Final Report of the Land-Based, Freshwater Testing of the Lye (NaOH) Ballast Water Treatment System

May 13, 2011

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EXECUTIVE SUMMARY

The Great Ships Initiative (GSI) provides independent, no-cost performance verification testing services to developers of ballast water treatment systems (BWTSs) and processes at a purpose-built, land-based ballast treatment test facility located in the Duluth-Superior Harbor of Lake Superior (Superior, WI). The GSI is capable of performing testing fully consistent with the requirements of the International Maritime Organization's (IMO's) International Convention for the Control and Management of Ships Ballast Water and Sediments (IMO, 2004) and the United States Environmental Protection Agency's (USEPA's) Environmental Technology Verification Program (ETV; NSF International, 2010). GSI procedures, methods, materials and findings are also publicly accessible on the GSI website (www.greatshipsinitiative.org).

In July 2010, GSI conducted a land-based performance evaluation test of a proposed BWTS developed by researchers from the U.S. Geological Survey's Leetown Science Center in Kearneysville, West Virginia. The proposed system involved application of sodium hydroxide (NaOH, in the same formulation used for lye or caustic soda) to ballast water to raise pH, followed by application of carbon dioxide (CO_2) as a neutralization step prior to discharge of the ballast water to the receiving system. The purpose of the land-based test of this system, consisting of four trials, was status testing for research and development. As such, the testing was based on, though not strictly consistent with, the IMO's G8 Guidelines for Approval of Ballast Water Management Systems (IMO, 2008a), the IMO's G9 Guidelines for Approval of Ballast Water Management Systems that make use of Active Substances (IMO, 2008b), and the USEPA's ETV Program *Generic Protocol for the Verification of Ballast Water Treatment Technology*, v.5.1 (NSF International, 2010).

During the test, the NaOH BWTS was evaluated for its ability to: (a) successfully treat ballast water without interruption, (b) successfully neutralize treated ballast water to achieve Wisconsin Department of Natural Resources (WIDNR) permitting levels for harbor discharge (i.e., pH 6-9), (c) meet discharge target values for water chemistry/quality and biology that are approximately consistent with the IMO Convention's Annex D-2 discharge standards, and (d) discharge water after two- or three-day retention periods that is environmentally benign (i.e., no residual toxicity) pursuant to USEPA water quality criteria.

The NaOH BWTS performed very well operationally and well enough biologically to warrant additional testing at the bench, land and ship-based scales. The system successfully treated ballast water without interruption, and successfully neutralized treated ballast water to achieve WIDNR permitting levels for harbor discharge (i.e., pH 6-9). The BWTS also significantly reduced live organism densities in treated discharge relative to control discharge in all size classes of organisms. Finally, in these tests, the BWTS performance met discharge target values that were approximately consistent with the IMO Convention's Annex D-2 discharge standards, though precision in this estimate was not possible given the research and development testing parameters. The only possible problem that this testing revealed was that the water discharged after two- or three-day retention periods was not entirely environmentally benign (i.e., with no residual toxicity at the 100 % effluent dilution), though the level of residual toxicity in 100 % effluent evident from these tests may not be of regulatory concern.

ACKNOWLEDGMENTS

The authors would like to express our sincere gratitude to the Great Ships Initiative (GSI) Advisory Committee which provides invaluable input and encouragement to the GSI project. We also wish to thank the ten United States and Canadian Great Lakes Ports which launched the GSI, and the Great Lakes Protection Fund which supported the initial scoping exercise. We sincerely thank the United States Department of Transportation, Maritime Administration, and National Oceanic and Atmospheric Administration for their substantial financial and in-kind support for the construction of the state-of-the-art GSI land-based testing facility. We thank the United States and Canadian St. Lawrence Seaway organizations, the Legislative Citizens Commission on Minnesota Resources, the University of Wisconsin-Superior, and the City of Superior, WI, for their active financial and/or in-kind support for GSI operations.

1.0. INTRODUCTION

In July 2010, the Great Ships Initiative (GSI) conducted a land-based evaluation of a proposed ballast water treatment system (BWTS) developed by researchers from the U.S. Geological Survey's Leetown Science Center (Kearneysville, WV). The BWTS involved application of sodium hydroxide (NaOH, in the same formulation used for lye or caustic soda) to ballast water on intake to increase the pH, followed by application of carbon dioxide (CO_2) as a neutralization step prior to discharge of the ballast water to the receiving system. The purpose of the evaluation was status testing for research and development of the proposed NaOH BWTS. The objectives of the evaluation were to determine the ability of the NaOH BWTS to: (a) successfully treat ballast water without interruption, (b) successfully neutralize treated ballast water to achieve Wisconsin Department of Natural Resources (WIDNR) permitting levels for harbor discharge (i.e., pH 6-9), (c) meet discharge target values for water chemistry/quality and biology that are approximately consistent with the IMO Convention's Annex D-2 discharge standards, and (d) discharge water after two- or three-day retention periods that was environmentally benign (i.e., no residual toxicity) pursuant to USEPA water quality criteria. The testing was based on, though not strictly consistent with, the IMO's G8 Guidelines for Approval of Ballast Water Management Systems (IMO, 2008a), the IMO's G9 Guidelines for Approval of Ballast Water Management Systems that make use of Active Substances (IMO, 2008b), and the USEPA ETV *Generic Protocol for the Verification of Ballast Water Treatment Technology*, v.5.1 (NSF International, 2010).

1.1. The Great Ships Initiative (GSI)

Great Ships Initiative (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, GSI has established superlative freshwater ballast treatment evaluation capabilities at three scales—bench, land-based, and on board ship.

GSI awards independent status-testing services at no-cost to developers of BWTSs and processes determined to be promising. GSI status-testing is performed at the scale appropriate to the state of development of the target treatment system, with the goal of facilitating the rapid progression of meritorious BWTSs through the research and development and approval processes to a market-ready condition.

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 quality assurance and quality control (QAQC) procedures and documentation (GSI, 2010a; GSI, 2010b). This attention to QAQC also assures high quality and credible evaluation of findings.

GSI has worked to standardize and calibrate its protocols to evaluate the performance of BWTSs with IMO guidelines, USEPA ETV Protocol, and other test facilities. GSI test protocols are as consistent as possible with the requirements of the IMO Convention for the Control and

Management of Ships' Ballast Water and Sediments (IMO, 2004) and United States federal requirements (NSF International, 2010). In particular, GSI testing directly supports the IMO's G8 Guidelines for Approval of Ballast Water Management Systems (IMO, 2008a), the IMO's G9 Guidelines for Approval of Ballast Water Management Systems that make use of Active Substances (IMO, 2008b), and the USEPA ETV Program's *Generic Protocol for the Verification of Ballast Water Treatment Technology*, v.5.1 (NSF International, 2010). GSI procedures, methods, materials and findings are also not proprietary, and are publicly accessible on the GSI's public website: www.greatshipsinitiative.org.

1.2. The NaOH (Sodium Hydroxide, Lye) Ballast Water Treatment System

Researchers from the U.S. Geological Survey's Leetown Science Center in Kearneysville, West Virginia developed the proposed system using sodium hydroxide (NaOH), in the formulation used for lye or caustic soda, for routine use as a BWTS. In 2008, GSI conducted bench-scale testing on the proposed NaOH BWTS and determined that pH levels of 11.5, 12.0, and 12.5 were effective at killing the broad range of aquatic organisms tested; especially adult rotifers (*Brachionus calyciflorus*), the cladoceran *Daphnia magna*, and *Eucyclops* copepods (GSI, 2009). These bench-scale findings were encouraging and land-based testing of a scaled-up model of the NaOH BWTS was proposed and awarded by GSI. Land-based tests utilized a version of the system that first increases the pH of ballast water on intake to pH 12, and then reduces the pH of the discharge water to less than 8.5 (but above 6.5) using carbon dioxide (CO_2). During retention, the ballast water remains at pH 12 and the pH is lowered just prior to discharge by recirculation between a Speece Cone-type carbonator and the ballast tank.

1.3. Relationship of GSI Testing to the IMO Convention's G8 and G9 Guidelines, and the USEPA Environmental Technology Verification Program's Protocol

The fundamental approach of GSI is to conduct independent, scientifically-sound, rigorous, and quality assured evaluations of BWTSs. At the same time, GSI seeks immediate relevance of its freshwater, land-based testing to regulatory processes such as those outlined in the IMO Convention and those under development domestically in the United States and Canada. To that end, GSI protocols are rooted in the essential features of the IMO's G8 Guidelines for Approval of Ballast Water Management Systems (IMO, 2008a), the IMO's G9 Guidelines for Approval of Ballast Water Management Systems that make use of Active Substances (IMO, 2008b), and the USEPA ETV Program's *Generic Protocol for the Verification of Ballast Water Treatment Technology,* v.5.1 (NSF International, 2010). All aspects of the GSI land-based facility testing infrastructure (e.g. flow rate, retention tank size, sample size, sample collection and analysis equipment and data logging) are directly consistent with these requirements. GSI also formally partners with the Maritime Environmental Resource Center (MERC; Solomons, MD), and other land-based test facilities, to ensure that GSI freshwater, land-based testing can be complemented by comparable brackish/salt water testing.

With respect to physical/chemical and biological characteristics of the intake stream, GSI is fortunate in that its feed water source (i.e., the Duluth-Superior Harbor of Lake Superior) naturally meets many of the IMO G8 and the USEPA ETV requirements for intake organism densities and physical/chemical conditions during the testing season (June to October, see Table 1). For those parameters that often do not naturally meet the IMO G8 and USEPA ETV requirements (e.g., total suspended solids, mineral matter, particulate organic carbon, and phytoplankton), GSI has the ability to augment intake water to achieve recommended IMO/ETV parameter levels (Table 1). Other parameters may occasionally fall below the challenge water requirements (i.e., zooplankton and heterotrophic bacteria, see Table 1), but GSI conducts IMO- and USEPA ETV-consistent tests only when they are sufficiently high. Though IMO and ETV protocols do not provide for them, GSI and the treatment system developer may also make a determination to set upper limits on certain water quality parameters, such as DOC concentrations, such that tests will be not be run when concentrations are exceedingly high, and these upper limits are reported in the test report. GSI conducts and documents frequent monitoring of water chemistry and biology to predict valid run conditions for GSI, IMO G8, and USEPA ETV performance evaluation/certification test trials.

Table 1. Comparison of USEPA ETV and IMO G8 Recommended Challenge Conditions to Ranges of Various Physical, Chemical, and Biological Parameters in Ambient[a] Water from the Duluth-Superior Harbor of Lake Superior (June – October).

Parameter	US EPA ETV[1]	Recommended IMO G8[2]	Duluth/Superior Harbor Ambient Ranges[a]
Temperature (°C)	4 – 35	No Requirement	4 - 30
Salinity (ppt)	< 1	Two salinities, >10 ppt difference	0 – 1
Total Suspended Solids (mg/L)	Min. 24	> 50	< 1 – 40
Mineral Matter (mg/L)	Min. 20	No Requirement	<1- 40
Particulate Organic Carbon (mg/L)	Min. 4	> 5	< 0.1 – 3
Dissolved Organic Carbon (mg/L)	Min. 6	> 5	6 – 30
Transmittance at 254 nm (%)[b]	No Requirement	No Requirement	14.0 – 68.5
Zooplankton (≥ 50 μm/m^3)	Min. 100,000	> 100,000	100,000 - 1,100,000
Phytoplankton (≥ 10 and < 50 μm/mL)	Min. 1000	> 1,000	25 – 4,500
Heterotrophic Bacteria (MPN[c]/mL)	Min. 1000	> 10,000	100 - 10,000

[a]Duluth-Superior Harbor ambient ranges were obtained from GSI monitoring data and records from June to October 2007 to 2010
[b]Measured on filtered Duluth-Superior Harbor water samples (May 2009 to October 2010)
[c]MPN = Most Probable Number

2.0. METHODS

Four NaOH BWTS trials took place at the GSI land-based test facility from July 6, 2010 to July 22, 2010. The experimental methods including procedures for sampling and analysis of each physical, chemical and biological parameter and variable are described below. All SOPs relevant to the NaOH BWTS tests are listed by analysis category in Appendix 1. Additional details on GSI's standard operating procedures (SOPs) can be found at www.greatshipsinitiative.org.

1 US Environmental Protection Agency, Environmental Technology Verification Program. Generic Protocol for the Verification of Ballast Water Treatment Technologies. Version 5.1. September, 2010.
2 IMO MEPC 57, Annex 3: Revised Guidelines for Approval of Ballast Water Management Systems (G8). April 4, 2008.

2.1. Experimental Design and Set-up

The NaOH BWTS test involved physical, chemical, and biological characterization of water samples upon uptake/intake of water, as well as, enumeration, sizing, and live/dead analysis of organisms in control and treated discharge water after a two- or three-day, in-tank holding time. The objective of the performance evaluation trials was to compare control (untreated) and treatment discharge in order to estimate the effects of the NaOH BWTS for its ability to: (a) successfully treat ballast water without interruption, (b) successfully neutralize treated ballast water to achieve WIDNR permitting levels for harbor discharge (i.e., pH 6-9), (c) meet discharge target values for water chemistry/quality and biology that are approximately consistent with the IMO Convention's Annex D-2 discharge standards, and (d) discharge water after two- or three-day retention periods that is environmentally benign (i.e., no residual toxicity) pursuant to USEPA water quality criteria.

Table 2 shows the schedule of the four trials, including the sequence of intake operations (simultaneous control and treatment) and discharge operations (sequential, treatment then control).

Table 2. Timing of Intake and Discharge Operations during the NaOH Ballast Water Treatment System Research and Development Trials at the GSI Land-Based RDTE Facility.

Trial	Treatment	Timing of Operation			
		Intake		Discharge	
A	Treatment	06 July 2010	11:08 – 12:08	8 July 2010	10:47-11:40
	Control				13:01-13:55
B	Treatment	09 July 2010	9:02 – 10:01	12 July 2010	10:53 – 11:45
	Control				13:18 – 14:12
C	Treatment	13 July 2010	9:02 – 10:02	16 July 2010	10:03 – 10:57
	Control				12:51 – 13:45
D	Treatment	19 July 2010	9:41 – 10:41	22 July 2010	10:12 – 11:07
	Control				12:33 – 13:27

2.1.1. Experimental Infrastructure: The GSI Land-Based Research, Development, Testing, and Evaluation (RDTE) Facility

The test reported here evaluated the performance of the NaOH BWTS at GSI's purpose-built, Land-Based Research, Development, Testing and Evaluation (RDTE) Ballast Treatment Test Facility located in Superior, WI in the Duluth-Superior Harbor of Lake Superior (Figures 1-3). Key features of the facility include:

- Four x 200 m^3 matched retention tanks with internal agitation for experimental water;

- Matched control and treatment intake flows up to 341 m^3/hour;
- Highly automated flow and pressure control, monitoring and data logging;
- A freshwater estuary with plentiful aquatic life as a water intake source;
- Capacity to amend intake water to intensify challenge conditions;
- Semi-automated and validated facility sanitation between trials;
- High quality in-line or in-tank sampling and/or spiking;
- On-site laboratory space for live analysis of organisms in the ≥ 10 μm and < 50 μm and ≥ 50 μm size classes;
- Capacity to test treatment systems that operate on intake, discharge, in-tank, or combinations thereof;
- Off-site whole effluent toxicity (WET) testing; and
- Easy plug-in connections for treatment systems.

Figure 1. Location of GSI's Land-Based RDTE Facility in Superior, Wisconsin.

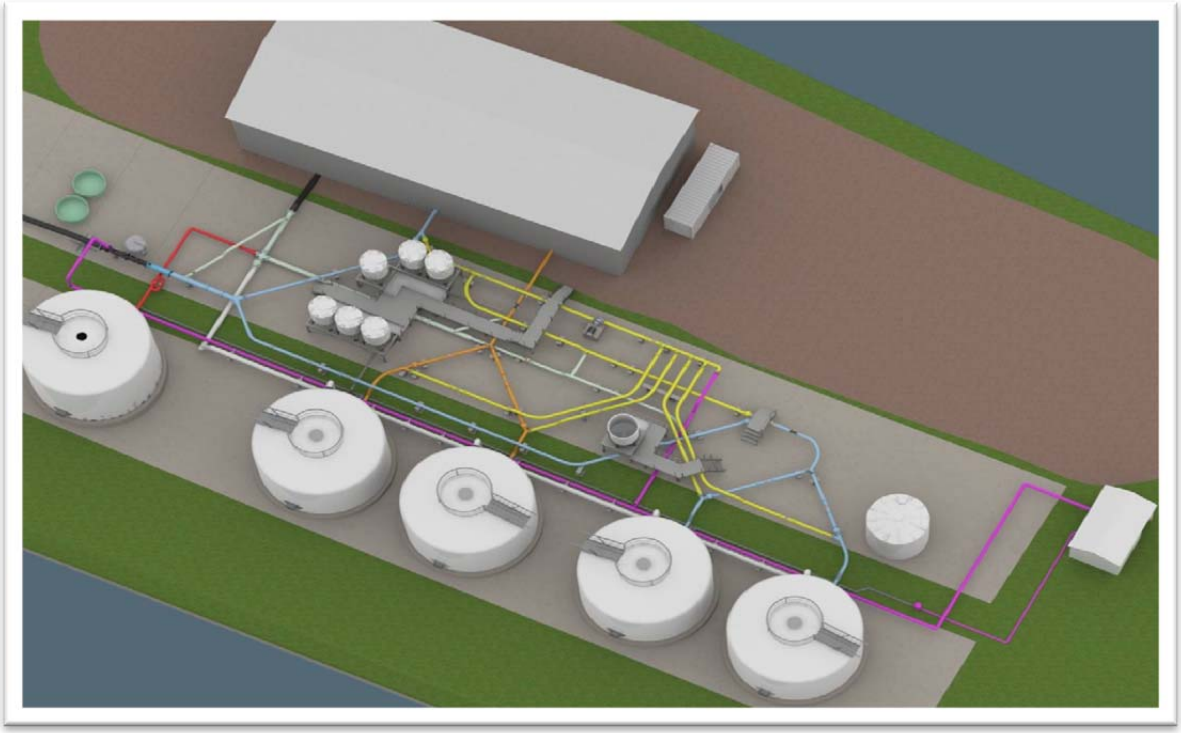

Figure 2. Computer-Generated Rendering of the GSI Land-Based RDTE Facility.

Figure 3. Photo of the GSI Land-Based RDTE Facility.

GSI's Land-Based RDTE Facility draws raw intake water from the Duluth-Superior Harbor at 400 m^3/hr to 680 m^3/hr. This main flow of intake water can be augmented with solids and/or organisms just prior to being split into control and treatment tracks (see injection points A and B; Figure 4).

A Y-split in the intake piping, just after a static mixer, simultaneously channels one half of the well-mixed flow (200 m^3/hr to 340 m^3/hr) to a treatment track and the other half (also 200 m^3/hr to 340 m^3/hr) to a matched control track (Figure 4). The treatment track directs water through the experimental BWTS and into a 200 m^3, cylindrical retention tank (Figure 4). The control track by-passes the treatment system and channels water directly into a matched control retention tank (Figure 4).

After a retention period, water is discharged sequentially from the treatment and control retention tanks at 200 m^3/hr to 340 m^3/hr. The water is directed either back to the Duluth-Superior harbor, to a 260-m^3 wastewater storage tank for subsequent discharge to the City of Superior sewer, neutralization, or circulated to a second set of facility retention tanks (Figure 4).

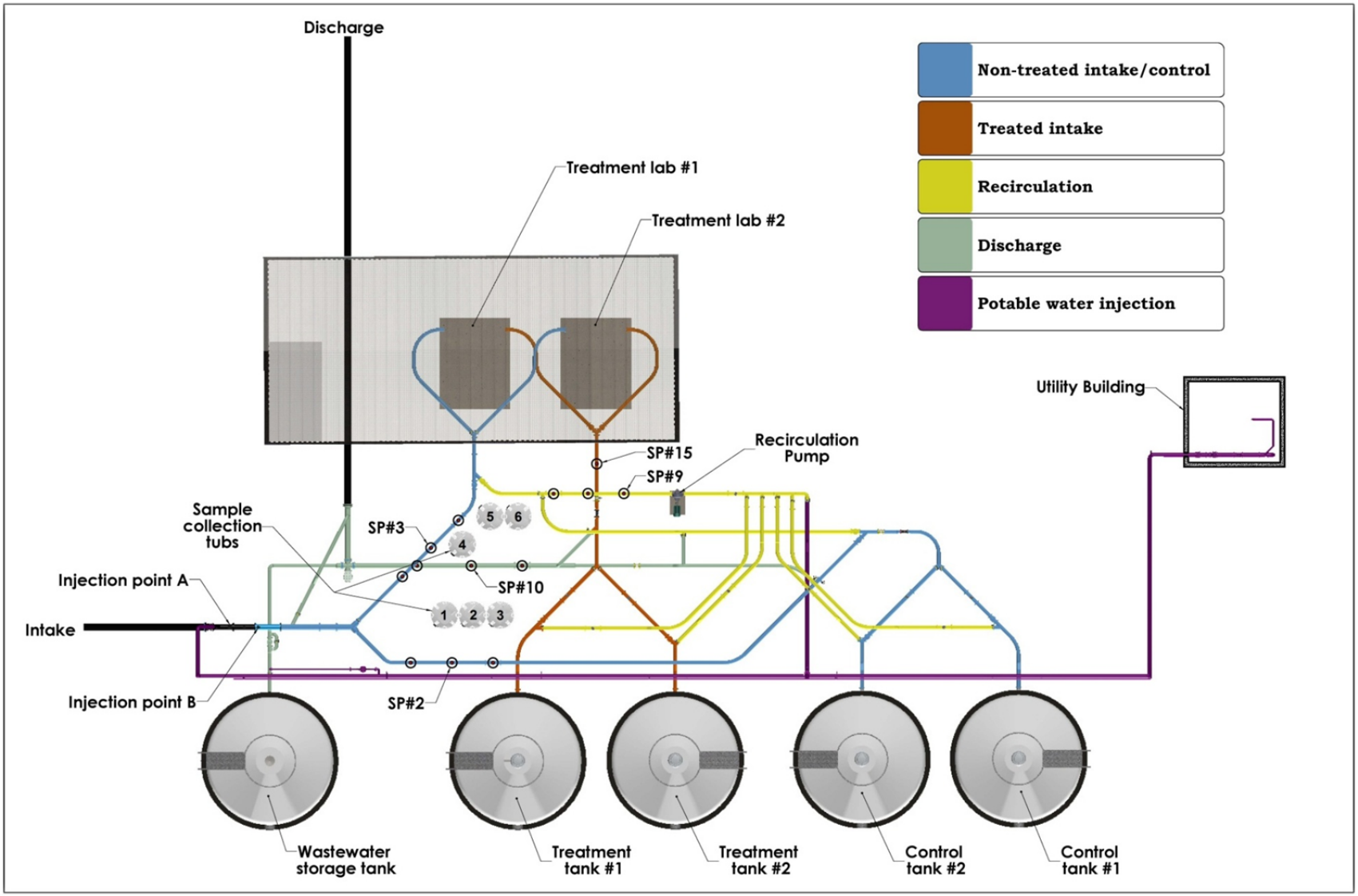

Figure 4. Simplified Schematic of the GSI Land-Based RDTE Facility Showing Location of Sample Points, Sample Collection Tubs, Injection Points, Retention Tanks, and Treatment and Control Tracks. Note: *Main intake and discharge lines are coded black.*

Water is sampled continuously throughout ballasting functions (i.e., intake, recirculation or discharge) through in-line sample points (SPs). Intake sampling takes place at paired intake sample points (SP#2 and SP#3) on the control and treatment tracks, respectively, and immediate post-treatment sampling occurs at SP#15 (Figure 4). Typically, discharge biological sampling is conducted at SP#9, with samples for water quality analysis collected at SP#10 (Figure 4). All these SPs consist of three identical sample ports spaced at regular intervals in a length of straight pipe consistent with IMO guidelines, with the exception of SP#15, which has only one sample port. Each port is fitted with a center-located, elbow-shaped pitot tube (90^{o}) which samples the water (Figure 5). This pitot design is based on one developed and validated analytically by the U.S. Naval Research Laboratory in Key West, Florida. The performance of the three identical sample ports at SP #2, 3, 9 and 10 was also validated empirically at GSI, and shown to produce equivalent, representative and unbiased samples of water flow.

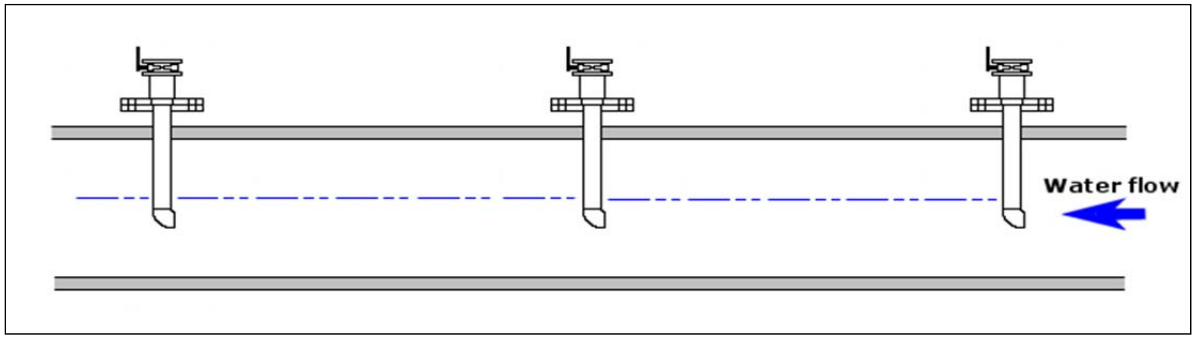

Figure 5. Simplified Schematic of a Sample Point (SP), Showing the Three Sample Ports.

Sample water drawn by sample ports is transferred simultaneously and continuously throughout ballasting operations (intake, recirculation or discharge) from the sample ports to replicate 3.8 m^3 sample collection tubs via clean 3.8 cm (internal diameter) flexible hoses and automated flow-controlled pneumatic diaphragm valves. The sample collection tubs, pictured in Figure 4, connect to the sample ports in the arrangement detailed Table 3. Though the same tubs serve as collection mechanisms for sample flow from more than one pitot, only one such pitot is used at a time during any given sample collection event. The naming convention for an individual pitot is: "SP number" plus "sample port letter". Sample collection tubs are labeled numerically 1-6.

Table 3. Intake and Discharge Sample Points (SPs) and their Corresponding Sample Port Pitots and Sample Collection Tubs.

	INTAKE							DISCHARGE					
	SP#2			SP#3			SP#15	SP#9			SP#10		
Sample Port Pitot	a	b	c	a	b	c	a	a	b	c	a	b	c
Sample Collection Tub	1	2	3	4	5	6	6	3 & 6	2 & 5	1 & 4	3 & 6	2 & 5	1 & 4

An on-site mobile field laboratory (Figure 6) and stationary laboratory (Figure 7) provide space to support time-sensitive analyses associated with the GSI land-based tests, including live analysis of phytoplankton and zooplankton. The laboratories are climate-controlled, and have enough bench space to allow for simultaneous analysis of samples by multiple personnel. All other analyses are conducted in laboratories of the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior; approximately three miles from the facility.

Figure 6. The GSI Mobile Field Laboratory.

Figure 7. The GSI Stationary Laboratory.

2.1.2. Challenge Conditions and Organism Injection Procedures

The expected ranges of physical, chemical and biological challenge conditions for the NaOH BWTS test trials appear in Table 4. Ambient Duluth-Superior Harbor water conditions were employed as the physical and chemical challenge conditions during all four trials. Biological challenge conditions were ambient during Trials A-C. During Trial D, organism densities in the smaller of the two plankton size classes (i.e., ≥ 10 and < 50 μm) were augmented to achieve greater than 1000 cells/mL on intake and thereby intensify challenge conditions. The phytoplankton injection procedure is detailed in *GSI/SOP/LB/G/O/5 – Procedure for Injecting Organisms and Solids into the GSI Land-Based RDTE Facility*. One to two days prior to the test trial, phytoplankton from the Duluth-Superior Harbor was collected and concentrated using 50- to 80 μm plankton nets towed from an outboard-powered boat. The concentrated phytoplankton was stored at the GSI Land-Based RDTE Facility in holding ponds equipped with aeration systems for less than 48 hours. Prior to injection, holding pond water containing concentrated phytoplankton was mixed, sampled, and analyzed for live cell density. In addition, a sample of Duluth-Superior Harbor water was collected to determine the ambient live cell density. Based on the density of cells in the holding ponds and ambient intake water, the volume of phytoplankton concentrate that was needed to achieve the desired density in intake water was calculated. This volume was added to the Organism Pressure Injection System (OPIS) vessel. The OPIS vessel was pressurized to 25 psi greater than the target system pressure. The phytoplankton concentrate was added at a constant rate to the intake water via the pressure differential for the entire duration of the intake procedure via Injection Point B (Figure 4). A static mixer installed in the main intake line just downstream of the two injection systems (SIS and OPIS) and prior to the main system "Y split" (Figure 4) ensured that the concentration of added phytoplankton was equivalent in the control and treatment tracks of the facility. Gentle agitators installed in the control and treatment retention tanks ensured that live organisms, especially less motile organisms that may settle to the bottom of the tank during the retention period, were accounted for to the greatest extent possible in the discharge water analysis (see *GSI/SOP/LB/G/O/7 – Procedure for Maintaining Solids Suspension in the GSI Land-Based RDTE Facility's Retention Tanks*).

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Table 4. Challenge Water Conditions for GSI Test Trials of the NaOH BWTS.

Parameter	Expected Ranges for GSI NaOH BWTS Challenge Water
Temperature (°C)	Ambient (4 – 30*)
Salinity (ppt)	Ambient (0 – 1*)
Total Suspended Solids, TSS (mg/L)	Ambient (≥ 1 – 40*)
Particulate Organic Carbon, POC (mg/L)	Ambient (< 0.1 – 3*)
Dissolved Organic Carbon, DOC (mg/L)	Ambient (6 – 30*)
Mineral Matter, MM (mg/L)	Ambient (≥ 1 – 40*)
Zooplankton (≥ 50 μm/m^3)	Ambient (100,000 – 1,100,000*)
Phytoplankton (≥ 10 and < 50 μm/mL)	Trial A-C - Ambient (> 25*) Trial D – Concentrated Ambient(≥ 1,000)
Heterotrophic Bacteria (MPN/mL)	Ambient (100 – 10,000 MPN/mL*)

*Duluth-Superior Harbor ambient ranges were obtained from GSI monitoring data and records from June to October 2007 to 2010.

2.1.3. Sodium Hydroxide (NaOH) and Carbon Dioxide (CO_2) Dosing and Operational Parameters

A 50 % by weight sodium hydroxide (NaOH) solution with a specific gravity of 1.53 was metered into the intake water in the treatment track (Figure 4) to achieve a pH of 12. The pH of the treatment water stream was monitored every ten seconds during the entire intake operation (i.e., before, during, and after NaOH injection) using an inline Signet pH sensor with built-in automatic temperature compensation (Georg Fischer Signet LLC; El Monte, CA) that was located downstream of the dosing equipment. The inline pH sensor was calibrated prior to each trial's intake operation according to the manufacturer's instructions, using a two-point calibration with pH 7 and 10 buffers. The NaOH dosing procedure began by partially closing the NaOH flow control valve (valve #9, Figure 8) in the main line of Treatment Lab #2 and opening the valves leading to the dosing pumps (Figure 8). This valve configuration created a pressure differential upon flow commencement that primed the two dosing pumps located in a side stream to the main line. Once the pressure differential reached 8-10 pounds per square inch, both centrifugal dosing pumps were started to provide a combined flow of 30-40 gallons per minute

(GPM). The supply valve from the NaOH tank was then opened and a flow of approximately 0.9 GPM was added into the 30-40 GPM from the dosing pumps by using a Venturi injector. A technician monitored the flow rate of NaOH and maintained it at 0.9 GPM during the dosing procedure. The dosing proceeded until 450 pounds (or approximately 35.2 gallons) of the 50 % by weight NaOH solution had been injected as measured from a Salter/Brecknell model SBI100 electronic scale, then the valve leading from the NaOH tank was closed and a bypass line was opened to flush the injection lines with Duluth-Superior Harbor water. The flushing of the NaOH dosing system continued for the remainder of the intake operation.

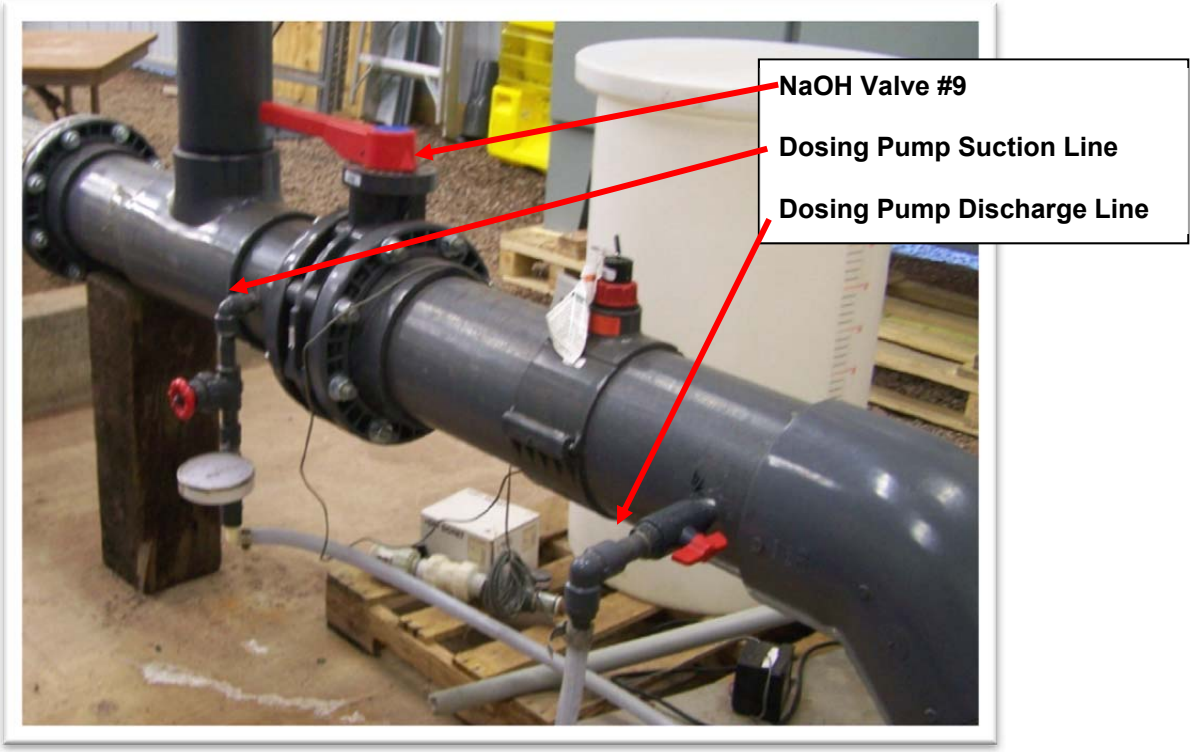

Figure 8. Photo of the NaOH Dosing Procedure Setup in Treatment Lab #2 of the GSI Land-Based Facility's Stationary Laboratory.

Following a two- or three-day retention time, the pH of the treated water was neutralized with carbon dioxide (CO_2) gas. An inline pH meter and Walchem pH sensor (Walchem; Holliston, MA) with built-in automatic temperature compensation were located on the inlet pipe of a Speece Cone; the pH probe was calibrated using fresh pH 7 and 10 buffers as per the calibration procedure outlined in the operator's manual. Water was removed from the treatment retention tank through an outlet centered in the base of the tank and plumbed from the existing 8" steel piping to a 6" diesel pump by flexible 4" PVC piping. The diesel pump drove the pH 12 water through a Speece Cone where the CO_2 gas was injected. The CO_2 was fed into the top of the Speece Cone so the inline rotameter read 15 standard cubic feet per minute (SCFM) which equated to a gas flow of 34 SCFM (by applying a pressure correction factor of 2.268). The pressure in the apex of the cone was kept at 18 pounds per square inch gauge (PSIG) and the flow through the cone was maintained between 560 and 600 GPM using a combination of

adjusting the discharge valve on the cone and the pump speed. The CO_2-enriched water was discharged subsurface back into the treatment retention tank using a PVC T-Joint that reduced the 6" feed to two, 3" jets oriented in opposite directions. The CO_2 flow continued at a rate of 15 SCFM until the inline pH probe indicated a pH of 8.5. Following, the flow of $CO_2$was cut off, and the neutralized water continued to recirculate for an additional 10 minutes to ensure the water was well mixed. Prior to the discharge operation, a 1 L sample was collected at mid-depth (i.e., using a Kemmerer Sampler) from the treatment retention tank, and measured using an Orion 3 Star pH Meter and pH Combination Electrode (Thermo Scientific) according to §2.2.1 below, to confirm the pH was 6.5-8.5.

Flow control valves and system logic assured that sample flow rates were equivalent and proportional to intake and discharge flow rates throughout each intake and discharge operation. Flow rates were recorded continuously every five seconds by automated, in-line sensors located on the control track, treatment track, and on the discharge line. Pressure readings were also recorded continuously at multiple points throughout the facility. These data, as well as, other operational and maintenance parameters (e.g., retention tank volume and volume sampled) were measured and recorded continuously using a Human Machine Interface (HMI) installed at the GSI Land-Based RDTE Facility. The HMI has a 15" color touch display and is capable of detailing valve positions, pressure from the pressure meters, fill level of the ballast retention tanks, and flow rates in the control and treatment lines, etc. The HMI console then saved the information to a specific file. An external computer, connected to the HMI, was used to store the data files.

2.1.4. Preventing Cross Contamination

To minimize potential cross contamination of the treatment discharge water between trials, prior to the first trial and after each test trial, the interior of the retention tanks were cleaned according to *GSI/SOP/LB/G/O/3 – Procedure for Cleaning and Verifying Cleanliness of the Retention Tanks and Piping at the GSI Land-Based RDTE Facility*. After each intake and discharge operation, the sampling equipment (i.e., sample collection tubs, drain spout hose and nozzle, plankton nets, etc.) was cleaned according to *GSI/SOP/LB/G/O/4 – Procedure for Cleaning Sampling Equipment at the GSI Land-Based RDTE Facility*. The GSI facility recirculation lines were flushed with potable water from an on-site potable water tank that had been verified to be free of living organisms. The flushing was undertaken after each intake and prior to each discharge operation. The thoroughness of the cleaning process was checked by partially filling a randomly selected treatment sample collection tub with potable water, draining that water through a 35 μm plankton net, and examining the filtrate for evidence of living organisms. The facility was deemed clean only if the filtrate water was completely free of live Duluth-Superior Harbor zooplankton visible with a compound microscope at a magnification of 40X to 100X (see *GSI/SOP/LB/G/O/3*). Nets and other sample collection equipment were likewise validated for cleanliness prior to each sample operation (see *GSI/SOP/LB/G/O/4*).

2.2. Water Quality Analysis

2.2.1. pH

Samples for pH analysis were collected during intake as follows:

- Three 1 L whole water samples were collected from the pre-treatment line (SP #3; Figure 4) at approximately 10, 30, and 50 minutes after the start of the intake procedure (which lasted approximately 57 minutes), and
- One 1 L whole water sample was collected from the treatment retention tank (Figure 4) after it was filled (i.e., using a Kemmerer Sampler) to confirm the pH was approximately 12.

The following samples were collected during discharge:

- Two 1 L whole water samples were collected from the treatment retention tank using a Kemmerer Sampler (Figure 4); one prior to neutralization of the treatment water via CO_2 injection and one after the neutralization procedure was completed,
- One 1 L whole water sample was collected from the treatment line approximately 30 minutes after the start of the discharge procedure via SP #10 (Figure 4, Trials A and B) and SP #15 (Figure 4, Trials C and D), and
- One 1 L whole water sample was collected from the control line approximately 30 minutes after the start of the control tank discharge procedure (SP #10, Figure 4).

Sample analysis was conducted according to *GSI/SOP/BS/RA/C/9– Procedure for pH Meter Calibration and pH Measurement*. Measurements were made using an Orion 3 Star pH Meter and pH Combination Electrode (Thermo Scientific). The pH electrode was calibrated daily prior to use with certified pH buffers 4, 7, and 10. In addition, a pH "Check Buffer" (i.e., pH 12.45 buffer) was used to verify the accuracy of the pH electrode following calibration at pH values greater than the most basic pH calibration buffer (i.e., pH > 10). Prior to Trial A, the Automatic Temperature Compensation (ATC) probe was calibrated; the display temperature was checked weekly during the testing and the ATC probe was recalibrated if needed.

Quality control measures consisted of collecting and analyzing one of the samples from all pH samples collected during the four trials in duplicate.

2.2.2. Total Suspended Solids (TSS), Including Mineral Matter (MM)

Samples for TSS analysis were collected during intake and discharge as follows:

- On intake, three 1 L whole water samples were collected from the pre-treatment line (SP #3; Figure 4) approximately 10, 30, and 50 minutes after the start of the intake procedure.
- On discharge, one or three 1 L whole water samples were collected from the treatment line approximately 30 minutes (i.e., one sample) or 10, 30, and 50 minutes

(i.e., three samples) after the start of the discharge procedure. Samples were collected using SP #10 (Figure 4) for Trials A and B and SP #15 (Figure 4) for Trials C and D. In addition, one or three 1 L whole water samples were collected from the control line approximately 30 minutes (i.e., one sample) or 10, 30, and 50 minutes (i.e., three samples) after the start of the control tank discharge procedure (SP #10, Figure 4).

Samples were collected in-line rather than from the sample collection tubs to avoid settling of suspended solids. This approach assured a more accurate measurement of solids and organic carbon in the intake water.

Sample analysis was conducted according to *GSI/SOP/BS/RA/C/8– Procedure for Analyzing Total Suspended Solids (TSS)*. The samples were vacuum filtered through pre-washed, dried, and pre-weighed Whatman 934-AH glass fiber filters. After the sample was filtered, it was dried in an oven and brought to constant weight. TSS values were determined based on the weight of particulates on the filter and the volume of water filtered.

Quality control measures consisted of collecting and analyzing one of the samples from all TSS samples collected during the four trials in duplicate.

Mineral matter is defined as the difference between TSS and particulate organic matter (measured as POC). Therefore, MM concentrations were determined in each sample collected during these trials on intake following analysis of TSS, and the determination of POC as calculated from the NPOC and DOC concentrations (see §2.2.3.).

2.2.3. Non-Purgeable Organic Carbon (NPOC) and Dissolved Organic Carbon (DOC), and Determination of Particulate Organic Carbon (POC) Concentrations

Samples for NPOC, DOC, and POC analysis were collected immediately after TSS sample collection during intake only as follows:

- Three 125 mL whole water samples were collected from the pre-treatment line (SP #3, Figure 4) approximately 10, 30, and 50 minutes after the start of the operation.

In these tests, NPOC was measured as a surrogate for total organic carbon (TOC), though it may be a slight underestimate of TOC. The analytical instrument used to measure NPOC purges the sample with air to remove inorganic carbon before measuring organic carbon levels in the sample. Thus, the NPOC analysis does not incorporate any volatile organic carbon which may be present in the sample.

Sample analysis was conducted according to *GSI/SOP/BS/RA/C/3– Procedures for Measuring Organic Carbon in Aqueous Samples*. Upon arrival at LSRI, an aliquot of each 125 mL sample was filtered through a Whatman GF/F filter and acidified with hydrochloric acid for analysis of DOC. The remaining portion of the sample was acidified with hydrochloric acid and analyzed for NPOC. A Shimadzu Total Organic Carbon Analyzer (Model TOC-5050A; Shimadzu

Scientific Instruments, Inc.; Columbia, MD) was employed for analysis of both NPOC and DOC. Concentrations of NPOC and DOC were determined based on a calibration curve developed on the instrument using organic carbon standards prepared from potassium hydrogen phthalate. Reported particulate organic carbon (POC) concentrations were determined as the difference between the NPOC and DOC values for a given sample.

Quality control measures consisted of collecting and analyzing two of the samples from all organic carbon samples collected during the four trials in duplicate. A TOC reference standard (NSI Solutions Inc., Raleigh, NC QCI-062, Lot #051210-09) was analyzed once during testing to confirm the accuracy of the data being generated.

2.2.4. Percent (%) Transmittance

An aliquot of the filtered portion of each sample collected for TSS analysis was analyzed to determine percent transmittance. Sample analysis was conducted according to *GSI/SOP/BS/RA/C/4 – Procedure for Determining Percent Transmittance (%T) of Light in Water at 254 nm*. A spectrophotometer set at 254 nm was used to measure %T of the filtered samples. Deionized water was used as a reference to adjust the spectrophotometer to 100 %T, and each filtered sample was measured in a pre-rinsed sample cuvette.

2.2.5. Water Quality Measurements using YSI Multiparameter Water Quality Sondes

Water quality was measured during each trial using calibrated YSI Multiparameter Water Quality Sondes (YSI 6600 V2-4 Sondes; YSI Incorporated; Yellow Springs, OH, USA). The Sondes were calibrated prior to each trial following *GSI/SOP/LB/G/C/4 - Procedure for Calibration, Deployment, and Storage of YSI Multiparameter Water Quality Sondes*. The YSI Sondes have multiple probes that are able to measure the following parameters: dissolved oxygen, specific conductivity, salinity, temperature, pH, turbidity, and total chlorophyll. Water quality parameters were measured from approximately 1 L samples of water from each sample collection tub sampled on intake and discharge. Samples were taken immediately following collection of phytoplankton and microbial samples, and each measurement was recorded on pre-printed datasheets. In addition, water quality parameters in the control and treatment retention tanks were measured at mid-depth every 15 minutes during the two- or three-day holding time. Prior to discharge of the respective tanks, the Sondes were removed and taken to the mobile laboratory where the data were later downloaded as test files to a laptop computer using EcoWatch® for Windows® Software (v.3.18, 14 April 2006; YSI Incorporated); the files were then translated to MS Excel files, which were stored on a laptop computer in the mobile laboratory and later uploaded to the GSI SharePoint intranet website.

2.3. Viable Organism Analysis

During these trials sample water for analysis of viable organisms was simultaneously and continuously collected from replicate sample ports into identical 3.8 m^3 sample collection tubs during each intake, treatment discharge, and control discharge operation (retention tank discharge was sequential, treatment then control). Volumes retained were always greater than

volumes recommended in the IMO Convention's G8 guidelines. The water in each sample collection tub constituted an independent, time-integrated, replicate sample of the 200 m^3 experimental water mass.

2.3.1. Organisms ≥ 50 µm in Minimum Dimension

2.3.1.1. Sample Collection

During the intake operation, i.e. the filling of the treatment and control 200 m^3 retention tanks, the following time-integrated sample volumes were collected and analyzed (additional samples were collected but were not analyzed) by continuous flow from the intake lines simultaneously:

- One 2 m^3 sample from the pre-treatment intake line, and
- One 2 m^3 sample from the control intake line.

The pre-treatment and control samples served as replicate intake sub-samples for each trial.

During discharge the following time-integrated sample volumes were collected and analyzed (additional samples were collected but were not analyzed):

- Two time-integrated samples of 2 m^3 each (total volume 4 m^3) were continuously collected from the treatment discharge line, and
- One 2 m^3 time-integrated sample was continuously collected from the control discharge line.

Flow control valves and system logic assured that sample flow rates were equivalent and proportional to intake and discharge flow rates throughout each operation. Immediately after the sample collection tubs were filled, the phytoplankton and microbial whole water samples were collected and Sonde readings recorded, followed by the zooplankton sample collection. The zooplankton samples were collected by draining the remaining volumes (i.e., 2 m^3 minus 5 L of rinse/Sonde water and the 1 L phytoplankton and microbial samples) from the sample collection tubs and concentrating through 35 μm (50 μm diagonal dimensions) plankton nets into 1 L cod-ends for microscopic examination. See *GSI/SOP/LB/RA/SC/6 - Procedure for Zooplankton Sample Collection*. On intake, the zooplankton sample collection order alternated between collecting the pre-treatment or the control sample first. After the first sample was collected and analyzed, then the second sample was collected (either the control or the pre-treatment) and analyzed. On discharge, the treatment and control samples were also collected sequentially.

2.3.1.2. Live/Dead and Size Analysis

All live/dead analysis was conducted according to *GSI/SOP/LB/RA/SA/2 - Procedure for Zooplankton Sample Analysis*, and took place within two hours of collecting and concentrating the individual samples. Microzooplankton (e.g., rotifers, copepod nauplii, and dreissenid veligers) and macrozooplankton (e.g., copepods, cladocerans, and insect larvae), all generally greater than or equal to 50 μm in minimum dimension, were analyzed simultaneously by

separate taxonomists. Microzooplankton subsamples were analyzed in a Sedgewick-Rafter counting chamber by examination under a compound microscope at a magnification of 40X to 100X. Macrozooplankton were analyzed in a Ward's Counting Wheel at a magnification of 20 to 30X using a dissecting microscope. Due to high densities, quantification of zooplankton in the control intake, pre-treatment intake, and control discharge samples required analysis of sub-samples and extrapolation to number live organisms per cubic meter. For these samples, a subsample was removed for analysis using a Henson-Stempel pipette. The dead organisms (i.e., those organisms that did not move or respond to stimuli) were enumerated, then all organisms in the sample were killed by adding 50 % (v/v) acetic acid solution (for microzooplankton) or Lugol's solution (for macrozooplankton) to the counting chamber/wheel and the total number of organisms was enumerated. The number of live organisms was quantified by subtracting the number of dead organisms from the total number of organisms in the counting chamber/wheel. The treatment discharge samples had lower densities allowing analysis of a greater proportion of the sample (see the "Results" section for the proportion of sample volumes analyzed). Therefore, the treatment discharge samples were split in half using a Folsom Plankton Splitter. Half of the sample was analyzed for macrozooplankton and the other half was examined for microzooplankton. Only live organisms were enumerated using standard movement and response to stimuli techniques.

Statistical analysis for the ≥ 50 μm size class for the four trials was conducted using SigmaStat, version 3.5 (Systat Software, Inc.; Chicago, IL USA). A One Way Analysis of Variance (ANOVA) was used to determine the differences in the mean values among the treatment groups if the data were normally distributed with equal variance. If the data did not meet the assumptions of the One Way ANOVA, the data were transformed using either log (base 10), log normal, or square root transformation and a One Way ANOVA was used to compare the transformed data. If transformation did not produce normally distributed data with equal variance, an appropriate non-parametric test was used. In all cases α=0.050.

Quality assurance measures during these trials included live/dead analysis of four intake (i.e., one pre-treatment and three control samples) and one control discharge sample by two separate taxonomists over the course of the four trials. The average percent similarity of taxonomic identification (live organisms only) and the average relative percent difference of the number of live organisms counted were calculated for all second analyses. In addition, all live organisms identified in the treatment discharge samples were recorded and verified to be live by a second taxonomist, and the minimum visible dimension was measured using an eyepiece micrometer and recorded. Those organisms that were determined to be less than 50 μm in minimum visible dimension were reported separately from the live zooplankton that did meet the size criterion detailed in Annex D-2 of the IMO Convention (IMO, 2004).

2.3.2. Organisms ≥ 10 and <50 µm in Minimum Dimension

2.3.2.1. Sample Collection

For live analysis of organisms ≥ 10 and < 50 μm in minimum dimension, during intake the following whole water samples were collected:

- One 1 L sample was collected immediately after filling from the pre-treatment sample collection tub (Tub #4, Figure 4), and
- One 1 L sample was collected from the control sample collection tub (Tub #1, Figure 4).

The pre-treatment and control samples served as replicate intake sub-samples for each trial.

During discharge:

- Three 1 L samples were collected from the three treatment sample collection tubs (Tubs #4-#6, Figure 4), and
- One 1 L sample was collected from the control tank via the sample collection tub (Tub #1, Figure 4).

The three, 1 L treatment discharge samples were composited for analysis. Analysis of all samples occurred on-site within 1.5 hours of sample collection, with samples stored in coolers during the interim. Prior to analysis, samples were concentrated through 10 μm mesh plankton netting and stored in a 25 mL sample container. See *GSI/SOP/LB/RA/SC/3 - Procedure for Algae/Small Protozoa Sample Collection*.

2.3.2.2. Sample Analysis

Sample analysis was conducted according to *GSI/SOP/LB/RA/SA/1 - Procedure for Algae/Small Protozoan Sample Analysis.* A 1.5 mL subsample of the concentrated sample was transferred to a 2 mL sample container, with 4 μL of fluorescein diacetate (FDA) viability stain stock solution added. The subsample was then allowed to incubate in the dark for 5 minutes. The 1.5 mL incubated sample was mixed and 1.1 mL was immediately transferred to a Sedgwick-Rafter cell, covered and placed on the stage of a microscope that was set for simultaneous observation using brightfield and epifluorescence. At least two horizontal transects were counted (an area known to reflect greater than 1 mL of original sample water), aiming for at least 100 entities (i.e., unicellular organism, colony or filament) counted. If time permitted, additional transects were counted to increase statistical power. Single cell entities and cells comprising colonial and filamentous entities were characterized as follows: alive = cells showing obvious green fluorescence from cell contents; dead = cells showing no or very little evidence of green fluorescence from cell contents; and ambiguous = cells or entities that cannot be clearly identified as alive or dead (were uncommon). Records were kept of transect lengths and widths so that the total counted area and volume analyzed could be calculated later.

Entities less than 10 μm in all visible dimensions or greater than 50 μm in minimum visible dimension were not counted. Counting and measurement of all other entities followed standard procedures for individuals (length and width), colonies (e.g., number of cells, cell length and width) and filaments (e.g., number of cells, cell length and width or total filament length if cells could not be discerned). The remaining concentrated sample in the 25 mL bottle was archived using a preservative (formalin or Lugol's) for long-term storage.

Statistical analysis for the ≥ 10- and < 50 μm size class for the four trials was conducted using SigmaStat, version 3.5 (Systat Software, Inc.; Chicago, IL USA). A One Way ANOVA was used to determine the differences in the mean values among the treatment groups if the data were normally distributed with equal variance. If the data did not meet the assumptions of the One Way ANOVA, the data were transformed using either log (base 10), log normal, or square root transformation and a One Way ANOVA was used to compare the transformed data. If transformation did not produce normally distributed data with equal variance, an appropriate non-parametric test was used. In all cases α=0.050.

Quality assurance measures included analysis of one intake sample and three discharge samples (i.e., two treatment and one control discharge) by two separate taxonomists using a dual-headed microscope (i.e., both taxonomists analyzed the same sample at the same time) over the four trials of the NaOH BWTS. In addition, subsample analysis was conducted on two treatment discharge samples (over the entire four-trial NaOH BWTS Test) by a single taxonomist (i.e., one taxonomist analyzed two separate aliquots from one sample) to determine within sample precision. The average percent similarity of taxonomic identification and the average relative percent difference of the number of live organisms counted were calculated for all second analyses.

2.3.3. Organisms < 10 μm in Minimum Dimension

Control and treatment samples for these trials were collected and analyzed for heterotrophic bacteria and three specific indicator organisms for waterborne pathogens: total coliform bacteria, *Escherichia coli* and *Enterococcus spp.*

2.3.3.1. Sample Collection

Whole water samples were collected as follows:

- On intake, three 1 L samples were collected immediately after filling the pre-treatment sample collection tub and collection of the ≥ 10 and < 50 μm size class sample (Tub #4, Figure 4).
- On discharge, three 1 L samples were collected immediately after tank discharge from the treatment sample collection tubs (Tubs #4-#6, Figure 4), and three 1 L samples were collected from the control retention tank via Tub #1 (Figure 4) after collection of the ≥ 10 and < 50 μm size class sample.

All samples were collected according to *GSI/SOP/LB/RA/SC/4 – Procedure for Microbial Sample Collection*, and were transported within one hour of collection in an insulated cooler to LSRI and analyzed as individual replicates.

2.3.3.2. Sample Analysis

Viable heterotrophic bacteria were enumerated according to *GSI/SOP/BS/RA/MA/1 – Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate® for HPC Method*.

This method utilizes the IDEXX SimPlate® for HPC Method (IDEXX Laboratories, Inc.; Westbrook, Maine), which is based on IDEXX Laboratories' patented multiple enzyme technology.

The most probable number (MPN) per 100 mL of total coliform bacteria, *E. coli* (*GSI/SOP/BS/RA/MA/4 - Procedure for the Detection and Enumeration of Total Coliforms and E. coli Using IDEXX's Colilert*®) and enterococci (*GSI/SOP/BS/RA/MA/3 - Procedure for the Detection and Enumeration of Enterococcus using Enterolert*™) were determined using Quanti-Tray/2000® with Colilert® and Enterolert™, respectively, which are both based on IDEXX's patented Defined Substrate Technology (DST®; IDEXX Laboratories, Inc.; Westbrook, Maine).

Statistical analysis for all four types of bacteria in the <10 μm size class (i.e., total coliform bacteria, *E. coli*, *Enterococcus spp*., and total heterotrophic bacteria) for the four trials was conducted using SigmaStat, version 3.5 (Systat Software, Inc.; Chicago, IL USA). A One Way ANOVA was used to determine the differences in the mean values among the treatment groups if the data were normally distributed with equal variance. If the data did not meet the assumptions of the One Way ANOVA, the data were transformed using either log (base 10), log normal, or square root transformation and a One Way ANOVA was used to compare the transformed data. If transformation did not produce normally distributed data with equal variance, an appropriate non-parametric test was used. In all cases α=0.050.

Quality control samples analyzed for each intake and discharge operation included a media blank and a positive control for *E. coli*/total coliforms and *Enterococcus spp*., and a media blank for heterotrophic bacteria. Quality assurance measures included analysis of at least 10 % of the samples in duplicate from the total number of samples collected over the four trials. The average relative percent difference of all duplicates analyzed during the trials was calculated separately for *E. coli*, *Enterococcus spp*., and heterotrophic bacteria.

2.4. Whole Effluent Toxicity (WET) Testing

GSI's whole effluent toxicity (WET) testing of the NaOH BWTS was conducted using three freshwater species as described in Table 5. The WET tests were conducted on *P. promelas* and *C. dubia* using Trial B treatment discharge, and on *P. promelas*, *C. dubia*, and *S. capricornutum* using Trial D treatment discharge.

The WET of treatment discharge water was determined using standard USEPA procedures (USEPA, 2002) following a three-day retention period in the land-based facility's 200 m^3 treatment retention tank (Figure 4). Sample water (i.e., 19 L), collected from one of the treatment discharge sample collection tubs using a 20 L, high-density, polyethylene container, was immediately transported to LSRI and used upon arrival to set up the WET tests. Following set up of the tests, the remaining sample water was stored at 4 °C in the dark to preserve as much of the initial water quality/chemistry properties as possible, and portions (i.e., 2 to 3 L) of the discharge sample water was warmed to 25 °C each day to serve as renewal water for the bioassay. Filtered Duluth-Superior Harbor water (i.e., filtered through a Whatman 934-AH Glass Microfiber Filter, 1.5 μm particle retention in liquid) served as the control, and treatments

consisted of 0 % treatment discharge water (i.e., filtered harbor water control), 100 % treatment discharge water, and a performance control (i.e., *Ceriodaphnia dubia* and *Pimephales promelas* culture water, and algae growth media for *Selenastrum capricornutum*). All tests were conducted in temperature-controlled incubators or water baths, or at ambient room temperature following the SOPs listed in Table 5. Differences in mean percent survival (for *C. dubia* and *P. promelas*), mean dry weight values (for *P. promelas*), mean *S. capricornutum* cell density, and mean number of *C. dubia* young per female between the 0 % and 100 % treatment discharge groups were analyzed for statistical significance at α=0.05 using a One-Way Analysis of Variance and a post hoc statistical comparison.

The WET tests were initiated with healthy, vigorous organisms. To determine the overall health of the test organisms, reference toxicant tests were performed with the cladoceran *Ceriodaphnia dubia* and the minnow *Pimephales promelas* prior to the start of each definitive test or at least once per month. In addition, a performance control was used for all species tested. The performance control consists of the normal culturing conditions for each species, providing the test organisms with the optimal environment for survival, growth, and reproduction. Therefore, the performance control, along with the reference toxicant tests, provides verification of the health of the test organisms. To determine the validity of the WET tests, percent survival of *C. dubia* and *P. promelas*, dry weights of surviving *P. promelas*, mean *S. capricornutum* cell density, and mean number of young per female *C. dubia* in the controls were compared to the test acceptability criteria published in the USEPA's *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (2002). Class I weights were used to verify the accuracy of the laboratory balance according to *GSI/SOP/BS/RA/GL/1 – Procedure for Verification of Laboratory Balances*. Daily or weekly calibration of test meters ensured optimal performance. The *P. promelas* drying process is verified by re-weighing a percentage of the fish after they have been dried for an additional length of time in the oven.

Table 5. Standard Operating Procedures Relative to Whole Effluent Toxicity (WET) Testing.

GSI SOP Code	Test Type	Test Species	Test Endpoint
GSI/SOP/BS/RA/WET/1	Short-Term, Chronic	Cladoceran (*Ceriodaphnia dubia)*	Survival and Reproduction
GSI/SOP/BS/RA/WET/2	Short-Term, Chronic	Fathead Minnow (*Pimephales promelas)*	Survival and Growth (growth measured via dry weight)
GSI/SOP/BS/RA/WET/3	Short-Term, Chronic	Green Alga (*Selenastrum capricornutum)*	Growth (measured via direct density counts)

2.5. Data Management

2.5.1. Data Recording

All biological and chemical data were recorded by hand (using indelible ink) on pre-printed data collection forms and/or in bound, uniquely-identified laboratory notebooks that were specific to the NaOH BWTS test. The data that were recorded on pre-printed data collection forms were secured in uniquely-identified three ring binders, specific to the type of data and to the treatment technology.

Biological and chemical data that were recorded by hand were entered into either a MS Access Database that was designed, developed, and is maintained by the GSI Database Manager (i.e., microbial, phytoplankton, and zooplankton data) or the data were entered into a MS Excel spreadsheet (i.e., water chemistry and WET test data; see *GSI/SOP/G/RA/DM/1 - Procedure for Data Entry, Data Quality Control, and Database Management*).

In-tank water quality data (e.g., temperature, pH, dissolved oxygen, specific conductivity, salinity, turbidity, and total chlorophyll) was measured continuously every fifteen minutes during each retention period and automatically recorded in a text file, which was later translated to a MS Excel spreadsheet. Facility data (e.g., flow rates and pressure measurements) were electronically recorded every five seconds during intake and discharge. This data was exported to MS Excel for subsequent analysis, and is stored by AMI Engineers on a secure network and on GSI SharePoint.

Following completion of the NaOH BWTS trials, a thorough review of all data sheets and laboratory notebooks was undertaken to ensure compliance with the documentation procedures outlined in all relevant GSI SOPs and in the GSI Land-Based Quality Assurance Project Plan (GSI, 2010a). A percentage of data that was recorded by hand and entered into MS Access or MS Excel was verified against the original raw data. This process also included verification of formulas and calculations (i.e., hand-calculation of data). The percentage of verified raw data ranged from 10 % to 100 % of the original raw data, depending on the data type. More detail on the GSI's data validation activities is additionally detailed in Section 7 of the GSI Quality Assurance Project Plan for Land-Based Tests (GSI, 2010a). This section also details the acceptable values, where appropriate, for the following quality objectives: accuracy, precision, completeness, comparability, representativeness, and sensitivity.

2.5.2. Data Processing and Storage

After examination and quality assurance analysis, the data distribution files from the MS Access database were posted to the LSRI's Local Area Network (LAN) in an organized hierarchical folder system. All electronic data files stored on the LSRI's secured LAN can be accessed only by GSI personnel. The GSI Database Manager is the single point of control for access to the LSRI LAN. The LSRI LAN is automatically backed up every 24 hours. A backup of the database was also made regularly to avoid any loss of data following computer/electronic glitches.

Electronic data files, including MS Excel files, are stored on the LSRI LAN as well as GSI's internal SharePoint website, which acts as a secondary data backup/storage mechanism. All original raw data will be stored in a climate-controlled, secure archive room at the LSRI for five years after this report is finalized.

3.0. RESULTS

Four trials (Trials A-D) of the NaOH BWTS's biological effectiveness were completed, with WET tests incorporated into two trials (Trial B and D). During these trials, there were no significant deviations from the above methods.

3.1. Intake and Discharge Challenge Conditions

3.1.1. Operational Conditions

Operational conditions, measured continuously during intake, for all four trials were extremely consistent with each other and between treatment and control tracks of the GSI Land-Based Facility. Flow rate was slightly below 200 m^3/hour and pressure in the facility lines was 30.4 – 31.6 psi (Table 6). On discharge, the flow duration, flow rate, and pressure was very similar between the treatment and control tracks (Table 7).

Table 6. Average Operational Parameters Measured During Ballasting Simulation of the Four Trials of the NaOH Ballast Water Treatment System.

Trial	Flow Duration (min)	Treatment Flow Rate (m^3/h)	Total Volume of Water Treated (m^3)	Control Flow Rate (m^3/h)	Pressure (psi)
A	56.9	198.6	188.3	199.1	30.4
B	56.5	198.9	187.3	198.9	31.0
C	56.5	198.2	186.6	199.3	31.3
D	56.5	198.4	186.8	199.5	31.6

Table 7. Operational Parameters Measured During the Deballasting Simulation for the Four Trials of the NaOH Ballast Water Treatment System.

	Treatment			Control		
Trial	Flow Duration (min)	Flow Rate (m^3/h)	Pressure (psi)	Flow Duration (min)	Flow Rate (m^3/h)	Pressure (psi)
A	53.3	200	30.2	52.6	198	28.9
B	52.3	197	31.2	54.1	195	31.1
C	54.2	196	30.9	54.7	195	31.4
D	54.5	195	31.5	54.0	192	31.4

3.1.2. Physical, Chemical and Biological Challenge Conditions

A summary of the physical/chemical conditions of intake water are provided in Table 8. The ambient TSS was characteristically low, ranging from 1.3 mg/L to 2.4 mg/L, and averaging 2.0 mg/L for the four trials. The NPOC was entirely DOC, as the average of both parameters was 13.4 mg/L throughout the four trials. The %T ranged from 24.8 to 29.4 in the first three trials, but in Trial D rose substantially (42.7 %T). The average % T across the four trials was 31.0 %T. The challenge water pH ranged from 7.75 to 7.99 during all four trials.

The average pH of the post-treatment water collected immediately prior to the water entering the treatment retention tank was 12.02, achieving the target pH of 12.00. Samples collected from the treatment retention tank just prior to neutralization had an average pH was 12.04, showing that the pH of the treated water did not change significantly during the holding time. After the neutralization process, samples measured from the treatment tank and in-line from the treatment discharge averaged 8.17 and 8.14, respectively). The neutralized discharge pH met the target value of less than 8.5, and was within the WIDNR permitting levels for discharge to the Duluth-Superior Harbor (i.e., pH 6 to 9).

Table 8. Average (± Std. Dev.) Challenge Water Quality during Four Trials of the NaOH Ballast Water Treatment System.

Parameter	Desired pH Value	Sample	Trial A	Trial B	Trial C	Trial D	Summary (*n*=4)
pH[1]	7-9 (Ambient)	Pre-Treatment Intake	7.76 ± 0.01	7.83 ± 0.04	7.99 ± 0.04	7.75 ± 0.02	**7.83 ± 0.11**
	12.00	Post-Treatment Intake	12.00	12.04	12.04	12.00	**12.02 ± 0.02**
	12.00	In-Tank Before Neutralization	12.06	12.07	11.99	12.02	**12.04 ± 0.04**
	≤ 8.5	In-Tank After Neutralization	8.27	8.21	7.81	8.39	**8.17 ± 0.25**
	7-9	Treatment Discharge	NA	8.22	7.81	8.40	**8.14 ± 0.30**
TSS (mg/L)	N/A	Intake	1.3 ± 0.2	2.0 ± 0.1	2.4 ± 0.1	2.1 ± 0.1	**2.0 ± 0.5**
NPOC (mg/L)	N/A	Intake	13.5 ± 0.5	15.3 ± 0.3	14.4 ± 0.2	10.3 ± 0.2	**13.4 ± 2.2**
DOC (mg/L)	N/A	Intake	13.7 ± 0.3	15.3 ± 0.1	14.3 ± 0.3	10.3 ± 0.2	**13.4 ± 2.2**
POC (mg/L)	N/A	Intake	-0.2 ± 0.7	-0.1 ± 0.2	0.1 ± 0.3	0.0 ± 0.4	**-0.1 ± 0.1**
%T (254 nm)	N/A	Intake	24.8 ± 0.5	26.9 ± 0.1	29.4 ± 0.2	42.7 ± 0.8	**31.0 ± 8.1**

[1]The sample temperature was measured simultaneously with sample pH measurements. The average sample temperature was 20.9 ± 1.8 °C in pre-treatment intake, 23.1 ± 1.9°C in post-treatment intake, 22.6 ± 1.5 °C in the treatment retention tank before and after neutralization, and 21.0 ± 1.0°C in treatment discharge.

The live plankton densities in intake and control discharge samples, for the ≥ 10 μm and < 50 μm size class and for the larger plankton size class (i.e., ≥ 50 μm), are summarized in Table 9. During all four trials, average intake densities of live zooplankton (i.e., those organisms ≥ 50 μm) ranged from 28,331/m^3 (Trial A) to 648,158/m^3 (Trial C), for an average of 291,229/m^3. The zooplankton community was comprised mainly of dreissenid mussel veligers; the rotifers *Keratella, Polyarthra,* and *Synchaeta*; calanoid and cyclopoid copepods; and the cladoceran *Bosmina.* The target intake density of ≥ 100,000/m^3 was achieved during all but the first trial (Trial A). The density of live organisms in the control discharge samples after the two- or three-day holding period ranged from 22,047/m^3 (Trial A) to 725,980/m^3 (Trial C), which was 78 % to 112 % of the starting densities. Control discharge densities in Trials B and C were slightly higher than their corresponding intake densities (1% to 12% respectively), likely due to reproduction of the rotifers (*Keratella spp.* and *Polyarthra spp.*) and the cladoceran *Bosmina*.

The live organism density for the ≥ 10 and < 50 μm organism size class on intake, consisting mainly of phytoplankton, ranged from 67.56 cells/mL (Trial A) to 661.91 cells/mL (Trial D, in which the intake stream was amended with concentrated harbor algae; Table 9). The community of protists comprised, in decreasing relative abundance, chain-forming diatoms (largely *Aulacoseira*), coccoid green algae (largely *Gonium*), filamentous blue-green algae (*Oscillatoria*), miscellaneous microflagellates, and free-living centric diatoms, such as *Cyclotella*. The density

of live phytoplankton in the control discharge samples ranged from 16.00 cells/mL (Trial C) to 275.95 cells/mL (Trial D), for an average of 93.43 cells/mL (Table 9). This represented 4 % to 42 % of the intake densities (Table 9). Although organism densities were lower in the control discharge samples, the relative abundance of taxa were similar to that observed in intake samples.

Table 9. Live Plankton Densities in Intake and Control Discharge During the Four NaOH Trials. Note: The Live Intake Densities are an Average of the Control Intake and the Pre-treatment Intake Samples.

Organism Size Category	Sample	Trial A	Trial B	Trial C	Trial D	Average ± Std. Dev. (*n*=4)
≥ 50 μm (#/m^3)	Intake	28,331	384,216	648,158	104,210	**291,229 ± 141,462**
	Control Discharge	22,047	389,885	725,980	102,478	**310,098 ± 159,531**
≥ 10 and < 50 μm (#cells/mL)	Intake	67.56	276.63	417.01	661.91	**355.78 ± 124.77**
	Control Discharge	27.44	54.31	16.00	275.95	**93.43 ± 61.37**

Concentrations of organisms in the < 10 μm size class in the intake and control discharge samples during the four NaOH Trials are provided in Table 10. Overall intake densities within this size class were highest during Trials C and D. Total coliform bacteria ranged from 204 MPN/100 mL in Trial A to 552 MPN/100 mL in Trial D. *E. coli* ranged from 41 MPN/100 mL (Trial A), to 107 MPN/100 mL (Trial C). *Enterococci* ranged from 35 MPN/100 mL (Trial A) to 164 MPN/100 mL (Trial D). Finally, total heterotrophic bacteria ranged from 400 MPN/mL in Trial A to 1240 MPN/mL in Trial B (Table 10). In the control discharge, indicator organisms for waterborne pathogens were more sparse, which is to be expected as the retention tank is not a favorable environment to support growth of these organisms. The total coliform bacteria ranged from 19 MPN/100 mL (Trial B) to 129 MPN/100 mL (Trial D), an overall decline of 78 % relative to intake. The overall average *E. coli* density was 6 MPN/100 mL. *Enterococci* ranged from 4 MPN/100 mL in Trial A to 143 MPN/100 mL in Trial B. Total heterotrophic bacteria ranged from 424 MPN/mL (Trial A) to 1833 MPN/mL (Trial D).

Table 10. Viable Microbial Densities (Average ± Standard Error of the Mean) in Intake and Control Discharge During the Four NaOH Trials.

< 10 μm Size Class Group	Sample	Trial A	Trial B	Trial C	Trial D	Summary (*n*=4)
Total Coliform Bacteria (MPN/100 mL)	Intake	239 ± 53	204 ± 20	536 ± 40	552 ± 46	**383 ± 93**
	Control Discharge	123 ± 32	19 ± 4	51 ± 2	129 ± 7	**81 ± 27**
E. Coli (MPN/100 mL)	Intake	41 ± 2	55 ± 4	107 ± 5	73 ± 5	**69 ± 14**
	Control Discharge	11 ± 2	1 ± 0.5[a]	3 ± 1	9 ± 1	**6 ± 2**
Enterococci (MPN/100 mL)	Intake	35 ± 7	40 ± 14	139 ± 26	164 ± 31	**95 ± 33**
	Control Discharge	4 ± 0.3	143 ± 88	68 ± 8	76 ± 3	**73 ± 28**
Total Heterotrophic Bacteria (MPN/mL)	Intake	400 ± 200	1240 ± 30	1033 ± 491	1117 ± 164	**948 ± 187**
	Control Discharge	424 ± 63	1225 ± 200	900 ± 321	1833 ± 67	**1096 ± 296**

[a] One or more values were below the limit of detection (LOD). Half the value of the LOD was used for calculations. See Appendix 2 for raw data.

3.1.3. In-Tank Water Quality

Control and treatment retention tank water quality data are presented in Table 11. Two values are reported for the treatment tank: the values measured prior to the start of the neutralization period, and the values measured after neutralization and just prior to the start of the discharge operation. Only data from Trials C and D are reported; the YSI Sondes were not calibrated prior to Trial A or Trial B; therefore, the accuracy of the water quality data from these trials cannot be assured.

Temperature and dissolved oxygen were similar in the control and treatment tanks throughout the entire holding period, including after neutralization of the treatment tank. However, several parameters were different as a result of the NaOH injection into the treatment track. As expected, the pH of the treatment tank water was significantly higher than the control tank water. In trials C and D, the average pH of water in the treatment tank prior to neutralization was 11.80 and 11.85, respectively. In contrast, the average pH of water in the control tank was 7.72 for Trial C and 7.45 for Trial D. The pH of water held in the treatment tank following neutralization with CO_2 was 7.57 and 8.18 for the two trials respectively, thereby meeting Wisconsin DNR permit requirements for discharge to the harbor.

In both trials reported in Table 11 (Trials C and D) the specific conductivity in the treatment tank prior to neutralization was on average 14.5 to 17.4 times higher, than in the control tank during the three-day holding period. The neutralization process decreased the specific conductivity by approximately half, but on average the post-neutralization conductivity just prior to treatment

discharge was still 6.6 to 7.6 times higher than in the control tank. In addition, the treatment tank salinity (as calculated by the YSI Sonde based on specific conductivity) increased as a result of the NaOH injection. On average, the salinity in the treatment tank prior to neutralization was 15.5 to 20.4 times higher than in the control tank. The neutralization process reduced the salinity by about half from the average salinity measured prior to CO_2 injection but the levels in the treatment tank just prior to discharge were still an average of eight times higher than the levels in the control tank just prior to discharge in both Trial C and D.

The turbidity of water held in the control and treatment tanks during Trial D was similar; however, during Trial C the turbidity reading for the control tank water as substantially higher than all other readings for control and as compared to the treatment tank water (i.e., average 12.7 NTU in the control tank and 1.8 NTU in the treatment tank), possibly indicating a problem with the Sonde probe post calibration. In both trials, the turbidity of the treatment tank did not change after the neutralization process.

Table 11. Control and Treatment Retention Tank Water Quality (Average ± Std. Dev.) During the Three-day Holding Period for Trials C and D of the NaOH BWTS Test.

Parameter	Retention Tank	Sample Period	Trial C	Trial D
Temperature (°C)	Control	Entire Retention	21.95 ± 0.24 (*n*=288)	21.33 ± 0.23 (*n*=283)
	Treatment	Before Neutralization	21.98 ± 0.25 (*n*=277)	21.47 ± 0.23 (*n*=276)
		Prior to Discharge	22.19 (*n*=1)	21.99 (*n*=1)
Specific Conductivity (mS/cm)	Control	Entire Retention	0.224 ± 0.001 (*n*=288)	0.179 ± 0.001 (*n*=283)
	Treatment	Before Neutralization	3.258 ± 0.016 (*n*=277)	3.113 ± 0.021 (*n*=276)
		Prior to Discharge	1.475 (*n*=1)	1.365 (*n*=1)
Salinity (ppt)	Control	Entire Retention	0.11 ± 0.00 (*n*=288)	0.08 ± 0.00 (*n*=283)
	Treatment	Before Neutralization	1.71 ± 0.01 (*n*=277)	1.63 ± 0.01 (*n*=276)
		Prior to Discharge	0.74 (*n*=1)	0.68 (*n*=1)
pH	Control	Entire Retention	7.72 ± 0.06 (*n*=288)	7.45 ± 0.02 (*n*=283)
	Treatment	Before Neutralization	11.80 ± 0.02 (*n*=277)	11.85 ± 0.02 (*n*=276)
		Prior to Discharge	7.57 (*n*=1)	8.18 (*n*=1)
Turbidity (NTU)	Control	Entire Retention	12.7 ± 0.6 (*n*=288)	0.6 ± 0.3 (*n*=283)
	Treatment	Before Neutralization	1.8 ± 0.2 (*n*=277)	0.8 ± 0.5 (*n*=276)
		Prior to Discharge	1.4 (*n*=1)	0.7 (*n*=1)

Parameter	Retention Tank	Sample Period	Trial C	Trial D
Dissolved Oxygen (% Saturation)	Control	Entire Retention	89.2 ± 3.1 (*n*=288)	83.1 ± 0.9 (*n*=283)
	Treatment	Before Neutralization	89.8 ± 2.2 (*n*=277)	83.3 ± 0.9 (*n*=276)
		Prior to Discharge	86.8 (*n*=1)	81.0 (*n*=1)
Dissolved Oxygen (mg/L)	Control	Entire Retention	7.80 ± 0.24 (*n*=288)	7.36 ± 0.10 (*n*=283)
	Treatment	Before Neutralization	7.78 ± 0.16 (*n*=277)	7.29 ± 0.10 (*n*=276)
		Prior to Discharge	7.53 (*n*=1)	7.06 (*n*=1)

3.2. Ballast Water Treatment System Biological Efficacy

Plankton densities and associated sample volumes relevant to live organisms in the ≥ 50 μm and ≥10 μm to < 50 μm size classes in control and treatment discharge samples from Trials A, B, C and D are summarized in Tables 12 to 16. In addition, live densities of four groups of bacteria (i.e., total coliform, *E. coli*, *Enterococcus spp.,* and total heterotrophic) in the <10 μm size class are reported in Tables 17 and 18. Percent reduction of live organism density in the treatment discharge as compared to the control discharge of organisms in the ≥ 50 μm, ≥ 10 and < 50 μm, and < 10 μm size classes is summarized in Table 19.

3.2.1. Organisms ≥ 50 µm in Minimum Dimension

Average live organism densities in treated discharge and total volume of treated discharge water analyzed during Trials A, B, C and D are reported in Table 12. Significant amounts of dead material in the treatment discharge samples limited the sample volume that could be analyzed for live organisms ≥ 50 μm in minimum dimension prior to maximum sample holding time (i.e., two hours). Sample volumes analyzed for live macrozooplankton ranged from 0.6 m^3 to 2.2 m^3 and sample volumes analyzed for live microzooplankton ranged from 0.1 to 0.3 m^3 (Tables 12 and 13). These low volumes resulted in low statistical certainty of density estimates.

Table 12. Live Treatment Discharge Densities and Treatment Discharge Sample Volume Analyzed Within the ≥ 50 μm Size Class During Four Trials of the NaOH Ballast Water Treatment System.

Treatment Discharge Density/ Vol. Analyzed	Trial A	Trial B	Trial C	Trial D	Avg. ± SEM
Density (#/m^3)	0.5	0.0	19	0.0	**4.9 ± 4.8**
Total Vol. Analyzed (m^3)	2.2 MacroZP 0.2 MicroZP	1.3 MacroZP 0.3 MicoZP	0.8 MacroZP 0.1 MicroZP	0.6 MacroZP 0.1 MicroZP	**1.2 ± 0.7 MacroZP** **0.2 ± 0.1 MicroZP**

Overall, the average live organism densities ≥ 50 μm in minimum dimension in treated discharge across all four trials was 4.9 live organisms/m^3 (Table 12). Table 13 provides the relative densities of live organisms across taxa in the treated discharge from the four trials. In Trial A, one live ostracod was observed in the treated discharge sample resulting in a density estimate of 0.5 live organisms per m^3 (Tables 12 and 13). There were no live organisms measuring ≥ 50 μm in minimum dimension observed in trials B or D treatment discharge (Table 12), however, there was one chironomid larvae each (both measuring 40 μm and in minimum dimension) observed in Trial B and Trial D treatment discharge resulting in a density estimate of 0.80 and 1.67 per m^3 respectively (Table 13). Trial C treated discharge had a total of 19.2 live zooplankton per m^3 ≥ 50 μm (Table 12), including chironomid larvae, planaria, copepods, and dreissenid larvae (Table 13).

Table 13. Live Zooplankton Densities Across Taxa in Treatment Discharge from Four Trials of the NaOH Ballast Water Treatment System.

Test Trials:	Trial A	Trial B	Trial C	Trial D
Total Vol. Treatment Discharge Analyzed, m^3:	**2.21 (0.24 MicroZP)**	**1.28 (0.30 MicoZP)**	**0.82 (0.14 MicroZP)**	**0.60 (0.08 MicroZP)**
≥ 50 μm (min. dimension)				
Taxa Group	**Avg. Density (per m^3)**	**Avg. Density (per m^3)**	**Avg. Density (per m^3)**	**Avg. Density (per m^3)**
Copepod: Calenoid/Cyclopoid			2.43	
Chironomid			4.86	
Planaria			4.86	
Ostracod	0.50			
Dreissenid (Zebra Mussel)			7.01	
Equal to or Greater than 50 μm (min. dimension) Total:	**0.50**	**0.00**	**19.16**	**0.00**
< 50 μm (min. dimension)				
Taxa Group	**Avg. Density (per m^3)**	**Avg. Density (per m^3)**	**Avg. Density (per m^3)**	**Avg. Density (per m^3)**
Chironomid		0.80	1.22	1.67
Less than 50 μm (min. dimension) Total:	**0.00**	**0.80**	**1.22**	**1.67**
< 50 μm (min. dimension); Observed but not Quantified				
Taxa Group	**Observations/ Comments**	**Observations/ Comments**	**Observations/ Comments**	**Observations/ Comments**
Eggs/Cysts		A few observed.	Many observed (~85,000/m^3 live).	Many observed.
Phytoplankton		A few observed with chlorophyll.		
Copepod Nauplii			Four live observed under dissecting scope.	

The results of the statistical comparison between live organism density in the treatment discharge and in the control discharge are shown in Table 14. The Kruskal-Wallis One Way ANOVA was used to compare the two groups, as the data were normally distributed but did not have equal variance and transformation of the data (e.g., log base 10, log normal, and square root) did not successfully produce data that met the assumptions of a One Way ANOVA. After four trials of the NaOH BWTS, overall live zooplankton density in the treated discharge was significantly (p = 0.029) lower than that of the control discharge.

Table 14. Result of Statistical Comparison of Live Zooplankton Density in Control Discharge to Treatment Discharge.

The hypothesis tested was that the NaOH BWTS significantly reduces the number of live organisms on discharge in comparison to untreated, control discharge.

Treatment Group	Mean Density (*n*=4)	Std. Dev.	SEM	*t*	*p*	Probability of Trial Resulting in No Difference
Control Discharge	310,098 live/m^3	319,063	159,531	26.000	0.029	1 in 34
Treatment Discharge	5 live/m^3	9	5			

3.2.2. Organisms ≥ 10 and < 50 µm in Minimum Dimension

In the ≥ 10 and < 50 μm size class, live organism densities ranged from 0.2 cell/mL (Trial B) to 2.5 cells/mL (Trial D), for an average of 1.0 cell/mL (Table 15). The volume of treatment discharge water analyzed was 5.5 mL to 8.9 mL (Table 15). While few in number, surviving organisms in treated water were taxonomically various including diatoms, green algae, blue-green algae, and protozoans.

Table 15. Live Treatment Discharge Density and Treatment Discharge Sample Volume Analyzed Within the ≥10 μm and < 50 μm Size Class during Four Trials of the NaOH Ballast Water Treatment System.

Treatment Discharge Density/ Vol. Analyzed	Trial A	Trial B	Trial C	Trial D	Avg. ± SEM
#cells/mL	0.3	0.2	1.0	2.5	**1.0 ± 0.5**
Total Vol. Analyzed (mL)	8.9	5.5	8.2	7.1	**7.4 ± 1.5**

The results of the One Way ANOVA are provided in Table 16 below. The data were not normally distributed; therefore, the data were transformed using log (base 10) transformation. Overall, the live phytoplankton density in the treatment discharge was significantly (p = 0.002) lower as compared to the untreated, control discharge.

Table 16. Result of Statistical Comparison of Live Phytoplankton Density in Control Discharge to Treatment Discharge.

The hypothesis tested was that the NaOH BWTS significantly reduces the number of live organisms on discharge in comparison to untreated, control discharge.

Treatment Group	Mean Density (*n*=4)	Std. Dev.	SEM	*t*	*p*	Probability of Trial Resulting in No Difference
Control Discharge	93 cells/mL	123	61	5.200	0.002	1 in 500
Treatment Discharge	1 cell/mL	1	0.5			

3.2.3. Organisms < 10 μm in Minimum Dimension

The density of live organisms in the < 10 μm size class in treatment discharge from the four NaOH BWTS trials are presented in Table 17. The total coliform bacteria concentrations in treated discharge ranged from less than the limit of detection (i.e., <1 MPN/100 mL) in Trials A, B, and D to a maximum of 2 MPN/100 mL in Trial C. For all four trials the *E. coli* density was <1 MPN/100 mL (i.e., the limit of detection) in treatment discharge. The live density of *Enterococci* ranged from 2 MPN/100 mL (Trials A and D) to 42 MPN/100 mL (Trial B) in treatment discharge. Total heterotrophic bacteria densities in treatment discharge ranged from 116 MPN/mL (Trial B) to 363 MPN/mL (Trial A), which is an average of 80 % less heterotrophic bacteria as compared to the control discharge.

Table 17. Live Density (Average ± Standard Error of the Mean) of Regulated Microbes in the Treatment Discharge from the Four Trials of the NaOH Ballast Water Treatment System.

<10 μm Size Class Group	Trial A	Trial B	Trial C	Trial D	Summary (*n*=4)
Total Coliform Bacteria (MPN/100 mL)	<1	<1	2 ± 2[a]	<1	**<1**
E. Coli (MPN/100 mL)	<1	<1	<1	<1	**<1**
Enterococci (MPN/100 mL)	2 ± 1[a]	42 ± 42[a]	6 ± 5	2 ± 1	**13 ± 10**
Total Heterotrophic Bacteria (MPN/mL)	363 ± 0	116 ± 6	222 ± 24	174 ± 21	**219 ± 53**

[a] One or more values were below the limit of detection (LOD). Half the value of the LOD was used for calculations. See Appendix 2 for raw data.

The results of the One Way ANOVA are provided in Table 18 below. There was a significant ($p < 0.05$) reduction in live organism density in the treatment discharge as compared to the control discharge for all groups analyzed except *Enterococcus spp*.; with reductions in total coliform bacteria being the most pronounced. The total coliform and *E. coli* data did not meet the assumptions of the One Way ANOVA. The total coliform data were transformed using log (base 10) transformation. Transformation (e.g., log (base 10), natural log, and square root) of the *E. coli* data did not produce normally distributed data with equal variance; therefore, the nonparametric Kruskal-Wallis One Way ANOVA on Ranks was used to compare differences between the treatment and control. The *Enterococcus spp.* data and the total heterotrophic data were analyzed using a One Way ANOVA.

Table 18. Results of Statistical Comparisons of Live Microbe Density in Control Discharge and Treatment Discharge in the <10-μm Size Class.
The hypothesis tested was that the NaOH BWTS significantly reduces the number of live organisms on discharge in comparison to untreated, control discharge.

Type of Bacteria	Treatment Group	Mean Density (*n*=4)	Std. Dev.	SEM	*t*	*p*	Probability of Trial Resulting in No Difference
Total Coliform Bacteria	Control Discharge	80.50 MPN /100 mL	54.19	27.10	7.881	0.0002	1 in 5000
	Treatment Discharge	0.88 MPN /100 mL	0.75	0.38			
E. coli	Control Discharge	6.00 MPN/100 mL	4.76	2.38	26.000	0.029	1 in 34
	Treatment Discharge	0.50 MPN/100 mL	0.00	0.00			
***Enterococcus* spp.**	Control Discharge	72.75	56.85	28.42	1.989	0.094	1 in 11
	Treatment Discharge	13.00	19.43	9.713			
Total Heterotrophic Bacteria	Control Discharge	1095.50	591.55	295.78	2.918	0.027	1 in 37
	Treatment Discharge	218.75	105.48	52.74			

3.2.4. Percent Reduction of Live Organisms in the Treatment Discharge

Percent reduction of live organism density in the treatment discharge as compared to the control discharge for all three regulated size classes was calculated by the following equation:

$$Percent\ Reduction\ in\ Treatment\ Discharge = \left[1 - \left(\frac{Treatment\ Discharge\ Density}{Control\ Discharge\ Density}\right)\right] \times 100\%$$

Table 19 summarizes the percent reduction in live organism density in treatment discharge for all four trials of the NaOH BWTS. The largest percent reduction of live organism density was seen in the ≥ 50 μm (zooplankton) and ≥ 10 μm and < 50 μm (phytoplankton) size classes. There was a reduction of 100 % live zooplankton in the treatment discharge as compared to the control discharge during Trials B and D. Trials A and C saw an approximate reduction of 100 % of the live zooplankton density in the treatment discharge, although 0.5 live/m^3 and 19 live/m^3 were counted in each trial, respectively. Trials B and D were also the most successful for phytoplankton with an approximate treatment discharge reduction of 100 % (5.5 live cells/mL were counted) in Trial B and 99 % reduction in Trial D. There was a reduction of 99 % and 94 % in Trials A and C, respectively, for an overall average of 98 % reduction during all four trials. The NaOH BWTS was less effective for the organisms in the < 10 μm size class. There was a greater than one-log reduction in live coliform bacteria density in treatment discharge as compared to control discharge (i.e., average of 98 % reduction in live density in treatment discharge). The live density of *E. coli*, *Enterococcus spp.*, and heterotrophic bacteria in treatment discharge was reduced less than one log as compared to the control discharge. There was an

overall average of 81 % reduction in live *E. coli*, 77 % reduction in live *Enterococcus spp.*, and 68 % reduction in live heterotrophic bacteria.

Table 19. Percent Reduction of Live Organism Density in Treatment Discharge as Compared to Control Discharge. Trial A had a Retention Time of Two Days; Trials B - D had a Retention Time of Three Days.

Organism Size Category	Group	Trial A	Trial B	Trial C	Trial D	Summary (*n*=4)
≥50 μm	Zooplankton	100%[a]	100%	100%[a]	100%	**100% ± 0%**
≥10 μm and <50 μm	Phytoplankton	99%	100%[a]	94%	99%	**99% ± 3%**
<10 μm[b]	Total Coliform Bacteria	100%[a,c]	97%[c]	96%	99%[c]	**98% ± 2%**
	E. coli	95%[c]	50%[c]	83%[c]	94%[c]	**81% ± 21%**
	Enterococci	50%	71%	91%	97%	**77% ± 21%**
	Total Heterotrophic Bacteria	14%	91%	75%	91%	**68% ± 36%**

[a] Live organisms were found during this trial. The percent reduction was rounded up to 100% although complete elimination of live organisms in treatment discharge was not observed.
[b] Percent reduction of live bacteria density in treatment discharge as compared to control discharge is based on the average (*n* = 3 samples) live organism density per trial in treatment and control discharge.
[c] Average (*n* =3) treatment discharge density values were below the limit of detection (LOD), which is 1 MPN/100 mL. Half the value of the LOD, or 0.5 MPN/100 mL was used for percent reduction calculations.

3.3. Whole Effluent Toxicity (WET) Testing

WET tests were conducted on *P. promelas* and *C. dubia* using Trial B treatment discharge, and on *P. promelas*, *C. dubia*, and *S. capricornutum* using Trial D treatment discharge. The performance controls (i.e., culture water for *P. promelas* and *C. dubia*, and algae media for *S. capricornutum*) met test acceptability criteria in all cases, with the exception of the *C. dubia* survival criterion in Trial B. The untreated filtered harbor water controls (0 % treatment discharge water) met the test acceptability criteria for all species tested during both Trials B and D.

The survival of *C. dubia* was only 70 % in the Trial B performance control (i.e., hard reconstituted culture water), and therefore these results were discarded and are not reported here. The WET test conducted on *C. dubia* using Trial D whole effluent showed no lethal effect (Table 20). However, there was a significant ($p<0.05$) reduction in the mean number of young produced per female in the 100 % Effluent group as compared to the 0 % Effluent group (Table 20). In Trial D, each female in the 100 % Effluent produced an average of 20 young as compared to 36 young per female in the 0 % Effluent (Table 20). This result indicates a potential effect of 100 % treatment discharge on cladoceran reproduction.

In the WET test conducted on the algal species *S. capricornutum* using treatment discharge from Trial D, the average cell density at test termination in the 100 % Effluent group (3,896,875 cells/mL) was significantly ($p<0.05$) higher as compared to the 0 % Effluent group (2,875,000

cells/mL), suggesting a possible effect of 100 % Effluent might be enhanced algal growth (Table 21).

Exposure to 100 % BWTS treatment discharge water did not affect *P. promelas* survival or growth in either Trial B or Trial D (Table 22). However, these organisms did display behavioral differences from their counterparts exposed to Control tank discharge. During both Trials B and D, organisms in treated discharge continuously swam in a circular pattern, an effect not observed in the harbor water control.

Table 20. Average (± Standard Error of the Mean) Survival and Reproduction of *C. dubia* Exposed to Whole Effluent Collected from NaOH Treatment Discharge During Trial D. Note: Statistical comparisons were made within one trial only, i.e., no comparisons were made between trials.

Treatment Group	TRIAL D	
	Survival (%)	No. Young per Female
C. dubia Culture Water (Performance Control)	100 ± 0.0	33 ± 3.2
0% Effluent (Untreated Harbor Water)	100 ± 0.0	36 ± 4.0
100% Effluent	90 ± 10.0	20 ± 3.4[a]

Test acceptability criteria: 80 % or greater survival and an average of 15 more young per female in the controls.
[a] The difference in average number of young per female is greater than would be expected by chance; the 100% Effluent group is statistically (p<0.05) less than the 0% Effluent group.

Table 21. Average (± Standard Error of the Mean) Final Density of *S. capricornutum* Exposed to Whole Effluent Collected from NaOH Treatment Discharge During Trial D.

Treatment Group	*S. capricornutum* Density (cells/mL)	CV (%)
Algae Growth Media (Performance Control)	2,240,625 ± 218,146	19.5
0% Effluent (Untreated Harbor Water)	2,875,000 ± 112,384	7.8
100% Effluent	3,896,875 ± 162,850[a]	8.4

Test acceptability criteria: Control flask must exceed $1 * 10^6$ cells/mL and not vary more than 20 % among replicates.
[a] The difference in average cell density is greater than would be expected by chance; the 100% Effluent group is statistically (p<0.05) greater than the 0% Effluent group.

Table 22. Average (± Standard Error of the Mean) *P. promelas* Survival and Dry Weight per Surviving Minnow Exposed to Whole Effluent Collected from NaOH Treatment Discharge During Trials B and D.

Treatment Group	TRIAL B		TRIAL D	
	Survival (%)	Dry Weight per Survivor (mg)	Survival (%)	Dry Weight per Survivor (mg)
Laboratory Water (Performance Control)	98 ± 1.7	0.45 ± 0.02	100 ± 0.0	0.54 ± 0.03
0% Effluent (Untreated Harbor Water)	100 ± 0.0	0.47 ± 0.01	100 ± 0.0	0.58 ± 0.02
100% Effluent	95 ± 3.2	0.53 ± 0.02	100 ± 0.0	0.57 ± 0.02

Test acceptability criteria: 80 % or greater survival in the controls; average dry weight per surviving organism in the controls equal to or exceeding 0.25 mg.

4.0. QUALITY MANAGEMENT

GSI uses a wide variety of quality management documents and records to implement its quality management system. These include quality system documentation (i.e., the GSI Quality Management Plan), project-specific documentation (i.e., Quality Assurance Project Plans), and routine procedures documentation (i.e., Standard Operating Procedures).

4.1. Quality Management Plan (QMP)

Detailed information on the structure and organization of GSI's quality system can be found in the GSI Quality Management Plan (GSI, 2010b). Electronic copies of this document are available upon request. The GSI QMP covers all aspects of GSI's commitment to quality including policies and procedures; criteria for and areas of application; roles, responsibilities, and authorities; assessment and response; and quality improvement. It is the framework for planning, implementing, documenting, and assessing the GSI's quality assurance and quality control (QAQC) activities.

4.2. Quality Assurance Project Plan (QAPP)

Additional information and details regarding the activities undertaken by GSI to assure the quality and credibility of its research at the Land-Based RDTE Facility can be found in GSI's Land-Based Quality Assurance Project Plan (GSI, 2010a). This document is available electronically upon request. The QAPP covers all aspects of quality assurance/quality control (QAQC), including data quality indicators, evaluation processes, performance measures and acceptance criteria; instrument certification and calibration; personnel training requirements; documents and records; data management; and QAQC assessments and response actions.

4.3. Standard Operating Procedures (SOPs)

SOPs are used to implement all GSI 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; operation of the GSI Land-Based RDTE facility; sample collection, labeling, analysis and custody; and safety. Appendix 1 provides a list of GSI SOPs relevant to land-based test activities.

5.0. DISCUSSION OF RESULTS

The NaOH BWTS operated effectively during the four trials conducted on the proposed $NaOH/CO_2$ treatment process. In particular, the BWTS led to highly significant reductions in live organism densities in all taxonomic categories evaluated in treated discharge relative to control discharge. It is not possible to assess performance against a discharge standard using the results from these land-based trials; the testing conditions and quality assurances were not at IMO- or ETV-consistent levels given the research and development objective of the test series.

In these tests, there was a nearly 100 % reduction in live organisms greater than 50 μm in minimum dimension (zooplankton) in the treatment discharge as compared to the control discharge, with zero live organisms found in Trials B and D and very few live organisms found in Trials A and C. The overall percent reduction of live phytoplankton density in the treatment discharge was greater than 97 % as compared to the control discharge density during the four trials of the NaOH BWTS. In terms of microbial organisms, any reductions caused by the treatment system in these tests were less than one log in magnitude. Coliform bacteria and *E. coli* appeared most sensitive to the BWTS of the organisms tested, decreasing these microbial organisms by an average of 98% and 81%, respectively across the four trials. The process had very little effect if any on total heterotrophic bacteria; though densities (i.e. MPN per mL) were on average lower in treated discharge than control discharge. The overall percent reduction was less pronounced for the *Enterococci* and total heterotrophic bacteria, with greater than 77 % and 67 % reduction as compared to the control, respectively.

Treatment effectiveness in this larger size class of organisms (greater than 50 μm in minimum dimension) appeared roughly consistent with IMO D-2 standards. That is, in three trials, the density estimates were well below IMO D-2 standards ($0.0/m^3$ to $0.5/m^3$; Trials A, B, and D). In one trial (Trial C), the estimate was clearly above it ($19/m^3$), but it was impossible to discern a difference between any of these estimates (above or below) and the 10 live organisms per m^3 benchmark from a statistical standpoint given the small sample volumes analyzed (Miller, *et al*, 2011). With no filter system associated with this treatment process, the detritus in the samples subject to analysis was too great to allow analysis of sufficient sample volumes in the time period required to afford a precise estimate of discharge densities. This problem will likely not go away when the treatment process is subjected to actual certification testing, setting up a situation in which many more trials or many more microscopists are needed to generate adequate statistical certainty.

The relatively high density value for live organisms from the ≥ 50 μm size class in the Trial C treatment discharge is most likely an artifact resulting from the extremely low sample volumes subject to analysis. To illustrate the effect of this sample condition, consider that Dreissenids figure prominently in Trial C treatment discharge, contributing to nearly one-half of the live organism density. However, due to the small amount of sample volume analyzed for microzooplankton (0.14 m^3), the discovery of just one live dreissenid during sample analysis accounted for that density estimate. It is interesting to note that the intake and control discharge density of organisms in the ≥ 50 μm size class was significantly higher in Trial C as compared to the other three trials, ranging from 2 - 23 times higher on intake and 2 - 33 times higher in the control discharge (Table 9), but this difference does not itself account for the disparity. Operational sources of error were likely not the cause. Cross contamination was prevented in Trial C, as well as the other three trials, and samples were analyzed to ensure no live organisms were present in the potable water source used for cleaning, and treatment tubs prior to each discharge operation (see Section 2.1.3.). In addition, a thorough review of the raw data did not reveal any potential contamination from the plankton net or sampling equipment, nor did the procedural audits conducted during the NaOH Trials point out any potential contamination from those sources. The density estimates for organisms in the ≥ 10 and < 50 μm size class in treated discharge were consistently low, but our ability to conclude performance within the IMO D-2 standard is nonetheless limited here by sample size as well (Miller *et al*., 2011).

WET test findings were indicative of chronic toxicity and behavioral effects of 100 % treatment discharge effluent water. Undiluted discharge from the treatment process led to inhibition of cladoceran reproduction, accelerated algal growth, and circular swimming in fish. It should be noted that 100 % effluent is not the condition that aquatic organisms will be subject to in reality at the point of ballast outfall since dilution occurs upon the ballast discharge making contact with the receiving system. Instead it is indicative of a potential for residual toxicity. More WET testing using additional dilution levels should be undertaken in any follow-up land-based tests to corroborate the findings and determine the percent dilution at which toxicity is observed, and at what dilution it no longer is detectable.

In addition, follow-up bench tests at GSI will seek to better elucidate the cause for this effect. The effects may be due to the increased conductivity or residual CO_2 from the neutralization process in the 100 % effluent water as compared to the harbor water control. Bench-scale tests should seek to rule out attributes in the treated water that could contribute to an effect in order to better isolate the causal agent(s).

6.0. CONCLUSIONS

The NaOH (Sodium Hydroxide, Lye) BWTS performed very well operationally and well enough biologically to warrant additional testing at the bench, land and ship-based scales. The system successfully treated ballast water without interruption, and successfully neutralized treated ballast water to achieve WIDNR permitting levels for harbor discharge (i.e., pH 6-9). The BWTS also significantly reduced live organism densities in treated discharge relative to control discharge in all size classes of organisms. Finally, in these tests, the BWTS performance met

discharge target values that were approximately consistent with the IMO Convention's Annex D-2 discharge standards, though precision in this estimate was not possible given the research and development testing parameters. This testing revealed that the water discharged after two- or three-day retention periods was not entirely environmentally benign (i.e., with no residual toxicity at the 100 percent effluent dilution), though the level of residual toxicity in 100 % effluent evident from these tests may not be of regulatory concern.

REFERENCES

Great Ships Initiative (2009). Technical Report on the Sodium Hydroxide (NaOH) Bench-Scale Test Findings. Available at: http://www.nemw.org/GSI/GSI-BS-P-TR-NaOH.pdf.

GSI (2010a). Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2010). Northeast-Midwest Institute, Washington, DC.

GSI (2010b). Great Ships Initiative (GSI) Quality Management Plan (QMP), 2010. Northeast-Midwest Institute, Washington, DC.

International Maritime Organization (IMO), 2004. International Convention for the Control and Management of Ships Ballast Water and Sediments. As adopted by consensus at a Diplomatic Conference at IMO, London, England, February 13 2004.

International Maritime Organization (2008a). Guidelines for Approval of Ballast Water Management Systems (G8), Resolution MEPC.174 (58). Adopted on 10 October 2008.

International Maritime Organization (2008b). Procedure for Approval of Ballast Water Management Systems that Make use of Active Substances (G9), Resolution MEPC.169 (57). Adopted on 04 April 2008.

NSF International (2010). Generic Protocol for the Verification of Ballast Water Treatment Technology. Prepared for the U.S. Environmental Protection Agency, Environmental Technology Verification Program in cooperation with the U.S. Coast Guard, Environmental Standards Division, Washington, DC, and the U.S. Naval Research Laboratory, Center for Corrosion Science and Engineering, Washington, DC. Version 5.1., September 2010.

USEPA, Office of Water (2002). Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Edition. Accessed from: http://www.epa.gov/waterscience/methods/wet/disk3/index.html.

APPENDIX 1.

List of GSI SOPs Relevant to the Land-Based Evaluation of the NaOH Ballast Water Treatment System.

SOP CODE	SOP TITLE	CATEGORY	SUBCATEGORY
GSI/SOP/G/A/RK/1	Procedure for Record Keeping	Administration	Record Keeping
GSI/SOP/G/RA/DM/1	Procedure for Data Entry, Data Quality Control and Database Management	Research Activities	Data Management
GSI/SOP/G/RA/SC/2	Procedure for Labeling Samples Collected at the GSI Land-Based RDTE Facility	Research Activities	Sample Custody
GSI/SOP/BS/RA/GL/1	Procedure for Verification of Laboratory Balances	Research Activities	General Laboratory
GSI/SOP/BS/RA/WET/1	Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to *Ceriodaphnia dubia*	Research Activities	Residual Toxicity
GSI/SOP/BS/RA/WET/2	Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Fathead Minnow (*Pimephales promelas*)	Research Activities	Residual Toxicity
GSI/SOP/BS/RA/WET/3	Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Green Alga (*Selenastrum capricornutum*)	Research Activities	Residual Toxicity
GSI/SOP/BS/RA/MA/1	Procedure For Quantifying Heterotrophic Plate Counts (HPCs) Using IDEXX's SimPlate® for HPC Method	Research Activities	Microbial Analysis
GSI/SOP/BS/RA/MA/3	Procedure for the Detection and Enumeration of *Enterococcus* Using Enterolert™	Research Activities	Microbial Analysis
GSI/SOP/BS/RA/MA/4	Procedure for the Detection and Enumeration of Total Coliforms and *E. coli* Using IDEXX's Colilert®	Research Activities	Microbial Analysis
GSI/SOP/BS/RA/MP/1	General Microbiology Preparation Procedures	Research Activities	Microbial Procedures
GSI/SOP/BS/RA/C/3	Procedures for Measuring Organic Carbon in Aqueous Samples	Research Activities	Chemistry
GSI/SOP/BS/RA/C/4	Procedure for Determining Percent Transmittance (%T) of Light in Water at 254 nm	Research Activities	Chemistry

SOP CODE	SOP TITLE	CATEGORY	SUBCATEGORY
GSI/SOP/BS/RA/C/8	Procedure for Analyzing Total Suspended Solids (TSS)	Research Activities	Chemistry
GSI/SOP/BS/RA/C/9	Procedure for pH Meter Calibration and pH Measurement	Research Activities	Chemistry
GSI/SOP/LB/G/O/1	Procedure for Operating the GSI Land-Based RDTE Facility	General	Operation
GSI/SOP/LB/G/O/2	Procedure for Sampling and Analyzing Treated Water in the Retention Tanks Prior to Discharge	General	Operation
GSI/SOP/LB/G/O/3	Procedure for Cleaning and Verifying Cleanliness of the Retention Tanks and Piping at the GSI Land-Based RDTE Facility	General	Operation
GSI/SOP/LB/G/O/4	Procedure for Cleaning Sampling Equipment at the GSI Land-Based RDTE Facility	General	Operation
GSI/SOP/LB/G/O/5	Procedure for Injecting Organisms and Solids into the GSI Land-Based RDTE Facility	General	Operation
GSI/SOP/LB/G/O/7	Procedure for Maintaining Solids Suspension in the GSI Land-Based RDTE Facility's Retention Tanks	General	Operation
GSI/SOP/LB/G/C/4	Procedure for Calibration, Deployment, and Storage of YSI Multiparameter Water Quality Sondes	General	Calibration
GSI/SOP/LB/G/S/1	Procedure for Ensuring Worker Health and Safety at the GSI Land-Based RDTE Facility	General	Safety
GSI/SOP/LB/RA/SC/1	Procedure for Collecting Biological Sample Water via In-Line Sample Ports	Research Activities	Sample Collection
GSI/SOP/LB/RA/SC/3	Procedure for Algae/Small Protozoa Sample Collection	Research Activities	Sample Collection
GSI/SOP/LB/RA/SC/4	Procedure for Microbial Sample Collection	Research Activities	Sample Collection
GSI/SOP/LB/RA/SC/6	Procedure for Zooplankton Sample Collection	Research Activities	Sample Collection
GSI/SOP/LB/RA/SA/1	Procedure for Algae/Small Protozoan Sample Analysis	Research Activities	Sample Analysis
GSI/SOP/LB/RA/SA/2	Procedure for Zooplankton Sample Analysis	Research Activities	Sample Analysis

APPENDIX 2.

Trimmed, Raw Microbial Analysis Data from Testing of Lye (NaOH) Ballast Water Treatment System

Trial	Sample Location	Sample Tub	Rep.	Total Coliform Bacteria	*E. coli*	*Enterococcus spp.*	Heterotrophic Bacteria
				MPN/100 mL	MPN/100 mL	MPN/100 mL	MPN/1 mL
A	PRE-TREATMENT INTAKE	4	1	179	42	21	200
			2	192	36	45	200
			3	345	44	40	800
	CONTROL DISCHARGE	1	1	186	12	4	514
			2	79	8	5	454
			3	105	12	4	303
	TREATMENT DISCHARGE	4	1	<1	<1	1	363
		5		<1	<1	4	363
		6		1	<1	1	363
B	PRE-TREATMENT INTAKE	4	1	210	50	35	1210
			2	166	52	19	1210
			3	236	62	67	1301
	CONTROL DISCHARGE	1	1	17	2	313	1150
			2	14	<1	101	923
			3	26	<1	16	1603
	TREATMENT DISCHARGE	4	1	<1	<1	<1	104
		5		<1	<1	126	124
		6		<1	<1	<1	120
C	PRE-TREATMENT INTAKE	4	1	548	113	173	1000
			2	461	111	155	1900
			3	598	96	88	200
	CONTROL DISCHARGE	1	1	55	4	84	800
			2	48	2	65	400
			3	51	3	56	1500
	TREATMENT DISCHARGE	4	1	6	<1	1	266
		5		<1	<1	15	183
		6		<1	<1	1	216
D	PRE-TREATMENT INTAKE	4	1	614	67	186	800
			2	461	69	204	1200
			3	579	82	103	1350
	CONTROL DISCHARGE	1	1	118	6	80	1900
			2	142	11	71	1700
			3	127	10	78	1900
	TREATMENT DISCHARGE	4	1	<1	<1	1	216
		5		<1	<1	2	155
		6		<1	<1	4	151