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

Sodium Chloride (NaCl) - Brine

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ABSTRACT

The Great Ships Initiative (GSI) conducts bench-scale (i.e., laboratory scale) research to aid developers of innovative technologies which could have application as ballast treatment systems. This report describes 2008 findings from bench-scale evaluations of a ballast treatment involving sodium chloride (NaCl), i.e., brine. The GSI undertook the evaluations at the request of its Advisory Committee to investigate the use of brine as a contingency treatment for ships reporting no ballast onboard but which have not purged unpumpable residuals enroute. Based on a review of the scientific literature, bench-scale research services were undertaken of NaCl in doses up to 75 ppt. In the case of brine, bench tests were undertaken by GSI exclusively to assist range-finding for effective doses, because there was adequate information about potential residual toxicity already in the literature. Please see www.greatshipsinitiative.org for more information about GSI's bench-scale testing program.

Brine was effective at killing a broad range of aquatic organisms, including adult rotifers, adult cladocerans and adult copepods which had 100 percent mortality after two hours exposure to 15 and 50 ppt. In contrast, the green alga, *Selenastrum* sp., was highly resistant, with 99 percent survival following 48 hours exposure to the highest salinity level tested (75 ppt). Brine at the higher doses tested also proved effective at killing benthic species in the presence of sediments in the sample. There was 0 percent survival for both the insect *Chironomus dilutus* and oligochaete *Lumbriculus variegates* in the 15 ppt and greater salinity doses following 48 hours exposure. These tests support the prospect that brine could provide additional protection against discharge of aquatic invasive species by ships in instances in which residual ballast has not been adequately flushed or treated.

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INTRODUCTION

This technical report presents quantitative and measured findings from GSI bench-scale evaluations of sodium chloride (NaCl), i.e., brine, as a possible ballast treatment with application to the Great Lakes. Brine is proposed as a contingency treatment for ships reporting no ballast onboard (NOBOB) which have not purged unpumpable residuals enroute. GSI undertook these bench-scale tests during 2008 at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior in Superior, Wisconsin. Tests focused on dose effectiveness, i.e., determination of the concentration of brine most harmful to a range of freshwater organisms under different water quality conditions. The GSI bench analysis did not include chemical degradation and residual toxicity tests because the dissociation of NaCl into its respective ions and residual toxicity under a number of dilution scenarios has been extensively researched. A summary of the GSI bench test findings for non-scientific audiences can be accessed at http://www.nemw.org/GSI/GSI-BS-P-FS-7.pdf.

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

BACKGROUND

The Great Ships Initiative

The Great Ships Initiative (GSI)[1] is a collaborative effort whose objective is to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System. To that end, the GSI established sophisticated independent third party ballast treatment evaluation capabilities at three scales—bench, land-based, and shipboard. Each scale is dedicated to addressing specific evaluation objectives:

- GSI Bench-Scale Tests
  - Range finding for effective doses under a range of ambient conditions;
  - Chemical degradation over time under a range of ambient conditions;
  - Detection of any residual toxicity under a range of ambient conditions; and
  - Confirmation of treatment process.

1 www.greatshipsinitiative.org

- GSI Land-Based Tests
    - Detection of scale-up, mechanical operation issues;
    - Effectiveness of a dose with respect to the full range of ambient organisms; and
    - Detection of any whole water effluent toxicity.

- GSI Shipboard Tests
    - Confirmation of biological and operational performance as expected in the ship environment; and
    - Confirmation of performance as expected under a broad range of ambient conditions.

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

GSI tests are objective third party assessments subject to rigorous quality assurance, quality control (QA/QC) procedures. They are completely independent of any vested interest in outcomes. All procedures, methods materials and findings are publicly posted on the GSI website (www.greatshipsinitiave.org). The GSI tests are supported by general project funds which derive from federal and state agency grants and contributions, Great Lakes port contributions, and in-kind contributions by the local government and universities. None of these funds come to the GSI with any strings (other than public disclosure).

To assure relevancy of test output, GSI test protocols are as consistent with the International Maritime Organization (IMO) Convention and federal requirements as practicable. In particular, bench testing directly supports IMO G9 evaluations, and land-based testing directly supports IMO G8 and G9 evaluations.

Ms. Allegra Cangelosi of the Northeast-Midwest Institute is the Principal Investigator and Manager of the GSI. Researchers from the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI), and the University of Minnesota-Duluth's Natural Resources Research Institute, among others, provide critical scientific and technical expertise and implementation services to GSI's biological research activities, and the GSI generally. Mr. Matthew TenEyck, of LSRI, leads the bench-testing for GSI. A GSI Advisory Committee comprising top-level officials of key stakeholder groups helps steer the GSI providing crucial assistance in making GSI award decisions and fund-raising. The GSI Advisory Committee includes elected leadership, environmental organizations, port directors and federal officials from the United States and Canada, and industry representatives. 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.

The largest contributor of GSI operating funds is the United States Department of Transportation, including its Maritime Administration, and the Saint Lawrence Seaway Development Organization. GSI also receives significant funds and in-kind contributions from the National Oceanic and Atmospheric Administration, the Canadian St. Lawrence Seaway Management Corporation, the City of Superior, Wisconsin, and approximately ten U.S. and Canadian ports in the Great Lakes.

GSI Bench Tests

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

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

GSI bench-scale dose effectiveness tests help determine the range of concentrations of an active substance that is harmful to a variety of robust freshwater zooplankton, algae and bacteria known to be relatively resilient to stressors. Dose effectiveness test results are expressed as percent survival, percent mortality, and percent hatch. Where applicable, they are also expressed in terms of a series of absolute quantifications: LC_{99}, i.e., the experimentally derived concentration of an active substance estimated to kill 99 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 treatment system involving active substances. No organisms are used in association with these analytical assays; instead test solutions are analyzed for their concentration of active substance (or active component of the substance). Test results are expressed as the percent change in active ingredient concentration.

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

ABOUT SODIUM CHLORIDE (NaCl)

At the request of its Advisory Committee, the GSI investigated the potential for using sodium chloride (NaCl), i.e., brine, as a contingency treatment for ships reporting no ballast onboard (NOBOB) which have not purged unpumpable residuals enroute. Tests evaluated brine effectiveness in doses up to 75 ppt, i.e., within the range determined to be effective at inactivating a range of freshwater aquatic organisms by Bradie *et al*. (2008).

METHODS

Standard Operating Procedures and Quality Assurance

GSI bench-scale evaluations are in keeping with GSI standard operating procedures (SOPs). In general, GSI SOPs are grounded in published standard methods and modified to reflect ballast treatment circumstances. They are also consistent with international and domestic guidelines where they exist. All GSI SOPs were peer-reviewed prior to acceptance and are subject to periodic review and revision to assure that the most up to date approaches are employed. In addition, all GSI bench-scale research activities comply strictly with a detailed Quality Assurance Project Plan (QAPP) which is consistent in format, detail and stringency with United States Environmental Protection Agency requirements. Table 1 outlines the GSI bench-scale SOPs utilized for the brine tests.

Tests were initiated with healthy, vigorous animals. Reference toxicant tests were performed with all test species prior to the start of the definitive test. Control charts are available upon request. Test conditions were monitored daily for parameters that might affect the outcome of the test (i.e., temperature, and dissolved oxygen). Daily and weekly calibration of test meters ensured optimal performance. The GSI Quality Assurance and Quality Control Officer performed inspections of logbooks, recorded measurements, and instrumentation used during the tests. Any deviations were discussed with the principal investigator and documented in the study logbook. Duplicate samples were analyzed and findings compared for percent similarity consistent with QAQC criteria.

Table 1. GSI Bench-Scale Standard Operating Procedures (SOPs) Utilized for Sodium Chloride (NaCl), i.e., Brine, Tests.

SOP Category	Subcategory	SOP Title	SOP Code
General	Administration	Procedure for Record Keeping	GSI/SOP/G/A/RK/1
General	Sample Custody	Procedure for Custody of GSI Bench-Scale Samples	GSI/SOP/G/RA/SC/1
General	Sample Custody	Procedure for Labeling Bench-Scale Samples	GSI/SOP/G/RA/SC/4
Research Activities	Culturing	Procedure for Culturing the Midge *Chironomus dilutus*	GSI/SOP/BS/RA/C/1
Research Activities	Culturing	Procedure for Culturing the Cladocerans *Daphnia magna* and *Ceriodaphnia dubia*	GSI/SOP/BS/RA/C/3
Research Activities	Culturing	Procedure for Culturing *Selenastrum Capricornutum*	GSI/SOP/BS/RA/C/4
Research Activities	Culturing	Procedure for Culturing the Oligochaete *Lumbriculus variegatus*	GSI/SOP/BS/RA/C/5
Research Activities	Culturing	Procedure for Culturing the Copepod *Eucyclops spp.*	GSI/SOP/BS/RA/C/6
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Copepod *Eucyclops spp.*	GSI/SOP/BS/RA/DE/1
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Daphnid *Daphia magna*	GSI/SOP/BS/RA/DE/2
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Treatment System Using the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/3
Research Activities	Dose Effectiveness	Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using Cysts of the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/4
Research Activities	Dose Effectiveness	Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using *Selenastrum capricornutum*	GSI/SOP/BS/RA/DE/5
Research Activities	Dose Effectiveness	Procedure for Exposing Test Organisms to an Active Substance	GSI/SOP/BS/RA/DE/7
Research Activities	Sediment Toxicity	Procedure for Conducting a 48-Hour Sediment Toxicity Test With *Chironomous dilutus*	GSI/SOP/BS/RA/ST/1
Research Activities	Sediment Toxicity	Procedure for Conducting a 48-Hour Sediment Toxicity Test With *Lumbriculus variegatus*	GSI/SOP/BS/RA/ST/2

General Methods

Testing Apparatus and Venue

Testing apparatus consisted of a set of four 300 mL borosilicate high-form beakers, three 250 mL Elenmeyer flasks, and several tissue culture plates which were housed within environmental chambers with controlled temperature and light regimes. The environmental chambers are located within a laboratory equipped with adequate ventilation, electrical connections, and climate control located at the LSRI testing facility.

Method of Preparing Sample Water

Sample water consisted of Duluth-Superior Harbor water collected from a depth of approximately 3 meters in the Duluth-Superior Harbor of Lake Superior. Alkalinity ranged from 65-69 mg/L as $CaCO_3$. Non-purgeable organic carbon ranged from 16-22 mg/L. The water is stained with tannins. Prior to use the water was filtered in sequence through a Whatman GF/B glass fiber filter followed by a Millipore 0.45 μm HA membrane filter.

Method of Salinity Adjustment

The salinity of the filtered Duluth-Superior Harbor water (FHW) was adjusted to 0.4, 1.4, 4.5, 15, 50 and 75 ppt. This was achieved by dissolving appropriate quantities of NaCl in a beaker containing FHW. Solutions were stirred until all the NaCl had dissolved. Aliquots of solutions were then removed and the salinity verified using a hand-held salinity refractometer. Filtered harbor water without the addition of NaCl was used as the control.

Method of Preparing Sediment Samples

Approximately 3 gallons of sediment from Duluth-Superior Harbor were collected from a depth of 3 meters using a hand held Eckman dredge. The sediment was placed into a pre-cleaned polyethylene bucket, transported to the laboratory and stored at 4.0 °C with approximately half an inch of overlying water and minimal head space. Prior to testing, the sediment was mixed for 15 minutes using a commercial drill equipped with a stainless steel mortar paddle. At 5 minute intervals, the sediment was briefly stirred manually to ensure further homogeneity. After homogenization, approximately 100 mL of sediment were added to a 300 mL high form beaker. Approximately 150 mL of test water were then added slowly down the side of the beaker to minimize the agitation of the underlying sediment.

Organisms

Freshwater organisms selected for these GSI dose effectiveness tests span a range of life stages and are known to be relatively resilient to stressors. They included juvenile (less than 24 hours old) cladocerans, adult copepods, newly hatched rotifers, rotifer resting eggs, and a green alga.

The effect of brine on two sediment dwelling freshwater organisms—juvenile (10-12 day old) insects and adult oligochaetes—was also investigated.

Exposures

Water and sediment samples with up to five salinity levels and a control (no salinity adjustment) were prepared and held at a temperature of 25.0 °C ± 1.0 °C. Animals were added to exposure treatments by gently transferring organisms from a clear Pyrex® sorting pan using a large bore dropper. All organism exposures except for those involving rotifer adults and rotifer resting eggs took place in complete darkness over 48 hours, with 50 mL of exposure solution in a 300 mL beaker, and three replicates. Tests on rotifer adults and rotifer resting eggs involved four replicates and 2.0 mL of exposure solution. In addition, adult rotifers were exposed in complete darkness for 24 hours due to their sensitivity. The rotifer resting eggs were exposed to continuous light to stimulate hatching.

Table 2 describes the exposure conditions across organism type while table 3 arrays the types and numbers of organisms analyzed, the exposure concentrations, and the number of replicates per dose effectiveness test. Test water was not renewed during the tests.

Mortality Measurement

In all non-sediment tests, periodic observations were made on mortality, and measured water quality parameters including temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness (data available on request). Mortality was measured by counting the number of dead organisms based on active and reactive mobility assessments at the appropriate time period. If organisms were immobile they were further examined under a dissecting microscope to check for internal movement or heart beat. If none was evident, the organism was deemed dead.

Survival in sediment tests was analyzed only once after the 48 hour exposure period because the analysis process was necessarily destructive of the samples in order to assure that burrowing organisms were counted.

Table 2. Exposure Conditions for GSI Dose-Effectiveness Tests on Zooplankton, Algae, Insects and Oligochaetes.

Organism	Exposure Volume per Replicate (mL)	Sediment Volume per Replicate (mL)	Exposure Duration (hr)	Light:Dark Cycle (hr)	Temperature (º C)
Cladoceran (*Daphnia magna*)	50	0	48	0:24	25±1.0
Copepods (*Eucyclops* spp.)	50	0	48	0:24	25±1.0
Newly hatched rotifers, (*Brachionus calyciflorus*)	2	0	24	0:24	25±1.0
Rotifer resting eggs, (*Brachionus calyciflorus*)	2	0	48	24:0	25±1.0
Green alga (*Selenastrum* spp.)	50	0	48	0:24	25±1.0
Insect (*Chironomus dilutus*)	150	100	48	0:24	25±1.0
Oligochaete (*Lumbriculus variegates*)	150	100	48	0:24	25±1.0

Table 3. Numbers and Types of Organisms, and Salinity Levels Used for GSI Dose-Effectiveness Tests on Zooplankton, Algae, Insects and Oligochaetes.

Organism Type	Species	Test Water Type	Initial Salinity Levels	No. of Organisms per Exposure /Control	No. of Replicates per Exposure /Control	Total Number of Organisms
Juvenile Cladocerans	*Daphnia magna*	FHW	0.41, 1.35, 4.5, 15 and 50 ppt, and a control (no salinity adjustment)	10	3	4 x 10 x 3 = 120
Adult copepods	*Eucyclops* spp.	FHW	0.41, 1.35, 4.5, 15 and 50 ppt, and a control (no salinity adjustment)	10	3	4 x 10 x 3 = 120
Adult rotifers	*Brachionus calyciflorus*	FHW	0.41, 1.35, 4.5, 15 and 50 ppt, and a control (no salinity adjustment)	5	4	4 x 5 x 4 = 80
Rotifer resting eggs	*Brachionus calyciflorus*	FHW	0.41, 1.35, 4.5, 15 and 50 ppt, and a control (no salinity adjustment)	20	4	4 x 20 x 4 = 320
Green alga	*Selenastrum spp.*	FHW	0.61, 2.03, 6.75, 22.5 and 75 ppt, and a control (no salinity adjustment)	200,000 cells/mL	3	4 x 200,000 x 3 = 2,400,000
Insect	*Chironomus dilutus*	FHW	0.41, 1.35, 4.5, 15 and 50 ppt, and a control (no salinity adjustment)	10	4	40
Oligochaete	*Lumbriculus variegates*	FHW	0.41, 1.35, 4.5, 15 and 50 ppt, and a control (no salinity adjustment)	10	4	40

Statistical Methods

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

FINDINGS

Results of these GSI bench-scale dose effectiveness tests on brine toxicity on robust species of freshwater organisms are presented in table 4. Brine proved ineffective at inactivating the algal test species—there was 99 percent survival at 48 hours exposure for the green alga *Selenastrum sp*. at the highest salinity level tested (75 ppt), and effective in the presence and absence of sediment at inactivating the zooplankton and benthic organisms tested. The relatively low dose of 15 ppt significantly reduced numbers of live organisms across all zooplankton groups tested—there was 0 percent survival at this dose after two hours for the cladoceran *Daphnia magna,* the copepod *Eucyclops sp*., and the adult rotifer *Branchionus calyciflorus*. Fifteen ppt salinity also resulted in 0 percent hatch of rotifer cysts after 48 hours.

In the presence of sediment, brine treatment within the proposed salinity range also was significantly effective at reducing live numbers of the insect *Chironomus dilutus* and oligochaete *Lumbriculus variegates* (table 5). There was 0 percent survival in both species after 48 hours at 15 ppt salinity. Survival of these two species may have been affected by the treatment prior to the 48 hour test conclusion, especially at the higher doses, but earlier observations were not taken as noted above.

Table 4. Percent Survival of Species (Standard Error) Following 24 and 48 Hours of Exposure to Sodium Chloride (NaCl) at Different Salinity Levels.

Major Taxonomic Group	Type	Species	Water Type	Salinity Level (ppt)	Survival (%)	
					24 Hours	48 Hours
Algae	Green alga	*Selenastrum sp.*	FHW	Control	100 (0)	100 (0)
				0.61	100 (0)	100 (0)
				2.03	100 (0)	100 (0)
				6.75	100 (0)	100 (0)
				22.5	100 (0)	100 (0)
				75	99 (0)	99 (0)
Zooplankton	Cladoceran	*Daphnia magna*	FHW	Control	97 (1.1)	97 (1.1)
				0.41	93 (2.1)	83 (2.8)
				1.35	100 (0)	83 (1.1)
				4.5	83 (2.8)	63 (2.8)*
				15	0 (0) §	N/A
				50	0 (0) §	N/A
	Copepod	*Eucyclops sp.*	FHW	Control	100 (0)	100 (0)
				0.41	100 (0)	100 (0)
				1.35	100 (0)	100 (0)
				4.5	43 (4.3)*	6 (2.1)*
				15	0 (0) §*	N/A
				50	0 (0) §*	N/A
	Rotifer	*Branchionus calyciflorus*	FHW	Control	100(0)	N/A
				0.41	100(0)	N/A
				1.35	100(0	N/A
				4.5	0 (0)*	N/A
				15	0 (0 §*	N/A
				50	0 (0) §*	N/A
		B. calyciflorus cysts ∞	FHW	Control	Not Measured	16 (4.3)
				0.41	Not Measured	11.3 (2.4
				1.35	Not Measured	13.8 (2.4)
				4.5	Not measured	15 (2.9)
				15	Not measured	0 (0)*
				50	Not measured	0 (0)*

§ Endpoint was 2 hours.
∞ Measured as percent hatch.
* The difference in the median values among the treatment groups are greater than would be expected by chance, i.e., there is a statistically significant difference ($p<0.05$).

Table 5. Percent Survival of *Chironomus dilutus* and *Lumbriculus variegatus* (Standard Error) Following 48 Hours of Exposure to Sodium Chloride (NaCl) at Different Salinity Levels in Sediment Collected from the Duluth-Superior Harbor.

Type	Species	Water Type	Salinity Level (ppt)	Survival (%)
				48 Hours
Insect	*Chironomus dilutus*	FHW	Control	80 (1.9)
			0.41	62 (2.4)
			1.35	74 (2.1)
			4.5	74 (2.1
			15	0 (2.5)*
			50	0 (0*
Oligochaete	*Lumbriculus variegatus*	FHW	Control	100 (0)
			0.41	94 (1)
			1.35	100 (0)
			4.5	100 (0)
			15	0 (0) *
			50	0 (0) *

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

Table 6 provides the lowest lethal concentrations across all test species that resulted in 99 percent mortality. Based on the LC_{99} values the adult rotifer is the most sensitive (4.9 ppt) while the insect *C. dilutus* is the least sensitive (44.1 ppt) (table 6). The cladoceran and the copepod had similar sensitivities (table 6).

Table 6. Exposure to Sodium Chloride (NaCl) at Different Salinity Levels that Resulted in 99 Percent Mortality (LC_{99}), the Lowest-Observed-Effect-Concentration (LOEC) and the No-Observed-Effect-Concentration (NOEC).

Type	Species	LC_{99} (ppt)	NOEC (ppt)	LOEC (ppt)
Algae	*Selenastrum sp.*	NC	NC	NC
Rotifer	*B. calyciflorus*	4.9	1.5	5.0
Cladoceran	*D. magna*	14.4	1.7	4.7
Copepod	*Eucyclops sp*	14.4	1.0	4.6
Oligocheate	*L variegatus*	12.5	3.6	12.6
Insect	*C. dilutus*	44.1	3.6	12.6

Quality Assurance/Quality Control

Approximately 10 % of the samples analyzed were collected and analyzed in duplicate. The results of the duplicate analysis were consistent with GSI QA/QC criteria and are provided in the following table (table 7).

Table 7. Duplicate Analysis of Brine Samples.

	Duplicate Agreement (%)
Mean	95
Standard Deviation	12
Maximum	100
Minimum	50
Number of duplicate samples	22

Survival in the controls for the zooplankton tests in DHW ranged from 97-100 % which is greater than the acceptable criteria of 80 % listed in the GSI QAPP and SOPs. However, there was an average of 70 % survival in the *C. dilutus* sediment test control. Though this level fell below acceptable criteria, treatment effects were detectable, so the experiment was not repeated.

CONCLUSION

In conclusion, the higher salinity levels were effective at killing the broad range of aquatic animals tested—including test species that spend part or all of their life cycle in the surface of sediments, but not the test green alga *Selenastrum sp*.

These tests support the prospect that brine could provide additional protection against discharge of aquatic invasive species by ships in instances in which residual ballast has not been adequately flushed or treated. It is unlikely, however, to supply a general purpose ballast treatment for ships that is adequately effective across the range of relevant taxonomic groups.

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