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

Hydrated Lime, $Ca(OH)_2$

May 13, 2011

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ABSTRACT

The Great Ships Initiative (GSI) conducts bench-scale (i.e., laboratory) research to aid developers of innovative technologies which could have application as ballast water treatment systems (BWTSs). This report describes 2009 findings from bench-scale evaluations of a treatment proposed by researchers from the U.S. Geological Survey's Leetown Science Center in Kearneysville, West Virginia. The treatment utilizes a calcium hydroxide ($Ca(OH)_2$) slurry, commonly known as hydrated lime, to raise the pH of the ballast water to a level greater than 11.5 and is intended for use as a routine ballast water treatment. GSI conducted range-finding tests on this proposed treatment to determine pH stability after dosing, dose effectiveness, and residual toxicity. Please see www.greatshipsinitiative.org for more information about GSI's bench-scale testing program.

In general, the higher two pH levels (12.0 and 12.2) were effective at significantly reducing live concentrations of the entire range of aquatic organisms tested in experimental water, with live densities of green algae, adult rotifers, adult copepods and < 24 hour old cladocerans falling to below 1 % within 48 hours. In many cases, the planned experiment duration (i.e., 24 or 48 hrs) was not met because complete mortality was observed within a matter of two hours. In the sediment dose effectiveness experiments, after the full 48 hours, *C. dilutus* had a reduced (68 %) survival in the pH 11.5 treatment, and a significantly reduced 25 % and 3 % survival in the pH 12.0 treatment and pH 12.2 treatment, respectively. Annelid *L. variegatus* was more sensitive, showing significant reductions at all three pH levels after 48 hours to 0% for the 12.0 and 12.5 pH exposures, and 3% for the 11.5 pH exposure. Percent survival for all three sensitive organisms tested in the residual toxicity experiments was high and ranged from 90 - 100 %.

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INTRODUCTION

This technical report presents quantitative findings from GSI bench-scale evaluations of a ballast water treatment system (BWTS) proposed by researchers from the U.S. Geological Survey's Leetown Science Center in Kearneysville, West Virginia, with possible application to the Great Lakes. The BWTS utilizes calcium hydroxide, $Ca(OH)_2$, commonly known as hydrated lime, to increase the pH of the ballast water and is intended for use as a routine ballast treatment. GSI undertook these bench-scale tests during 2009 at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior (UW-S) in Superior, Wisconsin. Preliminary testing of the system involved:

- pH stability tests, including determination of the influence of organic matter on the stability of pH over the course of 96 hours;
- Dose effectiveness tests, including determination of the effects of different levels of pH on a suite of freshwater organisms such algae, zooplankton and two species of benthic organisms; and
- Residual toxicity tests, including the determination of the effect on sensitive freshwater organisms after dilution of treated water.

BACKGROUND

Great Ships Initiative (GSI)

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

GSI awards its independent status-testing services to developers of BWTSs and processes determined to be promising. GSI status-testing is performed at the scale appropriate to the state of development of the target treatment system, with the goal of facilitating the rapid progression of meritorious BWTSs through the research and development and approval processes to a market-ready condition.

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

Organization

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

Ms. Allegra Cangelosi of NEMWI is GSI's Principal Investigator and Director (GSI PI). A GSI Advisory Committee comprising top-level officials of key stakeholder groups provides direct input to Ms. Cangelosi, advising on GSI award decisions, program direction, finances and fund-raising. The American Great Lakes Ports Association advises the project, assuring that the GSI is meeting the needs of the maritime industry; and coordinating maritime industry and supply chain outreach. Researchers from UW-S's Lake Superior Research Institute (LSRI) and the UM-D's Natural Resources Research Institute (NRRI), among others, provide critical scientific and technical expertise and implementation services to the GSI PI. Dr. Mary Balcer of LSRI is the project's lead zooplankton ecologist. She is also the team leader for LSRI staff engaged in GSI research activities. Dr. Euan Reavie of the NRRI leads all phytoplankton analysis and NRRI staff. Mr. Matthew TenEyck of LSRI leads all bench-testing and Whole Effluent Toxicity (WET) tests. Mr. Tom Markee of LSRI is responsible for GSI chemical analysis. Ms. Heidi Saillard of LSRI is responsible for GSI microbial analysis. Ms. Nicole Mays of NEMWI is GSI's Senior Quality Systems Officer and Ms. Kelsey Prihoda of LSRI is GSI's Senior QAQC Officer.

Projects and Activities

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

GSI Bench-Scale Tests

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

GSI Land-Based Tests

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

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

GSI Shipboard Tests

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

GSI BENCH-SCALE TESTS

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

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

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

GSI bench-scale chemical degradation tests determine the effect that various water quality or environmental parameters may have on the rate of chemical degradation of a BWTS involving active substances. No organisms are used in association with these analytical assays; instead test solutions are analyzed for their concentration of active substance (or active component of the substance). Test results are typically expressed as the percent change in active ingredient concentration.

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

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

METHODS

Treatment System

Treatment and Test Apparatus

In these tests, the treatment was direct addition of a calcium hydroxide $Ca(OH)_2$ slurry to filtered Duluth-Superior Harbor water (FHW). Specifically, the slurry was 35 % by weight $Ca(OH)_2$ which adjusted the pH of the FHW from its normal pH range of 7.6 - 8.0 to a pH of 11.5, 12.0, or 12.2.

Experiments took place in an LSRI laboratory equipped with adequate ventilation, electrical connections, and climate control. Test apparatus generally consisted of several 300 mL borosilicate high-form beakers housed within environmental chambers set to the appropriate temperature and light regime.

Treatment Application

To prepare the 35 % by weight $Ca(OH)_2$ slurry, $Ca(OH)_2$ was added to deionized water in the ratio of 3.5 g $Ca(OH)_2$ to 6.5 g water. Aliquots of the slurry were added to the test water by dipping a stir rod into the slurry and placing the tip of the stir rod with the slurry adhering to it into the test water. This process was continued until the target pH was achieved. The stir bar was then turned off, though still positioned in the test water, thereby allowing the larger clumps of $Ca(OH)_2$ to settle out. The overlying treated test water was then poured off for use in the experiments. This procedure produced cloudy test mixtures, indicating that the $Ca(OH)_2$ was not completely dissolved.

General Methods

Sample Water Preparation

Four experimental water qualities were prepared in the laboratory as follows:

- Laboratory water (LW): Treated Lake Superior water from the City of Superior that was passed through an activated carbon column. Alkalinity ranged from 45 - 50 mg/L as $CaCO_3$. Nonpurgeable organic carbon (NPOC) ranged from 0.5 - 2.0 mg/L. The water is transparent.
- High organic content laboratory water (HOC): LW as described above with addition of tannic (15 mg/L) and humic acids (5 mg/L) to adjust the NPOC to approximately 8 mg/L. The water is stained and has a lower light transmittance when compared to LW. The enriched NPOC level is also reflective of Duluth-Superior Harbor water.
- Filtered Duluth-Superior Harbor water (FHW): Harbor water collected from a depth of approximately 3 m in the Duluth-Superior Harbor and filtered through a Whatman 934-AH glass fiber filter prior to use. Alkalinity ranges from 65-69 mg/L as $CaCO_3$. NPOC range is 16-22 mg/L. The water is stained with tannins.
- 50 % high organic content water (HOC50): High organic content water as described above but diluted with an equal volume of LW.

Experimental water temperature was adjusted to the desired level of 25 ± 3 °C by placing the beaker containing the appropriate amount of water in an incubator set to the desired test temperature.

Chemical Analysis

Measurements of pH were made using an Orion Model 290A pH/ISE meter and a Corning combination pH electrode. The pH meter was calibrated daily prior to use using pH buffer solutions certified at pH 4.00, 7.00, and 10.00 (Fisher Scientific, Pittsburgh, PA). Buffers and samples were at room temperature when calibration was performed and pH measurements were conducted.

Experimental Methods

pH Stability Experiments

GSI bench-scale tests were undertaken to determine the effect of the above-described water qualities on the stability of pH. Tests were performed using LW, HOC water, and HOC50 water. In these experiments the pH of each water type was adjusted to 11.5 or 12.2; unadjusted water served as control. Triplicate 200 mL aliquots of each treatment and control water quality were transferred to 300 mL beakers, covered with a glass plate, and placed in an incubator set at 25 ºC and in complete darkness for the duration of the experiment (i.e., 96 hours). The pH was measured in replicate samples every 24 hours over the 96 hour test period.

Dose Effectiveness Experiments

GSI dose effectiveness tests measured the effect of three pH levels (11.5, 12.0, and 12.2) in FHW, and a control (no pH adjustment; natural pH of 7.6 - 8.0) on a range of freshwater organisms known to be relatively resilient to stressors. Test organisms included the green algae *Selenastrum capricornutum,* the adult rotifer *Branchionus calyciflorus,* resting eggs of *Branchionus calyciflorus,* adult copepods *Eucyclops sp.* and the cladoceran *Daphnia magna.*

All exposures, except for those involving algae, rotifer adults and rotifer resting eggs, took place in complete darkness over 48 hours, with 50 mL of exposure solution held in a 300 mL beaker, and three replicates per exposure. Tests with algae took place in an Erlenmeyer flask with a foam plug on a shaker table in complete darkness. Tests on rotifer adults and rotifer resting eggs involved four replicates and 2.0 mL of exposure solution. In addition, adult rotifers were exposed in complete darkness for 24 hours. The rotifer resting eggs were exposed to continuous light to stimulate hatching. All samples were held at a temperature of 25.0 ºC ± 3.0 ºC and test water was not renewed.

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

Table 1. Exposure Conditions for GSI Dose Effectiveness Tests on Algae and Zooplankton in Filtered Duluth-Superior Harbor Water.

Organism	Exposure Volume per Replicate (mL)	Exposure Duration (hr)	Light:Dark Cycle (hr)	Temperature (º C)
Green alga (*Selenastrum capricornutum*)	50	48	0:24	25 ± 3.0
Newly hatched rotifers, (*Brachionus calyciflorus*)	2	24	0:24	25 ± 3.0
Rotifer resting eggs,(*Brachionus calyciflorus*)	2	48	24:0	25 ± 3.0
Copepods (*Eucyclops* spp.)	50	48	0:24	25 ± 3.0
Cladoceran (*Daphnia magna*)	50	48	0:24	25 ± 3.0

Table 2. Numbers and Types of Organisms, and pH Levels Used for GSI Dose Effectiveness Tests on Algae and Zooplankton in Filtered Duluth-Superior Harbor Water.

Organism Type	Species	pH Levels	No. of Organisms per Exposure /Control	No. of Replicates per Exposure /Control
Green alga	*Selenastrum capriornutum*	11.5, 12.0, and 12.2, and a control (no pH adjustment; natural pH of 7.6 - 8.0)	10,000,000	3
Adult rotifers	*Brachionus calyciflorus*	11.5, 12.0, and 12.2, and a control (no pH adjustment; natural pH of 7.6 - 8.0)	5	4
Rotifer resting eggs	*Brachionus calyciflorus*	11.5, 12.0, and 12.2, and a control (no pH adjustment; natural pH of 7.6 - 8.0)	20	4
Adult copepods	*Eucyclops* spp.	11.5, 12.0, and 12.2, and a control (no pH adjustment; natural pH of 7.6 - 8.0)	10	3
< 24 hour old Cladocerans	*Daphnia magna*	11.5, 12.0, and 12.2, and a control (no pH adjustment; natural pH of 7.6 - 8.0)	10	3

Sediment Dose Effectiveness Experiments

Sediment dose effectiveness tests were conducted using two benthic organisms, *Chironomus dilutus* and *Lumbriculus variegatus*. In these tests,sediment from a depth of one meter was collected near Barker's Island on the Duluth-Superior Harbor of Lake Superior (Superior, WI), sieved to remove large debris and homogenized. Test units were prepared by placing 100 mL of sediment in 300 mL test beakers. Next 100 mL of untreated test water (i.e., FHW) were added gently so as not to mix the sediment into the water column. Ten 10-13 day-old *C. dilutus* larvae or ten *L. variegatus* organisms of mixed age were then randomly added to each beaker. Beakers were placed in an incubator set at 25.0 ºC ± 1.0 ºC in complete darkness to allow the organisms to acclimate and burrow into the test sediment for 24 hours.

After 24 hours, overlying water was siphoned from the exposure beakers. Next, 100 mL of control water (FHW) or 100 mL of FHW with pH adjusted to 11.5, 12.0, or 12.2 was added such that the floc on the surface of the sediment was mixed into the water column. Beakers were placed back into an incubator set at 25°C.

Periodic observations were made over a 48 hour test period, though definitive counts of mortality were not made until 48 hours because of destructive sampling. Water quality parameters including temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness were measured. Table 3 describes the exposure conditions, numbers of organisms analyzed, and the number of replicates per each sediment dose effectiveness test.

Table 3. Numbers and Types of Organisms, and pH Levels Used for GSI Sediment Dose Effectiveness Tests on Algae and Zooplankton in Filtered Duluth-Superior Harbor Water.

Species	pH Levels	Exposure Duration (hr)	No. of Organisms per Exposure /Control	No. of Replicates per Exposure /Control
Chironomus dilutus	11.5, 12.0, and 12.2, and a control (no pH adjustment; natural pH of 7.6 - 8.0)	48	10	4
Lumbriculus variegatus	11.5, 12.0, and 12.2, and a control (no pH adjustment; natural pH of 7.6 - 8.0)	48	10	4

Residual Toxicity Experiments

Residual toxicity tests were undertaken to explore toxicity that may be associated with degradation of $Ca(OH)_2$ following a dilution step of 1:100 or 1:1000. Test organisms included the daphnid *Ceriodaphnia dubia* (< 24 hours old), the fathead minnow *Pimephales promelas* (< 24 hours old), and the benthic amphipod *Hyalella azteca* (7 to 8 days old). The pH of FHW stock solutions was adjusted to 11.5 or 12.2 as described above (see General Methods) and diluted with FHW to neutralize the pH and achieve a ratio of 1:100 or 1:1000. It should be noted that the control (untreated FHW) was not diluted as this process would have not altered the sample's pH level. The test water was not renewed.

All exposures were conducted in a 16:8 light:dark cycle, were 48 hours in length, involved 50 mL of solution in a 300 mL beaker, and consisted of three replicates. Table 4 arrays the test conditions and number of organisms used in the residual toxicity experiments.

Table 4. Numbers and Types of Organisms, and pH Levels Used for GSI Residual Toxicity Tests in Filtered Duluth-Superior Harbor Water (FHW).

Major Taxonomic Group	Type	Species	Treatment Groups	No. of Organisms per Exposure /Control	No. of Replicates per Exposure /Control	Total Number of Organisms
Zooplankton	< 24 hour old daphnids	*Ceriodaphnia dubia*	No dilution (control), 1:100 dilution of pH 11.5 and 12.2, and 1:1000 dilution of pH 11.5 and 12.2	10	3	3 x 10 x 5? = 150
	Benthic amphipods	*Hyalella azteca*	No dilution (control), 1:100 dilution of pH 11.5 and 12.2, and 1:1000 dilution of pH 11.5 and 12.2	10	3	3 x 10 x 5 = 150
Fish	< 24 hour old fathead minnows	*Pimephales promelas*	No dilution (control), 1:100 dilution of pH 11.5 and 12.2, and 1:1000 dilution of pH 11.5 and 12.2	15	3	3 x 15 5 = 225

Statistical Analysis

Where applicable, statistical analysis was performed with SigmaStat (v. 3.5, Systat Software Inc., Chicago, IL) statistical software. Prior to conducting an analysis of variance (ANOVA), the data were tested for normality and homogeneity of variance. If data were normally distributed and homogenous, survival was analyzed by ANOVA followed by Holm-Sidak, Dunn's, or Tukey's methods of means comparison test to determine the difference from control data. Significance level was tested at $\alpha=0.05$.

FINDINGS

pH Stability

Results of GSI bench-scale tests on pH stability are presented in figure 1. The pH of the two higher pH-treatment samples (i.e., pH of 12.2) and controls remained relatively stable, regardless of water type, over the 96 hour test period (figure 1). In contrast, samples adjusted to the lower pH level of 11.5 decreased to approximately 9.0 over the 96 hour test period (figure 1), indicating that this pH level is less stable. Differences were also detected between water types at this lower pH level, with the pH of both HOC and 50 % HOC water samples decreasing more rapidly than the pH of LW samples (figure 1).

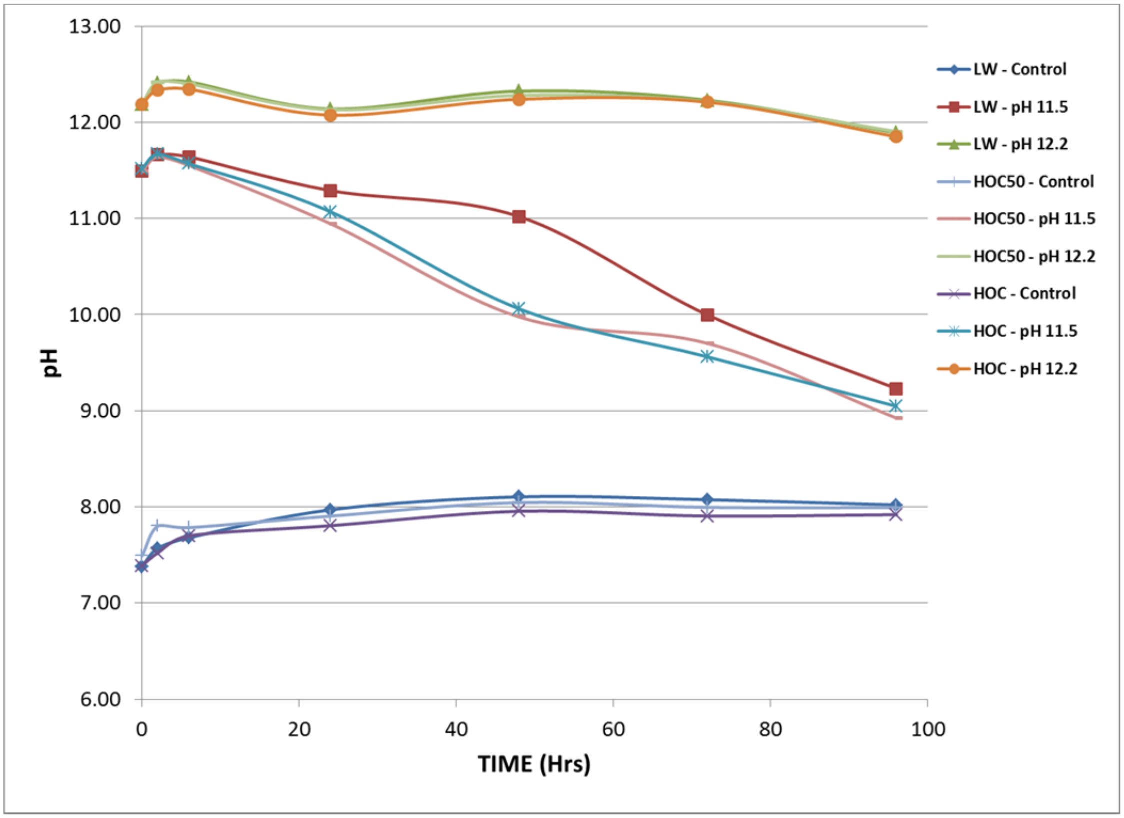

Figure 1. pH Stability of Two pH Treatments (11.5 and 12.2) and a Control (Natural pH of 7-8) in Laboratory Water (L), High Organic Content (HOC) Water and 50% HOC Water (HOC50) Over the 96 hour Test Period.

Dose Effectiveness Experiments

Results of GSI bench-scale dose effectiveness tests involving $Ca(OH)_2$ in FHW on the freshwater algae *Selenastrum* are presented in table 5. The three adjusted pH levels tested (i.e., 11.5, 12.0 and 12.2) had little effect on algae survival when measured immediately after exposure (i.e., at 0 hours). However, algae survival ranged from 0 – 33 % at 24 hours and 0 – 22 % at 48 hours, respectively, across all adjusted pH levels. Though complete mortality was not observed in treatments involving the lower pH level of 11.5, it should be noted that this pH level decreased to an average of 8.50 over the 48 hour test period (table 5). In contrast, the two higher pH levels (12.0 and 12.2) were more stable and remained near original levels over the same test period (table 5).

Table 5. Mean Percent Survival (Standard Deviation) of the Green Algae *Selenastrum capricornutum* Exposed to Three Levels of Adjusted pH in Filtered Duluth-Superior Harbor Water (FHW) Over a 48 Hour Exposure Period.

Treatment ID	Average pH during 0 hr exposure	Average pH during 24 hr exposure	Average pH during 48 hr exposure	Survival (%)		
				0 Hours	24 Hours	48 Hours
Control	7.76	8.10	8.15	99 (0.4)	100 (0)	100 (0)
11.5	11.57	10.39	8.50	100 (0)	*33 (18)	*22 (17)
12.0	11.99	11.78	11.77	100 (0)	*0 (0)	*0 (0)
12.2	12.23	12.18	12.11	100 (0)	*0 (0)	*0 (0)

*Holm-Sidak method indicates a statistically significant difference compared to the control (p<0.05)

Results of GSI bench-scale dose effectiveness tests involving $Ca(OH)_2$ on the freshwater rotifer *Brachionus calyciflorus* are presented in table 6. In FHW, survival ranged from 0 – 73 % at 24 hours for all adjusted pH levels tested and was 100 % at 24 hours in the unadjusted control. The pH of the 11.5 treatment samples declined to an average of 8.42 over the course of 24 hours, while the average pH of the higher two treatments (12.0 and 12.2) were 11.16 and 11.60, respectively, at 24 hours (table 6).

Table 6. Mean Percent Survival (Standard Deviation) of the Rotifer *Brachionus calyciflorus* Exposed to Various pH values in Filtered Duluth-Superior Harbor Water Over a 24 Hour Exposure Period.

Treatment ID	Average pH during 0 hr exposure	Average pH during 24 hr exposure	Survival (%)	
			0 Hours	24 Hours
Control	7.74	8.08	100 (0)	100 (0)
11.5	11.48	8.42	100 (0)	73 (14)
12.0	11.98	11.16	100 (0)	*0 (0)
12.2	12.20	11.60	100 (0)	*0 (0)

*Dunn's method indicates a statistically significant difference compared to the control (p<0.05)

Results of GSI bench-scale dose effectiveness tests involving $Ca(OH)_2$ on the freshwater daphnid *Daphnia magna* are presented in table 7. In FHW there was 0 % survival at two hours in all pH-adjusted treatment groups (p<0.05; table 7). In contrast, there was 100 % survival in the unadjusted control samples over the same time period (table 7).

Table 7. Mean Percent Survival (Standard Deviation) of the Daphnid *Daphnia magna* Exposed to Various pH Values in Filtered Duluth-Superior Harbor Water (FHW) Over a 2 Hour Exposure Period.

Treatment ID	Average pH during 0 hr exposure	Average pH during 2 hr exposure	Survival (%)	
			0 Hours	2 Hours
Control	7.76	8.08	100 (0)	100 (0)
11.5	11.57	10.74	100 (0)	*0 (0)
12.0	11.99	11.67	100 (0)	*0 (0)
12.2	12.23	12.05	100 (0)	*0 (0)

*Holm-Sidak method indicates a statistically significant difference compared to the control ($p<0.05$)

Results of GSI bench-scale dose effectiveness tests involving $Ca(OH)_2$ on cysts of the freshwater rotifer *Brachionus calyciflorus* are presented in table 8. When measured 48 hours after initial exposure, the two higher pH levels tested (12.0 and 12.2) had significantly ($p<0.05$) reduced cyst hatching compared to the control. There was an average of 60 % hatch in the control group, as compared to 39 % hatch in the pH 12.0 group and 21 % hatch in the pH 12.2 group (table 8). However, in contrast to the other dose effectiveness tests reported here, the average pH levels of the 11.5, 12.0, and 12.2 treatments at 48 hours were 8.49, 8.35, and 8.45, respectively, indicating that even the two higher treatments were less stable (table 8). This decline in pH across all samples is likely an artifact of the small test volume interacting with atmospheric carbon dioxide over the course of the 48 hour exposure.

Table 8. Mean Percent Hatch (Standard Deviation) of *Brachionus calyciflorus* Cysts Exposed to Various pH Values in Filtered Duluth-Superior Harbor Water (FHW) Over a 48 Hour Exposure Period.

TreatmentID	Average pH during 0 hr exposure	Average pH during 48 hr exposure	Hatch (%) at 48 Hours
Control	7.76	8.34	60 (16)
11.5	11.57	8.49	53 (22)
12.0	11.99	8.35	*39 (10)
12.2	12.23	8.45	*21 (7)

*Holm-Sidak method indicates a statistically significant difference compared to the control ($p<0.05$)

Results of GSI bench-scale dose effectiveness tests involving $Ca(OH)_2$ on the freshwater copepod *Eucyclops sp.* are presented in table 9. In FHW, the three adjusted pH levels tested had a significant effect on *Eucyclops* survival. There was 0 % survival across all pH-adjusted treatments when measured two hours following exposure (Table 9). In contrast, there was 100 % survival in the controls over the same test period. There was also little reduction in pH levels over the course of two hours, including for the lower pH of 11.5 (table 9).

Table 9. Mean Percent Survival (Standard Deviation) of the Copepod *Eucyclops sp.* Exposed to Various pH Values in Filtered Duluth-Superior Harbor Water (FHW) Over a 2 Hour Exposure Period.

Treatment ID	Average pH during 0 hr exposure	Average pH during2 hr exposure	Survival (%)	
			0 Hours	2 Hours
Control	7.76	8.06	100 (0)	100 (0)
11.5	11.57	10.77	100 (0)	*0 (0)
12.0	11.99	11.59	100 (0)	*0 (0)
12.2	12.23	12.05	100 (0)	*0 (0)

*Holm-Sidak method indicates a statistically significant difference compared to the control (p<0.05)

Sediment Dose Effectiveness Experiments

Results of GSI bench-scale sediment dose effectiveness tests involving $Ca(OH)_2$ in FHW on the benthic organisms *Chironomus dilutus* and *Lumbriculus variegatus* are presented in tables 10 and 11 respectively. Both species were exposed to three levels of adjusted pH (11.5, 12.0 and 12.2) and an unadjusted control, in the presence of sediment. Results of these tests indicate that *C. dilutus* survival was significantly (p<0.05) reduced in the higher pH treatments (12.0 and 12.2) compared to the control after 48 hours (table 10). Similarly, *L. variegatus* survival was significantly (p<0.05) reduced in the two higher pH treatments, but also in the 11.5 pH treatment (table 11). Control survival was 83 % for *C. dilutus* and 100 % for *L. variegatus* at 48 hours.

Table 10. Mean Percent Survival (Standard Deviation) of the Midge Larvae *Chironomus dilutus* Exposed to Various pH Values in Filtered Duluth-Superior Harbor Water (FHW) Over a 48 Hour Exposure Period.

Sample ID	Average pH during 0 hr exposure	Average pH during 24 hr exposure	Average pH during 48 hr exposure	Survival (%)	
				0 Hours	48 Hours
Control	7.74	7.60	7.63	100 (0)	83 (13)
11.5	11.48	9.40	8.40	100 (0)	68 (15)
12.0	11.98	11.25	9.83	100 (0)	*25 (24)
12.2	12.20	11.68	11.22	100 (0)	*3 (5)

*Holm-Sidak method indicates a statistically significant difference compared to the control (p<0.05)

Table 11. Mean Percent Survival (Standard Deviation) of the Annelid *Lumbriculus variegatus* Exposed to Various pH Values in Filtered Duluth-Superior Harbor Water (FHW) Over a 48 Hour Exposure Period.

Sample ID	Average pH during 0 hr exposure	Average pH during 24 hr exposure	Average pH during 48 hr exposure	Survival (%) 0 Hours	Survival (%) 48 Hours
Control	7.74	7.45	7.58	100 (0)	100 (0)
11.5	11.48	9.43	8.25	100 (0)	*3 (5)
12.0	11.98	11.24	9.82	100 (0)	*0 (0)
12.2	12.20	11.67	11.06	100 (0)	*0 (0)

*Holm-Sidak method indicates a statistically significant difference compared to the control (p<0.05)

Residual Toxicity Experiments

Results of GSI bench-scale residual toxicity tests involving $Ca(OH)_2$ in FHW on the daphnid *Ceriodaphnia dubia* (< 24 hours old), the fathead minnow *Pimephales promelas* (< 24 hours old), and the benthic amphipod *Hyalella azteca* (7 to 8 days old) are presented in tables 12 and 13 respectively. The initial pH of the control water in these tests was 7.76 and rose to 8.17 over the course of 48 hours (table 12). The average pH of the treatment samples ranged from 8.23 to 9.02 at 0 hours and 8.04 to 8.34 at 48 hours across all species tested in the 1:100 dilution (table 12). Initial pH values were slightly lower in the 1:1000 dilution when compared to the 1:100 dilution, and ranged from 7.77-7.82 at 0 hours to 8.06-8.17 at 48 hours (table 12). Percent survival for all three sensitive organisms tested was high and ranged from 90 - 100 % (table 13), with no statistically significant difference observed between the treatment and control samples for all three species tested.

Table 12. Average (n=3) pH Levels of Residual Toxicity Dilution Test Samples in Filtered Duluth-Superior Harbor Water (FHW) Over a 48 Hour Exposure Period.

	Initial pH	Dilution 1:100 0 hr	Dilution 1:100 24 hr	Dilution 1:100 48 hr	Dilution 1:1000 0 hr	Dilution 1:1000 24 hr	Dilution 1:1000 48 hr
C. dubia	Control	7.76		8.15		8.17	
	11.5	8.23	8.12	8.28	7.77	8.11	8.17
	12.2	9.02	8.21	8.34	7.82	8.18	8.25
H. azteca	Control	7.76		8.06		8.01	
	11.5	8.23	8.07	8.04	7.77	8.13	8.06
	12.2	9.02	8.13	8.21	7.82	8.06	8.11
P. promelas	Control	7.76		8.02		8.04	
	11.5	8.23	8.07	8.09	7.77	8.03	8.07
	12.2	9.02	8.11	8.19	7.82	8.00	8.10

Table 13. Average (Standard Deviation) Percent Survival of Species in Samples from Residual Toxicity Dilution Tests in Filtered Duluth-Superior Harbor Water (FHW) Over a 48 Hour Exposure Period.

	Initial pH	Dilution 1:100 % Survival	Dilution 1:1000 %Survival
C. dubia	Control	97 (5.8)	
	11.5	90 (10)	100 (0)
	12.2	100 (0)	100 (0)
H. azteca	Control	100 (0)	
	11.5	97 (5.8)	97 (5.8)
	12.2	100 (0)	100 (0)
P. promelas	Control	100 (0)	
	11.5	100 (0)	100 (0)
	12.2	100 (0)	98 (3.9)

GSI QUALITY MANAGEMENT

Standard Operating Procedures (SOPs)

Standard operating procedures (SOPs) are used to implement all GSI bench-scale test activities. This facilitates consistent conformance to technical and quality system requirements and increases data quality. The SOPs include both programmatic and technical processes and procedures such as organism culturing; sample collection, labeling, analysis and custody; and safety. GSI SOPs follow a common format and include specific QAQC procedures and metrics. They are grounded in published standard methods. They are also consistent with international and domestic guidelines where they exist. All GSI SOPs are subject to periodic review and revision to assure that the most up to date approaches are employed. Table 14 outlines the GSI SOPs utilized for these tests. Any deviations made to SOPs during the experiment were recorded and also approved by the GSI Lead On-Site Investigator for Bench-Scale Studies as soon as practicable, as well as communicated to the GSI Senior QAQC Officer.

Table 14. GSI Bench-Scale Standard Operating Procedures (SOPs) Utilized for Tests of the $Ca(OH)_2$ Bench-Scale BWTS.

SOP Category	Subcategory	SOP Title	SOP Code
General	Administration	Procedure for Record Keeping	GSI/SOP/G/A/RK/1
		Procedure for Data Entry, Data Quality Control and Database Management	GSI/SOP/G/RA/DM/1
Research Activities	Sample Custody	Procedure for Labeling GSI Bench-Scale Samples	GSI/SOP/G/RA/SC/4
	General Laboratory	Procedure for Verification of Laboratory Balances	GSI/SOP/BS/RA/GL/1
	Chemical Degradation	Bench-Scale Procedure for Examining the Aquatic Degradation of Active Substance(s) in a Ballast Treatment System (DRAFT)	GSI/SOP/BS/RA/CD/1
	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Copepod *Eucyclops spp.* (DRAFT)	GSI/SOP/BS/RA/DE/1
		Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Cladoceran *Daphia magna* (DRAFT)	GSI/SOP/BS/RA/DE/2
		Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/3
		Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using Cysts of the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/4
		Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using *Selenastrum capricornutum*	GSI/SOP/BS/RA/DE/5
	Residual Toxicity	Procedure for Measuring Acute Residual Toxicity Using *Ceriodaphnia dubia*	GSI/SOP/BS/RA/RT/1
		Procedure for Measuring Acute Residual Toxicity Using Fathead Minnows (*Pimephales promelas*)	GSI/SOP/BS/RA/RT/2
		Procedure for Measuring Acute Residual Toxicity Using the Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/RT/4
	Sediment Dose Effectiveness	Procedure for Conducting a 48-Hour Sediment Toxicity Test With *Chironomous dilutus* (DRAFT)	GSI/SOP/BS/RA/ST/1
		Procedure for Conducting a 48-Hour Sediment Toxicity Test With *Lumbriculus variegatus* (DRAFT)	GSI/SOP/BS/RA/ST/2

Quality Assurance/Quality Control (QA/QC)

Test conditions were monitored daily for changes in water quality parameters that might negatively affect the survival of the test organisms (i.e., temperature, hardness, alkalinity, conductivity, and dissolved oxygen). Daily and weekly calibration of test meters ensured optimal performance.

Data Management and Archiving

Data were recorded on data collection forms or in specific laboratory notebooks. All hard- and electronic-copies of data and records will be maintained by LSRI and archived for a period of five years.

CONCLUSION

In conclusion, the higher two pH levels (12.0 and 12.2) significantly reduced live concentrations of the entire range of aquatic organisms tested in experimental water, with green algae, adult rotifers, adult copepods and < 24 hour old cladocerans exhibiting less than 1 % survival 48 hours. In many cases, the experiment was cut short from the planned duration (i.e., 24 or 48 hrs) because complete mortality was observed within two hours.

In the sediment dose effectiveness experiments, after the full 48 hours, *C. dilutus* had a reduced (68 %) survival in the pH 11.5 treatment, and a significantly reduced 25 % and 3 % survival in the pH 12.0 treatment and pH 12.2 treatment, respectively. Annelid *L. variegatus* was more sensitive, showing significant reductions at all three pH levels after 48 hours to 0% for the 12.0 and 12.5 pH exposures, and 3% for the 11.5 pH exposure.

In all of the experiments, the pH level dropped over time due to contact with atmospheric carbon dioxide even though exposure containers were covered. This effect was greatest in those dose effectiveness experiments that used a small exposure volume (i.e. rotifers). The stability of the lowest level of pH tested (11.5) was somewhat inconsistent over the course of the experiments reported here perhaps due to differences in water types and insolubility of the lime slurry.

No residual toxicity was observed in any of the three species tested after either dilution ratio tested. The average pH of the treatment samples ranged from 8.23 to 9.02 at 0 hours the 1:100 dilution and ranged from 7.77 to 7.82 at 0 hours in the 1:1000 dilution. Hydrated lime met USEPA acceptable criteria of 6.5 - 9.0 at both dilutions levels tested. This latter property of the lime treatment process provides support for the application of the lime to yield the highest pH levels tested here, which were also the most stable and consistently effective.

Residual slurry particulates may be an operational and disposal issue in a shipboard application where there is limited mixing capacity. When hydrated lime is used to treat the ballast water in a full scale application, mixing apparati may be important to reduce settling.

This bench scale research gives support for further research on the proposal to use hydrated lime to treat organisms in ballast water. Its applicability could be general in scope, including the ballast onboard (i.e., BOB) and no ballast onboard (i.e., NOBOB) ship conditions, provided operational concerns, such as those noted above are addressed. Simple mixing devises installed in the ballast tanks to ensure residual slurry particles don't settle out may be an important step in this regard. The price of hydrated lime is much less expensive than sodium hydroxide and other biocides making further research warranted.