this pilot study.

5.2 Zequanox Mixing and Application

On treatment day, wind speeds were out of the west between 10 and 15 miles per hour. Waves were 1 to 2 feet in height and were approximately out of the southwest. These wind speeds are at or slightly above average for November and fall into the typical minimum/maximum range for the month, however they are higher than typical averages and average maximums for late July and early August in the region, when water temperatures are warmer (Cedar Lake Ventures n.d.).

The initial 20% w/v Zequanox stock solution proved to be too thick for the pump in the mixing system to transfer between the 1000 L tote on shore and the application boat, so the stock solution was diluted to 15%. During application, a layer of Zequanox could be seen (indicated by a cloudiness in the water) in the lower portion of the water column. There were instances where Zequanox could be seen mixing to the surface of the water, most notably coinciding with observed bubbles rising in the water at the start of the application and when the application system was stopped and then restarted towards the end of application (Figure 3).

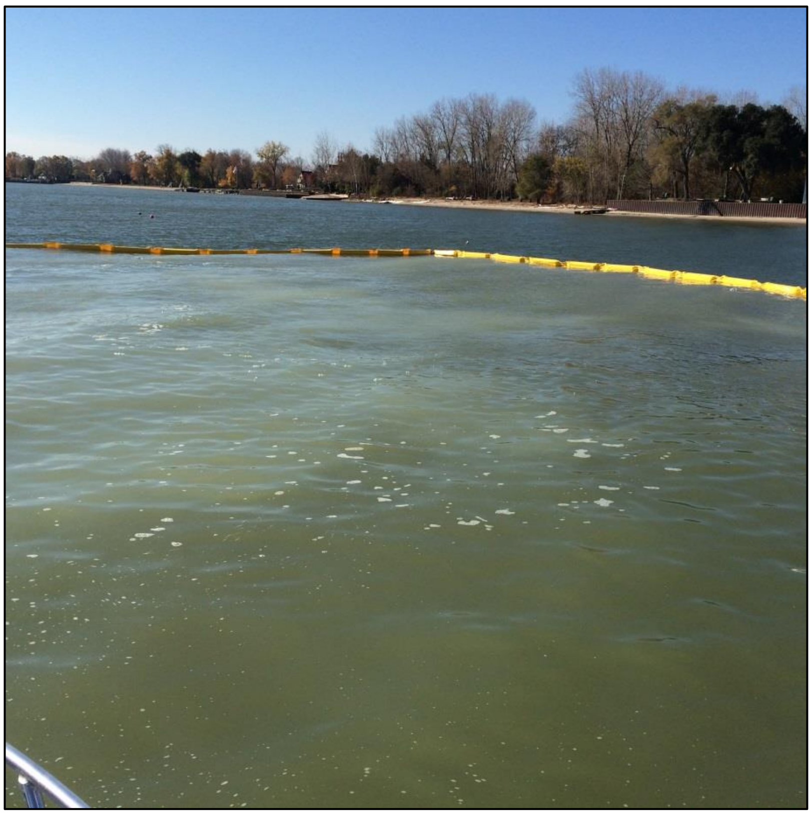

Figure 3 - View of the treatment area shortly after Zequanox application. Most of the cloudiness can be seen below the surface along with scattered plumes where Zequanox dispersed to the surface with bubbles. Photo credit: James Luoma, United States Geological

The pluming up of Zequanox during the application is thought to be due to unintentional air entrainment in the injection system. Air bubbles forced through the injection line outlets cause pressure fluctuations at the injection ports, and also injection of pressurized air to the benthos causes mixing of the water column as the air ascends. Charging (filling) the injection system with water prior to treatment to remove any air from the system and/or planning for a small additional amount of product will adjust for this source of water column mixing in future work.

5.3 Zequanox Concentrations in the Treatment Site

Turbidity levels within the treatment site after installation of the barriers (prior to treatment) were slightly higher than at the control site (approximately 10 NTU higher) due to the bottom of the barrier stirring up sediments (this difference was accounted for in calculating treatment concentrations). Immediately after application, a strong turbidity gradient was recorded with calculated concentrations at or near the targeted concentration of 100 mg a.i./L in the bottom layer (average 93.6 mg a.i./L). The middle and surface layer turbidity levels were slightly elevated indicating Zequanox concentrations of 14.9 mg a.i./L and 1.8 mg a.i./L, respectively. Over time, some mixing occurred with concentrations in the surface and middle layers increasing while concentrations in the bottom layer decreased. By 3 hours and 20 minutes after the Zequanox application, concentrations were evenly mixed across all depths (average 30.9 mg a.i./L) and remained stable at that level until the barriers were removed the following morning (Figure 4). During removal of the barriers, turbidity in the treated area could be seen quickly returning to appear the same as the surrounding untreated areas as the treated waters dispersed.

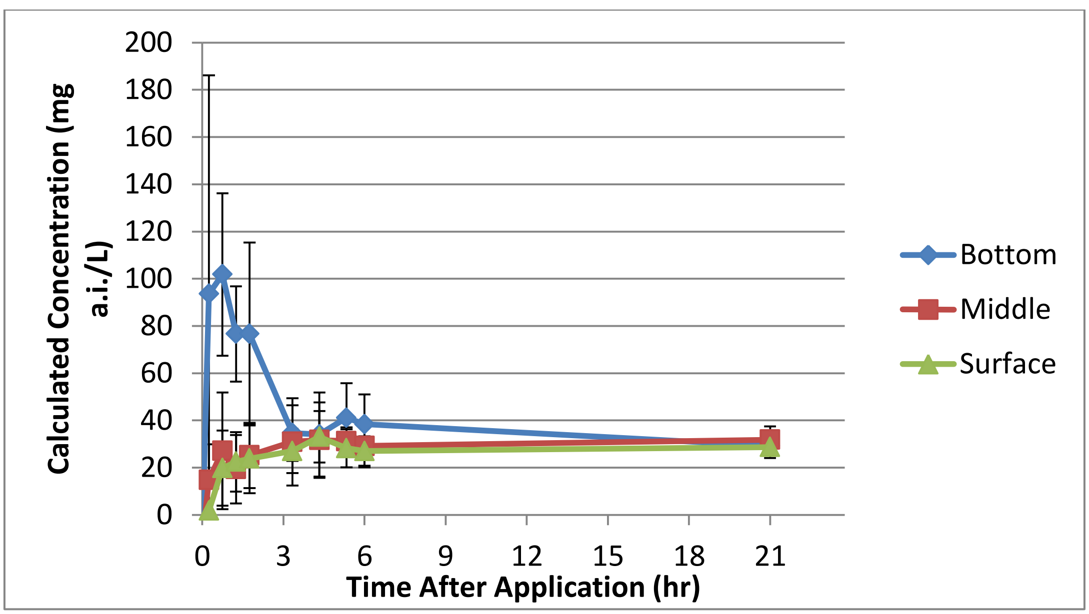

Figure 4 - Average calculated Zequanox concentrations with standard deviations in the bottom, middle, and surface layers of the treatment area.

In this study, a goal was set of maintaining at least 75% of the target treatment concentration in the bottom layer for 6 hours. This goal was set based on previous data from enclosed flow-through treatments, -- a use of Zequanox that has been registered for a number of years. Although treatment concentrations in the Lake Erie pilot study did not maintain concentrations for 6 hours(discussed further below), other previous work in open waters suggests that shorter exposure periods could still result in acceptable levels of mussel mortality. A Zequanox treatment in August 2013 at Deep Quarry Lake, Illinois maintained a stratified bottom treatment layer for at least 4.5 hours (calm conditions), and had acceptable mussel mortality to deem the treatment a success (Whitledge et al. 2015). Future work with stakeholders to develop acceptable mortality levels for a mussel control strategy in the Great Lakes along with additional studies evaluating minimum treatment times to reach the set mortality goals in peak mussel treatment months would be beneficial to establishing a Great Lakes treatment program.

5.4 Water Quality Monitoring

No significant difference in temperature, dissolved oxygen, conductivity, or pH was recorded between depths at either the treatment or control site. Between the two sites, the only difference noted was a slight variation in conductivity which is discussed below. Average water temperatures during treatment ranged from 8.5°C in the morning prior to treatment to a peak of 9.8°C during the day of the treatment. There was no significant difference in dissolved oxygen when comparing the treatment and control sites throughout the monitoring period. Dissolved oxygen in both sites generally remained between 11.4 milligrams per liter (mg/L) and 11.9 mg/L. Similarly, no differences in pH were recorded between the treatment and control site after the application of Zequanox with pH values ranging between 7.8 and 8.0. Differences in conductivity were recorded between treatment and control sites both prior to and during the Zequanox treatment, possibly due to the placement of the barrier around the treatment site. Treatment site conductivity ranged from 221.4 microsiemens/centimeter (µS/cm) to 247.4 µS/cm while average control site conductivity ranged from 236.6 µS/cm to 271.7 µS/cm. These values and intraday variations are within range of what has been recorded in the western basin of Lake Erie (Michigan Technological University 2014), indicating no distinct impact from the treatment to conductivity. A table with water quality monitoring results can be found in Appendix C.

6.0 Conclusions and Future Recommendations

The application technique evaluated was successful in applying a stratified Zequanox treatment in the bottom layer of the water column; however the use of a thick stock solution and barrier curtains were not sufficient to counteract the mixing that occurred from wind and waves in Lake Erie. The water quality monitoring results indicate that Zequanox treatments can be completed with no lasting impact to water quality.

Identification and consensus by shareholders of the levels of mussel control needed to achieve various management objectives in the Great Lakes are needed to guide treatment recommendations going forward. Research in the summer (the season of highest mussel activity and highest control potential) is recommended next in order to refine treatment period recommendations in high energy environments such as those in Lake Erie.

Additional development of barrier free treatment methods are also recommended. The current Zequanox formulation (a spray-dried powder) is developed to disperse readily for use in enclosed systems (for example, industrial cooling water systems). For open waters, development of an alternate formulation which is better able to target the benthos (where adult mussel populations are found), and limit dispersion over time into the upper layers, such as a sinking, slow dissolving granule, would be most beneficial.

7.0 References

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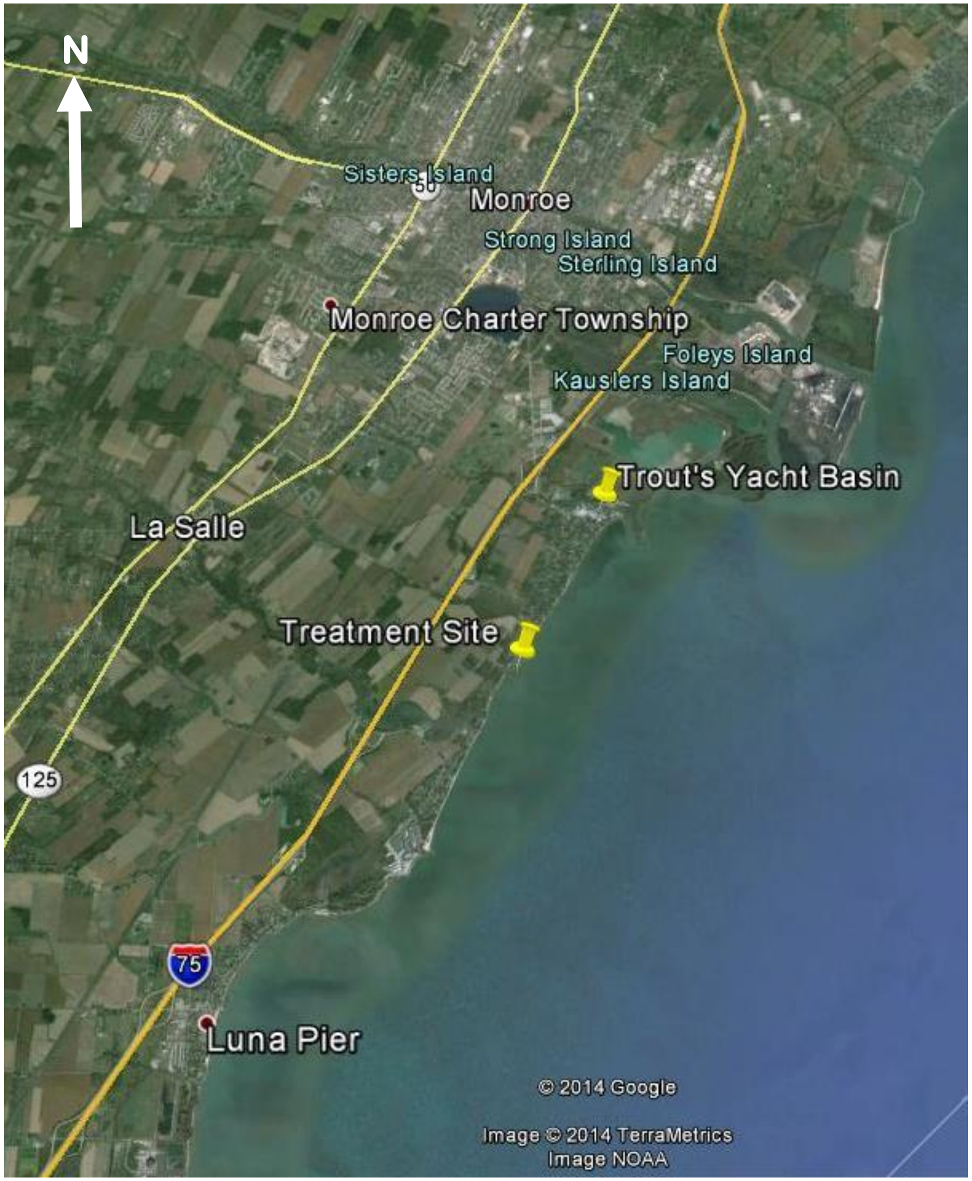
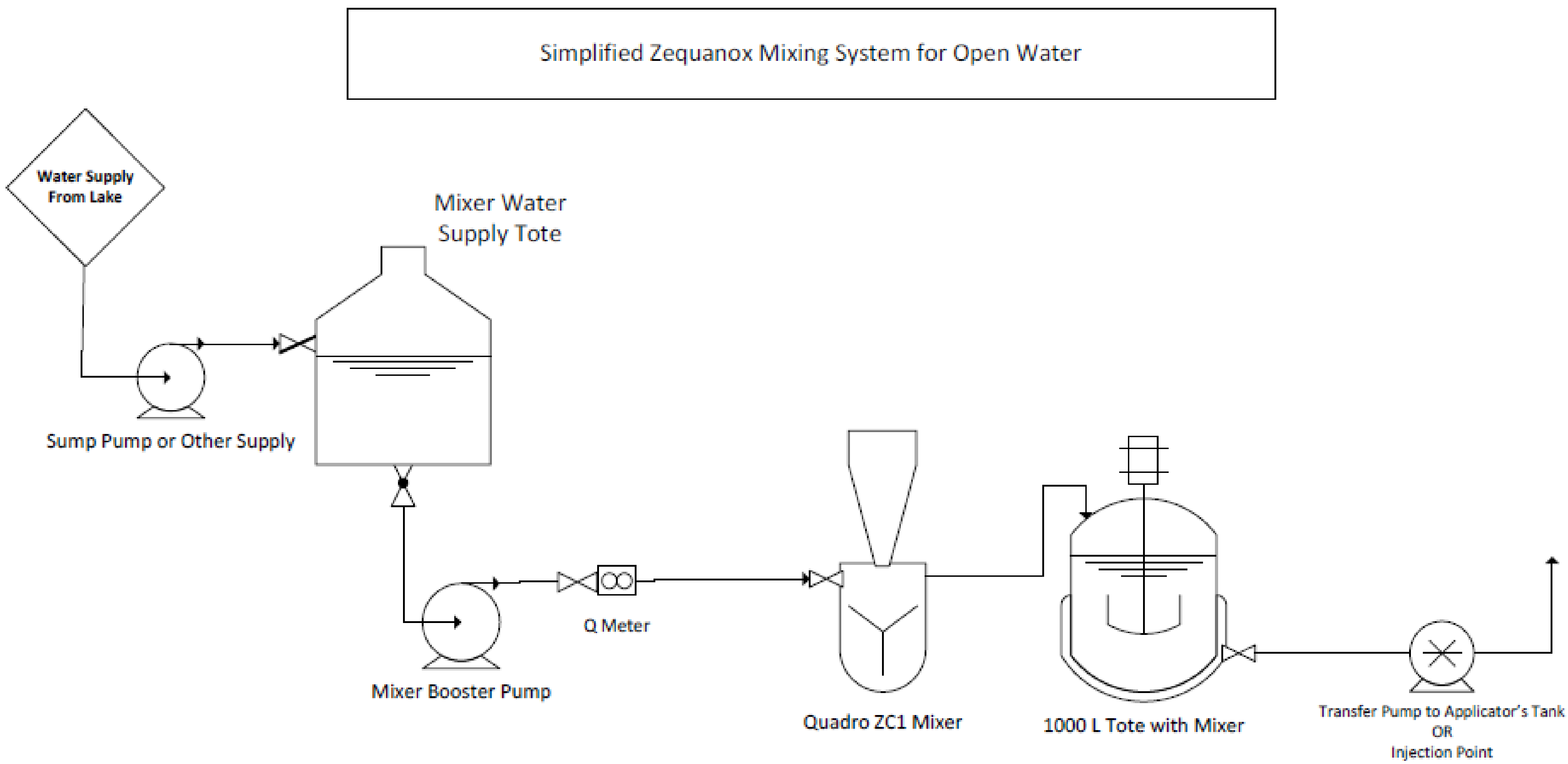
8.0 Appendix A – Treatment Area Maps



9.0 Appendix B – Mixing System Schematic



10.0 Appendix C – Water Quality Results Table

Time after Application (hh:mm)	Layer	Dissolved Oxygen (mg/L)		pH		Conductivity (µS/cm)	
		Treated	Control	Treated	Control	Treated	Control
Pre-treatment	Bottom	11.5 (0.0)	11.6	7.7 (0.1)	7.5	222.8 (0.8)	264.6
	Middle	11.6 (0.1)	11.7	7.7 (0.1)	7.5	222.7 (1.1)	262.4
	Surface	11.9 (.02)	12.4	7.7 (0.1)	7.5	222.4 (0.9)	262.3
0:15	Bottom	11.7 (0.0)	11.5	7.9 (0.0)	7.9	237.4 (4.5)	267.5
	Middle	11.7 (0.0)	11.6	7.9 (0.0)	7.9	227.3 (2.0)	267.4
	Surface	11.8 (0.1)	12.0	7.9 (0.0)	7.9	227.0 (1.1)	267.8
0:45	Bottom	11.6 (0.1)	11.5	7.8 (0.0)	7.9	239.0 (3.4)	267.1
	Middle	11.7 (0.0)	11.6	7.9 (0.0)	7.9	230.0 (1.4)	264.0
	Surface	11.8 (.01)	11.6	7.9 (0.0)	7.9	229.6 (1.4)	267.8
1:15	Bottom	11.7 (0.0)	11.6	7.9 (0.0)	7.9	233.7 (2.3)	258.0
	Middle	11.7 (0.0)	11.6	7.9 (0.0)	7.9	232.2 (3.7)	260.2
	Surface	11.8 (0.0)	11.9	7.9 (0.0)	7.9	231.0 (1.1)	262.0
1:45	Bottom	11.7 (0.0)	11.6	7.8 (0.0)	7.9	236.2 (0.8)	257.8
	Middle	11.7 (0.0)	11.6	7.9 (0.0)	7.9	232.9 (0.6)	258.5
	Surface	11.7 (0.1)	11.6	7.9 (0.0)	7.9	232.3 (0.6)	258.5
3:20	Bottom	11.7 (0.0)	11.9	7.8 (0.0)	7.8	236.1 (0.2)	256.4
	Middle	11.7 (0.0)	11.8	7.8 (0.0)	7.8	236.0 (0.2)	255.8
	Surface	11.7 (0.0)	11.4	7.8 (0.0)	7.8	236.2 (0.3)	255.8
4:20	Bottom	11.7 (0.0)	11.9	7.9 (0.0)	7.9	240.8 (2.3)	259.0
	Middle	11.7 (0.0)	11.8	7.9 (0.0)	8.0	237.6 (0.2)	236.6
	Surface	11.7 (0.0)	11.5	7.9 (0.0)	8.0	237.6 (0.2)	271.5
5:20	Bottom	11.7 (0.0)	11.8	7.9 (0.0)	7.9	238.6 (0.3)	265.5
	Middle	11.7 (0.0)	11.8	7.9 (0.0)	7.8	238.7 (0.3)	259.5
	Surface	11.7 (0.0)	11.4	7.9 (0.0)	7.8	238.7 (0.3)	255.3
6:00	Bottom	11.7 (0.0)	11.9	7.9 (0.0)	8.0	239.6 (0.8)	271.7
	Middle	11.7 (0.0)	11.8	7.9 (0.0)	8.0	239.0 (0.5)	269.4
	Surface	11.7 (0.0)	11.8	8.0 (0.1)	8.0	239.0 (0.7)	267.4
21:00	Bottom	11.4 (0.0)	11.4	7.9 (0.0)	8.0	246.6 (0.7)	261.6
	Middle	11.5 (0.0)	11.4	7.9 (0.0)	8.0	246.2 (0.4)	261.6
	Surface	11.5 (0.0)	11.5	7.9 (0.0)	8.0	246.1 (0.4)	261.6

Note: The values in the treated columns represent the average of the four water quality monitoring stations with standard deviations following in parentheses.