GREAT SHIPS INITIATIVE FINAL REPORT OF LAND-BASED FRESHWATER TESTING OF A BALLAST WATER TREATMENT INVOLVING SODIUM HYPOCHLORITE (NaOCl)

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Great Ships Initiative
Final Report of Land-Based Freshwater Testing of a Ballast Water Treatment Involving Sodium Hypochlorite (NaOCl)

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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) at the bench, land-based and shipboard scales. GSI has the expertise and resources to perform tests 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's Generic Protocol (ETV; USEPA, 2010). GSI performs formal verification tests appropriate to market-ready prototype BWTSs, and informal status testing for BWTSs that are still in the research and development stages. GSI procedures, methods, materials and findings are publicly accessible on the GSI website (www.greatshipsinitiative.org).

In early 2011, researchers from the National Parks of Lake Superior Foundation (NPLSF) in Marquette, Michigan, and the Michigan Technological University (MTU) in Houghton, Michigan, applied to GSI for land-based tests of a BWTS involving sodium hypochlorite (NaOCl), in the same formulation used for household bleach. The BWTS was proposed for emergency treatment of ballast water in tanks of Great Lakes vessels passing through the Welland Canal system of the St. Lawrence Seaway into the upstream lakes. The method involves multiple steps:

- Determination of the natural chlorine demand of the ballast water one day ahead of treatment application, i.e., prior to the vessel's entry into the Canal, for example, in Montreal, Quebec, Canada;
- Determination of the necessary volume of 6.15 % NaOCl solution to be added to the ballast water to overcome the natural chlorine demand and deliver a predetermined chlorine concentration;
- Mixing using a method designed by the researchers;
- Retention of the treated ballast water in tank for a predetermined length of time (i.e., exposure period);
- Determination of residual chlorine concentration, and determination and application of the amount of a neutralizer necessary to fully neutralize the treated water for safe discharge; and
- Verification of complete neutralization prior to the vessel's departure from the Canal system.

Tests took place at GSI's Land-Based Research, Development, Testing and Evaluation (RDTE) Facility in Superior, Wisconsin, in October 2011, with the goal of status testing for research and development purposes. 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 ETV Program's Generic Protocol for the Verification of Ballast Water Treatment Technology, v.5.1 (USEPA, 2010).

During the test, GSI implemented the entire proposed NaOCl BWTS method with the exception of the automated mixing system; trialing the mixing apparatus at a land-based facility would

offer little insight into its capability on board a ship in any case. GSI evaluated the BWTS for its ability to:

- Deliver the target concentration of chlorine (above natural chlorine demand) using a 6.15% NaOCl solution, and deliver the target concentration of neutralizer;
- Reduce densities of live organisms in intake water from prescribed threshold densities to below densities allowed by the Ballast Water Performance Standard of the IMO Convention (IMO, 2004); and
- Result in treatment water safe to discharge in terms of residual chlorine concentration and whole effluent toxicity (WET). Disinfection by products (DBPs) were also measured and reported.

The GSI test of the NaOCl BWTS yielded mixed results. In terms of operational performance, GSI was able to accurately dose a sampled volume of water with 6.15 % NaOCl solution to a predetermined chlorine concentration by factoring in the natural chlorine demand. The neutralization process recommended by the BWTS developer did require additional neutralizer additions, which could be problematic in an actual shipboard situation. More research is needed on the effect of temperature and water quality on the ability of sodium bisulfite ($NaHSO_3$) or a neutralization substitute to successfully neutralize NaOCl-treated water for BWTS application in the real-world. Second, the BWTS reduced live densities of organisms ≥ 50 μm which were adequately plentiful in the intake to meet IMO testing guidelines, relative to control discharge. But BWTS live discharge densities were well above the IMO benchmark (IMO, 2004). The BWTS did reduce live densities of organisms > 10 and <50 μm minimum dimension to below benchmark levels within the IMO Convention, but intake densities of these organisms also were below IMO testing guidelines due to the late season timing of the tests (IMO, 2004). Finally, the treated and neutralized discharge water was found to be safe to discharge (though, in some cases only after multiple neutralization steps) and free from toxicity in Whole Effluent Toxicity (WET) tests conducted by GSI. Measurable concentrations of DBP were found in the treatment discharge, specifically trihalomethanes (THM) and haloacetic acids (HAA). Overall, the GSI results show that the NaOCl BWTS both warrants and would benefit from further research and development on its potential as an emergency BWTS with relevancy in the Great Lakes.

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The authors would like to express our sincere gratitude to the Great Ships Initiative (GSI) Advisory Committee which provides invaluable input to the GSI. 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 for their active financial and/or in-kind support for GSI operations. Finally, we thank the treatment developers for their efforts in designing a proposed emergency treatment option applicable to the Great Lakes, and for their commitment to fact-based awareness of performance of the proposed system through having it tested at GSI's land-based testing facility.

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LIST OF ACRONYMS

%T: Percent Transmittance
BWT: Ballast Water Treatment
BWTS: Ballast Water Treatment System
CFU: Colony Forming Units
Cl_2: Chlorine
DBP: Disinfection Byproducts
DOC: Dissolved Organic Carbon
DOM: Dissolved Organic Matter
DPD: *N*, *N*-diethyl-*p*-phenylenediamine
ETV: Environmental Technology Verification
FDA: Fluorescein Diacetate
GSI: Great Ships Initiative
HAA: Haloacetic Acids
HCl: Hydrochloric Acid
HDPE: High Density Polyethylene
HMI: Human-Machine Interface
IMO: International Maritime Organization
LSRI: Lake Superior Research Institute
MM: Mineral Matter
MPN: Most Probable Number
MTU: Michigan Technological University
$Na_2S_2O_3$: Sodium Thiosulfate
$NaHSO_3$: Sodium Bisulfite
NaOCl: Sodium Hypochlorite
NEMWI: Northeast-Midwest Institute
NPLSF: National Parks of Lake Superior Foundation
NPOC: Non-Purgeable Organic Carbon
NR: No Requirement
NRRI: Natural Resources Research Institute
ODIS: Organism Diaphragm Injection System
POC: Particulate Organic Carbon
POM: Particulate Organic Matter
PVC: Polyvinyl Chloride
QA: Quality Assurance
QC: Quality Control
RDTE: Research, Development, Testing, and Evaluation
SP: Sample Port
THM: Trihalomethanes
TOC: Total Organic Carbon
TR: Trial
TRO: Total Residual Oxidants
TSS: Total Suspended Solids
UMD: University of Minnesota-Duluth
USEPA: United States Environmental Protection Agency
UWS: University of Wisconsin-Superior
WET: Whole Effluent Toxicity
WPDES: Wisconsin Pollution Discharge Elimination System

1. INTRODUCTION

In early 2011, the Great Ships Initiative (GSI) received an application from researchers at the National Parks of Lake Superior Foundation (NPLSF) in Marquette, Michigan, and the Michigan Technological University (MTU) in Houghton, Michigan, to undertake land-based tests of an emergency ballast water treatment system (BWTS) concept involving sodium hypochlorite (NaOCl) in the same formulation used for household bleach. The request was precipitated by 2008 bench-scale evaluations, conducted by GSI, of an earlier stage of this BWTS intended for use in emergency situations, and specifically to abate the ship-mediated movement of viral hemorrhagic septicemia (VHS) virus or similar pathogens. This early version BWTS was similar to, but not the same as, the version tested and reported on here. The earlier version involved dosing intake water with NaOCl solution at chlorine concentrations of up to 3.5 mg/L and involved injection of ascorbic acid (i.e., vitamin C) into the treated water prior to discharge to act as a dechlorination agent. These GSI bench tests were designed to assist with dose range-finding, determination of the rates of chemical degradation and neutralization, and the potential for residual toxicity. In these tests, GSI found that the degree of lethality associated with the proposed chlorine dose of up to 3.5 mg/L varied markedly with the chlorine demand of the test water and the type of species tested—adult rotifers (*Brachionus calyciflorus*), total coliforms, *E. coli* and *Enterococcus* were the most vulnerable; algae (*Selenastrum capricornutum*), copepods (*Eucyclops spp.*) and heterotrophic bacteria were the most resistant (GSI, 2009). GSI also detected no acute toxicity associated with the proposed chlorine dose range followed by neutralization with 9 mg/L ascorbic acid in any of the water types tested (GSI, 2009). Higher concentrations of ascorbic acid by itself, however, resulted in acute toxicity (GSI, 2009). No chronic residual toxicity was detected in GSI's limited toxicity analysis, but further testing was deemed necessary to conclude with confidence whether or not chronic toxicity would occur as a result of this treatment (GSI, 2009). Overall, the BWTS warranted further investigation for its potential application as an emergency BWTS, but required a method for accommodating to the wide range of water quality within which such a BWTS, including both its dosing and neutralization steps, would need to operate.

The BWTS developers sought to address this concern through the proposed BWTS version which GSI tested at its Land-Based Research, Development, Testing and Evaluation (RDTE) Facility and reports on here. Specifically, this version of the BWTS incorporates the following steps:

- Determination of the natural chlorine demand of the ballast water one day ahead of treatment application using a defined approach;
- Calculation of the volume of 6.15 % NaOCl solution which must be added into the ballast tank to overcome natural chlorine demand and achieve a predetermined available chlorine concentration;
- Addition of the volume of 6.15 % NaOCl solution when the ship enters a lock system in the St. Lawrence Seaway, and mixing using a portable mixing method. Note: the portable mixing system was not tested at the GSI Land-Based RDTE Facility as the facility's retention tanks are equipped with permanent in-tank mechanical agitators;
- Retention of the treated water for a minimum of seven hours;

- Assessment of residual chlorine, and addition of the calculated concentration of neutralizer (i.e., sodium bisulfite, $NaHSO_3$) which must be added to achieve desired levels of neutralization; and
- Confirmation that the desired level of neutralization has been achieved prior to the vessel's departure from the lock system.

Tests of this BWTS took place at GSI's Land-Based RDTE Facility in Superior, Wisconsin, during October 2011. GSI evaluated the BWTS for its ability to:

- Achieve the target concentration of chlorine (above natural chlorine demand) using 6.15% NaOCl solution, and achieve the target concentration of neutralizer in order to decrease total residual chlorine concentrations to below permitted discharge levels;
- Reduce densities of live organisms in intake water from prescribed threshold densities to below densities allowed by the Ballast Water Performance Standard of the International Maritime Organization's (IMO's) International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO, 2004); and
- Result in treatment water safe to discharge in terms of residual chlorine concentration and whole effluent toxicity (WET). Disinfection by products (DBP) were also measured and reported.

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 United States Environmental Protection Agency (USEPA), Environment Technology Verification (ETV) Program's Generic Protocol for the Verification of Ballast Water Treatment Technology, v.5.1 (USEPA, 2010). This report details methods and findings from these tests.

2. THE TESTING ORGANIZATION AND TESTING FACILITY

2.1. Overview

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 provides independent, no cost performance verification testing services to developers of BWTSs at a purpose-built, land-based ballast treatment test facility located in the Duluth-Superior Harbor on Lake Superior. GSI test protocols are consistent with the requirements of the IMO's International Convention for the Control and Management of Ships Ballast Water and Sediments (IMO, 2004), and are also consistent with the USEPA, ETV Program's Generic Protocol for the Verification of Ballast Water Treatment Technology, v. 5.1 (USEPA, 2010). GSI procedures, methods, materials, and findings are publicly accessible on the GSI website (www.greatshipsinitiative.org).

2.2. Organization

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

2.3. Testing Facility

The test reported on here was conducted at GSI's Land-Based RDTE Facility located in the Duluth-Superior Harbor on Lake Superior (Figure 1). Key features of the facility include:

- Four 200 m^3 matched retention tanks for experimental water, and a 260 m^3 wastewater storage tank;
- Connection to city water for facility cleaning and city sewer for disposal of spent experimental water;
- Matched (split) control and treatment intake flows up to 340 m^3/hour each;
- 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;
- Validated facility cleaning between trials;
- High quality in-line and/or in-tank sampling and/or spiking;
- On-site laboratory space for live analysis of phytoplankton and zooplankton;
- Capacity to test treatment systems that operate on intake, discharge, in-tank or combinations thereof;
- WET testing; and
- Easy plug-in connections for treatment systems.

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

GSI's Land-Based RDTE Facility draws raw intake water from Duluth-Superior Harbor at a rate of 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 2).

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 2). The treatment track directs water through the experimental BWTS (i.e., if in-line treatment is used) and into a 200 m^3 cylindrical treatment retention tank (Figure 2). The control track by-passes the BWTS and channels the water directly into a matched control retention tank (Figure 2). The cylindrical retention tanks have agitators to mix the water during retention and conical bottoms which allow nearly all of the sample water to be drained from them after retention.

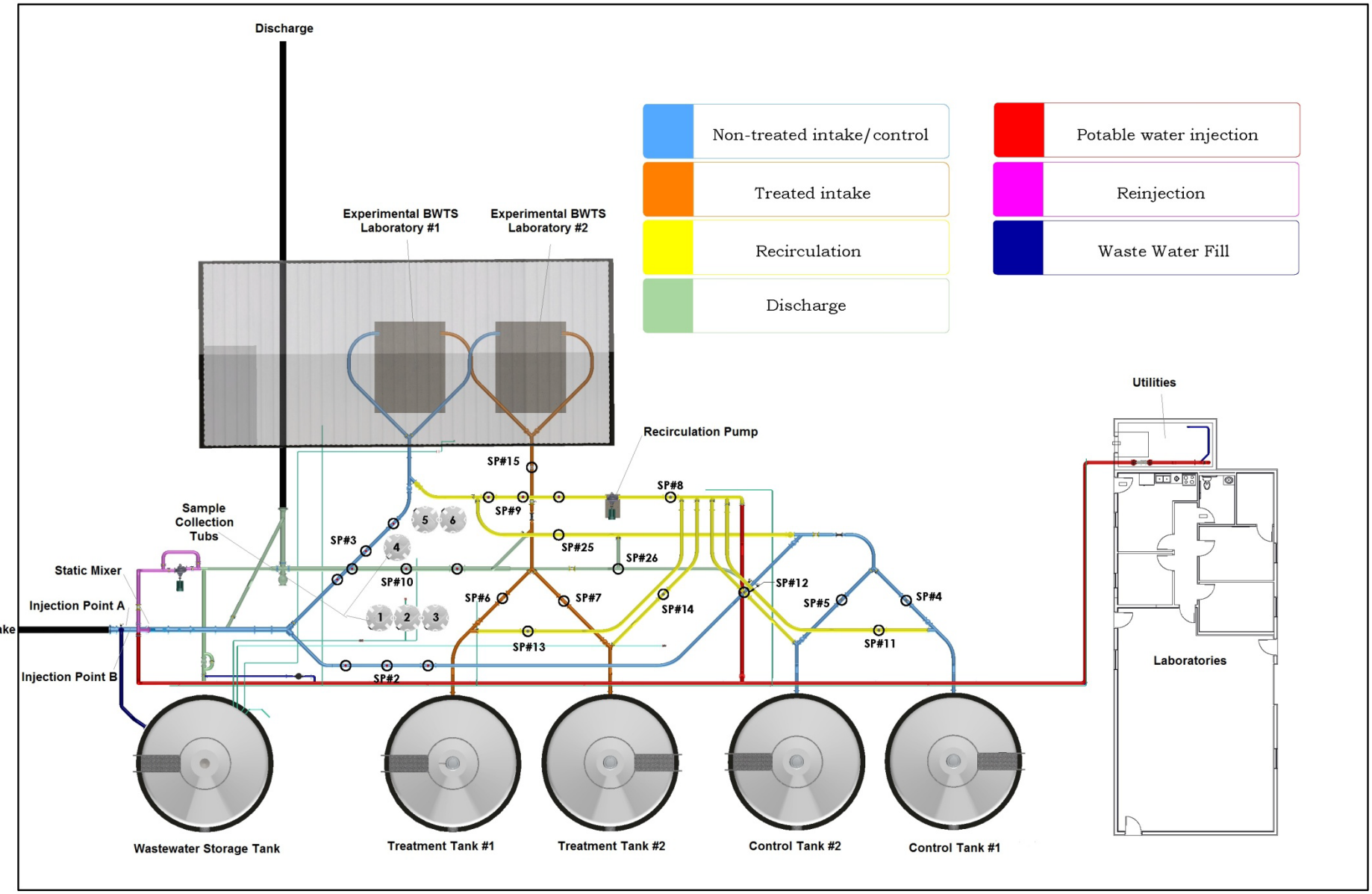
After a pre-determined retention period, water is discharged sequentially from the treatment and control retention tanks at a flow rate of up to 340 m^3/hr. The water is directed either back to the harbor, to a 260 m^3 wastewater storage tank for subsequent discharge to the City of Superior sewer (after any neutralization of treatment residuals or byproducts, if required), or recirculated to a second set of facility retention tanks (Figure 2). The treatment track water may be passed through the BWTS again on discharge or during recirculation (i.e., if in-line treatment is used).

Flow control valves and system logic assure that sample flow rates are equivalent and proportional to intake and discharge flow rates throughout each operation. Flow rates are recorded by automated meters located on the control track, treatment track, and on the discharge

line. Pressure readings are also recorded at multiple points throughout the facility. GSI measures and records these data and other operation and maintenance parameters using a Human-Machine Interface (HMI) installed at the facility. The HMI has a 15 inch color, touch display capable of detailing valve positions, pressure from the pressure meters, fill level of the retention tanks and flow rates in the control and treatment lines. The HMI reads and records data from all the positioners, limit switches, pressure meters, flow meters and level indicators every five seconds for the entire duration of the operational cycle. An external computer, connected to the HMI, is used to store the data files. Influent water quality is also monitored and recorded in the same manner using pH, dissolved oxygen, turbidity and temperature sensors installed in-line just prior to the experimental treatment system. (Note that influent water quality was not measured continuously in-line for the test reported here.)

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

Figure 2. 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.



3. THE BALLAST WATER TREATMENT SYSTEM

The NaOCl BWTS, in the same formulation used for household bleach (i.e., 6.15 % NaOCl solution), evaluated in these tests is an in-tank treatment designed for emergency treatment of ballast tanks in Great Lakes vessels as they pass through the Welland Canal system in the St. Lawrence Seaway. The BWTS is proposed for use by researchers from the NPLSF in Marquette, Michigan, and the MTU in Houghton, Michigan, and incorporates multiple steps:

(1) The natural chlorine demand of the ballast water is determined one day ahead of treatment application, i.e., prior to the vessel's entry into the Canal system, for example, in Montreal, Quebec, Canada, using an approach defined by the treatment developers;
(2) The appropriate volume of 6.15 % NaOCl solution is calculated to supply sufficient NaOCl to achieve 5 mg/L of chlorine above the natural chlorine demand after seven hours of treatment. This amount of 6.15 % NaOCl solution (as regular Clorox® Bleach) is then added into the ballast tank (manually) when the ship enters the Canal system;
(3) The treated ballast is mixed using an active automated method designed by the researchers, and retained in-tank for at least seven hours;
(4) Following retention, the residual chlorine concentrations of the treated water are determined, and based on this concentration, the necessary amount of neutralizer (in the form of sodium bisulfite, $NaHSO_3$) is added to the water to achieve full neutralization of the treated water;
(5) The neutralization process is verified by measuring Total Residual Oxidants (TRO, as chlorine) and ensuring that there is $\leq$ 0.038 mg/L TRO such that the water is safe for discharge to Wisconsin waters per requirements of the Wisconsin Department of Natural Resources (this permitted level may vary from state-to-state).

Note that this version of the BWTS used 40% (w/v) $NaHSO_3$ as the neutralization agent in contrast to the BWTS subject to GSI bench testing in 2008 which used ascorbic acid (i.e., vitamin C). See Appendix A for the analytical methods provided to GSI by the BWTS developer.

4. TEST CALENDAR, OBJECTIVES AND EXPERIMENTAL DESIGN

4.1. Test Calendar

GSI trials of the NaOCl BWTS began October 10, 2011, and ended October 21, 2011 (Table 1). There were four trials (n=4), undertaken at a rate of two per week. A trial was defined as one intake operation of ambient harbor organisms amended with concentrated harbor phytoplankton (i.e., organisms $\geq$ 10 μm and < 50 μm) to achieve at least 500 live cells/mL, a retention period of 18 - 24 hours, treatment with 6.15 % NaOCl solution (as regular Clorox® Bleach), a seven hour ($\pm$ 0.7 hours) exposure period, neutralization, post-neutralization retention (16 - 20 hours), and treatment and control tank discharge.

Table 1. Calendar of Activities for GSI Land-Based Testing of the NaOCl BWTS.

Week	Monday	Tuesday	Wednesday	Thursday	Friday
October 10 to 14, 2011	Facility Cleaning; Equipment Calibration; Phytoplankton Collection	**Trial 1 Intake & Chlorine Demand Determination** Facility Cleaning; Phytoplankton Collection	**Trial 1 Treatment & Neutralization;** **Trial 2 Intake & Chlorine Demand Determination** Facility Cleaning	**Trial 1 Discharge;** **Trial 2 Treatment and Neutralization** Facility Cleaning	**Trial 2 Discharge**
October 17 to 21, 2011	Facility Cleaning; Equipment Calibration; Phytoplankton Collection	**Trial 3 Intake & Chlorine Demand Determination** Facility Cleaning; Phytoplankton Collection	**Trial 3 Treatment & Neutralization;** **Trial 4 Intake & Chlorine Demand Determination** Facility Cleaning	**Trial 3 Discharge;** **Trial 4 Treatment and Neutralization** Facility Cleaning	**Trial 4 Discharge**

4.2. Test Objectives

Test objectives were to evaluate the BWTS with regard to:

- Operational efficacy, i.e., the ability to achieve the target concentration of 5 mg/L of chlorine above natural chlorine demand after seven hours of treatment with 6.15% NaOCl solution, and the target level of neutralization using defined procedures;
- Biological efficacy, i.e., the ability to reduce densities of live organisms in intake water from prescribed threshold densities to below densities allowed by the Ballast Water Performance Standard of the IMO Convention (IMO, 2004) as defined in terms of the three size classes of organisms: organisms $\geq$ 50 μm in maximum dimension on the smallest visible axis (generally defined by GSI as zooplankton); organisms $\geq$ 10 μm and $<$ 50 μm in maximum dimension on the smallest visible axis (generally defined by GSI as phytoplankton or protists), and organisms $<$ 10 μm in maximum dimension on the smallest axis (generally defined by GSI as bacteria); and
- Environmental acceptability, i.e., the ability to produce treatment discharge water that is safe as defined by acceptable residual chlorine concentrations and the absence of toxicity in standard WET evaluations of treated discharge[1].

4.3. Experimental Design

For each trial, one control retention tank and one treatment retention tank (Figure 2) were filled simultaneously with experimental water at a rate of 200 m^3/hr to an approximate volume of 100 m^3 each (i.e., half-full). During filling, continuous in-line samples were collected to assess

1 Concentrations of disinfection byproducts (DBP) were also measured and reported, but not compared to a metric for environmental acceptability in a discharge to an ambient system because there are currently none in place.

intake live densities of organisms. Immediately after intake, a water sample was collected in triplicate (i.e., 1 L each from the top, middle and bottom of the treatment tank using a Kemmerer sampler) with access gained through the top hatch of the tank, processed over a seven hour period and then analyzed for natural chlorine demand (see Section 6.3.1 of this report). Treatment and neutralization agents were applied to the treatment tank only:

- The volume of 6.15 % NaOCl solution was calculated consistent with the methods provided by the BWTS developers (see Appendix A) to achieve a desired concentration of 5 mg/L chlorine above the natural chlorine demand.
- Approximately 18 - 24 hours after intake, the calculated volume of 6.15 % NaOCl solution was slowly added through the top hatch of the treatment tank with the tank agitators set at 20 % power. The solution was poured into the treatment tank via a funnel with an attached flexible polyvinyl chloride (PVC) tube that was long enough to reach below the surface of the water.
- Approximately seven hours after dosing (i.e., to mimic the treatment time for ships passing through the Welland Canal locks), the TRO concentration (as mg/L of chlorine) in the treatment tank was measured, and the volume of neutralizing agent necessary was determined using the BWTS developer's recommended method (i.e, highest seven hour TRO concentration in the treatment tank plus 1.6 mg/L of neutralizing agent per mg/L of chlorine plus a 10 % margin of safety; see Appendix A).
- The treated water was then neutralized using 40 % (w/v) $NaHSO_3$ or $Na_2S_2O_3$ by adding the calculated volume of neutralizing agent to the retention tank (with the tank agitators still set at 20 % power). This was achieved by pouring the solution into a funnel with an attached, flexible PVC tube that reached below the surface of the water.
- The water was then retained and mixed for an additional 16 - 20 hours. During this retention period, samples for analysis of TRO were collected one hour after the tank neutralization process and just prior to scheduled discharge (i.e., at 15 - 19 hours), from the top, middle and bottom of the tank, as close to the outside wall as feasible. Samples were analyzed to determine if the chlorine concentration was below the maximum concentration allowed by GSI's Wisconsin Pollution Discharge Elimination System (WPDES) permit requirements, i.e., $\leq$ 0.038 mg/L or 38 μg/L of chlorine.
- If the chlorine concentration was above the maximum allowed by the WPDES permit, then a second or third neutralization attempt was made using the above neutralization procedure.

When the appropriate TRO concentration was achieved and verified in the neutralized water, both treatment and control tanks were discharged sequentially and sampled continuously during the process to determine live densities of organisms. Even though the chlorine concentration of the treatment water was within acceptable limits it was not discharged to the harbor but to the city sewer system in case WET analysis, which takes several days, showed toxicity. Discharge lines were thoroughly cleaned (usually on the day prior to discharge) following *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*. Data sheets were archived in GSI Sharepoint and directly shared with treatment developers.

5. CHALLENGE CONDITIONS AND PREPARATION

The objective of the NaOCl BWTS test was status testing for research and development purposes. 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 ETV Program's Generic Protocol for the Verification of Ballast Water Treatment Technology, v.5.1 (USEPA, 2010). GSI did not require the full suite of specific water quality and biological challenge conditions on intake due to the research and development nature of the test objective, and the late season timing of the tests. However, GSI did apply either the IMO or ETV threshold requirements for live densities on intake or discharge in all size classes (Table 2). Specifically, trials were considered valid if the pre-treatment (control) intake contained ≥ 100,000 live zooplankton per m^3 and ≥ 1,000 MPN/mL of heterotrophic bacteria *and* untreated control discharge water contained ≥ 100 live zooplankton per m^3 and ≥ 100 live phytoplankton per mL (Table 2). GSI, consistent with both IMO and ETV, applied no intake requirements for *E. Coli,* total coliform bacteria and *Entercoccus* (Table 2).

Table 2. Minimum Threshold Limits by Organism Size Class as Required by the ETV Protocol (USEPA, 2010) or IMO G8 Guidelines (IMO 2008a) and Applied to GSI Tests of the NaOCl BWTS.

Size Class of Organisms	Sample Type	ETV Protocol	IMO G8 Guidelines	GSI Target
≥ 50 *μ*m	Intake (Pre-Treatment)	≥ 100,000 organisms per m^3; 5 species across 3 phyla	≥ 100,000 per m^3 total density; at least 5 species from at least 3 different phyla/divisions	≥ 100,000 live zooplankton per m^3; at least 5 species from at least 3 different phyla/divisions
	Discharge (Control)	≥ 100 organisms per m^3	≥ 100 viable organisms per m^3	≥ 100 live organisms per m^3
≥ 10 and < 50 *μ*m	Intake (Pre-Treatment)	≥ 1,000 organisms per mL	≥ 1,000 individuals per mL; at least 5 species from at least 3 different phyla/divisions	At least 5 species from at least 3 different phyla/divisions
	Discharge (Control)	≥ 100 organisms per mL	N/A	≥ 100 live cells per mL
< 10 *μ*m – Heterotrophic bacteria	Intake (Pre-Treatment)	≥ 1,000 per mL as culturable aerobic heterotrophic bacteria	≥ 10,000 living bacteria per mL	≥ 1,000 MPN/mL
	Discharge (Control)	≥ 500 per mL as culturable aerobic heterotrophic bacteria	N/A	N/A
< 10 *μ*m – *Escherichia Coli*	Intake (Pre-Treatment)	N/A	N/A	N/A
	Discharge (Control)	N/A	N/A	N/A
< 10 *μ*m – Total Coliforms	Intake (Pre-Treatment)	N/A	N/A	N/A
	Discharge (Control)	N/A	N/A	N/A

< 10 μm – *Enterococci*	Intake (Pre-Treatment)	N/A	N/A	N/A
	Discharge (Control)	N/A	N/A	N/A

To achieve the control discharge density goal for phytoplankton of more than 100 live cells per mL, GSI augmented the intake water with concentrated harbor organisms. Typically, 500 live cells/mL or more in the intake stream deliver this threshold discharge density. The specific procedure for phytoplankton injection is detailed in *GSI/SOP/LB/G/O/5 – Procedure for Injecting Organisms and Solids into the GSI Land-Based RDTE Facility*. Briefly, one to two days prior to the intake operation, phytoplankton from the Duluth-Superior Harbor were collected and concentrated using 50 - 80 μm mesh plankton nets towed from an outboard-powered boat. The concentrated phytoplankton were stored for up to 96 hours at the GSI Land-Based RDTE Facility in holding ponds equipped with aeration systems. Prior to injection, the holding pond water was mixed (with in-pond circulators), sampled and analyzed for live cell density. In addition, a sample of Duluth-Superior Harbor water was collected to determine the ambient live phytoplankton density. Based on the density of cells in the holding ponds and ambient Duluth-Superior Harbor water, the volume of phytoplankton concentrate required to achieve 500 live cells/mL or more in intake water was calculated. The phytoplankton concentrate was added to the intake water at a constant rate for the entire duration of the intake procedure via an electric, double-acting diaphragm pump located at Injection Point B (Figure 2).

Operational effectiveness was determined by the extent to which the BWTS analytical procedures which took place throughout the tank retention period, delivered anticipated outcomes. Any changes to the procedure necessary to achieve operational objectives (such as discharge to the harbor in keeping with relevant regulatory requirements) were recorded. Environmental acceptability was determined through two replicate standard WET tests of treated discharge, and TRO analysis on treated discharge from each trial. Results of these tests were compared to prevailing standards. DBP concentrations were measured as well, but not for purposes of comparison against a standard, as none exists for discharges to ambient receiving systems.

6. SAMPLING AND ANALYSIS PLAN

6.1. Overview

Experimental water was sampled continuously and representatively throughout each intake and discharge operation via in-line sample points (SPs). Intake sampling took place on the treatment track (i.e., pre-treatment intake, SP#3; Figure 2), and control and treatment discharge sampling was conducted at SP#9 (Figure 2). The SPs consist of three identical sample ports spaced at regular intervals in a length of straight pipe. Each port is fitted with a center-located, elbow-shaped pitot tube (90 °) that samples the water. This pitot type is based on a design developed and validated analytically by the United States Naval Research Laboratory (NRL) in Key West, Florida (Richard *et al.*, 2008). The design and layout of these replicate sample ports were also validated empirically at GSI, and shown to produce equivalent, representative and unbiased samples of water flow.

Sample water for organisms in the three previously described size classes was drawn via the sample ports and transferred simultaneously and continuously 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 current pitot design can supply a volume of 2.0 - 3.6 m^3 via the recommended sub-isokinetic flow range during a typical intake/discharge operation; Richard *et al.*, 2008). As such, the water in each sample collection tub constituted an independent, time-integrated subsample of the experimental water mass. GSI has validated the independence and equivalency of these sample ports and sample collection tubs. Bacteria and phytoplankton samples were collected as whole water samples immediately after the sample tubs were filled. The remaining water was filtered through 35 μm mesh plankton nets to concentrate the zooplankton samples into 1 L cod ends.

Discrete grab (i.e., whole water) samples were collected via in-line sample ports for determination of total suspended solids (TSS), dissolved organic matter (DOM), particulate organic matter (POM), and percent transmittance (%T). Mineral matter (MM) was determined from the difference between TSS and POM values. These samples were collected from the pre-treatment water during intake and from treatment and control water during discharge. In addition, samples were collected from the top, middle and bottom of the treatment retention tank immediately after intake using a Kemmerer Sampler and analyzed for chlorine demand at LSRI's Chemistry Laboratory. After the appropriate volume of 6.15 % NaOCl solution had been manually poured into the treatment retention tank and the tank had been allowed to mix for at least one hour (i.e., until homogenous), three additional samples were collected using the Kemmerer Sampler from the tank and analyzed at LSRI's Chemistry Laboratory for TRO. After the seven hour exposure period, another three samples were collected from the treatment retention tank (i.e., from the top, middle and bottom) and analyzed for TRO to determine the appropriate volume of neutralizing agent to add for neutralization. Verification of neutralization was checked twice during each neutralization process: three samples for TRO analysis were collected from the treatment retention tank one hour after neutralization and a second set of three samples were collected just prior to the planned discharge.

Tables 3 - 5 list the operational, water quality, and biological samples that were collected on intake, during retention, and on discharge during each of the four trials in order to evaluate the operational and biological efficacy and environmental acceptability of the NaOCl BWTS. The sample collection and analytical methods for each sample type are described in Section 6.3. Table 6 lists the sample handling and storage requirements for the samples.

Table 3. Operational, Water Quality, and Biological Samples Collected during Intake as Part of the NaOCl BWTS Tests.

Treatment	Analysis Category	Parameter	Measurement Class	Sample Type	Instrument Type (Where Applicable)	Number of Samples	Sample Volume	Sample Location
Pre-Treatment Intake	Operational	BWTS Flow Rate	Core	In-Line, Continuous	In-Line Sensor	----	----	Pre-Treatment Line
		BWTS Pressure	Core					
		Sampling Flow Rate	Core					
		Sample Collection Tub Volume	Auxiliary	Calculated Based on Flow Rate (Flow meters accurate to ± 5 %)	Not Applicable (NA)			
		Retention Tank Volume	Auxiliary	Calculated Based on Flow Rate (Flow meters accurate to ± 5 %)	NA			
	Water Quality	Temperature	Auxiliary	Discrete Measurement from Sample Collection Tub	YSI Multiparameter Sonde	1	N/A	Tub #4 via SP#3a
		pH						
		Turbidity						
		Dissolved Oxygen						
		Salinity						
		Specific Conductivity						
		Total Chlorophyll						
		TSS and Percent Transmittance	Core	Discrete Grab	NA - TSS Spectrophotometer – Percent Transmittance	3 (Beginning, Middle, End)	0.9 L - 1 L	SP#3c
		POM and DOM	Core	Discrete Grab	TOC Analyzer	3 (Beginning, Middle, End)	100 mL - 125 mL	
	Biological	Organisms ≥ 50 μm	Core	Time-Integrated	NA	1	1.8 m^3 ± 5 %	Tub #4 via SP#3a
		Organisms ≥ 10 μm to < 50 μm	Core	Time-Integrated		1	0.9 L -1 L	
		Organisms < 10 μm	Core	Time-Integrated		3		

Table 4. Operational, Water Quality, and Biological Samples Collected during Retention as Part of the NaOCl BWTS Tests.

Treatment	Analysis Category	Parameter	Measurement Class	Sample Type	Instrument Type (Where Applicable)	Number of Samples	Sample Volume	Sample Location
Retention	Water Quality	Chlorine Demand	Core	Time-Integrated; Immediately after Intake (Time = 0 HRS)	Spectrophotometer (at 515 nm)	3 (Top, Middle, Bottom)	1 L	Treatment Retention Tank
		TRO	Core	Time-Integrated; ~1 HR after Treatment	Spectrophotometer (at 515 nm)		0.9 L - 1 L	
				Time-Integrated; ~6 HR after Treatment				
				Time-Integrated; ~1 HR after Neutralization				
				Time-Integrated; ~1 HR prior to Discharge				

Table 5. Operational, Water Quality, and Biological Samples Collected during Discharge as Part of the NaOCl BWTS Tests.

Treatment	Analysis Category	Parameter	Measurement Class	Sample Type	Instrument Type (Where Applicable)	Number of Samples	Sample Volume	Sample Location
Treatment	Operational	BWTS Flow Rate	Core	In-Line, Continuous	In-Line Sensor	----	----	Treatment Line
		BWTS Pressure	Core					
		Sampling Flow Rate	Core					
		Sample Collection Tub Volume	Auxiliary	Calculated Based on Flow Rate (Flow meters accurate to ± 5%)	Not Applicable			
		Retention Tank Volume	Auxiliary	Calculated Based on Flow Rate (Flow meters accurate to ± 5 %)	Not Applicable			
	Whole Effluent Toxicity (WET)	Whole Effluent	Core	Time-Integrated	NA	1	30 L	Tub #6 via SP#9a
	Disinfection Byproducts (DBP)	Trihalomethanes; Haloacetic Acids	Core	Discrete Grab	NA	2	40 mL and 250 mL	SP#15
	Water Quality	Temperature, pH, Turbidity, Dissolved Oxygen, Salinity, Specific Conductivity, and Total Chlorophyll	Core	Discrete Measurement from Sample Collection Tubs	YSI Multiparameter Sonde	1	N/A	Tub #4, #5, #6 via SP#9c/b/a
		TSS and Percent Transmittance	Core	Discrete Grab	NA - TSS Spectrophotometer – Percent Transmittance	3 (Beginning, Middle, End)	0.9 L - 1 L	SP#15
		POM and DOM	Core	Discrete Grab	TOC Analyzer	3 (Beginning, Middle, End)	100 mL - 125 mL	
	Biological	Organisms ≥ 50 μm	Core	Time-Integrated	N/A	1	> 3.6 m^3 ± 5% (2 tubs x 1.8 m^3/tub)	Tub #4 & #5 via SP#9c/b
		Organisms ≥ 10 μm to < 50 μm	Core			3	0.9 L -1 L, composite	Tub #4, #5, #6 via SP#9c/b/a
		Organisms < 10 μm	Core			3	0.9 L - 1 L	
Control	Operational	Sampling Flow Rate	Core	In-Line, Continuous	In-Line Sensor	----	----	Control Line
		Sample Collection Tub Volume	Auxiliary	Calculated Based on Flow Rate	Not Applicable			

Treatment	Analysis Category	Parameter	Measurement Class	Sample Type	Instrument Type (Where Applicable)	Number of Samples	Sample Volume	Sample Location
				(Flow meters accurate to ±0.5%)				
		Retention Tank Volume	Auxiliary	Calculated Based on Flow Rate (Flow meters accurate to ±0.5%)	Not Applicable			
	Water Quality	Temperature, pH, Turbidity, Dissolved Oxygen, Salinity, Specific Conductivity, and Total Chlorophyll	Core	Discrete Measurement from Sample Collection Tubs	YSI Multiparameter Sonde	1	N/A	Tub #1 via SP#9c
		TSS and Percent Transmittance	Core	Discrete Grab	NA - TSS Spectrophotometer – Percent Transmittance	3 (Beginning, Middle, End)	0.9 L - 1 L	SP#15
		POM and DOM	Core	Discrete Grab	TOC Analyzer	3 (Beginning, Middle, End)	100 mL - 125 mL	
	Biological	Organisms ≥ 50 μm	Core	Time-Integrated	N/A	1	1.8 m^3 ± 5% (1 tub x 1.8 m^3/tub)	Tub #1 via SP#9c
		Organisms ≥ 10 μm to < 50 μm	Core		N/A	1	0.9 L - 1 L	
		Organisms < 10 μm	Core		N/A	3		

Table 6. Sample Handling and Storage Requirements for Samples Collected during NaOCl BWTS Tests.

Parameter	Container	Minimum Sample Size	Sample Type	Processing/Preservation	Maximum Storage
Electronic Sample Collection Tub Data (pH, Temperature, Turbidity, Dissolved Oxygen, Total Chlorophyll, Specific Conductivity, and Salinity)	Not Applicable	Not Applicable	Discrete Measurement from Sample Collection Tub	Maintain digital archive.	Not Applicable
Whole Effluent Toxicity	2, 19 L HDPE Carboy	30 L	Time Integrated	Use immediately to set up WET Tests, and then refrigerate. Prior to renewals, warm whole effluent to approximately 25 °C.	Discard after WET Tests are terminated.
Total Suspended Solids	1 L HDPE	200 mL ± 1 %	Discrete Grab	Analyze immediately; or refrigerate.	7 days
Total Organic Carbon (as Non-Purgeable Organic Carbon)	125 mL Borosilicate Glass	100 mL	Discrete Grab	Add HCl to pH < 2 and analyze immediately or refrigerate until analysis.	28 days
Dissolved Organic Matter (as Dissolved Organic Carbon)	125 mL Borosilicate Glass	100 mL	Discrete Grab	Filter, add HCl to pH < 2 and analyze immediately or refrigerate until analysis.	28 days
Percent Transmittance at 254 nm	1 L HDPE	25 mL	Discrete Grab	Unfiltered Sample: Analyze immediately; or refrigerate.	24 hours
Chlorine Demand	1 L HDPE	1000 mL	Discrete Grab	Process and analyze immediately.	Analyze immediately
Total Residual Oxidants	1 L HDPE	250 mL	Discrete Grab	Process and analyze immediately.	Analyze immediately
<10 μm Size Class (Bacteria)	1 L Sterile PP	1000 mL	Time Integrated	Enumerate using appropriate media. Process and analyze immediately; or refrigerate.	24 hours
≥ 10 and < 50 μm Size Class (Phytoplankton)	1 L HDPE	1000 mL	Time Integrated	Stain with Fluorescein Diacetate. Process and analyze immediately. Preserve unanalyzed sample using Lugol's and/or formalin.	Process immediately
≥ 50 μm Size Class (Zooplankton)	1 L Cod End	1.8 m^3 concentrated to 1000 mL	Time Integrated	Observe with compound and dissecting microscope and probe organisms to determine live/dead status. Process and analyze immediately. Preserve unanalyzed sample using Lugol's solution.	Process immediately

[1]Total chlorophyll samples are collected as part of the calibration procedure for the YSI Multiparameter Water Quality Sondes (*see GSI/SOP/LB/G/C/4 – Procedure for Calibration, Deployment, and Storage of YSI Multiparameter Water Quality Sondes*). This sample can also be used for chlorophyll *a* analysis for calibration of the in-line chlorophyll *a* probe.

6.2. Operational Parameters

The valid ranges of measured operational parameters for the NaOCl BWTS trials are described in Table 7. During intake operations, the flow rate of water to the control and treatment retention tanks was set to achieve a goal of 180 – 210 m^3/hr, with a total sample volume of 95 – 105 m^3 per tank. Therefore, each intake operation was approximately 30 minutes in duration. In order to achieve recommended sub-isokinetic flow to the pitots leading to the sample collection tubs, a maximum volume of 1.8 m^3 could be collected per tub. Following two separate retention periods (i.e., after treatment and after neutralization), the treatment tank was discharged and then the control tank. The flow rate of water from the treatment and control retention tanks, flow rate to the sample collection tubs and sample collection tub volumes were the same for both intake and discharge (Table 7). The treatment and control discharge operations lasted approximately 30 minutes each.

Table 7. Valid Ranges of Operational Parameters for Tests of the NaOCl BWTS.

Intake Operations					
Parameter		**Control Tub**	**Pre-Treatment Tub**	**Control Retention Tank**	**Treatment Retention Tank**
Flow Rate (m^3/hr)	Valid Range of Avg.	3.0-3.6		180-210	180-210
Pressure (psi)[2]	Valid Range of Avg.	25-32			
Volume (m^3)	Valid Range	1.0-1.8		95-105	
Discharge Operations					
Parameter		**Control Tub**	**Treatment Tub**	**Control Retention Tank**	**Treatment Retention Tank**
Retention Time After Treatment* (hr)	Valid Range	Not Applicable		Not Applicable	6.3 – 7.7
Retention Time After Neutralization (hr)	Valid Range	Not Applicable		Not Applicable	16 - 20
Flow Rate (m^3/hr)	Valid Range of Avg.	3.0-3.6		180-210	
Pressure (psi)[3]	Valid Range of Avg.	25-32			
Volume (m^3)	Valid Range	1.0-1.8		> 80	

* Refers to time after treatment and neutralization of the Treatment Tank only, the Control Tank was neither treated nor neutralized.

6.3. Sample Collection and Analysis Methods

6.3.1. Water Chemistry/Quality Sample Collection, Handling, and Analysis

6.3.1.1. Chlorine Demand and Dosing Concentration Determination

Immediately after completion of the intake operation, three chlorine demand samples were collected from the top, middle and bottom of the treatment retention tank (and as close to the side of the tank as possible) using a Kemmerer water sampler and following the procedure outlined in the "Discrete Grab Water Chemistry Sample Collection from Retention Tanks" section of *GSI/SOP/LB/RA/SC/2, v.2 – Procedure for Collecting Water Chemistry Samples and Data* (Table 4). Samples were collected in 1 L high density polyethylene (HDPE) sample

2 Measured and recorded at the split of the control and treatment water.
3 Measured and recorded immediately after the recirculation pump.

bottles, previously prepared by soaking in a chlorine bleach solution and rinsing with copious amounts of deionized water to ensure that the sample containers did not have a chlorine demand of their own (Hach Company, 2011). The samples were transported to LSRI's Chemistry Laboratory in a cooler with ice packs, and were analyzed immediately (Table 6).

Consistent with the BWTS developer-recommended analytical methods (see Appendix A), the chlorine demand analysis was conducted via the "N,N-diethyl-p-phenylenediamine (DPD) Colorimetric Method for Determination of Oxidant Demand/Requirement", as outlined in *Standard Methods for the Examination of Water and Wastewater*, 21st Edition (Eaton *et al*. 2005). Sample pH and temperature were measured immediately upon receiving samples. The chlorine demand of the intake water was determined by adding 10 mg/L chlorine (as Regular Clorox® Bleach) to the three samples (measured using a graduated cylinder). The samples were then covered with aluminum foil to protect them from light and placed in an incubator set to the initial temperature ± 3 °C of the samples. After seven hours, pH and temperature were measured again, and the TRO concentration in each of the three samples was determined according to *GSI/SOP/BS/RA/C/2, v.2 – Procedure for Determining Total Residual Oxidants (TRO) in Water*. The chlorine demand of the intake water was determined by subtracting the TRO from the initial chlorine dose (as Regular Clorox® Bleach) of 10 mg/L. The step-by step procedure provided by the BWTS developer was as follows:

1. Determine the concentration of chlorine in Regular Clorox® Bleach by diluting a 175 μL aliquot of bleach to 100 mL using deionized (Milli-Q) water in a volumetric flask. This is the dosing solution with a nominal concentration of approximately 100 mg/L of chlorine.
    a. Dilute a 200 μL aliquot of the dosing solution to 10 mL using Milli-Q water in a volumetric flask.
    b. Determine the concentration of TRO (as mg/L of chlorine) in the dosing solution according to *GSI/SOP/BS/RA/C/2, v.2 – Procedure for Determining Total Residual Oxidants (TRO) in Water*.

2. Determine the chlorine demand of intake water:
    a. Take three, 1 L samples of water from the treatment retention tank (top, middle and bottom)
    b. Add 10 mg/L of chlorine per 1 L sample:

10 mg/L x 1g/1000mg = 0.01 grams CL_2
0.01 g CL_2/ (6.15 g CL_2/100ml) = 0.163 ml 6.15% NaOCl solution

    c. After seven hours (commensurate with the exposure period), measure TRO. Calculate chlorine demand of intake water:

Chlorine Demand = Initial chlorine dose – TRO

Following chlorine demand determination, the recommended volume of 6.15% NaOCl was added to the treatment retention tank to achieve a chlorine concentration of 5 mg/L above intake chlorine demand, according to the following equations:

Chlorine Demand + 5 mg/L = Chlorine dose
E.g., 3 mg/L + 5 mg/L = 8 mg/L

Example uses 100 m^3 treatment tanks and 8 mg/L dose:
100 m^3 x 1000 L/1 m^3 = 100,000 L
8 mg/L x 100,000 L x 1 g/1000 mg = 800 g CL_2
800 g CL_2/(6.15 g CL_2/100ml) x 1L/1000 ml = 13.0 L of 6.15% NaOCl solution

6.3.1.2. Total Residual Oxidants (TRO)

One hour after the addition of 6.15 % NaOCl solution to the treatment retention tank and approximately one hour prior to neutralizing the tank (i.e., at six hours following the dosing), three 1 L samples were collected from the top, middle and bottom of the treatment tank (as close to the outside wall as feasible) and analyzed for TRO by following the procedure outlined in the "Discrete Grab Water Chemistry Sample Collection from Retention Tanks" section of *GSI/SOP/LB/RA/SC/2, v.2 – Procedure for Collecting Water Chemistry Samples and Data* (Table 4). In addition, samples were collected one hour after neutralization and just prior to discharge from the same locations in the treatment retention tank and also analyzed for TRO (Table 4). Samples were collected in clean 1 L HDPE sample bottles, and transported to LSRI's Chemistry Laboratory in a cooler with ice packs and then analyzed immediately (Table 6).

The concentration of TRO was determined according to the procedure outlined in *GSI/SOP/BS/RA/C/2, v.2 – Procedure for Determining Total Residual Oxidants (TRO) in Water*. In this procedure, a Hach DPD Total Chlorine Reagent powder pillow was added to the water sample. If TROs were present, the sample developed a red/pink color that was proportional to the TRO concentration. A calibration curve was developed using chlorine standards reacted with the Hach DPD reagent, and measured on a spectrophotometer at 515 nm to determine the concentration of TRO in the sample; the measured TRO in the sample included all oxidative substances (i.e., may not have been just chlorine).

6.3.1.3. Disinfection Byproducts (DBP)

Two discrete whole water samples of 40 mL and 250 mL, respectively, were collected from the treatment retention tank on discharge from SP#15 (Figure 2 and Table 5) and sent to a contract laboratory, ALS Environmental (Middletown, Pennsylvania), for analysis of DBP, specifically THM and HAA. The THM were analyzed by USEPA Method 524.2 and the HAA by USEPA Method 552.2 (USEPA, 1995). Sample bottles, preservatives and coolers necessary for analysis and shipment were sent from ALS Environmental prior to collection.

6.3.1.4. Total Suspended Solids (TSS)

Three, 1 L discrete grab samples for TSS analysis were collected during intake at SP#3 (Figure 2), and from SP#15 during treatment and control discharge (Figure 2). Samples were collected at approximately 5 minutes after the start, at midpoint (~15 minutes after the start), and at approximately 5 minutes before the end of the intake/discharge operation (Tables 3 and 5). The

exact times of sample collection were recorded on the water chemistry sample collection datasheet following the procedure outlined in *GSI/SOP/LB/RA/SC/2 – Procedure for Collecting Water Chemistry Samples and Data*. Samples were transported to LSRI's Chemistry Laboratory in a cooler with ice packs, and were analyzed immediately (see Table 7 for sample handling and storage requirements).

Analysis of TSS was conducted according to *GSI/SOP/BS/RA/C/8 – Procedure for Analyzing Total Suspended Solids (TSS)*. In this procedure, accurately measured sample volumes (± 1 %) were vacuum filtered through pre-washed, dried and pre-weighed glass fiber filters (i.e. Whatman 934-AH). After each sample was filtered it was dried in an oven and brought to constant weight. Concentrations of TSS were determined based on the weight of particulates collected on the filter and the volume of water filtered.

6.3.1.5. Organic Carbon

Three, 125 mL discrete whole water samples for DOC and non-purgeable organic carbon (NPOC) analysis were collected during intake at SP#3 as detailed in Table 3, and from SP#15 during treatment and control discharge (Table 5). Samples were collected at 5 minutes after start, at midpoint (~15 minutes after the start), and at 5 minutes before end of the intake/discharge operation in 125 mL glass bottles prepared by soaking in Micro® cleaning solution (followed by hot water and deionized water rinses). The exact times of sample collection were recorded on the water chemistry sample collection datasheet following the procedure outlined in *GSI/SOP/LB/RA/SC/2 – Procedure for Collecting Water Chemistry Samples and Data*. Samples were transported to LSRI's Chemistry Laboratory in a cooler with ice packs, and were analyzed immediately (Table 6).

In these tests, NPOC was used as an alternative to 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 concentrations in the sample. Thus, the NPOC analysis may not incorporate volatile organic carbon which may be present in the sample. DOC was used as a surrogate measure for DOM. Similarly, POC was used as a surrogate measure for POM and was calculated as the difference between NPOC and DOC values for a given 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 (HCl) for analysis of DOC. The remaining portion of the sample was acidified with HCl and analyzed for NPOC. A Shimadzu Total Organic Carbon Analyzer (Model TOC-L) was used for analysis of both NPOC and DOC. Concentrations of NPOC and DOC were determined based on a calibration curve developed on the analyzer using organic carbon standards prepared from potassium hydrogen phthalate. Concentrations of POC were determined as the difference between the NPOC and DOC values for a given sample.

6.3.1.6. Mineral Matter (MM) Calculation

For the purposes of this test, MM was defined as the difference between TSS and POM (measured as POC). Therefore, MM concentrations were calculated from each water quality sample collected on intake following analysis of TSS and the determination of POC based on the NPOC and DOC concentrations as described above.

6.3.1.7. Percent Transmittance (%T, Filtered and Unfiltered)

Filtered and unfiltered %T sample analyses were conducted according to *GSI/SOP/BS/RA/C/4 – Procedure for Determining Percent Transmittance (%T) of Light in Water at 254 nm*. For analysis of the filtered aliquot, an appropriate volume of sample was filtered through a glass fiber filter (i.e., Whatman 934-AH). A PerkinElmer Lambda 35 UV-Vis Spectrophotometer was used to measure %T of the unfiltered and filtered sample aliquots. Deionized water was used as a reference to adjust the spectrophotometer to 100 %T, and then each unfiltered and filtered sample aliquot was analyzed in a pre-rinsed sample cuvette with a 1 cm path length.

6.3.1.8. Water Quality Measurements Using YSI Multiparameter Water Quality Sondes

Calibrated (i.e., according to *GSI/SOP/LB/G/C/4 - Procedure for Calibration, Deployment, and Storage of YSI Multiparameter Water Quality Sondes*) Multiparameter Water Quality Sondes (YSI 6600 V2-4 Multiparameter Sondes; YSI Incorporated; Yellow Springs, OH) were used to measure water quality parameters during sample collection on intake and discharge according to the procedure outlined in the "Discrete Measurement of Water Chemistry Parameters from Sample Collection Tubs" section in *GSI/SOP/LB/RA/SC/2 − Procedure for Collecting Water Chemistry Samples and Data*. The sondes were lowered into the midwater of the pre-treatment sample collection tub (Tub #4) on intake (Table 3), and the treatment and control sample collection tubs (Tubs #4, #5, #6, and Tub #1) on discharge (Table 5) and the following water quality parameters were measured: temperature, dissolved oxygen (mg/L and %), pH, turbidity, salinity, specific conductivity and total chlorophyll.

6.3.2 Whole Effluent Toxicity (WET) Sample Collection, Handling, and Analysis

Immediately after completion of the treatment discharge operation, approximately 38 L of water was collected in two, 19 L HDPE carboys from sample collection Tub #6 via SP#9a (Table 5). The WET tests were conducted following the procedures outlined in Table 8.

GSI's toxicity testing is designed to meet Section 5.2 of the "Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9)" (IMO, 2008b). GSI's preference is to include both a sub-lethal endpoint (growth or reproduction) and a survival endpoint is based on the USEPA's *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, 4th edition (USEPA, 2002).

Two WET trials were conducted during the NaOCl BWTS test; one using treatment discharge water from Trial 2 and one using treatment discharge water from Trial 4. The WET

tests were conducted in order to determine residual toxicity of the treated water post-neutralization at the point of discharge. Following collection (Table 5), sample water, stored in large 19 L HDPE containers, was immediately transported to the LSRI Toxicity Testing Laboratory and held at 4 °C in the dark to retain the integrity of the sample. The WET testing began immediately, with portions of the discharge sample warmed to 25 °C each day to serve as renewal water for the bioassay. A dilution series of 100 %, 80 %, 40 % and 0 % whole effluent was used for each species. Filtered Duluth-Superior Harbor water was used as the 0 % treatment and to prepare the dilutions. A performance control consisting of the culture water for each type of organism was used as a quality control (QC) measure to determine the health of the test organisms. All tests were conducted in temperature-controlled incubators, water baths or at ambient room temperature. Table 8 lists the test species used, test types and test endpoints.

Table 8. Whole Effluent Toxicity (WET) Test Standard Operating Procedures, Test Types, Test Species and Test Endpoints.

GSI SOP Code	Test Type	Test Species	Test Endpoint
GSI/SOP/BS/RA/WET/1	Chronic – Static renewal	*Ceriodaphnia dubia* (species of Cladoceran)	Reproduction
GSI/SOP/BS/RA/WET/2	Chronic – Static renewal	*Pimephales promelas* (fathead minnow)	Growth
GSI/SOP/BS/RA/WET/3	Chronic - Static	*Selenastrum capricornutum* (species of green algae)	Growth

6.3.3. Biological Sample Collection, Handling, and Analysis

Samples for analysis of biological efficacy were collected during intake and discharge as detailed in Tables 3 and 5. Time-integrated samples were collected from the sample collection tubs for analysis of organisms in all three size classes. On discharge, the treatment retention tank was always discharged first, followed by the control retention tank.

6.3.3.1. Organisms ≥ 50 μm

One time-integrated sample of approximately 1.8 m^3 was collected during intake (i.e., pre-treatment intake), and at least three time-integrated samples of approximately 1.8 m^3 each were collected during discharge (i.e., two treatment discharge samples and one control discharge sample) operations for analysis of organisms ≥ 50 μm in maximum dimension on the smallest axis (Tables 3 and 5). Multiple sample collection tubs were filled during each operation, but the following sample volumes were collected and analyzed:

- 1.8 m^3 pre-treatment water was sampled on intake, concentrated to 1 L, and 2 mL to 10 mL were subsampled for analysis;
- 1.8 m^3 control discharge water was sampled, concentrated to 1 L, and 2 mL to 15 mL were subsampled for analysis; and
- At least 3.6 m^3 treatment discharge water was sampled (i.e., two samples each of 1.8 m^3 concentrated to 1.5 – 2.0 L). The two samples were each concentrated to approximately 1 L, split in half for analysis of microzooplankton and macrozooplankton, and 12-16 mL of the microzooplankton split and 17 - 26 mL of the macrozooplankton

split were analyzed. This equates to 16.5 to 99 L of the original sample volume analyzed for microzooplankton and 299 to 1,364 L of the original sample volume analyzed for macrozooplankton for each sample.

Sample collection for the ≥ 50 μm size class began after samples were collected from the sample tubs for analysis of the ≥ 10 μm and < 50 μm size class, as well as the < 10 μm size class. The entire contents of each treatment and control sample collection tub were drained sequentially and concentrated through a 35 μm mesh (50 μm diagonal dimensions) plankton net fitted with a 1 L cod-end as described in *GSI/SOP/LB/RA/SC/6 - Procedure for Zooplankton Sample Collection*. During intake operations, one pre-treatment tub was drained and the sample concentrated and analyzed immediately (Table 3). During discharge operations, one treatment tub was drained and the sample concentrated and analyzed immediately (Table 5), and one additional treatment tub was drained and the sample concentrated and analyzed before draining, concentrating and analyzing one of the control discharge samples (Table 5). Unanalyzed samples (or portions of samples) were preserved using Lugol's solution.

Live/dead analysis of organisms ≥ 50 μm in challenge water (i.e., pre-treatment on intake) and in control discharge 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 other macroinvertebrates), all generally greater than 50 μm in maximum dimension on the smallest axis, 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 subsamples 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 this size class of organisms in both the challenge water on intake and control discharge samples required analysis of multiple sub-samples and extrapolation to the entire sample volume. 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 50 % (v/v) acetic acid solution was added to the counting chamber/wheel and the total number of organisms enumerated. The number of live organisms was calculated by subtracting the number of dead organisms in the counting chamber/wheel from the total number of organisms.

The treatment discharge samples had lower organism densities, allowing for analysis of a greater proportion of the total volume of sample collected. In this situation, samples were split in half using a Folsom Plankton Splitter, with half of the sample analyzed for macrozooplankton and the other half analyzed for both macrozooplankton and microzooplankton. During these analyses, only live organisms were enumerated using standard movement and response to stimuli techniques. To increase statistical accuracy, analyses continued until a minimum of 1 m^3 of initial sample was examined in its entirety or until more than 20 live organisms were counted.

6.3.3.2. Organisms ≥ 10 and < 50 µm

Immediately after filling the sample collection tubs, a 1 L whole water phytoplankton sample was collected (Tables 3 and 5) from the tub's bottom drainspout. Samples were immediately placed into a cooler containing an ice pack to protect the sample from exposure to sunlight and heat, and were processed and analyzed immediately at the GSI Land-Based RDTE Facility (Table 7). Unanalyzed samples (or portions of samples) were preserved using Lugol's solution.

Samples were analyzed for live organisms ≥ 10 μm to < 50 μm within 1.5 hours of sample collection, with samples stored in coolers during the interim. Prior to analysis, samples were concentrated through a 7 μm mesh plankton sieve, then backwashed and stored in a 25 mL sample container. Samples were analyzed according to *GSI/SOP/LB/RA/SA/1 - Procedure for Algae/Small Protozoan Sample Analysis*. Briefly, a 2 mL subsample of the concentrated sample was transferred to a 5 mL sample container, with 3-5 μL of fluorescein diacetate (FDA) viability stain stock solution added. The use of FDA as the primary stain for GSI analyses is based on a thorough investigation of several methods (see Reavie *et al*. 2010). The subsample was then allowed to incubate in the dark for 5 minutes. Then, the 2 mL incubated sample was mixed and 1.1 mL immediately transferred (using a pipette) to a Sedgwick-Rafter cell, which was covered and placed on the stage of a compound microscope set for simultaneous observation using brightfield and epifluorescence. Multiple transects were analyzed, aiming for at least 100 entities (i.e., unicellular organism, colony or filament) and ensuring at least 1 mL of original sample water was assessed analyzed (10 mL for treated samples). 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 (counted); dead = cells showing no or very little evidence of green fluorescence from cell contents (not counted); and ambiguous = cells or entities that cannot be clearly identified as alive or dead (uncommon). Records were kept of transect lengths and widths so that the total counted area and volume analyzed could be calculated. 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 cannot be discerned).

6.3.3.3. Organisms < 10 µm

Immediately after collection of the phytoplankton samples, one to three 1 L whole water microbial samples were collected (in sterile polypropylene bottles) and stored as described in *GSI/SOP/LB/RA/SC/4 – Procedure for Microbial Sample Collection* (Tables 3 and 5). Samples were transported within one hour of collection in an insulated cooler containing several ice packs to LSRI's Microbiology Laboratory and processed to commence analysis immediately upon arrival (Table 7).

To quantify culturable, aerobic, heterotrophic bacteria, subsamples were diluted in a 10-fold dilution series in sterile ambient Duluth-Superior Harbor water. The appropriate dilution and volume applied onto the media plate are detailed in *GSI/SOP/BS/RA/MA/1 – Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate® for HPC Method*. This procedure is based on IDEXX Laboratories' patented multiple enzyme technology (IDEXX Laboratories, Inc.; Westbrook, Maine) whereby a 1 mL subsample is placed on a SimPlate® with

media and incubated at 35 °C for 48-72 hours. Fluorescing wells are then counted and most probable number (MPN) calculated. Results are reported in MPN/mL, which correlates well with CFU/mL.

The density of *E. Coli* and total coliform bacteria (*GSI/SOP/BS/RA/MA/4 - Procedure for the Detection and Enumeration of Total Coliforms and E. coli Using IDEXX's Colilert®*) was determined using a method which is based on IDEXX's patented Defined Substrate Technology® (DST®). Colilert® media was added to 100 mL of sample and transferred to a Quanti-Tray®/2000. After incubation, fluorescing/yellow wells were counted as positive for *E. Coli*/ total coliforms, respectively. Results are reported in MPN/100mL which correlates well with CFU/100 mL. Note that analysis of total coliform bacteria is not an additional procedure, but a second result given from the Colilert® test conducted for *E. coli* analysis.

The density of *Enterococcus* spp. (*GSI/SOP/BS/RA/MA/3 - Procedure for the Detection and Enumeration of Enterococcus using Enterolert®* were determined using Quanti-Tray®/2000 and Enterolert®, which is also based on IDEXX's patented Defined Substrate Technology® (DST®). Enterolert® media was added to 100 mL of sample and transferred to a Quanti-Tray®/2000. After incubation, fluorescing wells were counted as positive for enterococci. Results are reported in MPN/100mL which correlates well with CFU/100mL.

7. DATA MANAGEMENT, ANALYSIS AND REPORTING

7.1. Water Quality, WET Test, and Biological Data

Water quality, WET test and biological sample collection and analysis data was recorded by hand (using indelible ink) on pre-printed data collection forms and/or in bound laboratory notebooks that were uniquely-identified (i.e., coded) and were specific to the NaOCl BWTS. Water quality, WET test and biological data that were recorded by hand were manually entered into either a Microsoft Access Database that was designed, developed and is maintained by the GSI Database Manager (according to *GSI/SOP/G/RA/DM/1 – Procedure for GSI Zooplankton Database Data Entry, Data Quality Control, and Database Management* or *GSI/SOP/G/RA/DM/3 – Procedure for GSI Phytoplankton Database Data Entry, Data Quality Control, and Database Management*), or the data were entered into a Microsoft Excel Spreadsheet (in the case of water quality and WET test data). See *GSI/SOP/G/RA/DM/2 – Procedure for General Data Entry using Microsoft® Excel* for details on water quality and WET test data entry and quality control.

Electronic data files specific to the GSI Access Database are stored on the LSRI's secured Local Area Network (LAN) that can be accessed only by relevant 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. All other electronic data files, including electronic copies of completed data collection forms and laboratory notebook pages, are stored on the GSI's internal SharePoint website.

7.2. Operation and Maintenance, and Other Data

Facility data (e.g., flow rates and pressure measurements) that were automatically recorded every five seconds by the HMI during intake and discharge operations were exported to Microsoft Excel for subsequent analysis, and are stored by AMI Engineers on a secure network. Files are also being stored on the GSI SharePoint website for additional archiving.

8. RESULTS

8.1. Experimental Conditions

8.1.1. Facility Operational Data

8.1.1.1. Intake Operational Data

Flow rates (m^3/hour) of control and treatment sample water during intake operations are shown in Figures 3-6. In all four trials, the goal average flow rate range of 180 - 210 m^3/hour to the treatment retention tank was achieved (as depicted by the horizontal lines in Figures 3-6). During Trials 1 and 2 the flow rate to the control retention tank also remained within the desired goal flow rate range of 180 to 210 m^3/hour (Figures 3 and 4, respectively). During Trials 3 and 4 however, there were issues with the control flow rate between the 5 and 15 minute mark, the result of a positioner malfunction that could not be addressed between the two trials (Figures 5 and 6, respectively). The impact of these two deviations from the goal average flow rate is likely minimal, since the NaOCl BWTS is an in-tank treatment. In addition, during Trial 4, the ponds containing the concentrated phytoplankton for injection were emptied 3 minutes prior to the end of the intake operation.

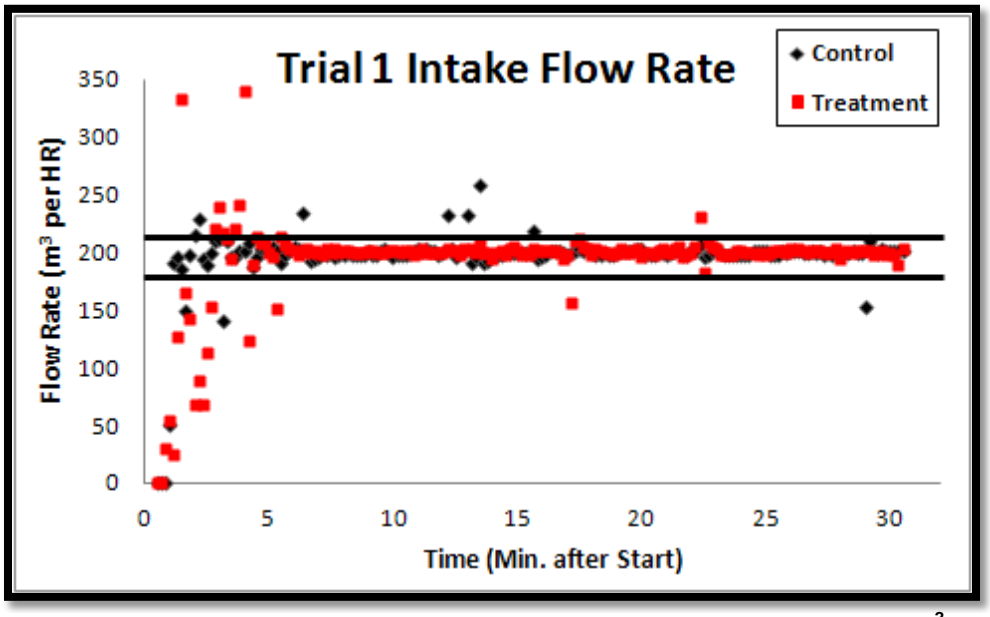


Figure 3. NaOCl BWTS Trial 1 Intake Control and Treatment Flow Rate (m^3/hr). Horizontal Lines on Graph Indicate the Goal Flow Rate Range for this Parameter.

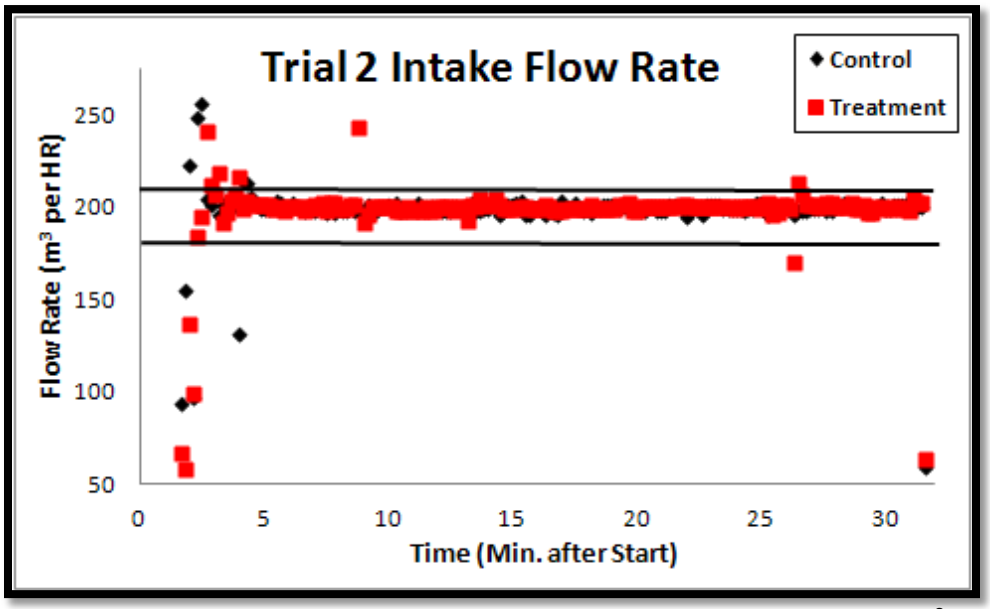


Figure 4. NaOCl BWTS Trial 2 Intake Control and Treatment Flow Rate (m^3/hr). Horizontal Lines on Graph Indicate the Goal Flow Rate Range for this Parameter.

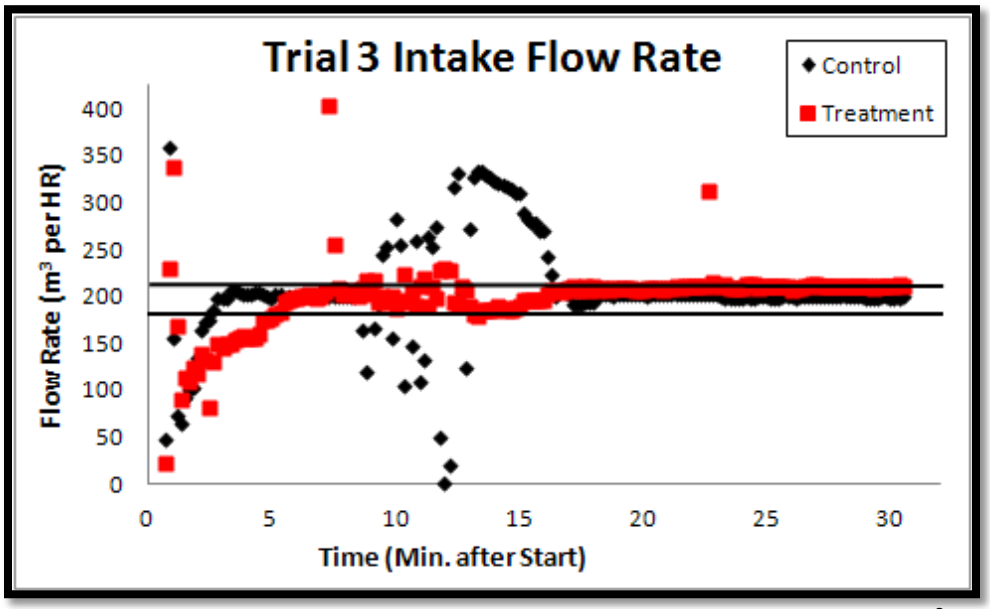


Figure 5. NaOCl BWTS Trial 3 Intake Control and Treatment Flow Rate (m^3/hr). Horizontal Lines on Graph Indicate the Goal Flow Rate Range for this Parameter.

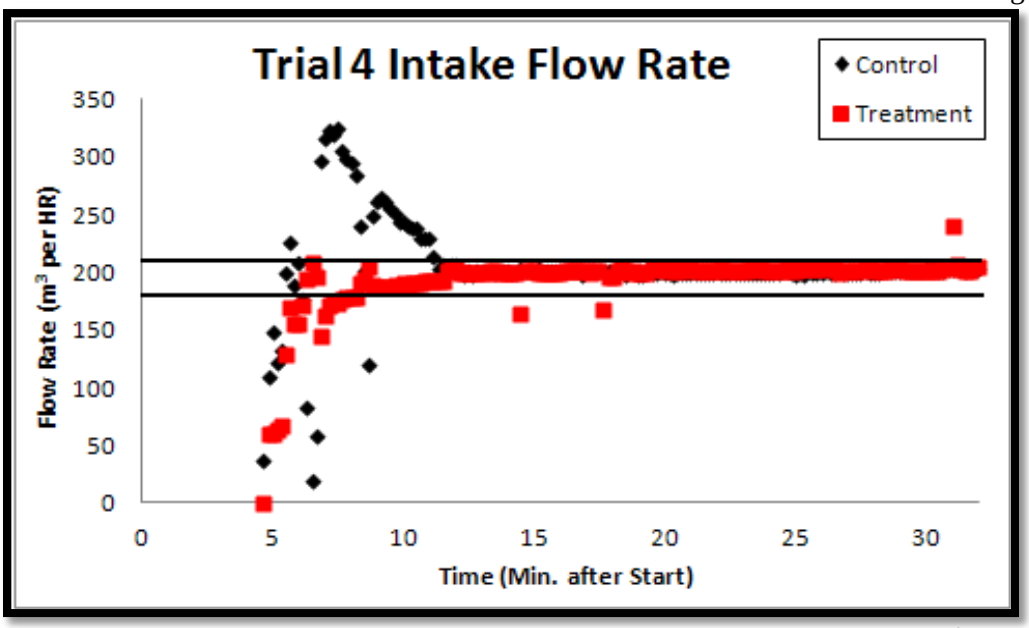


Figure 6. NaOCl BWTS Trial 4 Intake Control and Treatment Flow Rate (m^3/hr). Horizontal Lines on Graph Indicate the Goal Flow Rate Range for this Parameter.

8.1.1.2. Discharge Operational Data

Average flow rate (m^3/hour), pressure (bar) and total volume (m^3) of control discharge water during the NaOCl BWTS test are shown in Table 9. All measured parameters were within the valid range specified in the system's Test Plan (GSI, 2011c), and there were no deviations from this plan (GSI, 2011c).

Table 9. Operational Parameters Measured during Control Discharge Operations of the NaOCl BWTS (Trials 1 to 4); Average ± 1 Standard Deviation.

Parameter	Measurement Location	Valid Range	Trial 1	Trial 2	Trial 3	Trial 4	Test Average (*n*=4)
Average Flow Rate (m^3/hr)	Control Track	180 - 210	198 ± 22	195 ± 40	191 ± 40	194 ± 26	**195 ± 3.0**
	Sample Collection Tubs 1 & 2	3.0 - 3.6	3.5 ± 0.0	3.4 ± 0.0	3.4 ± 0.0	3.5 ± 0.0	**3.5 ± 0.1**
Average Pressure (Bar)	Post Recirculation Pump	1.7 - 2.2	2.0 ± 0.1	1.9 ± 0.4	1.9 ± 0.4	2.0 ± 0.3	**2.0 ± 0.1**
Total Volume (m^3)	Sample Collection Tubs 1 & 2	1.0 - 1.8	1.6 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	**1.7 ± 0.1**

Average flow rate (m^3/hour), pressure (bar), and total volume (m^3) of treatment discharge water during the NaOCl BWTS test are shown in Table 10. All measured parameters were within the valid range specified in the Test Plan (GSI, 2011c). Prior to Trial 1 discharge, a valve was partially opened on the treatment retention tank by operator error and 40 % of the water volume in the treatment retention tank was lost (i.e., total volume went from approximately 100 m^3 to 60 m^3); therefore, the length of the treatment discharge operation was 18 minutes; the collection

times of the discrete grab samples for water quality were updated accordingly (i.e., 3, 9, and 15 minutes after start). Treatment discharge operations during Trials 2 - 4 proceeded as planned.

Table 10. Operational Parameters Measured during Treatment Discharge Operations of the NaOCl BWTS Treatment Discharge (Trials 1 to 4; Average ± 1 Standard Deviation).

Parameter	Measurement Location	Valid Range	Trial 1	Trial 2	Trial 3	Trial 4	Test Average (*n*=4)
Average Flow Rate (m^3/hr)	Treatment Track	180 - 210	189 ± 69	197 ± 25	192 ± 32	191 ± 43	**192 ± 3.4**
	Sample Collection Tubs 4, 5, & 6	3.0 - 3.6	3.4 ± 0.0	3.5 ± 0.0	3.5 ± 0.0	3.4 ± 0.0	**3.5 ± 0.1**
Average Pressure (Bar)	Post Recirculation Pump	1.7 - 2.2	1.8 ± 0.4	2.1 ± 0.2	2.0 ± 0.3	2.1 ± 0.4	**2.0 ± 0.1**
Total Volume (m^3)	Sample Collection Tubs 4, 5, & 6	1.0 - 1.8	1.0 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.5 ± 0.0	**1.5 ± 0.3**

8.1.2. Water Quality

8.1.2.1. Intake Water Quality

Intake water quality measurements for NPOC, DOM as DOC, POM as POC and %T (both filtered and unfiltered), were consistent from trial to trial (Table 11). MM and TSS differed from week to week (i.e., Trials 1 and 2 were conducted in the first week; Trials 3 and 4 were conducted in the second week) due to the natural variation of the harbor water (Table 11).

Table 11. Average (± 1 Std. Dev.) Water Quality Measured from Grab Samples Collected during the Four Intake Operations of the NaOCl BWTS. TR = Trial. NR = No Requirement.

Parameter (Units)	Valid Range	TR1 Avg. (*n*=3)	TR2 Avg. (*n*=3)	TR3 Avg. (*n*=3)	TR4 Avg. (*n*=3)	Test Avg. (*n* = 4)
Non-Purgeable Organic Carbon (mg/L)	NR	9.3 ± 0.3	10.1 ± 0.4	8.3 ± 0.1	7.6 ± 0.2	**8.8 ± 1.1**
Dissolved Organic Matter (mg/L as DOC)	6 to 30	8.8 ± 0.0	9.6 ± 0.1	8.3 ± 0.1	7.4 ± 0.1	**8.5 ± 0.9**
Particulate Organic Matter (mg/L as POC)	<0.1 to 15	0.5 ± 0.3	0.5 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	**0.3 ± 0.2**
Mineral Matter (mg/L)	<1 to 40	1.6 ± 0.4	1.5 ± 0.6	4.0 ± 0.4	5.3 ± 0.3	**3.1 ± 1.9**
Total Suspended Solids (mg/L)	<1 to 40	2.1 ± 0.1	2.0 ± 0.3	4.0 ± 0.4	5.5 ± 0.4	**3.4 ± 1.7**
Transmittance – Unfiltered (% at 254 nm)	NR	38.1 ± 0.1	36.7 ± 0.7	40.5 ± 0.1	45.1 ± 0.2	**40.1 ± 3.7**
Transmittance – Filtered (% at 254 nm)	NR	39.8 ± 0.1	38.5 ± 0.1	43.0 ± 0.4	48.2 ± 0.1	**42.4 ± 4.3**

Intake water quality measurements from the sample collection tubs were consistent for all four trials for specific conductivity, salinity and dissolved oxygen (Table 12). Trials 1 and 2, which were conducted in the same week, had similar measurements for temperature and pH (Table 12). Temperature and pH measured during Trials 3 and 4 (also conducted in the same week) were similar to each other but differed from Trials 1 and 2 by as much as 5.16 °C and 0.97 pH unit (Table 12). Turbidity also varied between the two weeks of testing with an average of 2.4 NTU in the first week (Trials 1 and 2; Table 12) and an average of 4.9 NTU in the second week (Trials 3 and 4; Table 12). Total chlorophyll was higher for Trials 1 and 2 with an average of 6.75 μg/L (Table 12), which dropped to an average of 4.9 μg/L during the second week of testing (Trials 3 and 4; Table 12). These differences can all be attributed to natural variations in the weather and harbor water.

Table 12. Average (± 1 Std. Dev.) Water Quality Measured from Sample Collection Tubs using a YSI Multiparameter Water Quality Sonde during the Four Intake Operations. TR = Trial. NR = No Requirement.

Parameter (Units)	Valid Range	TR1	TR2	TR3	TR4	Test Avg. (*n* = 4)
Temperature (°C)	4 to 30	14.01	14.20	9.87	9.04	**11.78 ± 2.71**
Sp. Conductivity (mS/cm)	NR	0.073	0.075	0.090	0.085	**0.081 ± 0.008**
Salinity (ppt)	≤1	0.03	0.03	0.04	0.04	**0.04 ± 0.01**
pH	6 to 9	7.82	7.81	8.78	8.23	**8.02**
Turbidity (NTU)	NR	2.5	2.3	4.7	5.0	**3.6 ± 1.4**
Dissolved Oxygen (mg/L)	6 to 11	9.60	9.44	10.09	10.35	**9.87 ± 0.42**
Dissolved Oxygen (% Saturation)	NR	93.3	92.1	89.2	89.7	**91.1 ± 2.0**
Total Chlorophyll (μg/L)	NR	6.4	7.1	5.3	4.5	**5.8 ± 1.2**

8.1.2.2. Discharge Water Quality

Control and treatment discharge water quality measurements were consistent from trial to trial and between control and treatment with the exception of %T (Table 13). The %T in treatment discharge samples was consistently higher by ~10 % compared to that of the control discharge samples (Table 13).

Discharge water quality measurements from the sample collection tubs were also consistent between control and treatment for all four trials for specific conductivity, salinity and dissolved oxygen (Table 14). Trials 1 and 2, which were conducted in the same week, had similar measurements for temperature and pH between the control and treatment tubs (Table 14). Temperature and pH measured during Trials 3 and 4 (conducted in the same week) were similar to each other but differed from Trials 1 and 2 (Table 14). However, the average temperature of sample water in the control and treatment collection tubs across the entire testing period was similar (Table 14). Turbidity was consistently higher in the treatment tubs versus the control tubs

(Table 14). Total chlorophyll was higher in the control tubs compared to the treatment tubs over the course of the four trials (Table 14).

Table 13. Average (± 1 Std. Dev.) Control and Treatment Discharge Water Quality across Trials as Measured from Grab Samples. TR = Trial.

Parameter (Units)	TR1 Average (*n*=3)		TR2 Average (*n*=3)		TR3 Average (*n*=3)		TR4 Average (*n*=3)		Test Avg. (*n* = 4)	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Non-Purgeable Organic Carbon (mg/L)	9.1 ± 0.1	9.1 ± 0.8	9.4 ± 0.3	9.6 ± 0.0	8.6 ± 0.2	8.4 ± 0.0	7.8 ± 0.2	7.8 ± 0.2	**8.7 ± 0.7**	**8.7 ± 0.8**
Dissolved Organic Matter (mg/L as DOC)	9.0 ± 0.1	8.7 ± 0.1	9.2 ± 0.1	9.3 ± 0.1	8.5 ± 0.1	8.5 ± 0.1	7.5 ± 0.1	7.3 ± 0.0	**8.6 ± 0.8**	**8.5 ± 0.8**
Particulate Organic Matter (mg/L as POC)	0.1 ± 0.1	0.4 ± 0.9	0.2 ± 0.3	0.3 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.3 ± 0.2	0.5 ± 0.2	**0.2 ± 0.1**	**0.3 ± 0.2**
Mineral Matter (mg/L)	2.1 ± 0.3	2.0 ± 0.4	1.8 ±0.6	1.0 ± 0.7	2.9 ± 0.3	3.0 ± 0.7	2.9 ± 0.5	2.8 ± 0.6	**2.4 ± 0.6**	**2.2 ± 0.9**
Total Suspended Solids (mg/L)	2.2 ± 0.4	2.4 ± 0.9	2.0 ± 0.3	1.4 ± 0.6	2.9 ± 0.2	3.2 ± 0.8	3.2 ± 0.4	3.2 ± 0.5	**2.6 ± 0.6**	**2.6 ± 0.9**
Transmittance – Unfiltered (% at 254 nm)	37.4 ± 0.2	46.0 ± 0.2	36.5 ± 0.1	47.4 ± 0.3	40.2 ± 0.1	50.2 ± 0.1	45.2 ± 0.1	55.2 ± 0.1	**39.8 ± 3.9**	**49.7 ± 4.1**
Transmittance – Filtered (% at 254 nm)	39.7 ± 0.1	49.0 ± 0.1	38.6 ± 0.2	49.6 ± 0.4	42.8 ± 0.2	53.4 ± 0.3	48.2 ± 0.1	58.4 ± 0.1	**42.3 ± 4.3**	**52.6 ± 4.3**

Table 14. Average (± 1 Std. Dev.) Control and Treatment Discharge Water Quality as Measured from the Sample Collection Tubs using a YSI Multiparameter Water Quality Sonde. TR = Trial.

Parameter (Units)	TR1 Average		TR2 Average		TR3 Average		TR4 Average		Test Avg. (*n*=4)	
	Control (*n*=1)	Treatment (*n*=3)	Control (*n*=1)	Treatment (*n*=3)	Control (*n*=1)	Treatment (*n*=3)	Control (*n*=1)	Treatment (*n*=3)	Control	Treatment
Temperature (°C)	13.99	13.77 ± 0.01	13.07	13.06 ± 0.01	8.68	8.20 ± 0.02	8.29	7.81 ± 0.01	**11.01 ± 2.94**	**10.71 ± 2.84**
Sp. Conductivity (mS/cm)	0.073	0.098 ± 0.00	0.075	0.104 ± 0.000	0.090	0.123 ± 0.000	0.089	0.115 ± 0.000	**0.082 ± 0.009**	**0.110 ± 0.010**
Salinity (ppt)	0.03	0.05 ± 0.00	0.03	0.05 ± 0.00	0.04	0.06 ± 0.00	0.04	0.05 ± 0.00	**0.04 ± 0.01**	**0.05 ± 0.00**
pH	7.75	7.85	7.85	7.36	6.83	7.79	7.79	8.40	**7.31**	**7.71**
Turbidity (NTU)	3.5	7.23 ± 3.45	2.8	3.8 ± 1.2	4.7	5.7 ± 0.1	4.3	5.0 ± 0.3	**3.8 ± 0.8**	**5.4 ± 2.0**
Dissolved Oxygen (mg/L)	9.04	9.42 ± 0.02	9.06	8.92 ± 0.02	10.22	10.03 ± 0.05	10.47	10.11 ± 0.05	**9.70 ± 0.75**	**9.62 ± 0.51**
Dissolved Oxygen (% Saturation)	87.7	91.0 ± 0.2	86.1	84.8 ± 0.2	87.8	85.1 ± 0.2	89.3	84.9 ± 0.3	**87.7 ± 1.3**	**86.5 ± 2.7**
Total Chlorophyll (µg/L)	5.4	3.8 ± 2.2	5.5	4.8 ± 4.1	4.4	2.6 ± 0.1	4.2	2.0 ± 0.2	**4.9 ± 0.7**	**3.3 ± 2.3**

8.1.3. Biota in Pre-Treatment (Untreated) Intake and Control Discharge Samples

8.1.3.1. Organisms ≥ 50 µm

Live densities of organisms in the ≥ 50 µm size class sampled during intake (i.e., pre-treatment) were well above prescribed threshold densities allowed by the IMO G8 Guidelines (IMO, 2008a; Table 15). Live organism densities in this size class were highest during Trial 1 at 256,000/m^3 and lowest during Trial 4 at 188,000/m^3, with an overall average across trials of 221,000/m^3 (Table 15).

Intake samples did not always meet the IMO G8 Guidelines' minimum requirements for taxonomic diversity of at least five species from at least three different phyla/divisions present in all samples. Representatives from the phyla Arthropoda (i.e., cladocerans and copepods) and Rotifera were present in all trials, with the macrozooplankton community dominated by the cladoceran *Bosmina*, as well as cyclopoid and calanoid copepods. Several rotifer species in the genera *Keratella, Polyarthra, Conochilus* and *Synchaeta* were common microzooplankton in the samples. During Trials 2 and 4, a few individuals of the phyla Annelida and Platyhelminthes were found in the intake samples.

In control discharge samples, the density of live organisms ≥ 50 µm ranged from 114,000/m^3 (Trial 4) to 207,000/m^3 (Trial 3; Table 15). Across the four trials, the average control discharge live organism density was 172,000/m^3, exceeding the IMO Convention's minimum requirements for untreated discharge by over three orders of magnitude (IMO, 2004; Table 15).

8.1.3.2. Organisms ≥ 10 and < 50 µm

In the ≥ 10 and < 50 µm size class, live organism densities on intake ranged from 593 cells/mL (Trial 3) to 961 cells/mL (Trial 2), with an average of 798 cells/mL across the four trials (Table 15). Intake densities were below the threshold required by the IMO G8 Guidelines (IMO, 2008a). Low ambient densities in this size class are an expected artifact of tests that occur late in GSI's testing season (i.e., October), but, as noted below, live densities on discharge exceeded the IMO G8 Guideline of 100 live cells/mL (IMO, 2008a).

In terms of diversity, pre-treatment intake samples met the IMO G8 Guidelines' minimum requirements, with at least five species from at least three different phyla/divisions present in all samples (IMO, 2008a). In order of decreasing abundance, organisms in these intake samples were dominated by filamentous diatoms (*Aulacoseira*), coccoid green algae (*Pandorina*, *Eudorina*), globular and filamentous blue-green algae (*Microcystis*-like, *Lyngbya*, *Oscillatoria*), solitary centric diatoms (*Cyclotella*, *Stephanodiscus*) and ribbon-shaped diatom colonies (*Fragilaria*).

Live organisms in control discharge samples ranged from 462 cells/mL (Trial 4) to 750 cells/mL (Trial 1), with an average of 548 cells/mL across the four trials (Table 15). These densities were well above the IMO G8 Guidelines' minimum requirements for untreated discharge, indicating that compared to intake densities, attrition of organisms was minimal owing to tank retention

alone (IMO, 2008a; Table 15). The relative taxonomic composition of these samples was also similar to that of intake samples.

8.1.3.3. Organisms < 10 µm

Total culturable heterotrophic bacteria densities in pre-treatment samples on intake over the four trials were consistently above 1,000 MPN/mL, i.e., the minimum threshold limit applied by GSI to these tests and required by the ETV Protocol (USEPA, 2010) and ranged from an average of 1,220 MPN/mL (Trial 4) to 2,870 MPN/mL (Trial 1; Table 15). In all four trials, heterotrophic bacteria densities in control discharge were higher than intake densities, likely due to in-tank retention conditions conducive to growth. Control discharge densities ranged from 3,700 MPN/mL (Trial 1) to 10,300 MPN/mL (Trial 4), with an overall average of 5,440 MPN/mL (Table 15).

Pre-treatment intake densities of *E. coli*, total coliform bacteria and *Enterococcus* bacteria, though consistent across trials, were all quite low, likely due to the late-season timing of the tests at the GSI Land-Based RDTE Facility (i.e., mid-October). *E. coli* densities ranged from 22 MPN/100 mL (Trial 4) to 56 MPN/100 mL (Trial 2), with an average across trials of 37 MPN/100 mL (Table 15). On discharge, *E. coli* densities in control samples averaged between 10 MPN/100 mL (Trial 4) and 27 MPN/100 mL (Trial 2), with an average across trials of 19 MPN/100 mL (Table 15).

Total coliform densities ranged between 135 MPN/100 mL (Trial 4) and 243 MPN/100 mL (Trial 1) in pre-treatment intake, and between 88 MPN/100 mL (Trial 2) and 109 MPN/100 mL (Trial 3) in control discharge (Table 15). Pre-treatment/Control samples averaged across trials equaled 184 MPN/100 mL on intake and 99 MPN/100 mL on discharge.

Enterococcus results were very similar to those for *E. coli*, with densities in pre-treatment intake samples low, ranging from 18 MPN/100 mL (Trial 1) to 38 MPN/100 mL (Trial 2; Table 15). Average across trials was 26 MPN/100 mL. Control discharge densities of *Enterococcus* ranged from 13 MPN/100 mL (Trial 3) to 56 MPN/100 mL (Trial 4), with an average across trials of 26 MPN/100 mL (Table 15).

Table 15. Densities (Average ± 1 Std. Dev.) of Live Organisms by Size Class in Pre-Treatment Intake and Control Discharge Samples Collected during the Four Trials of the NaOCl BWTS. Reported Densities for Live Organisms < 10 μm are Average ± 1 Standard Error of the Mean.

Size Class of Organisms	Sample Type	Target Minimum Density	Trial 1 (*n* = 1*)	Trial 2 (*n* = 1*)	Trial 3 (*n* = 1*)	Trial 4 (*n* = 1*)	Test Avg. (*n* = 4)
≥ 50 μm	Intake (Pre-Treatment)	100,000 /m^3	256,000 /m^3	194,000 /m^3	244,000 /m^3	188,000 /m^3	221,000 ± 34,000 /m^3
	Discharge (Control)	100 /m^3	197,000 /m^3	168,000 /m^3	207,000 /m^3	114,000 /m^3	172,000 ± 42,000 /m^3
≥ 10 and < 50 μm	Intake (Pre-Treatment)	N/A	796 /mL	961 /mL	593 /mL	841 /mL	798 ± 77 /mL
	Discharge (Control)	100 /mL	750 /mL	500 /mL	481 /mL	462 /mL	548 ± 68 /mL
< 10 μm – Heterotrophic bacteria	Intake (Pre-Treatment)	1,000 CFU/mL	2,870 ± 410 MPN/mL	1,330 ± 696 MPN/mL	2,720 ± 721 MPN/mL	1,220 ± 130 MPN/mL	2,030 ± 440 MPN/mL
	Discharge (Control)	N/A	3,700 ± 100 MPN/mL	3,930 ± 1300 MPN/mL	3,870 ± 722 MPN/mL	10,300 ± 2540 MPN/mL	5,440 ± 1,610 MPN/mL
< 10 μm – *Escherichia Coli*	Intake (Pre-Treatment)	N/A	33 ± 1 MPN/100 mL	56 ± 6 MPN/100 mL	39 ± 3 MPN/100 mL	22 ± 2 MPN/100 mL	37 ± 7 MPN/100 mL
	Discharge (Control)	N/A	15 ± 2 MPN/100 mL	27 ± 1 MPN/100 mL	22 ± 4 MPN/100 mL	10 ± 1 MPN/100 mL	19 ± 4 MPN/100 mL
< 10 μm – Total Coliforms	Intake (Pre-Treatment)	N/A	243 ± 25 MPN/100 mL	169 ± 14 MPN/100 mL	187 ± 15 MPN/100 mL	135 ± 3 MPN/100 mL	184 ± 23 MPN/100 mL
	Discharge (Control)	N/A	109 ± 9 MPN/100 mL	88 ± 3 MPN/100 mL	109 ± 16 MPN/100 mL	88 ± 8 MPN/100 mL	99 ± 6 MPN/100 mL
< 10 μm – *Enterococci*	Intake (Pre-Treatment)	N/A	18 ± 9 MPN/100 mL	38 ± 2 MPN/100 mL	26 ± 5 MPN/100 mL	21 ± 4 MPN/100 mL	26 ± 4 MPN/100 mL
	Discharge (Control)	N/A	16 ± 2 MPN/100 mL	20 ± 3 MPN/100 mL	13 ± 2 MPN/100 mL	56 ± 6 MPN/100 mL	26 ± 10 MPN/100 mL

* *n* = 3 for Heterotrophic bacteria, *E. coli*, Total Coliforms and *Enterococci*.

8.2. Experimental Outcomes

8.2.1. Operational Efficacy

8.2.1.1. Determination of Chlorine Demand

The chlorine demand of water sampled from the treatment retention tank and analyzed consistent with methods provided by the BWTS developers ranged from 8.42 mg/L in Trial 1 to 6.84 mg/L in Trial 4, indicating a decrease in demand over the two-week test period (Table 16). The initial pH of these samples was very similar across trials ranging from 7.32 in Trial 1 to 7.54 in Trial 4 (Table 17). The initial temperature of the samples was less similar across trials ranging from a high of 18.3 °C in Trial 2 to a low of 13.0 °C in Trial 4, likely the result of the ambient harbor water being subject to changing weather conditions over the two week test period (Table 17). Final pH and temperature values were slightly higher than initial values across all four trials, with final pH measurements on average 0.2 pH units higher than initial measurements and final temperatures on average 1.4 °C higher than initial temperatures (Table 17).

Table 16. Chlorine Demand of Untreated Retention Tank Sample Water across Trials Measured as Total Residual Oxidants (TRO) Seven Hours after Spiking with Clorox® Bleach (i.e., 10 mg/L of Chlorine).

Trial	Treatment Tank Sample Location	TRO (mg/L as Cl_2)	Chlorine Demand = Initial Chlorine Dose – TRO (mg/L)
Trial 1	Top	1.64	8.36
	Middle	1.56	8.44
	Bottom	1.54	8.46
	Average (*n*=3) ± 1 Std. Dev.	**1.58 ± 0.06**	**8.42 ± 0.06**
Trial 2	Top	2.57	7.43
	Middle	2.49	7.52
	Bottom	2.32	7.68
	Average (*n*=3) ± 1 Std. Dev.	**2.46 ± 0.13**	**7.54 ± 0.13**
Trial 3	Top	2.53	7.47
	Middle	2.39	7.61
	Bottom	2.45	7.55
	Average (*n*=3) ± 1 Std. Dev.	**2.45 ± 0.07**	**7.55 ± 0.07**
Trial 4	Top	3.16	6.84
	Middle	3.20	6.80
	Bottom	3.13	6.88
	Average (*n*=3) ± 1 Std. Dev.	**3.16 ± 0.04**	**6.84 ± 0.04**

Table 17. Initial (i.e., Zero Hour) and Final (i.e., Seven Hour) pH and Temperature of Untreated Retention Tank Sample Water Used for Chlorine Demand Determination across Trials.

Trial	Treatment Tank Sample Location	Initial pH	Initial Temp. (°C)	Final pH	Final Temp. (°C)
Trial 1	Top	7.35	16.2	7.50	18.9
	Middle	7.32	16.3	7.46	18.9
	Bottom	7.33	16.7	7.55	18.8
	Average (*n*=3) ± 1 Std. Dev.	**7.33 ± 0.02**	**16.4 ± 0.3**	**7.50 ± 0.05**	**18.9 ± 0.1**
Trial 2	Top	7.47	18.0	7.64	18.9
	Middle	7.41	18.3	7.70	18.7
	Bottom	7.36	18.3	7.68	19.1
	Average (*n*=3) ± 1 Std. Dev.	**7.41 ± 0.06**	**18.2 ± 0.2**	**7.67 ± 0.03**	**18.9 ± 0.2**
Trial 3	Top	7.52	13.6	7.70	14.5
	Middle	7.49	13.5	7.74	15.0
	Bottom	*	*	7.76	14.8
	Average (*n*=3) ± 1 Std. Dev.	**7.51 ± 0.02 (*n*=2)**	**13.6 ± 0.1 (*n*=2)**	**7.73 ± 0.03 (*n*=3)**	**14.8 ± 0.3 (*n*=3)**
Trial 4	Top	7.50	13.0	7.71	15.0
	Middle	7.48	13.1	7.68	15.0
	Bottom	7.54	13.0	7.63	15.1
	Average (*n*=3) ± 1 Std. Dev.	**7.51 ± 0.03**	**13.0 ± 0.1**	**7.67 ± 0.04**	**15.0 ± 0.1**

*Not enough sample volume available to make this determination.

8.2.1.2. Determination of Chlorine Dosing Concentration

The target 7-hour chlorine concentration in the treatment retention tank (i.e., 5 mg/L above natural chlorine demand) was calculated consistent with the BWTS developer's methods and using the highest chlorine demand found in the samples (Table 18). Following dosing with the calculated volume of 6.15 % NaOCl solution, the concentration of chlorine (measured as TRO) in the treatment retention tank one hour post-dose ranged from an average of 6.27 mg/L (Trial 1; Table 18) to 6.62 mg/L (Trial 2; Table 18). Approximately six hours after the addition of the pre-determined volume of 6.15 % NaOCl solution, the TRO of water sampled from the treatment retention tank had decreased, ranging from an average of 5.21 mg/L (Trial 1; Table 18) to 6.37 mg/L (Trial 2; Table 18). Thus, the TRO concentration in the treatment tank met the minimum target of 5 mg/L at the end of the 7-hour contact period.

Table 18. Goal 7 Hour Chlorine Concentration, Volume of 6.15 % NaOCl Solution Added to the Retention Tank, and Average Residual Chlorine Concentration 1 and 6 Hours Post-Dosing (Measured as Total Residual Oxidants, TRO) across Trials (± Standard Deviation).

Trial	Highest Concentration of Chlorine Demand Measured	Goal Chlorine Concentration = Concentration of Chlorine Added (5 mg/L + Chlorine Demand	Volume of 6.15 % NaOCl Solution added to the Retention Tank	Average TRO at 1 Hour (*n*=3)	Average TRO at 6 Hours (*n*=3)
Trial 1	8.46 mg/L	13.46 mg/L	21.9 L	6.27 ± 0.11 mg/L	5.21 ± 0.11 mg/L
Trial 2	7.68 mg/L	12.68 mg/L	20.5 L	6.62 ± 0.11 mg/L	6.37 ± 0.04 mg/L
Trial 3	7.61 mg/L	12.61 mg/L	20.3 L	6.61 ± 0.07 mg/L	5.90 ± 0.14 mg/L
Trial 4	6.88 mg/L	11.88 mg/L	19.3 L	6.49 ± 0.13 mg/L	6.24 ± 0.05 mg/L

8.2.1.3. Determination of Neutralizer Dosing Concentration

In Trial 1, 40 % (w/v) sodium thiosulfate ($Na_2S_2O_3$) was used to neutralize the NaOCl-treated water stored in the treatment retention tank; the shipment of $NaHSO_3$ was not received by GSI in time for this trial and a more readily available substitute was used. The 40 % (w/v) $Na_2S_2O_3$ was added to the treatment retention tank based on the developer-provided ratio for $Na_2S_2O_3$ solution of 1.6 mg/L per mg/L of chlorine plus a 10 % safety factor (see Appendix A). One hour following neutralization with 2.3 L of the, TRO concentration was, on average, well below the target of $\leq$ 0.038 mg/L (Table 19). The concentration of chlorine in the tank decreased even further following 16-20 hours retention (i.e., to 0.02 mg/L; Table 19) such that the neutralized water was deemed safe to discharge.

As per the developer's proposed treatment method, 40% (w/v) $NaHSO_3$ was used to neutralize the treated water in Trial 2. The same ratio of 1.6 mg/L per mg/L of chlorine plus a 10 % safety factor was used to calculate the required volume of solution. One hour following neutralization, the TRO concentration of water in the treatment tank was higher than the allowable discharge concentration of $\leq$ 0.038 mg/L. Another calculation and subsequent addition of 40% (w/v) $NaHSO_3$ was conducted. TRO was measured again one hour post-neutralization with TRO concentrations found to be still above the target (Table 19). A third calculation/addition of 40% (w/v) $NaHSO_3$ was required before the TRO concentration of the neutralized water was deemed safe for discharge (Table 19).

Since Trial 2's neutralization step was problematic, GSI increased the ratio of 40% (w/v) $NaHSO_3$ to calculate the volume of neutralizer required for Trial 3 and used the ratio of 1.7 mg/L per mg/L of chlorine plus 10 % margin of safety. However, the TRO concentration in the neutralized water was still above the target 16-20 hours post-neutralization such that a second neutralization attempt was necessary (Table 19).

In Trial 4 GSI again amended the neutralization determination calculation by increasing the ratio of 40% (w/v) $NaHSO_3$ to 1.8 mg/L per mg/L of chlorine plus a 10 % margin of safety. This ratio also proved inadequate to neutralize the treated water, and a second neutralization dose was necessary (Table 19).

Table 19. Volume of Neutralizer Added to the Treated Retention Tank and Total Residual Oxidant Concentration (as mg/L Chlorine; Average ± 1 Std. Dev., *n*=3) Post-Dosing for each Neutralization Step across Trials. TR=Trial.

TR	Neutralizer; All 40 % (w/v)	Time After Neutralization:	1 HR	16-20 HR	Time After 2nd Neutralization:	1 HR	Time After 3rd Neutralization:	1 HR
		Vol. (L) Added; 1st Attempt	TRO Conc. (mg/L Cl_2)	TRO Conc. (mg/L Cl_2)	Vol. (L) Added; 2nd Attempt	TRO Conc. (mg/L Cl_2)	Vol. (L) Added; 3rd Attempt	TRO Conc. (mg/L Cl_2)
1	$Na_2S_2O_3$	2.3	0.03 ± 0.00	0.02 ± 0.002	Not Needed	N/A	N/A	N/A
2	$NaHSO_3$	2.8	0.42 ± 0.01	0.30 ± 0.00	0.1	0.11 ± 0.01	0.1	0.02 ± 0.00
3	$NaHSO_3$	2.8	0.41 ± 0.05	0.30 ± 0.00	0.2	0.03 ± 0.00	Not Needed	N/A
4	$NaHSO_3$	3.1	0.34 ± 0.03	0.29 ± 0.00	0.2	0.03 ± 0.00	Not Needed	N/A

During Trial 4, the samples collected for initial TRO determination (i.e., collected one hour post-neutralization) were left to sit overnight at room temperature and analyzed again the following morning concurrent with the samples taken from the treatment tank 16-20 hours post-neutralization. The chlorine concentration of the samples left to sit at room temperature overnight was found to be less than half that of the samples collected from the treatment tank and analyzed immediately (Table 20).

Table 20. Comparison of TRO Concentration in Treatment Retention Tank Samples Collected during Trial 4 and Left to Sit at Room Temperature Overnight Compared to Samples Left in the Treatment Retention Tank Overnight.

Treatment Tank Sample Location	TRO Conc. (as mg/L Cl_2) of Samples Left in the Treatment Tank Overnight	TRO Conc. (as mg/L Cl_2) of Samples Left to Sit Overnight at Room Temperature
Top	0.29	0.13
Middle	0.29	0.13
Bottom	0.29	0.13
Average TRO (*n*=3) ± 1 Std. Dev.	**0.29 ± 0.00**	**0.13 ± 0.00**

8.2.2. Biological Efficacy

8.2.2.1. Organisms ≥ 50 µm

Densities of live organisms ≥ 50 μm in the treatment discharge ranged from 132/m^3 (Trial 2) to 1,338/m^3 (Trial 1), with an overall average of 642/m^3 across trials (Table 21). Though these concentrations represent reductions compared to control discharge densities, they are two to three orders of magnitude above the < 10 organisms per m^3 ballast water performance standard requirement of the IMO Convention (IMO, 2004).

In all four trials, the cladoceran *Bosmina* was the dominant taxa in the macrozooplankton community of the treatment discharge. The soft-bodied rotifers in the genus *Synchaeta* and *Conochilus* were some of the most abundant live organisms in the treatment discharge microzooplankton community, along with copepod nauplii and loricate rotifers in the genus *Keratella*. The relative density of the rotifers and nauplii varied with each trial. In Trials 1 and 2 *Synchaeta* was most abundant; *Keratella* and nauplii were most abundant in Trial 3 and Trial 4, respectively.

8.2.2.2. Organisms ≥ 10 and < 50 µm

Densities of live organisms ≥ 10 and < 50 μm in the treatment discharge were few, ranging from 1 cell/mL (Trial 2) to 6 cells/mL (Trial 1), and averaging less than 4 cells/mL across the four trials (Table 21). Densities were therefore within the < 10 organisms per mL ballast water performance standard requirement of the IMO Convention for this size class of organisms (IMO, 2004). In terms of diversity, chain forming diatoms, coccoid green algae, and small flagellates were the dominate taxa remaining in the samples.

8.2.2.3. Organisms < 10 µm

Densities of heterotrophic bacteria in treated discharge samples ranged from 130 MPN/mL (Trial 3) to 564 MPN/mL, with an average across trials of 321 MPN/mL (Table 21). These densities are lower than intake and control discharge densities, but there is no IMO standard against which to compare them. *E. coli* and *Enterococcus* densities were less than the limit of detection (i.e., < 1 MPN/100 mL) across all trials and well within the performance standard of the IMO Convention (IMO, 2004), but intake densities were also quite low, so no conclusion on BWTS effectiveness relative to these organism category can be drawn from these results. The BWTS did not result in complete mortality of total coliform bacteria present; discharge densities ranged from 2 MPN/100 mL (Trial 3) to 13 MPN/100 mL, but there is no IMO benchmark against which to compare these levels (Table 21).

Table 21. Densities of Live Organisms (Average ± 1 Std. Dev.) by Size Class in Treated Discharge Samples Collected during the Four Trials of the NaOCl BWTS. Densities of Organisms <10 μm are Reported as Average ± 1 Standard Error of the Mean.

Size Class of Organisms	IMO Standard	Trial 1	Trial 2	Trial 3	Trial 4	Test Avg. (n = 4)
≥ 50 μm (n = 2)	< 10 /m^3	1,338 ± 687 /m^3	132 ± 30 /m^3	768 ± 13 /m^3	333 ± 32 /m^3	642 ± 534 /m^3
≥ 10 and < 50 μm (n = 1)	< 10 /mL	6 /mL	1 /mL	4 /mL	4 /mL	4 ± 2 /mL
< 10 μm – Heterotrophic bacteria (n = 3)	N/A	373 ± 17 MPN/mL	219 ± 53 MPN/mL	130 ± 36 MPN/mL	564 ± 48 MPN/mL	321 ± 95 MPN/mL
< 10 μm – *Escherichia Coli* (n = 3)	< 250 CFU/100 mL	<1 MPN/100 mL	<1 MPN/100 mL	<1 MPN/100 mL	<1 MPN/100 mL	<1 MPN/100 mL
< 10 μm – Total Coliforms (n = 3)	N/A	13 ± 4 MPN/100 mL	7 ± 2 MPN/100 mL	2 ± 1 MPN/100 mL	4 ± 2 MPN/100 mL	6 ± 2 MPN/mL
< 10 μm – *Enterococci* (n = 3)	< 100 CFU/100 mL	<1 MPN/100 mL	<1 MPN/100 mL	<1 MPN/100 mL	<1 MPN/100 mL	<1 MPN/100 mL

8.2.3. Environmental Acceptability

8.2.3.1. Residual Chlorine

Results from the treatment retention tank neutralization indicate compliance with GSI's WPDES permit requirements of < 0.038 mg/L total residual chlorine in the treated discharge (Table 19). However, all treated discharge except that from Trial 1 (in which GSI deviated from the developer recommended approach) required multiple applications of neutralizer to achieve this result. In Trial 1, 40% (w/v) $Na_2S_2O_3$ (sodium thiosulfate) was applied as the neutralizing agent due to shipping delays making the 40% (w/v) $NaHSO_3$ (sodium bisulfate) called for by the BWTS developer unavailable. In Trial 1, addition of the pre-determined volume of 40% (w/v) $Na_2S_2O_3$ to the treatment water resulted in an average chlorine concentration of 0.03 mg/L (as TRO) at one hour and 0.02 mg/L (as TRO) after 16-20 hours (Table 19). However, the remaining three trials which adhered to the BWTS procedure by using 40% (w/v) $NaHSO_3$, required multiple attempts at neutralization and required deviations from the Test Plan (GSI, 2011c; Table 19) to achieve environmentally acceptable discharge. The same neutralization ratio which achieved neutralization in Trial 1 (i.e., 1.6 mg/L of neutralizer per mg/L of chlorine plus a 10 % margin of safety) resulted in inadequate neutralization in Trial 2. In that Trial, three attempts at neutralization were required before safe discharge was feasible (Table 19). GSI increased the neutralization ration in Trial 3 to 1.7 mg/L of 40% (w/v) $NaHSO_3$ per mg/L of chlorine plus a 10 % margin of safety. Trial 3 still required two attempts at neutralization before the water was deemed safe to discharge (Table 19). In Trial 4 the ratio of neutralizing agent was increased

again to 1.8 mg/L of 40% (w/v) $NaHSO_3$ per mg/L of chlorine plus a 10 % margin of safety, and also required two attempts at neutralization (Table 19).

8.2.3.2. Whole Effluent Toxicity (WET)

Results of WET tests involving effluent from Trial 2 are presented in Tables 22 – 28. Effluent water collected immediately upon discharge was not acutely or chronically toxic to sensitive freshwater organisms (Tables 23, 26, and 28). There was 100 % survival rate observed for both *P. promelas* and *C. dubia* in the 100 % whole effluent treatment (Tables 26 and 28, respectively). The *S. capricornutum* final cell density of $1.3*10^6$ cells/mL was not significantly reduced compared to the filtered Duluth-Superior Harbor water control (Table 23). No statistically significant reduction in mean individual dry weight for the *P. promelas* and no statistically significant reduction in mean number of young per female for the *C. dubia* when compared to the filtered Duluth-Superior Harbor water control were observed (Tables 26 and 28, respectively).

Results of WET tests involving effluent from Trial 4 are presented in Tables 29 – 35. Effluent water collected immediately upon discharge was not acutely or chronically toxic to sensitive freshwater organisms (Tables 30, 33, and 35). A 100 % survival rate was observed for *P. promelas* and *C. dubia* in the 100 % whole effluent treatment (Tables 33 and 35, respectively). The *S. capricornutum* final cell density of $9.0*10^5$ cells/mL was not significantly reduced compared to the filtered Duluth-Superior Harbor water control (Table 30). No statistically significant reduction in mean individual dry weight for the *P. promelas* and no statistically significant reduction in mean number of young per female for the *C. dubia* when compared to the filtered Duluth-Superior Harbor water control were observed (Tables 33 and 35, respectively).

Table 22. Average Values (Minimum, Maximum) for Water Chemistry Parameters of Exposure Solutions Used to Conduct the *S. capricornutum* WET Test (TRIAL 2 EFFLUENT).

Treatment Group	Day 0 TRO (mg/L as Cl_2)	Temp. (°C)	Day 0 Dissolved Oxygen (mg/L)	pH	Day 0 Conductivity (µS/cm)	Day 0 Hardness (mg/L $CaCO_3$)	Day 0 Alkalinity (mg/L $CaCO_3$)
***Selenastrum* Performance Control**	<0.005	24.5 (23.6, 25.5)	7.2	7.70 (7.58, 8.02)	92.9	14.4	13.0
0 % Effluent Control	<0.005	24.5 (23.2, 25.2)	8.6	8.33 (8.07, 8.70)	282	82.9	70.2
40 % Effluent	0.02	24.5 (23.2, 25.1)	8.7	8.25 (7.86, 8.65)	312	82.8	68.0
80 % Effluent	0.02	24.5 (23.7, 25.1)	8.7	8.18 (7.72, 8.71)	338	82.9	64.8
100 % Effluent	0.03	24.9 (24.4, 25.1)	9.0	8.06 (7.55, 8.61)	356	82.7	65.2

Table 23. 96 Hour Mean (*n*=4) Cell Density of the Green Algae *S. capricornutum* After Exposure to Whole Effluent Collected from Treatment Sample Collection Tub #6 (TRIAL 2 EFFLUENT).

Treatment Group	Average Cells/mL ± Std. Error	Comments
***Selenastrum* Performance Control**	1,635,000 ± 163,079	The cell density of EPA Nutrient Media (i.e., performance control) is significantly greater (p<0.05) than that of Filtered Duluth-Superior Harbor Water (i.e., test control).
0 % Effluent Control	877,678 ± 20,386	The control for this WET test (i.e., Filtered Duluth-Superior Harbor Water) did not pass the acceptance criterion for 96-hour cell density (must be greater than 1 x 10^6 cells/mL). Results of statistical analysis should be interpreted cautiously.
40 % Effluent	1,167,500 ± 95,601	The differences in mean cell density compared to the 0% Effluent Control is not greater than would be expected by chance; there is not a statistically significant difference.
80 % Effluent	1,088,333 ± 3 7,822	The differences in mean cell density compared to the 0% Effluent Control is not greater than would be expected by chance; there is not a statistically significant difference.
100 % Effluent	1,352,500 ± 81,208	The differences in mean cell density compared to the 0% Effluent Control is not greater than would be expected by chance; there is not a statistically significant difference.

Table 24. Average Values (Minimum, Maximum) for Water Chemistry Parameters of Stock Solutions Used to Conduct the *C. dubia* and *P. promelas* WET Tests (TRIAL 2 EFFLUENT).

Treatment Group	TRO (mg/L as Cl_2)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Conductivity (µS/cm)	Day 0 Hardness (mg/L $CaCO_3$)	Day 0 Alkalinity (mg/L $CaCO_3$)
***P. promelas* Performance Control**	<0.005	23.5 (23.3, 23.7)	8.1 (7.6, 8.4)	7.99 (7.88, 8.05)	134 (128, 162)	48.8	52.2
***C. dubia* Performance Control**	<0.005	25.2 (23.9, 26.4)	7.9 (7.7, 8.1)	8.49 (8.44, 8.53)	550 (531, 576)	176.6	113.4
0 % Effluent Control	0.01 (0.01, 0.02)	25.2 (24.1, 26.2)	9.6 (9.2, 10.0)	7.94 (7.88, 7.99)	206 (191, 217)	51.4	60.4
40 % Effluent	0.02 (0.02, 0.02)	24.5 (23.7, 25.2)	9.0 (8.7, 9.2)	7.81 (7.73, 7.86)	227 (226, 228)	67.5	63.6
80 % Effluent	0.02 (0.02, 0.03)	24.3 (23.6, 25.0)	9.2 (8.8, 9.5)	7.66 (7.58, 7.76)	256 (251, 258)	67.8	57.4
100 % Effluent	0.02 (0.02, 0.03)	24.3 (23.5, 26.7)	9.7 (8.7, 10.5)	7.53 (7.44, 7.65)	274 (270, 281)	66.3	57.2

Table 25. Average Values (minimum, maximum) for Water Chemistry Parameters of Exposure Solutions (Day 1 – Day 7) Used to Conduct *P. promelas* WET Test (TRIAL 2 EFFLUENT).

Treatment Group	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Day 7 Hardness (mg/L $CaCO_3$)	Day 7 Alkalinity (mg/L $CaCO_3$)
***P. promelas* Performance Control**	23.6 (21.7, 24.9)	6.5 (6.0, 6.9)	7.68 (7.60, 7.77)	46.4	48.8
0 % Effluent Control	23.5 (20.0, 24.7)	6.6 (6.0, 6.8)	7.82 (7.71, 7.90)	Sample was Lost.	61.0
40 % Effluent	23.9 (23.2, 25.2)	6.3 (5.5, 7.1)	7.78 (7.64, 7.88)	67.1	61.2
80 % Effluent	23.9 (23.2, 24.9)	6.0 (5.0, 7.0)	7.70 (7.55, 7.84)	67.0	58.2
100 % Effluent	23.6 (23.1, 24.6)	6.2 (5.6, 7.0)	7.73 (7.62, 7.88)	67.3	58.6

Table 26. *P. promelas* Mean (*n*=4) Percent Survival and Average Weight per Individual after Exposure to Whole Effluent Collected from Treatment Sample Collection Tub #6 (TRIAL 2 EFFLUENT).

Treatment Group	Percent Survival ± Std. Error	Mean Average Weight/Fish (mg) ± Std. Error	Comments
***P. promelas* Performance Control**	100 ± 0	0.37 ± 0.01	The differences in the mean values of survival and average weight per fish compared to the 0% Effluent Control are not greater than would be expected by chance; there is not a statistically significant difference.
0 % Effluent Control	100 ± 0	0.38 ± 0.01	-----
40% Effluent	100 ± 0	0.39 ± 0.01	The differences in the mean values of survival and average weight per fish compared to the 0% Effluent Control are not greater than would be expected by chance; there is not a statistically significant difference.
80% Effluent	100 ± 0	0.42 ± 0.01	The differences in the mean values of survival and average weight per fish compared to the 0% Effluent Control are not greater than would be expected by chance; there is not a statistically significant difference.
100% Effluent	100 ± 0	0.39 ± 0.01	The differences in the mean values of survival and average weight per fish compared to the 0% Effluent Control are not greater than would be expected by chance; there is not a statistically significant difference.

Table 27. Average Values (Minimum, Maximum) for Water Chemistry Parameters of Exposure Solutions (Day 1 – Day 7) Used to Conduct *C. dubia* WET Test (TRIAL 2 EFFLUENT).

Treatment Group	Temperature (°C)	pH	Day 7 Hardness (mg/L $CaCO_3$)	Day 7 Alkalinity (mg/L $CaCO_3$)
***C. dubia* Performance Control**	24.6 (23.9, 25.5)	8.50 (8.45, 8.61)	163.2	108.6
0 % Effluent Control	24.1(23.4, 24.9)	8.29 (8.17, 8.53)	67.4	60.2
40 % Effluent	24.1(23.5, 24.7)	8.23 (8.15, 8.44)	66.8	58.0
80 % Effluent	24.6 (24.0, 25.1)	8.20 (8.11, 8.38)	67.5	57.2
100 % Effluent	24.4 (23.7, 24.9)	8.21 (8.13, 8.40)	67.5	57.6

Table 28. *C. dubia* Mean (*n*=10) Percent Survival and Total Number of Offspring Produced in a Three-brood WET Test after Exposure to Whole Effluent Collected from Treatment Sample Collection Tub #6 (TRIAL 2 EFFLUENT).

Treatment Group	Percent Survival ± Std. Error	Average Total Number of Young per Female ± Std. Error	Comments
***C. dubia* Performance Control**	80 ± 13.3	3.5 ± 1.5	The performance control for this WET test (i.e., *C. dubia* Culture Water) did not pass the acceptance criterion for reproduction (must have a minimum average total of 15 young per female). The results of the performance control are reported, but were excluded from the statistical analysis.
0% Effluent Control	90 ± 10.0	16.8 ± 2.9	-----
40% Effluent	90 ± 10.0	13.2 ± 2.7	The differences in the mean values of survival and average number of young per female are not statistically different compared to the 0% Effluent Control.
80% Effluent	100 ± 0.0	17.6 ± 2.1	The differences in the mean values of survival and average number of young per female are not statistically different compared to the 0% Effluent Control.
100% Effluent	100 ± 0.0	18.7 ± 1.3	The differences in the mean values of survival and average number of young per female are not statistically different compared to the 0% Effluent Control.

Table 29. Average Values (Minimum, Maximum) for Water Chemistry Parameters of Exposure Solutions Used to Conduct *S. capricornutum* WET Test (TRIAL 4 EFFLUENT).

Treatment Group	TRO (mg/L as Cl_2)	Temp. (°C)	Day 0 Dissolved Oxygen (mg/L)	pH	Day 0 Conductivity (µS/cm)	Day 0 Hardness (mg/L $CaCO_3$)	Day 0 Alkalinity (mg/L $CaCO_3$)
***Selenastrum* Performance Control**	<0.005	23.7 (21.2, 24.7)	6.5	7.71 (7.54, 7.89)	47	14.3	10.8
0 % Effluent Control	0.01	24.3 (22.9, 25.1)	9.9	8.24 (8.01, 8.49)	228	74.6	62.2
40 % Effluent	0.01	24.6 (23.9, 25.1)	8.9	8.20 (7.85, 8.52)	275	77.2	63.4
80 % Effluent	0.02	24.5 (23.6, 25.6)	8.9	8.13 (7.71, 8.51)	309	77.3	64.2
100 % Effluent	0.02	24.6 (24.0, 25.0)	8.9	8.06 (7.59, 8.47)	325	78.3	64.2

Table 30. 96 Hour Mean (*n*=4) Cell Density of the Green Algae *S. capricornutum* after Exposure to Whole Effluent Collected From Treatment Sample Collection Tub #6 (TRIAL 4 EFFLUENT).

Treatment Group	Average Cells/mL ± Std. Error	Comments
***Selenastrum* Performance Control**	2,425,000 ± 101,678	There is a significant difference ($p<0.05$) in mean cell density compared to the 0% Effluent Control.
0 % Effluent Control	1,383,125 ± 129,395	-----
40 % Effluent	1,468,125 ± 140,684	The differences in mean cell density compared to the 0% Effluent Control is not greater than would be expected by chance; there is not a statistically significant difference.
80 % Effluent	1,187,500 ± 82,601	The differences in mean cell density compared to the 0% Effluent Control is not greater than would be expected by chance; there is not a statistically significant difference.
100 % Effluent	904,523 ± 61,058	The differences in mean cell density compared to the 0% Effluent Control is not greater than would be expected by chance; there is not a statistically significant difference.

Table 31. Average Values (Minimum, Maximum) for Water Chemistry Parameters of Stock Solutions Used to Conduct the *C. dubia* and *P. promelas* WET Tests (TRIAL 4 EFFLUENT).

Treatment Group	TRO (mg/L as Cl_2)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Conductivity (µS/cm)	Day 0 Hardness (mg/L $CaCO_3$)	Day 0 Alkalinity (mg/L $CaCO_3$)
P. promelas Performance Control	<0.005	23.4 (23.1, 23.7)	7.9 (7.3, 8.3)	7.85 (7.63, 8.02)	130 (120, 147)	46.6	48.4
C. dubia Performance Control	<0.005	23.2 (22.5, 23.9)	8.5 (8.2, 8.8)	8.47 (8.45, 8.50)	554 (544, 565)	166.4	111.6
0 % Effluent Control	0.01 (0.01, 0.01)	25.0 (23.7, 27.1)	10.2 (9.8, 10.9)	7.94 (7.90, 8.00)	161 (159, 168)	63.6	54.2
40 % Effluent	0.01 (0.01, 0.02)	25.3 (23.8, 27.1)	9.5 (9.2, 9.7)	7.79 (7.76, 7.81)	192 (191, 193)	63.7	55.6
80 % Effluent	0.02 (0.01, 0.02)	25.1 (23.4, 27.1)	9.7 (9.1, 9.9)	7.63 (7.58, 7.70)	229 (228, 229)	66.2	54.2
100 % Effluent	0.02 (0.02, 0.03)	25.2 (23.6, 26.8)	10.5 (9.2, 11.0)	7.48 (7.41, 7.54)	246 (245, 247)	66.6	55.2

Table 32. Average Values (Minimum, Maximum) for Water Chemistry Parameters of Exposure Solutions (Day 1 – Day 7) Used to Conduct *P. promelas* WET Test (TRIAL 4 EFFLUENT).

Treatment Group	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Day 7 Hardness (mg/L $CaCO_3$)	Day 7 Alkalinity (mg/L $CaCO_3$)
***P. promelas* Culture Performance Control**	24.4 (23.4, 25.1)	6.8 (6.1, 7.4)	7.69 (7.56, 7.83)	46.5	54.0
0 % Effluent Control	24.5 (23.4, 25.2)	6.6 (5.7, 7.3)	7.75 (7.60, 7.85)	61.1	57.0
40 % Effluent	24.4 (23.4, 25.1)	6.5 (5.7, 6.9)	7.76 (7.62, 7.86)	62.6	56.2
80 % Effluent	24.8 (24.1, 25.7)	6.3 (5.8, 6.9)	7.72 (7.59, 7.86)	62.7	55.3
100 % Effluent	24.7 (23.7, 25.5)	6.4 (5.8, 7.5)	7.70 (7.62, 7.82)	64.6	55.4

Table 33. *P. promelas* Mean (*n*=4) Percent Survival and Average Weight per Individual after Exposure to Whole Effluent Collected from Treatment Sample Collection Tub #6 (TRIAL 4 EFFLUENT).

Treatment Group	Percent Survival ± Std. Error	Mean Average Weight/Fish (mg) ± Std. Error	Comments
***P. promelas* Performance Control**	100 ± 0	0.42 ± 0.02	The differences in the mean values of survival and average weight per fish compared to the 0% Effluent Control are not greater than would be expected by chance; there is not a statistically significant difference.
0 % Effluent Control	98 ± 3	0.43 ± 0.01	-----
40 % Effluent	100 ± 0	0.44 ± 0.02	The differences in the mean values of survival and average weight per fish compared to the 0% Effluent Control are not greater than would be expected by chance; there is not a statistically significant difference.
80 % Effluent	101 ± 3	0.42 ± 0.02	The differences in the mean values of survival and average weight per fish compared to the 0% Effluent Control are not greater than would be expected by chance; there is not a statistically significant difference.
100 % Effluent	100 ± 0	0.44 ± 0.01	The differences in the mean values of survival and average weight per fish compared to the 0% Effluent Control are not greater than would be expected by chance; there is not a statistically significant difference.

Table 34. Average Values (Minimum, Maximum) for Water Chemistry Parameters of Exposure Solutions (Day 1 – Day 7) Used to Conduct *C. dubia* WET Test (TRIAL 4 EFFLUENT).

Treatment Group	Temperature (°C)	pH	Day 7 Hardness (mg/L $CaCO_3$)	Day 7 Alkalinity (mg/L $CaCO_3$)
***C. dubia* Performance Control**	24.1 (23.0, 25.2)	8.42 (8.35, 8.50)	165.8	113.4
0 % Effluent Control	24.3 (22.9, 25.7)	8.17 (8.08, 8.32)	60.2	57.0
40 % Effluent	24.4 (23.2, 25.5)	8.11 (7.99, 8.23)	61.9	56.1
80 % Effluent	24.3 (22.6, 25.7)	8.13 (8.07, 8.22)	63.0	55.9
100 % Effluent	24.3 (21.9, 25.8)	8.13 (8.03, 8.21)	65.6	55.4

Table 35. *C. dubia* Mean (*n*=10) Percent Survival and Total Number of Offspring Produced in a Three-Brood WET Test after Exposure to Whole Effluent Collected from Treatment Sample Collection Tub #6 (TRIAL 4 EFFLUENT).

Treatment Group	Percent Survival ± Std. Error	Average Total Number of Young per Female ± Std. Error	Comments
***C. dubia* Performance Control**	70 ± 13	4.5 ± 1.1	The performance control for this WET test (i.e., *C. dubia* Culture Water) did not pass the acceptance criteria for survival (≥80%) or reproduction (must have a minimum average total of 15 young per female). The results of the performance control are reported, but were excluded from the statistical analysis.
0 % Effluent Control	90 ± 10	26.0 ± 4.5	-----
40 % Effluent	100 ± 0	29.1 ± 3.5	The differences in the mean values of survival and average number of young per female are not statistically different compared to the 0% Effluent Control.
80 % Effluent	100 ± 0	31.1 ± 2.8	The differences in the mean values of survival and average number of young per female are not statistically different compared to the 0% Effluent Control.
100 % Effluent	100 ± 0	21.0 ± 1.6	The differences in the mean values of survival and average number of young per female are not statistically different compared to the 0% Effluent Control.

8.2.3.3. Disinfection Byproducts (DBP)

Measurable concentrations of many of the THM and HAA were found in the neutralized treatment discharge (Table 36). Total THM concentrations ranged from 165 μg/L to 415 μg/L (Table 36). Total HAA concentrations ranged from 181 μg/L to 292 μg/L (Table 36). Trichloroacetic acid was the major component of HAA with dichloroacetic acid also having a sizeable contribution (Table 36).

Table 36. Summary of Disinfection Byproducts (DBP) Present in Treatment Discharge by Trial. ND = Not Detected.

		Trial 1 (μg/L)	Trial 2 (μg/L)	Trial 3 (μg/L)	Trial 4 (μg/L)
Trihalomethanes (THM)	Bromodichloromethane	11.4	16.9	11.0	8.8
	Bromoform	ND	ND	ND	ND
	Chlorodibromomethane	ND	0.55	0.66	0.66
	Chloroform	362	397	190	154
	Total Trihalomethanes	**375**	**415**	**202**	**165**
Haloacetic Acids (HAA)	Bromochloroacetic	5.9	7.4	6.2	4.5
	Dibromoacetic	ND	ND	ND	ND
	Dichloroacetic	95.6	103	63.4	57.9
	Monobromoacetic	ND	ND	ND	ND
	Monochloroacetic	6.2	9.5	ND	4.5
	Trichloroacetic	184	163	131	114
	Total Haloacetic Acids	**292**	**283**	**201**	**181**

9. DISCUSSION OF RESULTS

The low variability between chlorine concentrations (measured as TRO) in samples collected from the top, middle and bottom of the treatment retention tank indicates that the GSI retention tank agitators succeeded in assuring the water was well mixed for the treatment effectiveness tests. The effectiveness of the BWTS developer's mixing system will need to be similarly validated on board all relevant types of vessels.

The BWTS developer's methods for determining chlorine demand proved effective at calculating the appropriate volume of 6.15 % NaOCl solution to be added to the GSI Land-Based RDTE Facility's retention tanks in order to achieve the 7-hour target available treatment concentration of 5 mg/L of chlorine. The BWTS developer's methods for neutralizing the treated water required multiple neutralization steps, which may not be ideal in an actual shipboard situation. The ratio of 1.6 mg/L neutralizing agent per mg/L of chlorine (plus a 10 % margin of safety) worked well if the neutralizing agent was 40% (w/v) $Na_2S_2O_3$. However, this theoretical calculation did not work well in practice when using 40% (w/v) $NaHSO_3$, the agent recommended by the BWTS developer. The $NaHSO_3$ may have been reacting with other residual oxidants in the test waters or treatment retention tank, thereby diminishing the chlorine neutralization effect. It may be possible to add a neutralizer demand test to the procedures in order to ensure neutralization. A higher safety margin for the dose may also work given the relatively small amount of neutralizer needed in the additional neutralization steps. The

solubility properties of the DPD reagent used in the TRO analysis also appeared sensitive to temperature; colder temperature water samples had to be warmed slightly for the reagent to dissolve effectively.

In terms of biological efficacy, the treatment met the IMO performance benchmark in the context of these tests for phytoplankton but not zooplankton. Live densities of organisms generally ≥ 50 μm in size in treatment discharge were lower than control discharge densities, but well above the IMO ballast water performance standard requirement of < 10 live organisms/m^3. In comparison, live densities of phytoplankton (i.e., organisms generally ≥ 10 and < 50 μm) in the treatment discharge were few, and well within the < 10 organisms per mL benchmark set by IMO.

Few solid conclusions about BWTS effectiveness on organisms < 10 μm can be drawn from these tests. Test results show both *E. coli* and *Enterococcus* bacteria densities in treatment discharge were less than the limit of detection (i.e., < 1 MPN/100 mL) across all trials, and their respective IMO benchmarks. However, initial (i.e., pre-treatment intake) densities of *E. coli* and *Enterococcus* spp. were also well below the IMO benchmark. Subtle reductions evident in densities of total culturable heterotrophic bacteria and total coliform bacteria cannot be associated with a performance standard.

In terms of environmental acceptability, the WET dose-response evaluations undertaken in these tests showed no toxic effects on *C. dubia, P. promelas*, or *S. capricornutum*. These preliminary results suggest no residual toxicity (acute or chronic) associated with organism exposure to the NaOCl BWTS treatment discharge as whole effluent. Two classes of DBP were in treatment discharge samples collected during all four trials of the NaOCl BWTS: THM and HAA. GSI did not measure DBPs in control discharge during these trials, however, results from a previous land-based BWTS performance evaluation showed that the concentration of THM and HAA in untreated harbor water were less than the limit of detection (< 2.0 μg/L and < 11.0 μg/L, respectively; GSI, 2010).

10. CONCLUSIONS

The performance of the NaOCl BWTS was evaluated in terms of operational and biological efficacy, and environmental acceptability during four trials conducted in October 2011 at the GSI Land-Based RDTE Facility. The GSI test yielded mixed results relative to GSI's test objectives. In terms of operational performance, GSI was able to accurately dose a sampled volume of water with 6.15 % NaOCl solution to a predetermined chlorine concentration by factoring in the natural chlorine demand. The neutralization process recommended by the BWTS developer was problematic in that it required numerous iterations, and a deviation from the protocol, for the GSI team to achieve neutralization within the time-frame available for the test. More research and development are needed on the effect of temperature and water quality on the ability of 40% (w/v) $NaHSO_3$ to successfully neutralize NaOCl-treated water. Second, the BWTS reduced live densities of organisms ≥ 50 μm, which were adequately plentiful in the intake to meet IMO testing guidelines, relative to control discharge. But live densities of these organisms in BWTS discharge were well above the IMO standard (IMO, 2004). The BWTS did reduce live densities of organisms > 10 and <50 μm minimum dimension to below benchmark levels within the IMO

Convention, but intake densities of these organisms also were below IMO testing guidelines due to the late season timing of the tests (IMO, 2004). . These tests produced no conclusive results for BWTS effectiveness on organisms < 10 μm in size (i.e., bacteria and viruses) due to low intake densities for organisms in this size class for which a standard exists. Finally, the treated and neutralized discharge water was found to be safe to discharge (though, in some cases only after multiple neutralization steps) and free from residual toxicity in these tests. However, measurable concentrations of DBP were found in the treatment discharge, specifically THM and HAA. Overall, the NaOCl BWTS both warrants and requires further research and development on its potential application as an emergency BWTS with relevancy in the Great Lakes.

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APPENDIX A – BWTS Sodium Hypochlorite Dosing Standard Operating Procedure Imitating Emergency Treatment of ships in Travel Status through the Great Lakes.

Materials:

Hose, funnel, and containers for holding biocide and neutralizers.
6.15% Bleach
40% bisulfite solution (instructions are for bisulfite, follow SOP for a different neutralizer such as thiosulfate to account for differences in concentration and reaction).
Colorimetric equipment, including glassware and reagents – Any will do, but we have attached information for the Hach Colorimeter II
Glassware – size depends on the volume of water to be treated.

Procedure:

1. Use safety measures as prescribed by the MSDS sheets for the chlorine.
2. Determine ambient chlorine demand of harbor water
    a. Take three 1 L samples of test tank water from the tanks.
    b. Add 10mg/L NaOCl per 1 L sample

10 mg/L x 1g/1000mg = 0.01 grams CL_2 $\frac{0.01\text{ g } CL_2}{6.15\text{ g } CL_2/100\text{ml}}$ = 0.163 ml 6.15% NaOCl solution

    c. After 24 hours of an un-agitated sample, measure total residual chlorine (TRC). Any method may be used, but if using the Hach Pocket Colorimeter II refer to the attached manual. If less time is used to determine demand, the sample container must be mixed and held for at least 7 hours. Monitor drop in chlorine at 1, 3 and 7 hours using wither method.
    d. Subtract the TRC from the initial chlorine dose (10mg/L) to get the ambient chlorine demand of harbor water

Ambient Chlorine Demand = Initial chlorine dose – TRC

3. Dose test tanks with 5 mg/L above ambient chlorine demand.

Ambient Chlorine Demand + 5mg/L = Chlorine dose *E.g., 3 mg/L + 5 mg/L = 8 mg/L*

    a. Add 5 mg/L to the ambient chlorine demand to determine desired chlorine dose

b. Calculate amount of NaOCl solution needed
    i. Take the chlorine dose times the amount of ballast water to treat in liters divide by 1000 divide by 6.15 times 100 divided by 1000 to get the liters of 6.15% solution to add to the ballast water.

> *Example uses 100 m^3 treatment tanks and 8 mg/L dose:*
> 100 m^3 x 1000 L/1 m^3 = 100,000 L
> 8 mg/L x 100,000 L x 1 g/1000 mg = 800 g CL_2
> $\frac{800\text{ g } CL_2}{6.15\text{ g } CL_2/100\text{ml}}$ x 1L/1000 ml = 13.0 L of 6.15% NaOCl solution

    ii. When treating multiple tanks, make the calculation for each tank separately.
c. Dose the tanks with the appropriate chlorine dose using the funnel and hose.
d. Triple rinse the hose and funnel into the final tank.

4. Mix each tank.
5. Let each tank sit for 7 hours (or longer, if time allows).
6. Neutralize tanks using 40% $NaHSO_3$ solution
    a. Measure the Total Residual Chlorine (TRC)
    b. Calculate amount of neutralizer. The dosing rate is 1.6 mg/L of $NaHSO_3$ per mg/L CL2 removed based on stoichiometric requirements plus 10% margin of safety. Take the TRC times 1.6 divide by the quantity of ballast water in liters divided by 1000 divided by 40 times 100 divided by 1000 to get the amount of 40% $NaHSO_3$ solution to add.

> *Example uses 100 m^3 (100,000 L) treatment tanks and 2 mg/L TRC:*
> 2 mg/L CL_2 x $\frac{1.6\text{mg/L } NaHSO_3}{1.0\text{mg/L } CL_2}$ = 3.2 mg/L $NaHSO_3$
> 3.2 mg/L $NaHSO_3$ x 100,000 L x 1 g/1000mg = 320 g $NaHSO_3$
> $\frac{320\text{ g } NaHSO_3}{40\text{ g } NaHSO_3/100\text{ml}}$ x 1 L/1000 ml = 0.8 L of 40% $NaHSO_3$ solution

    c. Dose the tanks with the appropriate chlorine dose using the funnel and hose.
    d. Triple rinse the hose and funnel into the final tank.
7. Mix each tank.
8. Let each tank sit for 7 hours. Monitor residual at 1, 3 and 7 hours to determine if tank can be discharged earlier.
9. Measure the Total Residual Chlorine using the same methods as above.
10. If TRC is above allowable discharge level, re-neutralize the tanks using the above procedure.
11. Discharge treatment water.