GREAT SHIPS INITIATIVE
BENCH-SCALE TEST FINDINGS
Technical Report - Public

Ozone (O_3) and Sonic Energy

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ABSTRACT

The Great Ships Initiative (GSI) conducts bench-scale (i.e., laboratory scale) research to aid developers of innovative technologies which could have application as ballast treatment systems. This report describes 2008 findings from bench-scale evaluations of a ballast treatment system proposed by Environmental Technologies Inc., of Ellisville, Missouri. The treatment system—a combination of ozone and sonic energy—is intended for use as a routine ballast treatment. GSI tested the proposed system to assist range-finding for effective doses, determination of chemical degradation, and the potential for residual toxicity. Please see www.greatshipsinitiative.org for more information about GSI's bench-scale testing program.

The ozone treatment component of the system proved effective at the bench scale at inactivating all species tested, including algae and bacteria, within the proposed dose range in laboratory water (LW) only. In challenge water (CW), the same species treated with the same level of ozone and sonic energy were highly resilient with a rate of survival ranging from 73 – 100 %, while the microbes showed no effect of treatment at all. The applied sonic energy led to no additional mortality across all species tested.

Chemical degradation experiments indicated that ozone did not degrade dramatically in low NPOC water (LW), but degraded rapidly to non-detect in high NPOC water (CW). Thus, in the conditions in which ozone was effective (LW), it did not degrade, and significant residual toxicity was observed. Survival of sensitive species in "post-discharge" water was 0 % at 24 hours for all species tested.

These results suggest that ballast treatments involving ozone, to be promising in the Great Lakes region, like chlorine-related treatments, should include a mechanism to assure treatment effectiveness in high NPOC water where ozone degradation is rapid, and a means of neutralizing residual toxicity in low NPOC water, where ozone degradation is slow. The question of reaction by-products resulting from ozone treatment was not investigated in this study, but should be prior to application to a larger scale.

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INTRODUCTION

This technical report presents quantitative and measured findings from GSI bench-scale evaluations of ozone (O_3) in combination with sonic energy as a possible ballast treatmentsystem with application to the Great Lakes. The system, developed by Environmental Technologies Inc., of Ellisville, Missouri, is proposed for routine use as a ballast treatment. GSI undertook these bench-scale tests during 2008 at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior in Superior, Wisconsin. Tests included range-finding evaluations of dose effectiveness, chemical degradation and residual toxicity. A summary of these findings for non-scientific audiences can be accessed at http://www.nemw.org/GSI/GSI-BS-P-FS-3.pdf.

BACKGROUND

Great Ships Initiative (GSI)

GSI is a regional effort devoted to ending the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System and globally. In support of that goal, the GSI has established superlative freshwater ballast treatment evaluation capabilities at three scales—bench, land-based, and on board ship.

GSI status testing is performed at the scale appropriate to the treatment state of development, with the goal of helping meritorious ballast treatment systems to progress as rapidly as possible to an approval-ready and market-ready condition. Developers of ballast water treatment systems apply for GSI research services online, and awards are offered based on an objective external review process, regardless of the state of development of the proposed treatment.

GSI tests are third party assessments, i.e., GSI has no involvement, intellectual or financial, in the mechanics, design or market success of the actual treatment systems it tests. To ensure that GSI tests are uncompromised by any real or perceived individual or team bias relative to test outcomes, GSI test activities are subject to rigorous QAQC procedures and documentation. This attention to QAQC assures high quality and credible evaluation of GSI and its findings.

GSI tests are supported by general project funds which derive from federal and state agency grants, Great Lakes port contributions, and in-kind contributions by the local government and universities. None of these funds come to the GSI with any strings other than timely public disclosure of methods and findings.

Organization

The GSI is a project of the Northeast-Midwest Institute (NEMWI)—a Washington, D.C-based private, non-profit, and non-partisan research organization dedicated to the economic vitality, environmental quality, and regional equity of Northeast and Midwest states. The project is carried out collaboratively with contracting entities including the University of Wisconsin-Superior (UW-S), AMI Consulting Engineers, Broadreach Services, and the University of Minnesota-Duluth (UM-D).

Ms. Allegra Cangelosi of NEMWI is the GSI Principal Investigator and Project Manager (GSI PI). A GSI Advisory Committee comprises top-level officials of key stakeholder groups, and provides direct input to Ms. Cangelosi, advising GSI award decisions, program direction, finances and fund-raising. The American Great Lakes Ports Association advises the project, assuring that the GSI is meeting the needs of the maritime industry; and coordinating maritime industry and supply chain outreach. Researchers from LSRI and the UM-D's Natural Resources Research Institute (NRRI), among others, provide critical scientific and technical expertise and implementation services to the GSI PI. Dr. Mary Balcer of the LSRI is the project's lead zooplankton ecologist. She is also the team leader for the UWS-LSRI staff engaged in GSI research activities. Dr. Euan Reavie of the NRRI leads all phytoplankton analysis and NRRI staff. Mr. Matthew TenEyck of LSRI leads all bench-testing and Whole Effluent Toxicity (WET) tests. Ms. Heidi Saillard of LSRI is responsible for GSI microbial analysis. Mr. Tyler Schwerdt of AMI Consulting Engineers provides engineering expertise and services in support of GSI testing activities.

Projects and Activities

GSI's current suite of projects and activities includes independent third party ballast treatment evaluations at three scales—bench, land-based, and shipboard. Each scale is dedicated to addressing specific evaluation objectives. These include:

GSI Bench-Scale Tests

- Range finding for effective treatment dose against diverse freshwater taxa and water quality conditions;
- Generation of freshwater relevant chemical degradation curves; and
- Estimation of residual toxicity given diverse freshwater taxa and water quality conditions.

GSI Land-Based Tests

- Pre-certification testing, i.e., operational and biological performance (including residual toxicity) status-testing given scale-up and a range of challenge conditions; and

- Certification/verification testing, i.e., formal assessment of performance against IMO and other discharge standards.

GSI Shipboard Tests

- Confirmation of biological and operational treatment performance as expected in the ship environment;
- U.S. Coast Guard Shipboard Technology Evaluation Program (STEP) testing;
- Shipboard type approval testing;
- Ship discharge monitoring; and
- Methods development.

GSI BENCH-SCALE TESTS

GSI bench-scale tests take place year-round at the LSRI of the University of Wisconsin-Superior in Superior, Wisconsin. The LSRI is amply equipped with staff expertise and resources to conduct the tests, and has a long history of successfully undertaking similar tests.

The overarching goals of GSI bench testing are to explore dose-effectiveness, chemical degradation, residual toxicity, and/or sensitivity to challenge conditions of a proposed treatment method about which little is known. To that end, the tests are "range-finding" missions, to determine the optimal treatment dose/intensity that would maximize effectiveness and minimize residual toxicity. Findings help treatment developers better design an effective system and/or to move to the next stage of treatment evaluation. The tests are also a form of trouble-shooting to encounter possible problems with the proposed treatment in advance of more extensive and larger scale tests.

GSI bench-scale dose effectiveness tests help determine the range of concentrations of an active substance that is harmful to a variety of robust freshwater zooplankton, algae and bacteria known to be relatively resilient to stressors. Dose effectiveness test results are expressed as percent survival, percent mortality, and percent hatch. Where applicable, they are also expressed in terms of a series of absolute quantifications: LC_{99}, i.e., the experimentally derived concentration of an active substance estimated to kill 99 % of the test population following 24 or 48 hours of continuous exposure; No Observed Effect Concentration (NOEC), i.e., the highest concentration of an active substance shown to have no significantly adverse effect on the test population compared to controls; and Lowest Observed Effect Concentration (LOEC), i.e., the lowest concentration of an active substance known to have a significantly adverse effect on the test population compared to controls.

GSI bench-scale chemical degradation tests determine the effect that various water quality or environmental parameters may have on the rate of chemical degradation of a treatment system involving active substances. No organisms are used in association with

these analytical assays; instead test solutions are analyzed for their concentration of active substance (or active component of the substance). Test results are expressed as the percent change in active ingredientconcentration.

GSI bench-scale residual toxicity tests help estimate the effect that treated water (following neutralization of the active substance, a degradation period, or no treatment at all) may have on non-target organisms in the receiving system. These test results are expressed in a manner similar to those for dose effectiveness assays. The principal difference between these tests and dose effectiveness tests is that the concentration of the active substance is adjusted to be consistent with potential discharge levels, and the tests are performed on sensitive organisms rather than robust species.

Please note that GSI's bench-scale tests do not by themselves provide adequate information to assess a prospective ballast treatment's ability to meet a particular discharge standard or to achieve environmental soundness under shipboard application. Instead, these tests provide initial insights for developers of treatment systems into possible strengths and weaknesses of the proposed treatment—information that can be used by developers to better design a more effective system and/or to move to the next stage of treatment evaluation. The tests are also a form of trouble-shooting to encounter possible problems with the proposed treatment in advance of more extensive and larger scale tests.

METHODS

Treatment System

Test Apparatus

In these tests, the treatment system consisted of an ozone generator and a 250 mL sonic energy flow cell reactor fitted with a sonic energy generator. A pump with a nominal flow rate of 4.0 gallons per minute (gpm) at 60 psi was used to draw test water from a 1000 L plastic influent tank through the treatment system and into a discharge tank, also with a capacity of 1000 L and made of plastic (figure 1).The influent tank served as a reservoir for the various water qualities and test organisms (with the exception of larger zooplankton, see below); the discharge tank was used to capture post-treatment effluent.

Test water was added to the influent tank via hot and cold water taps (figure 1). Both taps were fitted with an activated carbon filter to remove residual concentrations of chlorine. A three-way valve, installed in the intake line just after the influent tank was used to feed the larger zooplankton (i.e., copepods and cladocerans) into the influent line (figure 1). These organisms were held in a 2.0 L zooplankton dosing container positioned above the influent tank (figure 1). Following treatment, the larger zooplankton were captured in the discharge line using a 20 μm mesh net suspended in a 113 L container (figure 1). A

whole water sample was used to collect the smaller species (e.g. microbes and algae; figure 1).

A Venturi inlet port was used to inject ozone into the influent water. An ozone analyzer connected via a sample port located in the gaseous ozone generator line allowed ozone levels to be monitored through quantification of the amount of gaseous ozone generated.

Sample infrastructure was connected via 1.0 inch (ID) flexible PVC tubing (figure 1), with the distance from the point of ozone injection to the point of sample collection approximately 14 feet. Experiments took place in a LSRI laboratory equipped with adequate ventilation, electrical connections, and climate control.

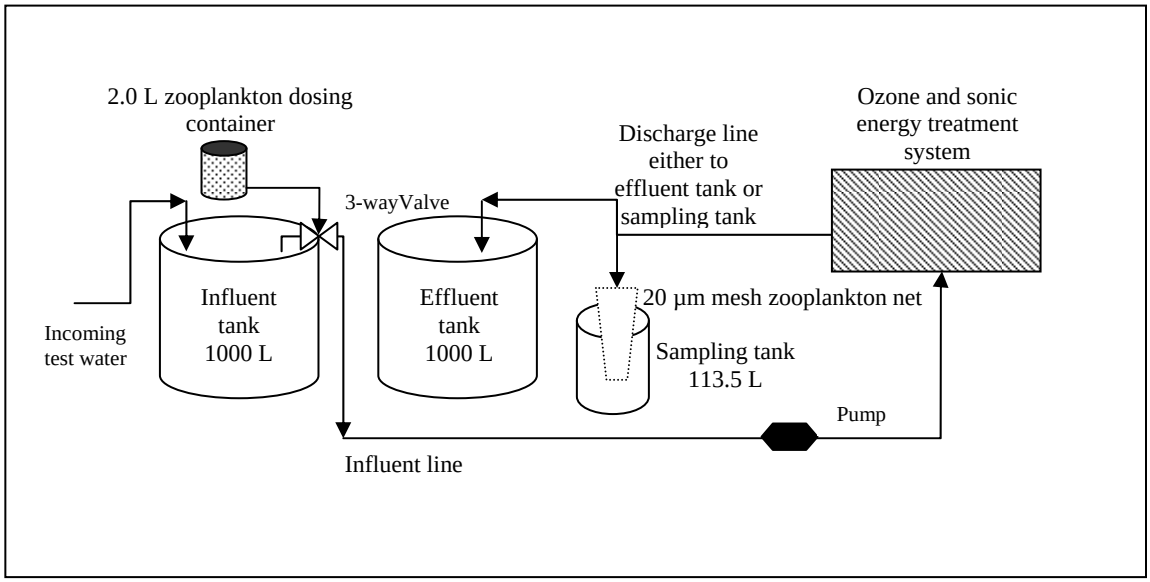

Figure 1. Schematic of general testing apparatus (not to scale).

Treatment Application

Differing amounts of ozone and sonic energy in 50 % increments were added to the test water by adjusting the settings of the ozone generator and sonic generator. The highest setting of both instruments was presumed to achieve 100 % output, and half-way to the full setting was presumed to achieve 50 % output. Using this approach, the following treatments were applied:

- 100 % ozone output and 100 % sonic output,
- 100 % ozone output and 50 % sonic output,
- 50 % ozone output and 100 % sonic output,
- 100 % ozone output and 0 % sonic output,
- 0 % ozone output and 100 % sonic output, and/or
- 0 % ozone output and 0 % sonic output (i.e., a control).

Prior to sample collection, the pump was turned on and the flow rate measured and recorded. The flow rate over the course of all experiments averaged 4.2 gallons per minute with an ozone retention time of 3.6 seconds. The oxygen flow to the ozone analyzer was set to 8.0 standard cubic feet per hour (SCFH). Once the gaseous ozone analyzer reached a stable reading (approximately 30-45 seconds) a water sample was collected at the end of the 14 foot hose. The applicant confirmed that the system was operating properly prior to commencement of the test.

It should be noted that while there was a method to calculate the applied ozone level to the influent, i.e., based on data collected from the gaseous ozone analyzer, ozone weight (%), and an average flow rate of 4.2 gpm with ozone retention of 3.6 seconds, it was not possible to determine how much of that applied ozone infiltrated the water column. As such, we report applied ozone versusmeasured ozone concentrations for effluent only (see results). With respect to sonic energy, only nominal values (full output versus 50 % output) were available for benchmarking applied sonic energy levels. It was also not possible to measure the consistency of the applied sonic energy level.

General Methods

Sample Water Preparation

Experimental water qualities were prepared in the laboratory as follows:

- Laboratory water (LW): Treated Lake Superior water from the City of Superior that was passed through an activated carbon column. Alkalinity ranged from 45-50 mg/L as $CaCO_3$. Total organic carbon ranged from 0.5-2.0 mg/L. The water is transparent.
- Challenge Water (CW): LW as described above with addition of tannic (15 mg/L) and humic acids (5 mg/L) to adjust the non-purgeable organic carbon (NPOC) to approximately 8 mg/L. The water is stained and has a lower light transmittance when compared to LW. The enriched NPOC level is also reflective of Duluth-Superior Harbor water.
- Salt water (SW): LW as described above with the addition of a commercially prepared salt mix to adjust the salinity to 32.0 ppt.
- Brackish water (BW): LW as described above with the addition of a commercially prepared salt mix to adjust the salinity to 16.0 ppt.

Sample Collection and Processing

Whole water samples were collected at the end of the 14 foot collection hose after the ozone analyzer reached a stable readingover the course of 15 minutes. The process involved rinsing a 1.0 L Teflon beaker twice with test water prior to collection of the sample. After rinsing, a large enough sample volume was collected to enable subsequent analysis of organisms and/or physical/chemical parameters.

In the case of larger organisms, i.e., zooplankton, organisms were captured in the discharge line using a 20 μm mesh net suspended in a 113 L container (figure 1). Organism exposure occurred while sample water passed through the treatment system and until the organisms could be rinsed with LW—a period of approximately five minutes. Organisms were gently rinsed with LW and directed off the sieve into a zooplankton counting wheel. This process took approximately 5 minutes from the time of collection and was considered the time point in which the exposure to the ozone was stopped. From the counting wheel, the organisms were randomly placed into their observation containers. Organisms were transferred with minimal amounts of treated test water to reduce exposure to residual treatment products after passing through the treatment system. In the case of bacteria, exposure to the ozone was neutralized using sodium thiosulfate already contained in the IDEXX containers.

Sample Analysis

Samples were analyzed for various physical/chemical parameters including concentrations of ozone, non-purgeable organic carbon (NPOC), total residual oxidants (TRO), and percent transmittance at 254 nm (%T). In some cases, i.e., for chemical degradation tests, samples were also analyzed for bromide and bromate.

In dose effectiveness and residual toxicity tests, organism mortality was measured by counting the number of dead organisms based on active and reactive mobility assessments at the appropriate time period. If organisms were immobile they were further examined under a dissecting microscope to check for internal movement or heart beat. If none was evident, the organism was deemed dead.SYTOX® Green, a nucleic acid stain that penetrates cells with compromised plasma membranes (i.e. dead cells) but does not enter live cells was used to check the survivorship of the algae.

Statistical Methods

At each time point (i.e., 0, 24, and 48 hours), treatment group means were compared using a one-way repeated measures analysis of variance (RM ANOVA). If the data passed the normality and equal variance tests, Dunnett's method for pairwise multiple comparisons was used to determine if significant (i.e., $p<0.05$) differences existed between the treatment group means at each time point. If the data did not pass the normality test, the data were compared using Kruskal-Wallis ANOVA on Ranks and Dunn's Method for pairwise comparisons to determine if significant differences existed between the treatment group ranks at each time point.

Experimental Methods

DoseEffectiveness Experiments

GSI dose effectiveness tests measured the effects of various combinations of ozone and sonic energy on a range of freshwater organisms known to be relatively resilient to stressors. Test organisms included the green algae *Selenastrum capricornutum*, the adult

rotifer *Branchionus calyciflorus,* resting eggs of *Branchionus calyciflorus,* adult copepods *Eucyclops sp.* and the cladoceran *Daphnia magna.*

Dose effectiveness was examined in two water qualities (LW and CW) held at a temperature of 25.0 ºC ± 1.0 ºC. Test water was not renewed.All exposures except for those involving rotifer adults and rotifer resting eggs took place in complete darkness over 48 hours, with 50 mL of exposure solution in a 300 mL beaker, and three replicates. Tests on rotifer adults and rotifer resting eggs involved four replicates and 2.0 mL of exposure solution. In addition, adult rotifers were exposed in complete darkness for 24 hours due to their sensitivity. The rotifer resting eggs were exposed to continuous light to stimulate hatching.

In all cases, periodic observations were made on mortality, and measured water quality parameters including temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness. Table 1 describes the exposure conditions across organism type while table 2 arrays the types and numbers of organisms analyzed, the ozone and sonic energy output levels, and the number of replicates per dose effectiveness test.

Table 1. Exposure Conditionsfor GSI Dose-Effectiveness Tests on Algae and Zooplankton.

Organism	Exposure Volume per Replicate (mL)	Exposure Duration (hr)	Light:Dark Cycle (hr)	Temperature (º C)
Green alga (*Selenastrum* spp.)	50	48	0:24	25±1.0
Newly hatched rotifers, (*Brachionus calyciflorus*)	2	24	0:24	25±1.0
Rotifer resting eggs,(*Brachionus calyciflorus*)	2	48	24:0	25±1.0
Copepods (*Eucyclops* spp.)	50	48	0:24	25±1.0
Cladoceran (*Daphnia magna*)	50	48	0:24	25±1.0

Table 2. Numbers and Types of Organisms, and Ozone and Sonic Energy Output Levels Used for GSI Dose-Effectiveness Tests on Algae and Zooplankton.

Organism Type	Species	Test Water Types	Ozone and Sonic Energy Output Levels (%)	No. of Organisms per Exposure /Control	No. of Replicates per Exposure /Control	Total Number of Organisms
Green alga	*Selenastrum spp.*	LW and CW	100 % ozone and 100 % sonic energy, 100 % ozone and 50 % sonic energy, 50 % ozone and 100 % sonic energy, 100 % ozone and 0 % sonic energy (LW only), 0 % ozone and 100 % sonic energy (LW only), and a control (0 % ozone and 0 % sonic energy)	200,000 cells/mL	3	4 x 200,000 x 3 = 2,400,000
Adult rotifers	*Brachionus calyciflorus*	LW and CW	100 % ozone and 100 % sonic energy, 100 % ozone and 50 % sonic energy, 50 % ozone and 100 % sonic energy, and a control (0 % ozone and 0 % sonic energy)	5	4	4 x 5 x 4 = 80
Rotifer resting eggs	*Brachionus calyciflorus*	LW and CW	100 % ozone and 100 % sonic energy, 100 % ozone and 50 % sonic energy, 50 % ozone and 100 % sonic energy, and a control (0 % ozone and 0 % sonic energy)	20	4	4 x 20 x 4 = 320
Adult copepods	*Eucyclops* spp.	LW and CW	100 % ozone and 100 % sonic energy, 100 % ozone and 50 % sonic energy, 50 % ozone and 100 % sonic energy, and a control (0 % ozone and 0 % sonic energy)	10	3	4 x 10 x 3 = 120
Juvenile Cladocerans	*Daphnia magna*	LW and CW	100 % ozone and 100 % sonic energy, 100 % ozone and 50 % sonic energy, 50 % ozone and 100 % sonic energy, and a control (0 % ozone and 0 % sonic energy)	10	3	4 x 10 x 3 = 120

Dose effectiveness tests were also conducted to determine if various combinations of ozone and sonic energywere harmful to bacteria. Test species included ambient bacteria assemblages, and ambient bacteria assemblages spiked with test organisms (*Escherichia coli* and *Enterococcus faecalis*). Tests evaluated three combinations of treatment (100 % ozone and 100 % sonic energy, 100 % ozone and 50 % sonic energy, and 50 % ozone and 100 % sonic energy) against a control (0 % ozone and 0 % sonic energy) at a temperature of 25.0 °C ± 1.0 °C. Samples were analyzed following 0, 24 and 48 hours of exposure and two water qualities were used—LW and CW.

E. coli and *Enterococcus* were enumerated using Colilert® and Enterolert™ assays using Quanti-Tray/2000 and the Quanti-Tray sealer from IDEXX laboratories. The Colilert® test has detection limits for total coliforms and *E. coli* of 1 colony forming unit (cfu) per 100 mL, and the Enterolert™ test can detect Enterococcus at 1 cfu/100 mL. Both tests use Defined Substrate Technology® (DST) in which the bacteria metabolize the enzymes in the specific media causing the sample to fluoresce. Heterotrophic plate count (HPC) bacteria were analyzed using SimPlate for HPC medium which uses IDEXX's Multiple Enzyme Technology™ (MET™). Results are expressed as Most Probable Number (MPN) per 100 mL for the Colilert® and Enterolert™ tests and MPN per 1 mL for the SimPlate tests. MPN is a common method of obtaining quantitative data on concentrations of discrete items from positive/negative (incidence) data and in this case is directly related to cfu.

Chemical Degradation Experiments

GSI bench-scale chemical degradation tests were undertaken to determine the effect that various water quality or environmental parameters have on the rate of chemical degradation of ozone.In these tests, samples were analyzed for their concentration of physical/chemical parameters including ozone, NPOC, TROs and %T.

The concentration of ozone in effluent samples was determined by addition ofa reagent solution containing potassium indigo trisulfonate to samples immediately following collection. If ozone was present, the indigo was decolorized with the decrease in absorbance linear to the increase in ozone concentration. Absorbance was then determinedby a Spectrophotometer set at 600 nm using a 1 cm cuvette.

Organic carbon analysis was conducted on a Shimadzu Model TOC-5050A Total Organic Carbon Analyzer. Before analysis, the samples were acidified with 0.2 % concentrated hydrochloric acid. Samples were then purged with high purity air to remove the inorganic carbon and purgeable organic carbon and injected into the analyzer. An organic carbon stock solution was prepared by dissolving 0.2125 g of oven dried anhydrous potassium hydrogen phthalate (KHP) in deionized water, adding 125 μL of concentrated hydrochloric acid, and diluting to 1000 mL with deionized water. This organic carbon stock solution had a concentration of 1000 mg/L carbon. A series of working standards (i.e., 1.0, 5.0, 10.0 and 100 mg/L C) were prepared from the organic carbon stock. Each standard was made to be 0.2 % in hydrochloric acid. The standards were used to generate a calibration curve which was then used to determine the concentration of organic carbon in the samples.

Concentrations of TROs were determined by adding sample water to a beaker containing the contents of a Hach DPD Total Chlorine Reagent powder pillow. If TROs were present, the water turned a red color which was proportional to the TRO concentration. A calibration curve, prepared using chlorine standards reacted with the Hach DPD reagent, was used to determine the concentration of TROs (as chlorine) in the sample. The %T of samples was determined at 254 nm using a UV-VIS spectrophotometer. The UV-VIS spectrophotometer was turned on, wavelength set to 254 nm, allowed to warm up, and

readout mode set to %T. Deionized water was placed in the reference and sample cuvettes and the readout adjusted to 100 %T. Following, the cuvettes were rinsed two times prior to the %T of the sample being read and recorded.

Experiments were also undertaken to determine concentrations of known toxic constituents in sample water following treatment with a range of ozone and sonic energy output scenarios. In these tests, one SW and two CW effluent samples were measured for their concentrations of the disinfection byproducts bromide and bromate. All three samples involved a treatment application consisting of 100 % ozone and 100 % sonic energy. Samples were sent to MetrohmUSA Inc., in Riverview, Florida for analysis.

Residual Toxicity Experiments

Residual toxicity tests were undertaken to explore toxicity that may be associated with degradation by-products of ozone. Test organisms included the daphnid *Ceriodaphnia dubia* (<24 hours old), the fathead minnow *Pimephales promelas* (<24 hours old), newly hatched rotifers (*Brachionus calyciflorus*), and the benthic amphipod *Hyalella azteca*. Tests evaluated one combination of treatment (100 % ozone and 100 % sonic energy) against a control (0 % ozone and 0 % sonic energy) at a temperature of 25.0 °C ± 1.0 °C intwo water qualities—LW and CW. The test water was not renewed.

All exposures were conducted in a 16:8 light:dark cycle, were 48 hours in length, involved 50 mL of solution in a 300 mL beaker, and consisted of three replicates with the exception of the rotifer adults. The rotifer adult exposure consisted of eight replicates, with 2.0 mL of solution, and was 24 hours in duration. Table 3 arrays the test conditions and number of organisms used in the residual toxicity experiments.

Table 3. Numbers and Types of Organisms, and Ozone and Sonic Energy Output Levels Used for GSI Residual Toxicity Tests.

Major Taxonomic Group	Type	Species	Test Water Types	Ozone and Sonic Energy Output Levels (%)	No. of Organisms per Exposure /Control	No. of Replicates per Exposure /Control	Total Number of Organisms
Zooplankton	Adult daphnids	*Ceriodaphnia dubia*	LW and CW	100 % ozone and 100 % sonic energy and a control (0 % ozone and 0 % sonic energy)	10	3	3 x 10 x 4 = 120
	Newly hatched rotifers	*Brachionus calyciflorus*	LW and CW	100 % ozone and 100 % sonic energy and a control (0 % ozone and 0 % sonic energy)	5	4	3 x 5 x 4 = 60
	Benthic amphipod	*Hyalella azteca*	LW and CW	100 % ozone and 100 % sonic energy and a control (0 % ozone and 0 % sonic energy)	10	3	3 x 10 x 4 = 120
Fish	Juvenile fathead minnows	*Pimephales promelas*	LW and CW	100 % ozone and 100 % sonic energy and a control (0 % ozone and 0 % sonic energy)	15	3	3 x 15 x 3 = 135

FINDINGS

Applied vs. Measured Ozone Concentrations

Applied ozone concentrations were calculated based on data collected from the gaseous ozone analyzer, ozone weight (%), and an average flow rate of 4.2 gpm with ozone retention of 3.6 seconds. However, not all of the applied ozone infiltrated the water. As such effluent samples were measured for their ozone content. Figures 2 and 3 present the applied ozone concentration versus the measured ozone concentration as determined in effluent samples for experiments involving LW and CW, respectively. As indicated, there was much variation in the amount of applied ozone for the two settings, i.e., 100 % and 50 %. The 100 % setting resulted in a range of applied ozone of 3.08-5.29 mg/L in LW and 4.37-5.49 mg/L in CW, while the 50 % setting resulted in a range of applied ozone of 1.84-2.08 mg/L in LW and 1.66-4.41 mg/L in CW.

There was a significant difference in the measured ozone concentrations for the two water qualities at the two applied ozone settings employed ($p<0.001$). The measured concentration of ozone in LW was approximately 40 - 50 % of the applied dose, while the measured concentration of ozone in CW was below detection, i.e., less than 0.05 mg/L, regardless of the applied dose.

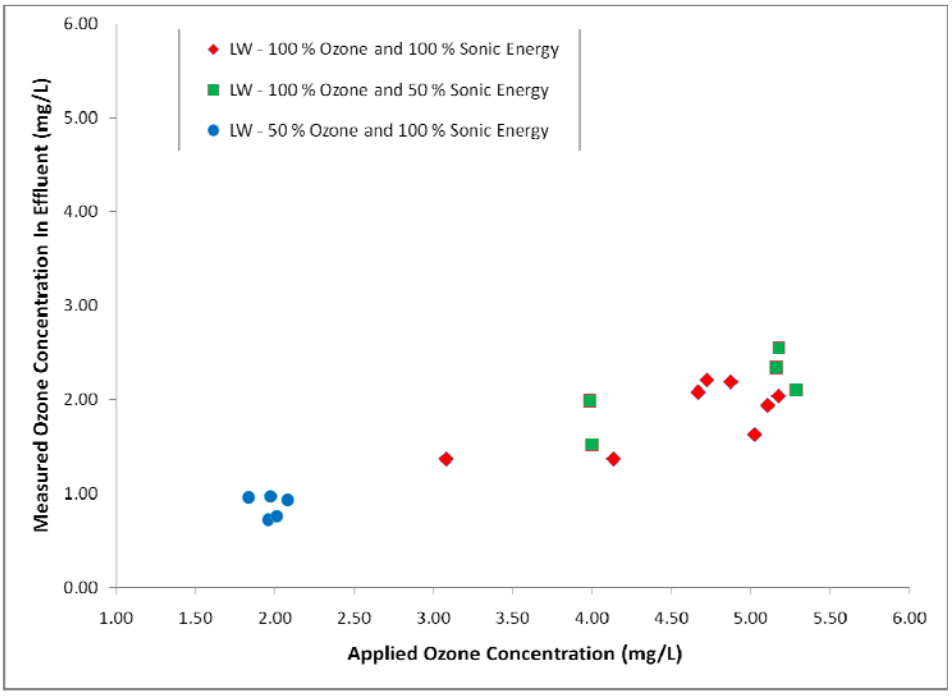

Figure 2. Applied ozone concentration versus measured ozone concentration as determined in treated effluent samples for experiments involving laboratory water (LW).

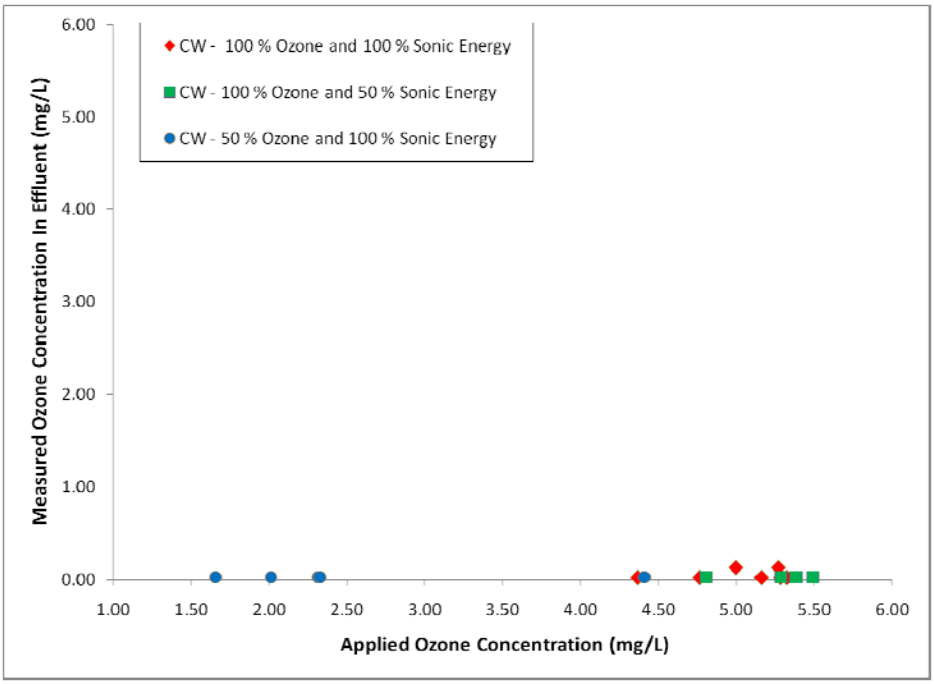

Figure 3. Applied ozone concentration versus measured ozone concentration as determined in treated effluent samples for experiments involving challenge water (CW).

Dose Effectiveness Experiments

Algae and Zooplankton

Results of GSI bench-scale dose effectiveness tests involving ozone and sonic energy toxicity on robust species of freshwater algae and zooplankton are presented in tables 4-6. Ozone in LW proved extremely effective at inactivating the green algal test species *Selenastrum sp*. There was 0 percent survival of the species immediately following application of the treatment, regardless of the application level (i.e., 50 or 100 %; table 4). There was also no regrowth of the species following 24 hours holding time (table 4). In contrast, ozone in CW had no effect on the test species (table 4). There was 100 percent survival of *Selenastrum sp*. following 48 hours exposure at both treatment levels (table 4).

Sonic energy had no effect on the green algal species irrespective of water type. There was 100 % survival of the species 24 hours following application of 100 % sonic energy in both LW and CW (table 4).

Table 4. Percent Survival (Standard Error) of the Green Alga *Selenastrum sp*. Following 0, 24 and 48 Hours of Exposure to Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Survival (%)		
	Ozone	Sonic Energy	0 Hours	24 Hours	48 Hours
LW	0	0	99 (0.8)	100 (0)	99 (0.3)
	100	100	0 (0)*	0 (0)*	N/A
	100	50	0 (0)*	0 (0)*	N/A
	100	0	0 (0)*	0 (0)*	N/A
	50	100	0 (0)*	0 (0)*	N/A
	0	100	100 (0)	100 (0.3)	Not Measured
CW	0	0	100 (0)	100 (0.2)	100 (0.2)
	100	100	100 (0)	100 (0)	100 (0)
	100	50	100 (0)	100 (0)	100 (0)
	50	100	100 (0)	100 (0)	100 (0)

* The difference in the median values among the treatment groups are greater than would be expected by chance, i.e., there is a statistically significant difference ($p<0.05$).

Ozone in LW also proved extremely effective at inactivating the adult rotifer species *Branchionus calyciflorus*. There was 0 percent survival of the species immediately following application of the treatment, regardless of the application level (i.e., 50 or 100 %; table 5). In comparison, percent survival for the species in CW ranged from 73 to 92 % following 24 hours of exposure (table 5).

Table 5. Percent Survival (Standard Error) of the Adult Rotifer *Branchionus calyciflorus* Following 0 and 24 Hours of Exposure to Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Survival (%)	
	Ozone	Sonic Energy	0 Hours	24 Hours
LW	0	0	100 (0)	98 (3)
	100	100	0 (0)*	Not Measured
	100	50	0 (0)*	Not Measured
	50	100	0 (0)*	Not Measured
CW	0	0	100 (0)	93 (4)
	100	100	100 (0)	73 (5)*
	100	50	100 (0)	92 (5)
	50	100	100 (0)	75 (5)*

* Significantly significant difference comparing treatments versus control group using Bonferroni t-test ($p < 0.05$).

Table 6 describes results of dose effectiveness experiments involving resting eggs of the rotifer species *Branchionus calyciflorus*. Results are expressed in terms of percent hatch. Unfortunately there were extremely low levels of percent hatch in LW control samples. These samples did not meet QAQC requirements. Therefore no LW samples are included in the results. For tests involving CW, the percent hatch ranged from 24 – 99 % following 48 hours exposure to the various levels of treatment (table 6).

Results for adult copepods and the cladoceran *Daphnia magna* are also not reported. There were high rates of mortality in the control samples for both these species regardless of water quality. Both these species are large in size compared to the green alga and rotifers. It is likely the increased mortality resulted from physical effects of the treatment apparatus, rather than the treatment itself.

Table 6. Percent Hatch (Standard Error) of Resting Eggs of the Rotifer *Branchionus calyciflorus* Following24 and 48 Hours of Exposure to Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Survival (%)	
	Ozone	Sonic Energy	24 Hours	48 Hours
CW	0	0	93 (7)	93 (7)
	100	100	76 (5)	75 (5)
	100	50	98 (7)	99 (6)
	50	100	19 (4)	24 (4)*

* Significantly significant difference comparing treatments versus control group using Bonferroni t-test ($p < 0.05$).

Microbes

In the microbial dose effectiveness experiments, all three treatment variations tested, i.e., 100 % ozone and 100 % sonic energy, 100 % ozone and 50 % sonic energy, and 50 % ozone and 100 % sonic energy, were effective at inactivating *Enterococcus, E. coli*, and heterotrophic bacteria in LW, reducing populations at all time points compared to controls (tables 7-9). In contrast, none of the treatment variations were effective at inactivating the three species in CW at any time point. The heterotrophic populations in all CW samples, including the controls, actually increased over the 48 hour exposure period.

Table 7. Most Probable Number (Standard Error) of *Enterococcus faecalis* per 100 mL Following 0, 24 and 48 Hours of Exposure to Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Most Probable Number per 100 mL		
	Ozone	Sonic Energy	0 Hours	24 Hours	48 Hours
LW	0	0	5.4E+07 ± 4.9E+06	3.5E+07 ± 8.5E+06	4.4E+07 ± 4.6E+04
	100	100	<1 ± NC	<1 ± NC	<1 ± NC
	100	50	<1 ± NC	<1 ± NC	<1 ± NC
	50	100	<1 ± NC	2.3 * ± NC	<1 ± NC
CW	0	0	3.7E+07 ± 3.8E+06	3.8E+07 ± 1.5E+06	4.4E+07 ± 0
	100	100	>2420 ± NC	5.7E+07 ± 1.1E+07	3.8E+07 ± 4.5E+06
	100	50	>2420 ± NC	2.6E+07 ± 7.1E+04	3.3E+07 ± 2.6E+06
	50	100	>2420 ± NC	3.9E+07 ± 2.3E+06	3.1E+07 ± 8.3E+06

NC - Not calculable.
* One value was lower than limit of detection, half of the detection limit was used in calculation.

Table 8. Most Probable Number (Standard Error) of *Escherichia Coli* per 100 mL Following 0, 24 and 48 Hours of Exposure to Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Most Probable Number per 100 mL		
	Ozone	Sonic Energy	0 Hours	24 Hours	48 Hours
LW	0	0	2.8E+07 ± 4.9E+05	3.1E+07 ± 8.3E+06	2.1E+07 ± 1.1E+06
	100	100	<1 ± NC	<1 ± NC	<1 ± NC
	100	50	<1 ± NC	<1 ± NC	<1 ± NC
	50	100	<1 ± NC	<1 ± NC	<1 ± NC
CW	0	0	3.4E+07 ± 7.1E+06	3.0E+07 ± 9.9E+06	3.6E+07 ± 7.3E+06
	100	100	>2420 ± NC	2.9E+07 ± 4.5E+06	2.3E+07 ± 8.4E+06
	100	50	>2420 ± NC	4.6E+07 ± 7.5E+06	2.9E+07 ± 0.0E+00
	50	100	>2420 ± NC	2.2E+07 ± 8.5E+04	2.7E+07 ± 2.3E+05

NC - Not calculable.

Table 9. Most Probable Number (Standard Error) of Heterotrophic Bacteria per 1 mL Following 0, 24 and 48 Hours of Exposure to Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Most Probable Number (MPN) per 1 mL		
	Ozone	Sonic Energy	0 Hours	24 Hours	48 Hours
LW	0	0	1.1E+06 ± 5.4E+05	5.4E+05 ± 3.5E+04	1.1E+06 ± 3.3E+05
	100	100	<2 ± NC	<2 ± NC	<2 ± NC
	100	50	<2 ± NC	<2 ± NC	45 ± NC
	50	100	<2 ± NC	<2 ± NC	NC
CW	0	0	8.7E+05 ± 1.8E+04	7.3E+05 ± 1.1E+05	>7.4E+06 ± NC
	100	100	>738± NC	1.1E+06 ± 2.5E+05	3.4E+06 ± 7.5E+05
	100	50	>738± NC	1.0E+06 ± 1.2E+05	2.8E+06 ± 5.1E+05
	50	100	>738± NC	8.0E+05 ± 3.4E+05	1.8E+06 ± 6.6E+05

NC - Not calculable.

Chemical Degradation Experiments

Concentrations of NPOC and TROs in effluent samples, as well as % Transmittance, were measured for the range of ozone and sonic energy output scenarios analyzed. The concentration of NPOC in LW was extremely low, ranging from 0.1 to 3.3 mg/L, while the concentration of NPOC in CW was high, ranging from 6.9 to 12.3 mg/L. Ozone did not degrade dramatically in low NPOC water, but did degrade rapidly in high NPOC water (figures 4 and 5), indicating a relationship between the presence of NPOC and the rate of ozone degradation. The concentration of TROs in CW was also extremely low, ranging from below detection (i.e., < 0.05 mg/L) to 0.12 mg/L, while the concentration of TROs in LW was much higher, ranging from 1.65 to 5.48 mg/L. TROs are reported in mg/L as chlorine. The percent transmittance (% T) at 254 nm of the two water qualities also differed markedly. The % T of CW ranged from 67.7 to 75.1 %, while the % T of LW was much higher, ranging from 94.8 to 102.9 %.

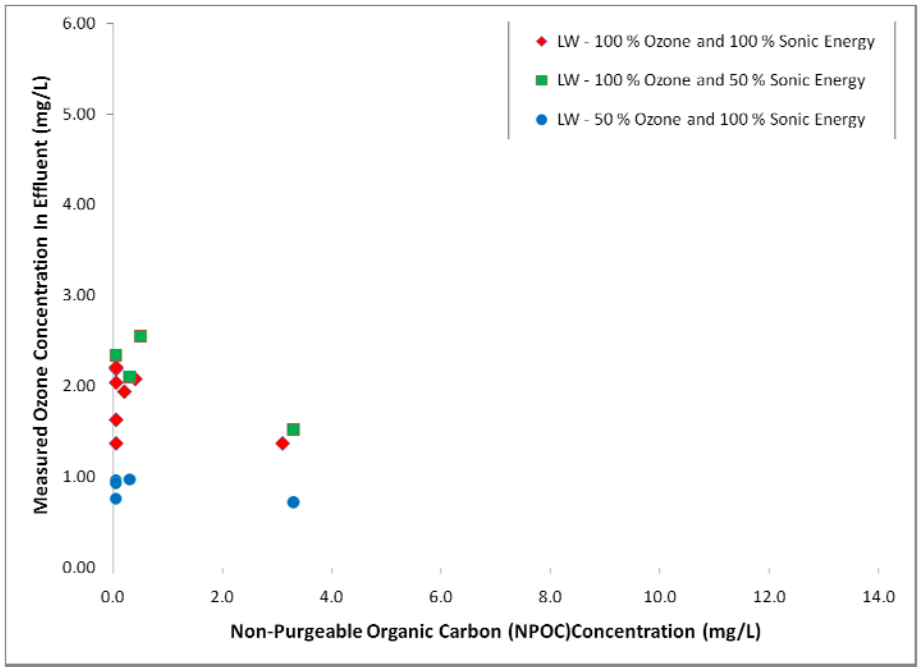

Figure 4. Concentration of non-purgeable organic carbon (NPOC) versus measured ozone concentration as determined in treated effluent samples for experiment involving laboratory water (LW).

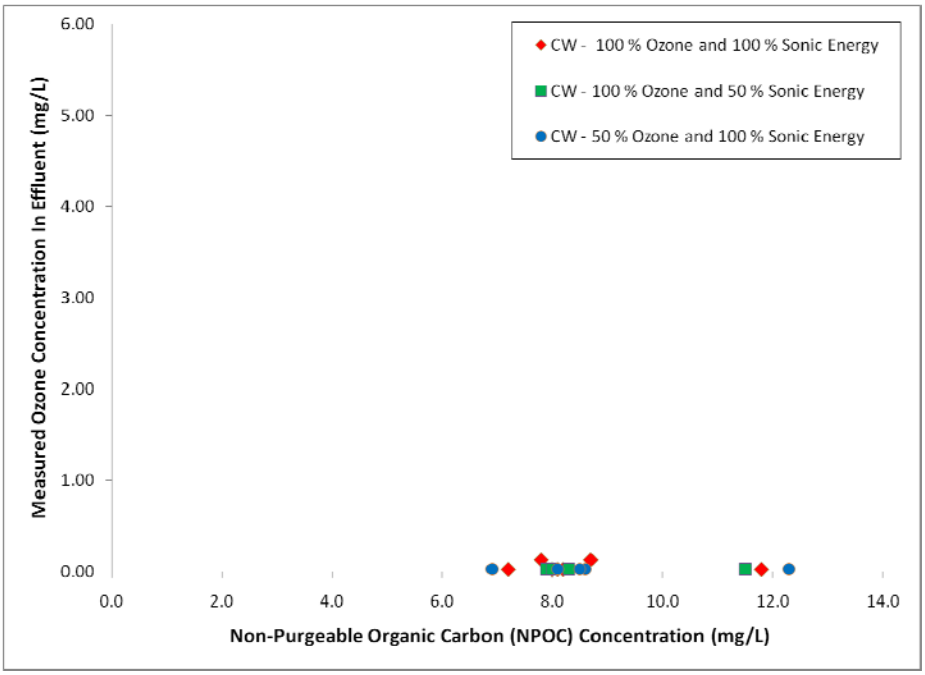

Figure 5. Concentration of non-purgeable organic carbon (NPOC) versus measured ozone concentration as determined in treated effluent samples for experiment involving challenge water (CW).

For the samples analyzed for their concentrations of the disinfection by-products bromide and bromate, results are presented in table 10. The concentration of bromide in SW was higher (i.e., approximately 75 times higher) than the concentration of bromide in CW. The concentration of bromate in SW was also higher than that of the CW sample It is likely that ozone will convert some of the bromide to bromate, depending on the treatment reaction conditions, as the amount of bromate formed is influenced by the quantity of bromide in water.

Table 10. EffluentConcentrations of Bromide and Bromate in Saltwater (SW) and Challenge Water (CW) Experiments involving 100 % Ozone and 100 % Sonic Energy.

Water Type	Ozone Output (%)	Sonic Energy Output (%)	Concentration of Bromide (μg/L)	Concentration of Bromate (μg/L)
SW	100	100	62,780	82.01
CW	100	100	840	1.95
CW	100	100	840	2.06

Residual Toxicity Experiments

Results of GSI bench-scale residual toxicity tests involving 100 % ozone in combination with 100 % sonic energy on the adult rotifer *Brachionus calyciflorus*, the benthic amphipod *Hyalella azteca*, the cladoceran *Ceriodaphnia dubia* and the fathead minnow *Pimephales promelas* are presented in tables 11-14. Ozone and sonic energy in LW demonstrated residual toxicity. There was 0 % survival in the treated LW for all species tested at 24 hours; the corresponding controls had a survival range of 93 - 100 %. In contrast, survival in the treated CW at 24 hours was 100 % for all species tested. However, in this water type, there was no survival in the control water of *C. Dubia* and a lower survival (40 %) of *H. azteca* at 48 hours possibly indicating sensitivity to the tannic acid. As such, these two tests need to be repeated.

Table 11. Percent Survival (Standard Error) at 0 and 24 Hours of the Adult Rotifer *Brachionus calyciflorus* Exposed to the Effluent of Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Survival (%)	
	Ozone	Sonic Energy	0 Hours	24 Hours
LW	0	0	103 (5)	98 (7)
	100	100	100 (0)	0 (0)
CW	0	0	100 (0)	93 (5)
	100	100	100 (0)	100 (0)

Table 12. Percent Survival (Standard Error) at 0, 24 and 48 Hours of the Amphipod *Hyalella azteca* Exposed to the Effluent of Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Survival (%)		
	Ozone	Sonic Energy	0 Hours	24 Hours	48 Hours
LW	0	0	100 (0)	93 (2)	93 (2)
	100	100	100 (0)	0 (0)	Not Measured
CW	0	0	100 (0)	100 (0)	40 (9)
	100	100	100 (0)	100 (0)	100 (0)

Table 13. Percent Survival (Standard Error) at 0, 24 and 48 Hours of the Cladoceran *Ceriodaphnia dubia* Exposed to the Effluent of Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Survival (%)		
	Ozone	Sonic Energy	0 Hours	24 Hours	48 Hours
LW	0	0	100 (0)	100 (0)	100 (0)
	100	100	100 (0)	0 (0)	Not Measured
CW	0	0	100 (0)	0 (0)	Not Measured
	100	100	100 (0)	100 (0)	97 (2)

Table 14. Percent Survival (Standard Error) at 0, 24 and 48 Hours of the Fathead Minnow *Pimephales promelas* Exposed to the Effluent of Various Levels of Ozone and Sonic Energy in Two Water Qualities (Laboratory Water and Challenge Water).

Water Type	Treatment (%)		Survival (%)		
	Ozone	Sonic Energy	0 Hours	24 Hours	48 Hours
LW	0	0	100 (0)	100 (0)	100 (0)
	100	100	102 (1)	0 (0)	Not Measured
CW	0	0	100 (0)	100 (0)	100 (0)
	100	100	100 (0)	100 (0)	100 (0)

GSI QUALITY MANAGEMENT

Standard Operating Procedures (SOPs)

Standard operating procedures (SOPs) were used to implement test activities. This facilitates consistent conformance to technical and quality system requirements and increases data quality. The SOPs include both programmatic and technical processes and procedures such as organism culturing; sample collection, labeling, analysis and custody; and safety. GSI SOPs follow a common format and include specific QAQC procedures and metrics. They are grounded in published standard methods. They are also consistent with international and domestic guidelines where they exist. All GSI SOPs are subject to periodic review and revision to assure that the most up to date approaches are employed. Table 15 outlines the GSI SOPs utilized for these ozone and sonic energy tests. Any deviations made to SOPs during the experiment were recorded and also approved by the GSI Lead On-Site Investigator for Bench-Scale Studies as soon as practicable, as well as communicated to a GSI QAQC officer.

Table 15. GSI Bench-Scale Standard Operating Procedures (SOPs) Utilized for Ozone (O_3) and Sonic Energy Tests.

SOP Category	Subcategory	SOP Title	SOP Code
General	Administration	Procedure for Record Keeping	GSI/SOP/G/A/RK/1
General	Administration	Procedure for Data Entry, Data Quality Control and Database Management	GSI/SOP/G/RA/DM/1
Research Activities	Sample Custody	Procedure for Custody of GSI Samples	GSI/SOP/G/RA/SC/1
Research Activities	Sample Custody	Procedure for Labeling GSI Bench-Scale Samples	GSI/SOP/G/RA/SC/4
Research Activities	Chemistry	Procedure for Analyzing the Concentration of Ozone in Water	GSI/SOP/BS/RA/C/1
Research Activities	Chemistry	Procedure for Determining Total Residual Oxidants (TRO) in Water	GSI/SOP/BS/RA/C/2
Research Activities	Chemistry	Procedures for Measuring Organic Carbon in Aqueous Samples	GSI/SOP/BS/RA/C/3
Research Activities	Chemistry	Procedure for Determining Percent Transmittance (%T) of Light in Water at 254 nm	GSI/SOP/BS/RA/C/4
Research Activities	Culturing	Procedure for Culturing the Freshwater Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/CU/2
Research Activities	Culturing	Procedure for Culturing the Cladocerans *Daphnia magna* and *Ceriodaphnia dubia*	GSI/SOP/BS/RA/CU/3
Research Activities	Culturing	Procedure for Culturing *Selenastrum capricornutum*	GSI/SOP/BS/RA/CU/4
Research Activities	Culturing	Procedure for Culturing the Copepod *Eucyclops spp.*	GSI/SOP/BS/RA/CU/6
Research Activities	Chemical Degradation	Procedure for Examining the Aquatic Degradation of Active Substance(s) in a Ballast Treatment System	GSI/SOP/BS/RA/CD/1
Research Activities	Chemical Degradation	Procedure for Examining the Degradation of Active Substance(s) in a Ballast Treatment System using Large-Volume Influent and Effluent Tanks	GSI/SOP/BS/RA/CD/2
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Copepod *Eucyclops spp.*	GSI/SOP/BS/RA/DE/1
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Cladoceran *Daphnia magna*	GSI/SOP/BS/RA/DE/2
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/3
Research Activities	Dose Effectiveness	Procedure for Assessing DoseEffectiveness of a Ballast Treatment System Using Cysts of the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/4
Research Activities	Dose Effectiveness	Procedure for Assessing DoseEffectiveness of a Ballast Treatment System Using *Selenastrum capricornutum*	GSI/SOP/BS/RA/DE/5
Research Activities	Dose Effectiveness	Procedure for Exposing Test Organisms to a Ballast Treatment System Using Large Volume Influent and Effluent Tanks	GSI/SOP/BS/RA/DE/6

Research Activities	Dose Effectiveness	Procedure for Exposing Test Organisms to an Active Substance	GSI/SOP/BS/RA/DE/7
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using *Ceriodaphnia dubia*	GSI/SOP/BS/RA/RT/1
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using Fathead Minnows (*Pimephales promelas*)	GSI/SOP/BS/RA/RT/2
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using the Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/RT/4
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/RT/5
Research Activities	Microbiology	Procedure For Quantifying Heterotrophic Plate Counts (HPCs) Using IDEXX's SimPlate® for HPC Method	GSI/SOP/BS/RA/MA/1
Research Activities	Microbiology	Procedure for the Detection and Enumeration of Enterococcus using Enterolert™	GSI/SOP/BS/RA/MA/3
Research Activities	Microbiology	Procedure for the Detection and Enumeration of Total Coliforms and E. coli Using IDEXX's Colilert®	GSI/SOP/BS/RA/MA/4

Quality Assurance/Quality Control (QA/QC)

Analytical

Test conditions were monitored daily for parameters that might affect the outcome of the test (i.e., temperature, and dissolved oxygen). Daily and weekly calibration of test meters ensured optimal performance.

Approximately 10 % of all samples were collected and analyzed in duplicate. The results of the duplicate analysis were consistent with GSI QA/QC criteria and are provided in the following table (table 16).

Table 16. Ozone and Sonic Energy Duplicate Sample Agreement and Spike Recovery

Water Type	Ozone (%) and Sonic Energy (%)	Duplicate Agreement Ozone	TRO	NPOC	%T @ 254 nm
BW	50 % Ozone and 100 % Sonic Energy	93.0 %	100.0 %	--	--
LW	100 % Ozone and 100 % Sonic Energy	99.4 %	94.8 %	--	99.6 %
LW	100 % Ozone and 100 % Sonic Energy	92.9 %	89.7 %	100.0 %	99.3 %
LW	50 % Ozone and 100 % Sonic Energy	95.9 %	92.0 %	100.0 %	100.0 %
LW	100 % Ozone and 0 % Sonic Energy	91.6 %	88.6 %	80.6 %	99.9 %
	Mean ± Std. Dev.	94.6 ± 3.1 %	93.0 ± 4.6 %	93.5 ± 11.2 %	99.7 ± 0.3 %
Sample ID		Spike Recovery		NPOC	
LW	100 % Ozone and 0 % Sonic Energy			94.5 %	
LW	100 % Ozone and 100 % Sonic Energy			106.5 %	
	Mean ± Std. Dev.			100.5 ± 8.5 %	

Phytoplankton and Zooplankton

Toxicity tests were initiated with healthy and vigorous plant and animals to the best of the researcher's ability. Reference toxicant tests were performed with all test species prior to the start of the definitive test.

Microbes

Three samples, one influent and two effluent, were collected for each of the microbial test runs and subsequently analyzed at 0, 24, and 48 hours using the Colilert®, Enterolert™, and SimPlate for HPC methods. Over the 48 hour period, eight samples (11 %) were analyzed in duplicate for Colilert® and Enterolert™ and fourteen (19 %) samples were analyzed in duplicate using SimPlate for HPC. Duplicate analyses and replicate samples yielded acceptable results within the quality control limits for the study. All media blanks for the Colilert®, Enterolert™, and SimPlate tests were negative. Some of the 48 hour HPC results appeared to be high, likely the result of regrowth stemming from an incomplete ozone inactivation as well as the presence of organic matter in the CW.

Data Audits, Management and Archiving

Data were recorded on data collection forms or in specific laboratory notebooks. The GSI QA/QC Officer performed inspections of datasheet, logbooks, recorded measurements, and instrumentation used during the tests. All hard- and electronic-copies of data and records will be maintained by LSRI and archived for a period of five years.

CONCLUSION

In conclusion, the ozone and sonic energy treatment proved effective at the bench scale at inactivating all species tested, including algae and bacteria, within the proposed dose range in laboratory water (LW) only. In challenge water (CW), the same species treated with the same level of ozone and sonic energy were highly resilient with a rate of survival ranging from 73 – 100 %, while the microbes showed no effect of treatment at all.

The applied sonic energy led to no additional mortality across all species tested. The treatment developer hypothesizes that at least some of the problem resides in the fact that the treatment system applied the sonic energy before the ozone in the flow-through sequence. Based on the GSI results, he theorizes that better outcomes would be achieved with the sonic energy process following the ozone, allowing the O_3 to initiate damage to the organisms, and the sonic energy to complete it.

Chemical degradation experiments indicated that the measured ozone in the LW was 40 to 50 % of the applied ozone. In the CW the amount of residual ozone in the water was a direct function of the amount of NPOC contained in the test water. Ozone did not degrade dramatically in low NPOC water, but degraded rapidly to non-detect in high NPOC water.

In the conditions in which ozone was effective (LW), it did not degrade, and significant residual toxicity was observed. Survival was 0 % at 24 hours for all species tested.

These results suggest that ballast treatments involving ozone, to be promising in the Great Lakes region, like for chlorine-related treatments, should include a mechanism to assure treatment effectiveness in high NPOC water where ozone degradation is rapid, and a means of neutralizing residual toxicity in low NPOC water, where ozone degradation is slow.